nature microbiology

ARTICLES

https://doi.org/10.1038/s41564-018-0337-x

The neuroactive potential of the human gut microbiota in quality of life and depression

Mireia Valles-Colomer[1,2], Gwen Falony[1,2], Youssef Darzi[1,2], Ettje F. Tigchelaar[3], Jun Wang[1,2], Raul Y. Tito[1,2,4], Carmen Schiweck[5], Alexander Kurilshikov[3], Marie Joossens[1,2], Cisca Wijmenga[3,6], Stephan Claes[5,7], Lukas Van Oudenhove[7,8], Alexandra Zhernakova[3], Sara Vieira-Silva[1,2,9] and Jeroen Raes[1,2,9]*

The relationship between gut microbial metabolism and mental health is one of the most intriguing and controversial topics in microbiome research. Bidirectional microbiota–gut–brain communication has mostly been explored in animal models, with human research lagging behind. Large-scale metagenomics studies could facilitate the translational process, but their interpretation is hampered by a lack of dedicated reference databases and tools to study the microbial neuroactive potential. Surveying a large microbiome population cohort (Flemish Gut Flora Project, $n = 1{,}054$) with validation in independent data sets ($n_{total} = 1{,}070$), we studied how microbiome features correlate with host quality of life and depression. Butyrate-producing *Faecalibacterium* and *Coprococcus* bacteria were consistently associated with higher quality of life indicators. Together with *Dialister*, *Coprococcus* spp. were also depleted in depression, even after correcting for the confounding effects of antidepressants. Using a module-based analytical framework, we assembled a catalogue of neuroactive potential of sequenced gut prokaryotes. Gut–brain module analysis of faecal metagenomes identified the microbial synthesis potential of the dopamine metabolite 3,4-dihydroxyphenylacetic acid as correlating positively with mental quality of life and indicated a potential role of microbial γ-aminobutyric acid production in depression. Our results provide population-scale evidence for microbiome links to mental health, while emphasizing confounder importance.

Neural, endocrine and immune communication lines tightly link the human gut microbiota with the host central nervous system. Communication along these lines has been suggested to be bidirectional, with the gut microbiota playing an active role in processes linked to brain development and physiology, psychology and behaviour[1]. This role would not be limited to modulation of host neural, hormonal and immune responses[2], but also encompasses regulation of intestinal epithelium and blood–brain barrier permeability[3] and both production and degradation of neuroactive compounds[4]. Mediators of microbiota–gut–brain communication affected by microbial metabolism include short-chain fatty acids (for example, butyrate), neurotransmitters (for example, serotonin and γ-aminobutyric acid (GABA)), hormones (for example, cortisol) and immune system modulators (for example, quinolinic acid).

Advances in sequencing technology enabled the exploration of the role of the gut microbiota in a broad range of neurological and psychiatric disorders and diseases including larger-scale analysis of self-reported conditions[5] or clinical studies of depression[6–9], Alzheimer's disease[10] and Parkinson's disease[11]. While such pioneering studies generated the first candidate pathology-associated taxa, they were generally underpowered or did not take into account the confounding effects of microbiome covariates[12]. Complementary to disease association studies, rodent models have been used to explore a potential causative role of the microbiota in behavioural alterations. Unfortunately, translation of model-based preclinical findings to the complex human phenotype has been shown to be far from straightforward[13]. With only a limited number of exceptions[8,14], sequencing-based analyses of microbiota alterations in neurological pathologies have focused on taxonomic composition. Functional interpretation of metagenomes in a microbiota–gut–brain context remains challenging and is hampered by the lack of a dedicated reference database of gut microbial neuroactive metabolic potential.

In this study, we first assess gut microbiota compositional covariation with quality of life (QoL) indicators and general practitioner-reported depression in the Belgian Flemish Gut Flora Project (FGFP) population cohort ($n = 1{,}054$)[15]. We validate results both in the Dutch LifeLines DEEP (LLD) cohort with associated QoL and self-reported depression metadata ($n = 1{,}063$)[16,17] and in previously published case–control studies on depression[6–9]. To facilitate the functional analyses of the gut microbiota neuroactive metabolic potential, we develop a module-based analytical framework enabling targeted profiling of the microbial pathways involved in neuro-microbiome mediator metabolism. Through reference genome mining, we catalogue the neuroactive potential of gut isolates. Finally, application of these gut–brain modules in a shotgun-sequenced subset of the FGFP ($n = 150$) and validation in the LLD metagenomes data set ($n = 1{,}063$), and among a patient

[1]Department of Microbiology and Immunology, Rega Institute for Medical Research, KU Leuven-University of Leuven, Leuven, Belgium. [2]VIB Center for Microbiology, Leuven, Belgium. [3]Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands. [4]Research Group of Microbiology, Department of Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium. [5]Department of Neurosciences, Psychiatry Research Group University of Leuven, Leuven, Belgium. [6]K. G. Jebsen Coeliac Disease Research Centre, Department of Immunology, University of Oslo, Oslo, Norway. [7]University Psychiatric Center KU Leuven, KU Leuven-University of Leuven, Leuven, Belgium. [8]Laboratory for Brain-Gut Axis Studies, Translational Research Center for Gastrointestinal Disorders, Department of Clinical and Experimental Medicine, KU Leuven-University of Leuven, Leuven, Belgium. [9]These authors contributed equally: Sara Vieira-Silva, Jeroen Raes. *e-mail: jeroen.raes@kuleuven.be

group suffering from treatment-resistant major depressive disorder (TR-MDD; $n=7$), allows us to link microbiota neuroactive capacity with QoL and depression.

Results and discussion

QoL in a Flemish population cohort. QoL was assessed in the FGFP ($n=1,054$)[15] using the RAND-36 health-related quality of life survey[18] (Supplementary Table 1), currently the most widely used QoL questionnaire[19]. It covers eight health concepts (four mental followed by four physical scores): role limitations caused by emotional health problems; social functioning; emotional well-being; vitality; physical functioning; role limitations caused by physical health; body pain; and general health perception. Each score ranges from 0 to 100, with higher scores defining more favourable health states. From these, two additional summary scores are derived:[20] a mental and a physical component summary. The FGFP RAND score distributions fell within population norms (Supplementary Table 2). Individuals with general practitioner-reported diagnosis of depression ($n=121$, 11.5%) displayed lower RAND scores than others, regardless of antidepressant treatment ($n_{treated}=52$; Wilcoxon rank-sum test, false discovery rate (FDR) <0.1; Supplementary Fig. 1; Supplementary Table 3).

QoL covariation with microbiota composition replicates across independent cohorts. We first explored gut microbiota covariation with QoL in the context of previously identified microbiome covariates, including age, sex, body mass index (BMI), stool consistency (Bristol stool scale (BSS)—a proxy for transit time[21]) and gastrointestinal diseases (inflammatory bowel disease (IBD), gastrointestinal cancer)[15,16]. A confounder analysis revealed multiple associations between established microbiome covariates and QoL, with most RAND scores being significantly lower in women and participants reporting gastrointestinal disease (Wilcoxon rank-sum test, FDR <0.1; Supplementary Table 4). While age and BMI were found to be negatively associated with physical health scores, they correlated positively with several RAND indicators for mental health (Spearman's correlation, FDR <0.1; Supplementary Table 4).

Next, we set off to determine the proportion of inter-individual variation in overall microbiota composition that can be explained by QoL (distance-based redundancy analysis (dbRDA), genus-level Aitchison distance). All RAND scores explained moderate but significant proportions of the FGFP microbiota compositional variation (dbRDA, adjusted R^2 range $=0.08$–0.35%, FDR <0.1; Fig. 1a and Supplementary Table 5). A multivariate approach showed that although RAND scores had partially overlapping explanatory power, three RAND scores (body pain, physical functioning and general health perception) provided additional contributions to inter-individual microbiota variation beyond anthropometrics and gastrointestinal covariates (stepwise dbRDA, total $R^2=2.8\%$; Fig. 1b and Supplementary Table 5). Besides being linked to community-wide variation, QoL indicators were also associated with the relative abundances of specific taxa. Fitting generalized linear models (GLMs) between RAND scores and single taxa, while partialling out anthropometric and bowel covariate contributions, we found ten genus abundances significantly correlated with QoL (GLM, FDR <0.1; Fig. 1c and Supplementary Table 6). Among those, the positive associations between several QoL scores and *Faecalibacterium* and *Coprococcus* and the negative association between physical functioning and *Flavonifractor* were validated in the LLD cohort ($n=1,063$; GLMs, $P<0.05$; Fig. 1c and Supplementary Table 6). *Faecalibacterium* and *Coprococcus* produce butyrate[22], a short-chain fatty acid that strengthens the epithelial defence barrier and reduces intestinal inflammation[23], and both have been reported to be depleted in IBD[24] and depression[7,8]. In the LLD data set, their relative abundances effectively correlated with stool butyrate concentrations ($n=1,063$; $\rho=0.33$, FDR $=4.83\times10^{-27}$ and $\rho=0.13$, FDR $=2.64\times10^{-5}$, respectively; faecal metabolites were not quantified in the FGFP cohort). By contrast, *Flavonifractor* was reported to be increased in major depression disorder patients[7]. Here we associate the relative abundances of microbial genera with QoL scores. In a pilot study in a closed experimental setting, Li et al.[25] linked mood scores over time to taxon abundances in three individuals. While not the same, mood states have been correlated with mental QoL[26]. However, the health-associated *Faecalibacterium* negatively correlated with mood scores contrarily to the positive association to mental QoL detected here. Still, their reported *Parabacteroides* association matched our positive association to emotional role (Fig. 1c and Supplementary Table 6).

***Coprococcus* and *Dialister* are consistently depleted in depression across cohorts.** Depression is the most prevalent mental disorder in industrialized societies:[27] estimates of prevalence of depressive disorders in Belgium range from 5 to 15%[28]. Accordingly, general practitioner-reported depression was the most prevalent psychiatric disorder in the FGFP cohort (11.5%) explaining 0.13% of microbiota compositional variation. Similarly, antidepressant use was a significant covariate individually (adjusted $R^2=0.08\%$), but did not contribute beyond diagnosis; it was therefore not selected by the optimal multivariate dbRDA model (Fig. 1b and Supplementary Table 5). We identified four taxa significantly depleted in participants with depression/undergoing antidepressant treatment (Fig. 1c). However, we found antidepressant use to be an important confounder of these specific genus-level findings; only *Coprococcus* and *Dialister* remained significant after partialling out effects of antidepressant use (Fig. 1c and Supplementary Table 7). Both associations were validated in the LLD validation data set (self-reported history of depression, $P<0.05$; Supplementary Table 7), also after deconfounding for antidepressant medication. Differential effects on the gut microbiota have been reported as associated to the mechanisms of action of specific classes of antidepressants[29,30]; however, our cross-sectional data sets are insufficiently powered to investigate these observations in more detail. Interestingly, *Coprococcus* and *Dialister* both featured among the seven genera positively associated with QoL scores.

Antidepressant medication is an important source of inter-study variation. Four recent clinical studies examined the gut microbiota composition in individuals with MDD ($n=34$–58) and matched controls ($n=18$–63)[6–9]. Although all studies reported depression-associated alterations in the relative composition of the microbiota, results are conflicting, potentially reflecting insufficient power due to low sample size and/or inadequate/incomplete confounder analysis. For instance, regarding the potential association between depression and microbiota diversity, Jiang et al.[7] reported a positive correlation and Kelly et al.[9] the opposite, while both Naseribafrouei et al.[6] and Zheng et al.[8] did not observe any significant association—the latter in agreement with our results. Out of the studies' collective catalogue of 36 reported genus-depression associations, only 7 could be replicated in the FGFP data set when partialling out the covariate effects (Supplementary Table 8). Featuring among replicated depression-associated taxa was *Lactobacillus*, previously suggested to be implicated in gut–brain communication[8] and to have positive effects on stress and cognition in animal models, although not translating well to humans[13]. In agreement with Zheng et al.[8], we observed increased *Lactobacillus* relative abundances in participants with depression. However, this association was no longer significant when controlling for antidepressant treatment, confirming that medication is a substantial confounder in association studies[15,31]. *Dialister* and *Coprococcus*, the two genera we identified to covary both with RAND scores and (treatment-free) depression status (Fig. 1c) were also observed to do so in at least one of the

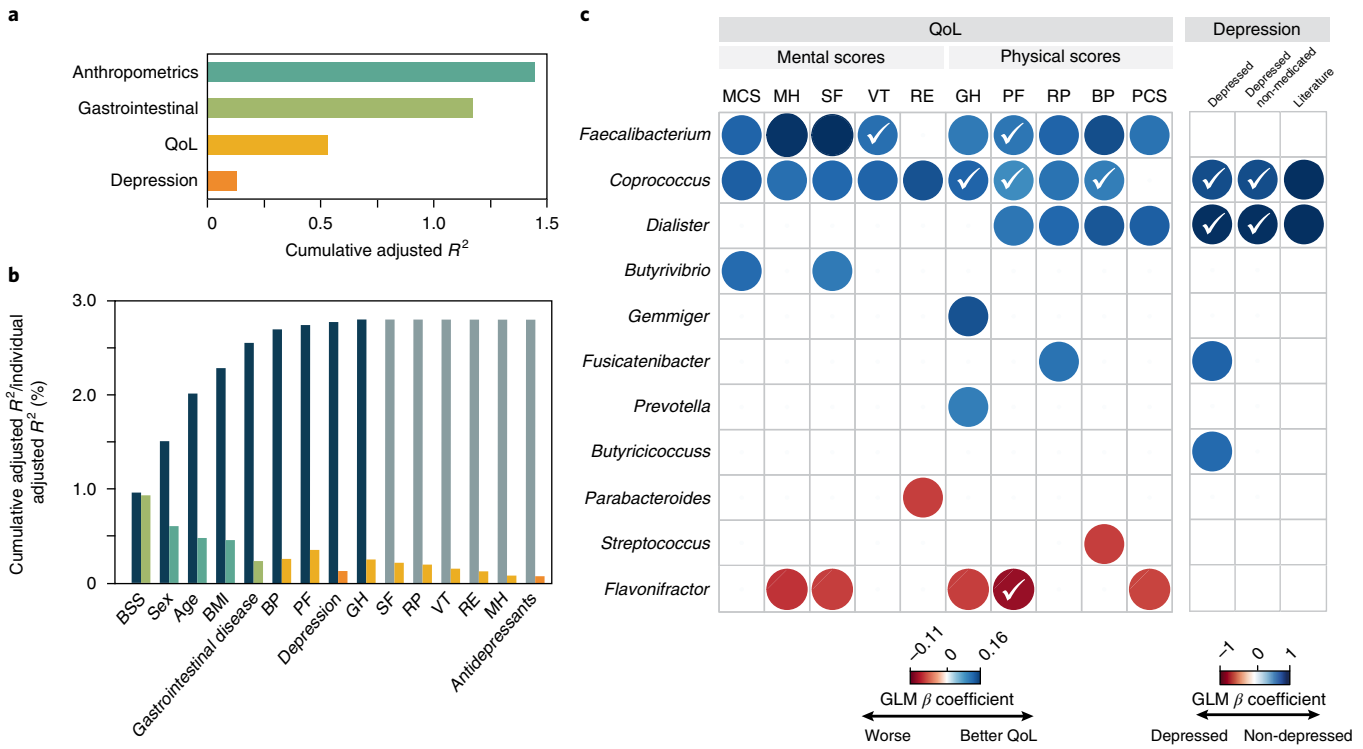

Fig. 1 | Ecosystem-wide and specific effects of QoL variables on microbiome variation. a, Combined explanatory power of FGFP covariates pooled in predefined categories on microbiome community variation (stepwise dbRDA on Aitchison distance; $n=1{,}054$). Anthropometrics: age, sex, BMI; gastrointestinal parameters: BSS and gastrointestinal disease; depression: diagnosis of depression and use of antidepressants. **b**, Cumulative effect sizes of FGFP covariates on microbiome community variation (left bars; stepwise dbRDA on Aitchison distance; grey, variables not entering the dbRDA model; $n=1{,}054$) compared to individual effect sizes assuming covariate independence (right bars). **c**, Associations between QoL scores or depression and bacterial genera after partialling out the effect of the main microbiota covariates (anthropometric and gastrointestinal parameters; GLMs, FDR < 0.1) in the FGFP cohort ($n=1{,}054$). Validation of QoL associations in the LLD data set ($n=1{,}063$; tick marks indicate successful validation) and validation of depression associations in the non-medicated subset of FGFP patients (depressed non-medicated) and in published case–control depression studies[6–9] (literature). Positive correlations (or taxa elevated in the non-depressed group) are displayed in blue and negative correlations in red. Colour intensity is proportional to standardized GLM β coefficients. MCS, mental component summary; MH, emotional well-being; SF, social functioning; VT, vitality; RE, role limitations caused by emotional health problems; GH, general health perception; PF, physical functioning; RP, role limitations caused by physical health; BP, body pain; PCS, physical component summary.

replicated reports[6–9]. Hence, these taxa can be regarded as potential leads for psychobiotics[32]—live organisms that, when ingested in adequate amounts, confer health benefits in patients suffering from psychiatric illness—and main targets for follow-up research.

Depression and lower QoL are associated with the *Bacteroides* enterotype 2 in the FGFP cohort. Next, we assessed enterotype distribution (identified using Dirichlet multinomial mixtures (DMMs)[33]; Supplementary Fig. 2; details in Methods) in relation to QoL scores and diagnosis of depression in the FGFP data set. All ten QoL scores were distributed unevenly across enterotypes (Kruskal–Wallis test, FDR < 0.1; Supplementary Table 9 and Fig. 2a), all reflecting lower QoL in the recently described, potentially dysbiotic *Bacteroides* enterotype 2[34] compared to *Prevotella*, *Bacteroides* enterotype 1 and Ruminococcaceae (post hoc Dunn's test, FDR < 0.1; Supplementary Table 9). *Bacteroides* enterotype 2, shown to harbour reduced microbial load, has increased prevalence among patients with Crohn's disease[34]. In the FGFP data set, enterotype distribution varied with depression status (χ^2 test, $P=7.87\times10^{-4}$; Fig. 2b), where depression diagnosis corresponded to higher prevalence of *Bacteroides* enterotype 2 samples (26 versus 13%; pairwise χ^2 tests, FDR < 0.1; Supplementary Table 9). To our knowledge, this is the first description of an association between an enterotype and mental health status, and while the lack of statistically significant DMM enterotype clustering in the LLD data set impeded replicating the results in this cohort, our results align with the previously reported assumption of the potentially dysbiotic nature of *Bacteroides* enterotype 2[34].

Gut–brain modules characterize the neuroactive potential of gut microbiota. To study the gut microbiota–brain interaction beyond taxonomic associations, we developed a module-based analytical framework enabling targeted profiling and interpretation of metagenomic data in the context of microbiota–gut–brain communication. This framework describes the microbial pathways that metabolize molecules that have the potential to interact with the human nervous system (neuroactive compounds). From literature review, we curated and annotated 56 gut–brain modules (GBMs), each corresponding to a single neuroactive compound production or degradation process (details in Methods; Supplementary Table 10 and Supplementary Data 1). To validate the framework, we assessed GBM detection in the genomes of microorganisms isolated from the human gastrointestinal tract included in the Integrated Microbial Genomes[35] (IMG) database ($n=532$). GBM detection captured the majority of taxa experimentally shown to produce or degrade corresponding neuroactive compounds (median detection sensitivity = 70%; Supplementary Table 11) and identified additional genera (median = 7) encoding thus far experimentally unassessed neuroactive metabolism.

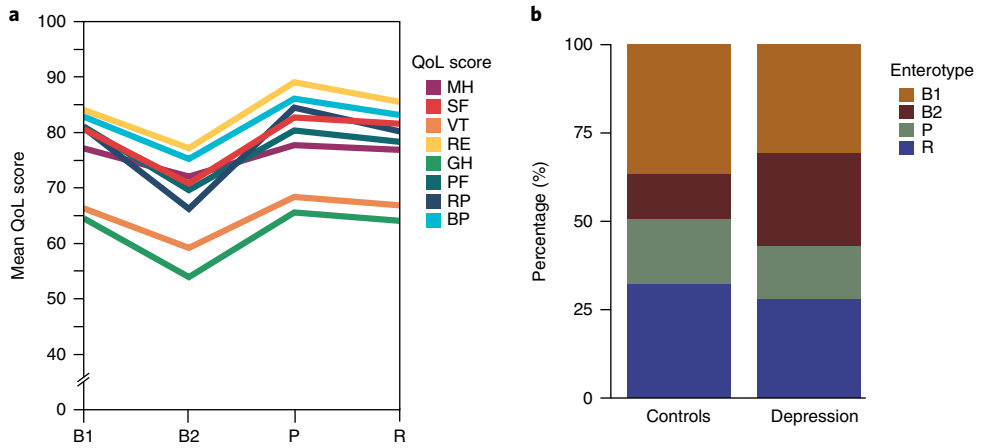

Fig. 2 | *Bacteroides* enterotype *2* association with lower QoL and depression status in the FGFP cohort. a, Mean QoL (RAND) scores in the four DMM enterotypes (B1, *Bacteroides* enterotype 1; B2, *Bacteroides* enterotype 2; P, *Prevotella;* R, Ruminococcaceae); Kruskal–Wallis test (FDR < 0.1 for all scores; Supplementary Table 9) with post hoc Dunn's test (FDR < 0.1 for associations between *Bacteroides* enterotype 2 and other enterotypes; Supplementary Table 9) in the FGFP cohort ($n = 1{,}054$). MH, emotional well-being; SF, social functioning; VT, vitality; RE, role limitations caused by emotional health problems; GH, general health perception; PF, physical functioning; RP, role limitations caused by physical health; BP, body pain. **b**, Enterotype distribution in FGFP individuals with general practitioner-reported depression ($n = 151$) versus controls ($n = 933$) (χ^2 test, $\chi^2 = 16.77$, $P = 7.87 \times 10^{-4}$). *Bacteroides* enterotype 2 was the only one with a different distribution in cases versus controls (pairwise χ^2 tests; $\chi^2 = 5.21$, FDR $= 8.99 \times 10^{-2}$; Supplementary Table 9).

The analysis of GBM prevalence in IMG genomes of human gut isolates allowed us to construct the first catalogue of gut microbiota neuroactive potential (Supplementary Table 12). While 4 GBMs (for example, synthesis of the anti-inflammatory and analgesic compound S-adenosylmethionine, or degradation of the neurotoxin quinolinic acid) were essentially ubiquitous (present in > 90% of gut microbial genomes), 14 were only rarely observed (prevalence < 5% of the genomes; including dopamine, acetylcholine, kynurenine, histamine, and serotonin II synthesis; Fig. 3a). A subset of the GBMs ($n = 39$) were non-randomly distributed across the microbial phylogenetic tree (phylogenetic inertia, Pagel's lambda (λ), FDR < 0.1; Supplementary Table 13). Of these, 34 were associated to specific bacterial phyla (Fisher's test, FDR < 0.1; Supplementary Fig. 3), the strongest association being histamine synthesis to *Fusobacterium* (Fisher's $R^2 = 0.72$, FDR $= 1.85 \times 10^{-22}$). Indeed, although not described in the literature, 3 out of the 18 *Fusobacterium* genomes analysed carried the potential to synthesize histamine. Only 30 out of 532 gut-associated IMG reference genomes encoded more than one rare GBM. Among the neuroactive specialists, (opportunistic) pathogens such as *Pseudomonas aeruginosa* and *Yersinia enterocolitica*, which are known to respond to gut neurotransmitters[36] (Fig. 3a), are featured.

In humans, the neurotransmitter serotonin is found in the highest concentrations in the gastrointestinal tract, where it is involved in the regulation of gastrointestinal secretion, motility and pain perception[37]. Gut microorganisms both modulate host serotonin biosynthesis[38] and produce serotonin[2]. The latter biosynthetic pathways are not yet fully elucidated, but two metabolic routes have been proposed[39]: decarboxylation of tryptophan to tryptamine followed by hydroxylation (plant-like pathway); and hydroxylation to 5-hydroxytryptophan and then decarboxylation (animal-like pathway). While the plant-like GBM (serotonin synthesis II) was characterized as rare, the animal-like GBM (serotonin synthesis I) was present in almost 20% of IMG gut-associated genomes (Fig. 3a). Serotonin synthesis has been experimentally observed in strains belonging to ten different gut-associated genera (Supplementary Table 11), only half of which were validated by GBM-based genome analysis, due to limited representative genomes. Additional genera, such as *Akkermansia*, *Alistipes* and *Roseburia* were identified as potential serotonin producers using the animal-like GBM (Supplementary Table 12).

Comparison of GBM prevalence in IMG genomes of gut-associated ($n = 532$) versus free-living ($n = 1{,}501$; classification as in IMG version 4.0, see Methods) taxa[35] allowed us to identify traits potentially involved in intestinal host–microbe interaction. Thirteen GBMs were significantly more prevalent in the genomes of gut-associated microorganisms (χ^2 test, FDR < 0.1); 25 were over-represented in microorganisms annotated as free-living (Fig. 3b and Supplementary Table 13). Among the top five most strongly host-associated GBMs, two were ubiquitously encoded by colon bacteria (>80%; acetate and glutamate synthesis I). By contrast, two were detected in less than a third of genomes of gut-associated species (tryptophan degradation and GABA synthesis III), while still displaying a wide phylogenetic range (Fig. 3c). Interestingly, all three currently characterized GABA synthesis pathways were significantly more prevalent in gut microorganisms. GABA is known to be produced in large amounts by intestinal bacteria[40], playing a role in intracellular pH homeostasis and energy generation[41]. Phylogenetically most widespread was the decarboxylation of glutamate to GABA, which is part of the GABA shunt pathway, notably implicated in bacterial survival in the extreme acidity of the stomach[42]. GABA-binding proteins, suggested to play a role in bacterial and inter-domain communication[41], are also widespread in gut-associated bacteria.

As gene mobility is an important driver of microbe–microbe and host–microbe interactions, with mobile elements like plasmids transferring functions promoting cooperation and/or virulence[43], we assessed GBM presence in IMG plasmid sequences[35]. GBMs were only exceptionally detected (8 GBMs, Supplementary Table 14, 2 of which are part of the 13 associated with the human gut environment). For the majority (62.5%), not even a single pathway step was encoded on the 1,150 plasmids tested, possibly reflecting the difficulty of horizontal transfer of complex traits involved in gut–brain communication.

Associations of microbiota neuroactive potential with QoL and depression. To determine whether neuroactive compound metabolism is associated with QoL and depression, we applied the GBM

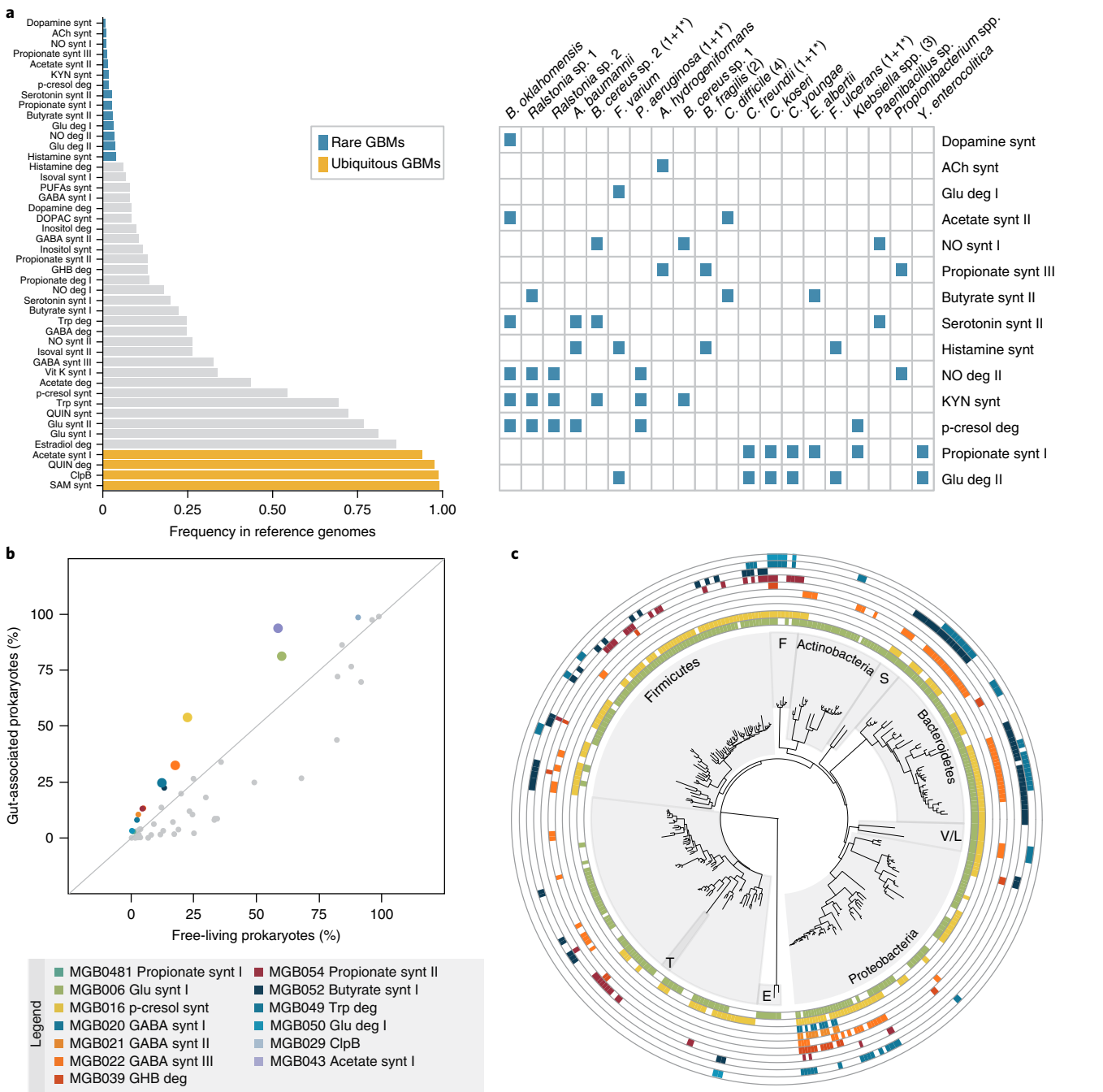

Fig. 3 | GBM distribution in microbial genomes. a, Left panel: GBM detection frequency in human gut-associated microbial genomes ($n=532$). Rare (present in <5% of genomes) and ubiquitous GBMs (present in >90% genomes) are highlighted, while others are in grey. Right panel: microbial strains encoding two or more rare GBMs. Full GBM and strain names can be found in Supplementary Tables 10 and 12, respectively. The numbers in parentheses correspond to the number of strains encoding the same GBMs (*, genome unclassified at the species level). Ach, acetylcholine; deg, degradation; GHB, γ-hydroxybutyric acid; Glu, glutamate; Isoval, isovaleric acid; KYN, kynurenine; NO, nitric oxide; PUFAs; polyunsaturated fatty acids; QUIN, quinolinic acid; SAM, s-adenosyl methionine; synt, synthesis; Trp, tryptophan; Vit K, vitamin K. **b**, GBM distribution in gut-associated species ($n=532$) versus in free-living species ($n=1,501$). GBMs possibly involved in host-microbiota interactions (FDR < 0.1 in χ^2 test) are coloured (larger dots: top 5 GBMs most significantly associated with a human gut-associated habitat), while others are in grey. **c**, Phylogenetic distribution of host-associated GBMs in gut reference genomes (16S rRNA gene phylogenetic tree). Only GBMs with an effect size > 0.1 and present in < 90% of genomes are shown ($n=10$). F, Fusobacteria; S, Synergistetes; V/L, Verrucomicrobia/Lentisphaerae; E, Euryarchaeota; T, Tenericutes. The concentric circles have the order and colour of the legend.

framework to 150 FGFP shotgun metagenomes, including 80 patients diagnosed as depressed ($n=40$ taking antidepressants) and 70 healthy controls. Groups were balanced based on age, sex, BMI and BSS (Wilcoxon rank-sum test for numerical variables and χ^2 test for binary variables, $P>0.05$). While exploratory associations with QoL were assessed on the whole GBM framework ($n=56$), a more targeted analysis was performed on the subset of GBMs covering metabolites associated with depression in the existing literature ($n=24$; Supplementary Table 10). QoL and depression-associated GBMs were subsequently validated in the LLD metagenomic data set ($n=1,063$)[16]. Depression-related GBMs were additionally validated in an independent set of individuals with a diagnosis of

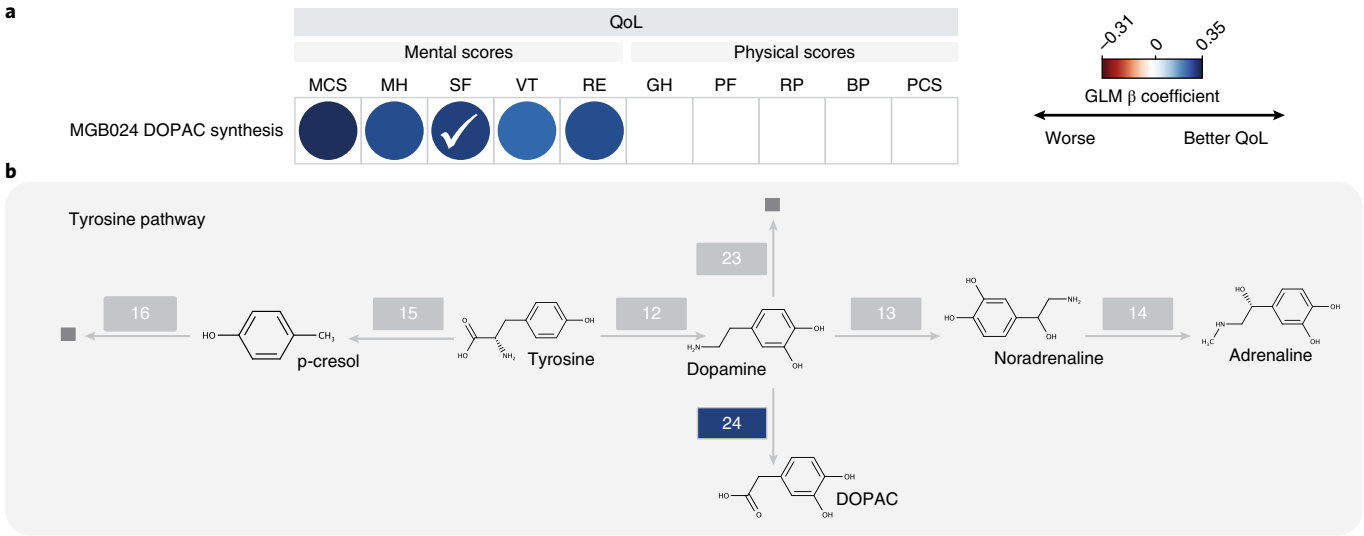

Fig. 4 | Association of the DOPAC synthesis GBM with mental QoL. a, Association between DOPAC synthesis and QoL indicators (GLMs partialling out anthropometric and gastrointestinal covariates, FDR < 0.1; Supplementary Table 15) in the FGFP data set ($n = 150$). Tick marks indicate successful validation in the LLD data set ($n = 1{,}063$). Positive associations are displayed in blue. Colour intensity is proportional to standardized GLM β coefficients. **b**, GBMs in the tyrosine pathway. GBMs are displayed as boxes with ID numbers. The DOPAC synthesis GBM is highlighted in blue. For simplicity, metabolites with no evidence of neuroactivity are represented with filled grey squares (see Supplementary Data 1 for the full GBM description).

TR-MDD ($n = 7$) balanced by age, sex and BSS to the FGFP healthy subset ($n = 70$); QoL information was not recorded in the TR-MDD data set.

In the FGFP data set, we detected three GBMs covarying with various mental QoL scores (Spearman's test on centred log-ratio-transformed data, FDR < 0.1; Supplementary Table 15), namely synthesis of 3,4-dihydroxyphenylacetic acid (DOPAC; positive correlation), isovaleric acid synthesis potential II (via α-keto-acid decarboxylase pathway; positive correlation), and histamine synthesis potential (negative correlation). However, only the association between DOPAC synthesis—a metabolite of dopamine— and the RAND social functioning score was replicated in the LLD data set (GLM on centred log-ratio-transformed data, standardized β coefficient = 0.065, $P = 4.25 \times 10^{-2}$; Supplementary Table 15 and Fig. 4a). While the first step for DOPAC synthesis from dopamine (Fig. 4b) is conversion to the endogenous neurotoxin 3,4-dihydroxyphenylacetaldehyde, DOPAC—the end product of the module—has anti-proliferative activity on colon cancer cells[44]; reduced DOPAC levels in cerebrospinal fluid have been proposed as a biomarker for Parkinson's disease[45]. In the FGFP data set, DOPAC synthesis potential was associated with enterotype distribution (Kruskal–Wallis test, $\chi^2 = 21.72$, FDR = 1.49×10^{-4}) being lower in the *Bacteroides* enterotypes 1 and 2, and *Prevotella* community types compared to Ruminococcaceae-enterotyped samples. Of note, the DOPAC synthesis potential was most strongly associated with the relative abundance of *Coprococcus* (Spearman's test on centred log-ratio-transformed data, $\rho = 0.29$, FDR = 2.99×10^{-4}; association validated in the LLD: $\rho = 0.11$, $P = 5.45 \times 10^{-4}$). DOPAC synthesis by *Coprococcus* has not been described previously and was not detected in our analyses of reference genomes; only the second step of the GBM, conversion of 3,4-dihydroxyphenylacetaldehyde to DOPAC, was encoded in the analysed genomes of *Coprococcus comes* and *Coprococcus catus*. However, we found the genus to be positively correlated with both mental and physical RAND scores. While covariation does not imply that *Coprococcus* strains can actually synthesize DOPAC, these results suggest that other mechanisms besides butyrate production could be responsible for the beneficial association of *Coprococcus* with QoL.

Two GBMs in the glutamate pathway, namely glutamate degradation I (to crotonyl-coenzyme A and acetate) and GABA synthesis III (GABA shunt pathway), tended to be respectively depleted and increased in participants with depression (Wilcoxon rank-sum test on centred log-ratio-transformed data, $r = -0.17$, $P = 3.33 \times 10^{-2}$ and $r = 0.17$, $P = 3.55 \times 10^{-2}$) in the FGFP data set. Although these associations were not significant after correction for multiple testing, decreased glutamate degradation potential in participants with a diagnosis of depression was validated in the TR-MDD data set ($r = 0.23$, $P = 3.98 \times 10^{-2}$; no GBM depression association was replicated in the LDD self-reported depression data set; Supplementary Table 16). While statistically not significant, both observations are intriguing. Indeed, GABA is the main inhibitory neurotransmitter in the brain and alterations in GABA signalling have been linked to anxiety and depression, while in the periphery it acts as a visceral pain inhibitor[46]. Increased GABA levels in the blood of patients with MDD have been reported[47], as well as a role of microbially produced GABA in gut–brain communication[4]. In turn, glutamate acts as an excitatory neurotransmitter in the brain, and comparatively higher levels have been reported in peripheral blood of participants with MDD[48]. Of note, both pathways were among the subset of host-associated GBMs in our analysis in reference genomes. While follow-up case–control research (preferentially combined with metabolomic assessment of actual levels of neuroactive compounds) is needed to investigate whether microbial glutamate metabolism contributes to depression, our analyses give an idea of the sample sizes required to allow detecting differences in GBM proportional abundances between study groups. For example, to detect fluctuations in the glutamate degradation I module ($r = -0.17$ and s.d. = 0.49, FDR < 0.1, with type II error rate $\beta = 0.2$), a minimal estimated sample size[49] of $n = 2 \times 262$ would be needed. Although considerable, such numbers are not outside the feasibility range of clinical microbiome studies.

Conclusions

Analysis of a large faecal microbiome population study and validation of several public and newly sequenced data sets allowed us to establish significant covariation of gut microbiota composition with QoL indicators as well as depression status. Our approach does

not allow testing for causality nor directionality of microbiota–gut–brain axis interactions; however, it provides a panel of stringent associations taking into account the compositionality of microbiome data and potential confounding effects of antidepressant medication. The contribution of QoL indicators and depression to overall microbiota community variation was in the range of (and added to) known major microbiome covariates. While *Coprococcus* and *Dialister* were both found to be positively associated with QoL and depleted in treatment-free depression, others, including *Butyricicoccus*, were found to be linked to antidepressant treatment. In terms of microbial community constellations, individuals classified in the previously reported low-microbial-density *Bacteroides* enterotype 2 displayed lower QoL and higher prevalence of depression. However, statistical support for enterotyping was not available in the LLD validation data set. By introducing a module-based analytical framework that facilitates microbiota–gut–brain focused analysis and interpretation of metagenomic data sets, we catalogued the neuroactive potential of colon isolates, identifying omnipresent traits as well as pathways with limited distribution. We showed that several microbial pathways, including GABA and tryptophan metabolism, are enriched in human gut-associated microorganisms, indicating a potential role in host–microbe symbiosis. In addition, three GBMs correlated with QoL, including synthesis of the dopamine metabolite DOPAC. The GBM framework presented is a valuable tool to study microbiota alterations, facilitating the translation and subsequent interpretation of shotgun metagenomic data in a gut–brain axis context.

Methods

Cohorts. *FGFP data set.* Samples were selected from the FGFP[15] data set ($n=1{,}054$) after excluding individuals without general practitioner-reported depression but who were taking medication labelled as 'antidepressant' by the Anatomical Therapeutic Chemical (ATC) Classification System (ATC code N06A). Eighty patients with general practitioner-reported depression ($n=40$ undergoing antidepressant treatment) and 70 healthy controls (without a history of gastrointestinal disease or cancer) balanced on age, sex, BMI and BSS were selected for shotgun sequencing. QoL was assessed based on the RAND 36-Item Health Survey 1.0[18] (not included in the original Falony et al.[15] publication). This survey consists of 36 items from which 10 scores are calculated. Scores range from 0 to 100, with higher scores representing better health. Aggregated mental and physical scores were calculated as described by Ware et al.[20], using the Dutch population norms[50] (since no Belgian/Flemish norms are available). Current depression status was evaluated by each participant's general practitioner, as well as IBD and (history of) gastrointestinal cancer, and antidepressant usage in the last six months. Commercial drug names were converted to ATC codes[51] (N06AA: non-selective monoamine reuptake inhibitors; N06AB: selective serotonin reuptake inhibitors; N06AX: other antidepressants). Gastrointestinal transit time was assessed using the BSS of the analysed sample.

LLD validation data set. In the LLD data set, 1,063 samples[16] were selected after excluding individuals lacking a self-reported history of depression but who were taking medication labelled as 'antidepressant' by the ATC Classification System (ATC code N06A). QoL was determined as in the FGFP data set, while a history of self-reported depression was assessed using the following question: 'Could you indicate which of the following disorders you have or have had? Depression'. Although the LLD RAND survey was released in the cohort description[17], no analysis in relation to microbiota composition had been performed. IBD and (a history of) gastrointestinal cancer and antidepressant use in the last three months were also self-reported. Commercial drug names were converted to ATC codes[51]. Gastrointestinal transit time was assessed using a seven-day average of the BSS.

TR-MDD validation data set. Seven samples from patients with TR-MDD, diagnosed by a psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), fourth edition, text revision[52] diagnostic criteria for moderate-to-severe MDD, without mood-incongruent psychotic features (diagnosis code 296.32 or 296.33) or moderate-to-severe type II bipolar depression (diagnosis code 296.89) based on clinical assessment, and a Hamilton Rating Scale for Depression score of ≥17 were included. Patients were recruited by the KU Leuven University Psychiatric Centre and must have had an inadequate response to at least two antidepressant pharmacotherapies (including tricyclic antidepressants or combinations of selective serotonin reuptake inhibitors/serotonin-norepinephrine reuptake inhibitors with low doses of antipsychotics). Gastrointestinal disease (IBD, irritable bowel syndrome, gastrointestinal cancer) was an exclusion criterion. Current and a history of antidepressant use was recorded by a psychiatrist. Gastrointestinal transit time was assessed with the BSS of the analysed sample. Participants were age-, sex- and BSS-balanced to the 70 FGFP healthy controls selected for shotgun sequencing.

Determination of short-chain fatty acid levels. Butyrate levels in the LLD data set ($n=1{,}063$) were determined by gas chromatography–mass spectrometry in the 'Dr Stein & Colleagues' medical laboratory (Maastricht, the Netherlands) as described in Mujagic et al.[53].

Microbiome data sequencing and preprocessing. *FGFP data set.* To analyse microbiota taxonomic composition, faecal DNA extraction, library preparation and 16S ribosomal RNA (rRNA) gene sequencing of the FGFP dual-index data set was performed as described in Tito et al.[54]. 16S rRNA data preprocessing was performed using LotuS[55] version 1.565, to demultiplex sequencing reads, and the DADA2[56] pipeline version 1.6.0; taxonomy assignment was carried out with the RDP classifier[57] version 2.12, using the default parameters. To analyse the microbiota neuroactive potential, shotgun sequencing of the FGFP data set was performed using the Illumina HiSeq 2500 System (151 base pair paired-end reads; Novogene (HK) Company Limited) to obtain 5 Gb raw data/sample. Paired-end reads were first quality trimmed with Trimmomatic[58] version 0.32, using the ILLUMINACLIP:trimmomatic-0.32/adapters/NexteraPE-PE.fa:2:30:10:2, MAXINFO:40:0.70, HEADCROP:15 and MINLEN:40 options. The high-quality reads were then decontaminated from PhiX and human sequences using DeconSeq[59] version 0.4.3; broken pairs were fixed using a custom Biopython[60] script (available from https://github.com/raeslab/raeslab-utils/). The resulting paired-end and single-end reads were mapped on the integrated gene catalogue[61] using the Burrows–Wheeler Aligner[62], and the mapping was summarized into functional profiles using featureCounts[63] version 1.5.3, with the --minOverlap=40 –pO parameters.

LLD data set. To analyse the taxonomic composition of microbiota, faecal DNA extraction and library preparation, 16S rRNA gene sequencing and 16S data processing was performed as described in Zhernakova et al.[16]. To analyse the neuroactive potential of the microbiota, LLD[16] shotgun metagenomes were processed using the workflow described for the FGFP data set, with the Trimmomatic MAXINFO parameters adjusted to MAXINFO:40:0.80.

TR-MDD data set. DNA extraction, library preparation and shotgun sequencing were performed following the protocols described for the FGFP data set.

Statistical analyses. All statistical analyses and graphical representations were performed in R[64], using the packages vegan[65], phyloseq[66], CoDaSeq[67], QuantPsyc[68], DirichletMultinomial[69], ggplot2[70], phytools[71] and corrplot[72]. For the appropriate analysis of microbiota compositional data, abundance matrices were centred log-ratio-transformed using the codaSeq.clr function in the CoDaSeq[67] R package, using the minimum proportional abundance detected for each taxon for the imputation of zeros. Only samples with >10,000 reads ($n=1{,}054$) and genera with a relative abundance >0.001 ($n=169$) in the FGFP data set were included in the 16S data analysis.

Microbiota community variation explained by metadata variables. The contribution of metadata variables to microbiota community variation was determined by dbRDA on genus-level Aitchison distance (Euclidian distance between samples after centred log-ratio transformation, as recommended for compositional data) with the capscale function in the vegan R package[65]. Correction for multiple testing (Benjamini–Hochberg procedure, FDR) was applied and significance was defined at FDR < 0.1.

The cumulative contribution of metadata variables was determined by forward model selection on dbRDA with the ordiR2step function in vegan, with variables that showed a significant contribution to microbiota community variation in the previous step. The RAND mental and physical component summary scores were not included due to high collinearity with other scores (Pearson's $|r|>0.8$).

Association of bacterial genera and GBM with metadata variables. Genera with a mean abundance >0 ($n=59$) and GBMs with mean abundance >−4.5 in centred log-ratio-transformed data ($n=36$) were included in the analysis (threshold corresponding to GBM relative abundance >0.0001% before centred log-ratio transformation). Taxa unclassified at the genus level were excluded. For a targeted analysis of GBM association with depression, the GBMs were filtered to the ones describing the metabolism of compounds involved in depression ($n=24$; Supplementary Table 10).

In the general population data sets (FGFP and LLD 16S data sets and LLD shotgun data set), associations between taxa or GBM abundances and mental health variables after partialling out the effects of the main microbiota covariates were assessed by fitting GLMs on centred log-ratio-transformed data with the glm R function. RAND scores (Gaussian, link = identity) and diagnosis of depression (binomial, link = logit; logistic regression) were used as response variables, and microbiota covariates as explanatory variables. The significance of deconfounded

microbiota contribution to psychological variable prediction was assessed by performing log-likelihood (χ^2) tests on nested GLMs, as follows:

[null model] glm0 = RV ≈ age + sex + BMI + BSS + gastrointestinal disease

[alternative model] glm1 = RV ≈ age + sex + BMI + BSS + gastrointestinal disease + G_i

where RV is the response variable (either RAND scores or depression diagnosis) and G_i is the genera or GBM relative abundance matrix, with 'i' being the taxon or module index.

Standardized GLM regression coefficients were calculated using the lm.beta R function (QuantPsyc package[68]). The Benjamini–Hochberg procedure (FDR) was used to correct for multiple testing of taxon or GBM metadata associations, with significance defined as FDR < 0.1. Associations found in the FGFP discovery data set were considered validated in the other data sets when $P < 0.05$.

In the case–control data sets balanced by microbiota covariates (FGFP and TR-MDD shotgun data sets), associations between continuous and two-level categorical variables (for example, depression diagnosis versus genus relative abundance) were analysed with Wilcoxon rank-sum tests, while associations between continuous variables (for example, RAND scores versus genus relative abundance) with Spearman's non-parametric correlation tests. The Benjamini–Hochberg procedure (FDR) was used to correct for multiple testing of taxon or GBM metadata associations, with significance defined as FDR < 0.1. Associations found in the FGFP discovery data set were considered validated in the other data sets when $P < 0.05$.

Enterotyping. Enterotyping (or community typing) based on the DMM approach was performed in R using the DirichletMultinomial[69] package as described by Holmes et al.[33] on the FGFP genus-level abundance matrix ($n = 1{,}054$) rarefied to 10,000 reads. The optimal number of Dirichlet components based on the Bayesian information criterion was four (Supplementary Fig. 2a; mean probability for community-type assignation = 0.98 and s.d. = 0.067). No statistical support for $N > 2$ DMM clusters was found in the LLD data set, thus enterotype-metadata associations could not be replicated in the Dutch data set. The four FGFP clusters were named *Prevotella* (19% of samples), *Bacteroides* 1 (36%), *Bacteroides* 2 (14%) and Ruminococcaceae (31%), as described by Vandeputte et al.[34]. The first has a high relative abundance of *Prevotella* and the fourth has the highest genus-level richness, while the two others are dominated by the *Bacteroides* genus. *Bacteroides* 2 enterotyped samples had lower relative abundances of the genus *Faecalibacterium* compared to *Bacteroides* 1 (Supplementary Fig. 2b), in addition to harbouring a reduced microbial load[34].

GBM assembly. A metabolic reconstruction framework specific for translating shotgun metagenomic data into microbial neuroactive metabolic potential was assembled based on extensive literature (> 300 peer-reviewed papers) and database (MetaCyc[73]) review. A set of 56 GBMs was assembled, each corresponding to a process of synthesis or degradation of a neuroactive compound by members of the gut microbiota. Module structure follows the Kyoto Encyclopedia of Genes and Genomes (KEGG) database syntax[74] as previously constructed for the gut microbial metabolic food chain[75]. Overlap with modules currently included in the KEGG database (release 7 (1/16)) is documented in Supplementary Data 1. The set of manually curated GBMs focuses on prokaryotic reactions as described in literature reports (citations provided in Supplementary Data 1) on microbial neuroactive compound synthesis/degradation. Each GBM is delimited by its input and output compounds and encompasses all enzymes (orthologue groups) to perform the reaction steps of all alternate pathways. When different pathways for synthesis/utilization of a certain compound exist in prokaryotes, different GBMs were assembled for each. For each enzyme, the most specific prokaryotic orthologous group containing all taxa that were experimentally proven to perform the function was selected, using the KEGG[76], TIGRFAM[77] and eggNOG version 3.0[78] orthology databases in order of preference. The module set facilitates specific analyses of gut microbiota variation in the context of its potential association with QoL and nervous system pathologies.

GBMs were annotated for function, pathway, structure and potential to cross the intestinal epithelium and the blood–brain barrier (admetSAR[79]) (Supplementary Table 10). The complete description of the 56 GBMs, together with the database and literature references used to assemble each module, can be found in Supplementary Data 1 and http://raeslab.org/software/gbms.html; they are free to download and use as a resource for bioinformatic pipelines. The GBM framework will be regularly updated based on progress in the literature and databases.

GBM refinement. The phylogenetic distribution of each GBM in the IMG version 4.0 genomes isolated from the human gastrointestinal tract ($n = 532$)[35] was compared with the corresponding list of microbial genera that were experimentally shown to perform the corresponding metabolic process. When necessary, the GBM was refined by fine-tuning the orthologous groups to maximize detection accuracy (Supplementary Table 11).

GBM detection. GBM abundances were derived from an orthologue abundance table using Omixer-RPM version 1.0 (https://github.com/raeslab/omixer-rpm) as described in Vieira-Silva et al.[75]. A web application is also available from GOmixer (http://www.raeslab.org/gomixer/). GBM coverage is calculated as the number of pathway steps for which at least one of the orthologous groups is found in a genome, divided by the total number of steps constituting the GBM. GBM presence in microbial genomes is defined with a detection threshold of at least 66% coverage, to provide tolerance to miss-annotations and missing data in incomplete (draft) genomes; a GBM is considered present in a genus of bacteria if it is found in at least one of the genomes of the genus.

Microbial genomes and plasmid sequences. Prokaryotic genomes isolated from the human gastrointestinal tract ($n = 532$, encompassing 260 species; IMG field: 'Body Site' containing 'Gastrointestinal tract') and plasmid sequences ($n = 1{,}150$), were retrieved from IMG version 4.0[35]. Genome taxonomic annotation was obtained from NCBI Taxonomy[80] (https://www.ncbi.nlm.nih.gov/taxonomy). To compare GBM distribution in human gastrointestinal isolates versus free-living species (χ^2 test), 1,501 genomes of free-living prokaryotic species (field: 'Biotic Relationship' containing 'Free living' AND 'Body Site' equal '−1' AND 'Ecosystem' does not equal 'Host-associated') were also retrieved from IMG version 4.0.

Phylogenetic tree and GBM phylogenetic inertia. 16S rRNA gene sequences were retrieved for a randomly selected member of each species in microbial reference genomes ($n = 260$) from the SILVA database[81] (www.arb-silva.de; release 121); 735 rRNA gene sequences with one representative per genus not represented in the reference genomes were added to increase taxon sampling and avoid long-branch attraction artefacts. Sequence alignment, removal of poorly aligned regions and tree reconstruction were performed as described in Vieira-Silva et al.[75]. Tree graphical representation was done using GraPhlAn[82]. Phylogenetic inertia—the degree to which GBM presence in genomes is correlated to the phylogenetic tree topology of the reference genomes–was calculated with Pagel's λ using the phytools R package.

Ethical compliance. All study procedures are compliant with all relevant ethical regulations. FGFP procedures were approved by the medical ethics committee of the University of Brussels–Brussels University Hospital (approval no. 143201215505, 5 December 2012). A declaration concerning the FGFP privacy policy was submitted to the Belgian Commission for the Protection of Privacy. The LLD study was approved by the institutional review board of University Medical Center Groningen, reference no. M12. 113965. The TR-MDD study was approved by the KU Leuven Medical Ethics Committee (S59102; EudraCT no. 2016-001715-21). Written informed consent was obtained from all participants.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Code availability

A custom Biopython script to fix broken pairs in metagenomic sequences is publicly available at https://github.com/raeslab/raeslab-utils/. The code to compute GBM abundances from an ortholog abundance table is freely available at: https://github.com/raeslab/omixer-rpm, and a web application is also available at http://www.raeslab.org/gomixer/.

Data availability

FGFP 16S sequencing data and metadata on the microbiota covariates used in this study are available at the European Genome-phenome Archive (EGA, https://www.ebi.ac.uk/ega/), accession no. EGAS00001003296. The LLD sequence data and age and sex information per sample are also available at the EGA with accession no. EGAS00001001704; the rest of the microbiota covariates can be requested from the Lifelines cohort study (https://lifelines.nl/lifelines-research/access-to-lifelines) following the standard protocol for data access. FGFP and TR-MDD shotgun sequencing data and metadata are available at the EGA (accession no. EGAS00001003298).

Received: 5 August 2017; Accepted: 5 December 2018;
Published online: 04 February 2019

References

1. Cryan, J. F. & Dinan, T. G. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* **13**, 701–712 (2012).
2. O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G. & Cryan, J. F. Serotonin, tryptophan metabolism and the brain–gut–microbiome axis. *Behav. Brain. Res.* **277**, 32–48 (2015).
3. Braniste, V. et al. The gut microbiota influences blood–brain barrier permeability in mice. *Sci. Transl. Med.* **6**, 263ra158 (2014).
4. Lyte, M. & Brown, D. R. Evidence for PMAT- and OCT-like biogenic amine transporters in a probiotic strain of *Lactobacillus*: implications for interkingdom communication within the microbiota–gut–brain axis. *PLoS ONE* **13**, e0191037 (2018).

5. Mcdonald, D. et al. American Gut: an open platform for citizen science. *mSystems* **3**, e00031-18 (2018).
6. Naseribafrouei, A. et al. Correlation between the human fecal microbiota and depression. *Neurogastroenterol. Motil.* **26**, 1155–1162 (2014).
7. Jiang, H. et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav. Immun.* **48**, 186–194 (2015).
8. Zheng, P. et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol. Psychiatry* **21**, 786–796 (2016).
9. Kelly, J. R. et al. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *J. Psychiatr. Res.* **82**, 109–118 (2016).
10. Hill, J. M., Clement, C., Pogue, A. I., Bhattacharjee, S. & Zhao, Y. et al. Pathogenic microbes, the microbiome, and Alzheimer's disease (AD). *Front. Aging Neurosci.* **6**, 127 (2014).
11. Burokas, A., Moloney, R. D., Dinan, T. G. & Cryan, J. F. Microbiota regulation of the mammalian gut–brain axis. **91**, 1–62 (2015).
12. Falony, G., Vieira-Silva, S. & Raes, J. Richness and ecosystem development across faecal snapshots of the gut microbiota. *Nat. Microbiol.* **3**, 526–528 (2018).
13. Kelly, J. R. et al. Lost in translation? The potential psychobiotic *Lactobacillus rhamnosus* (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain Behav. Immun.* **61**, 50–59 (2017).
14. Bedarf, J. R. et al. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients. *Genome Med.* **9**, 39 (2017).
15. Falony, G. et al. Population-level analysis of gut microbiome variation. *Science* **352**, 560–564 (2016).
16. Zhernakova, A. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* **352**, 565–569 (2016).
17. Tigchelaar, E. F. et al. Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics. *BMJ Open* **5**, e006772 (2015).
18. Hays, R. D., Sherbourne, C. D. & Mazel, R. M. The RAND 36-Item Health Survey 1.0. *Health Econ.* **2**, 217–227 (1993).
19. Hays, R. D. & Morales, L. S. The RAND-36 measure of health-related quality of life. *Ann. Med.* **33**, 350–357 (2001).
20. Ware, J. E., Keller, S. D. & Kosinski, M. *SF-36: Physical and Mental Health Summary Scales* (Health Institute, New England Medical Center, Boston, 1994).
21. Lewis, S. J. & Heaton, K. W. Stool form scale as a useful guide to intestinal transit time. *Scand. J. Gastroenterol.* **32**, 920–924 (1997).
22. Rivière, A., Selak, M., Lantin, D., Leroy, F. & De Vuyst, L. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front. Microbiol.* **7**, 979 (2016).
23. Louis, P., Hold, G. L. & Flint, H. J. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Microbiol.* **12**, 661–672 (2014).
24. Gevers, D. et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* **15**, 382–392 (2014).
25. Li, L. et al. Gut microbes in correlation with mood: case study in a closed experimental human life support system. *Neurogastroenterol. Motil.* **28**, 1233–1240 (2016).
26. Watten, R. G., Syversen, J. L. & Myhrer, T. Quality of life, intelligence and mood. *Soc. Indic. Res.* **36**, 287–299 (1995).
27. National Collaborating Centre for Mental Health, National Institute for Health and Clinical Excellence, Royal College of Psychiatrists, British Psychological Society *Depression: the Treatment and Management of Depression in Adults* (British Psychological Society and Royal College of Psychiatrists, London, 2010).
28. Bruffaerts, R., Bonnewyn, A. & Demyttenaere, K. The epidemiology of depression in Belgium. A review and some reflections for the future [Article in Dutch]. *Tijdschr. Psychiatr.* **50**, 655–665 (2008).
29. Maier, L. et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* **555**, 623–628 (2018).
30. Cussotto, S. et al. Differential effects of psychotropic drugs on microbiome composition and gastrointestinal function. *Psychopharmacology* https://doi.org/10.1007/s00213-018-5006-5 (2018).
31. Forslund, K. et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* **528**, 262–266 (2015).
32. Dinan, T. G., Stanton, C. & Cryan, J. F. Psychobiotics: a novel class of psychotropic. *Biol. Psychiatry* **74**, 720–726 (2013).
33. Holmes, I., Harris, K. & Quince, C. Dirichlet multinomial mixtures: generative models for microbialmetagenomics. *PLoS ONE* **7**, e30126 (2012).
34. Vandeputte, D. et al. Quantitative microbiome profiling links gut community variation to microbial load. *Nature* **551**, 507–511 (2017).
35. Markowitz, V. M. et al. IMG 4 version of the integrated microbial genomes comparative analysis system. *Nucleic Acids Res.* **42**, D560–D567 (2014).
36. Lyte, M. & Ernst, S. Catecholamine induced growth of gram negative bacteria. *Life Sci.* **50**, 203–212 (1992).
37. McLean, P. G., Borman, R. A. & Lee, K. 5-HT in the enteric nervous system: gut function and neuropharmacology. *Trends Neurosci.* **30**, 9–13 (2007).
38. Yano, J. M. et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264–276 (2015).
39. Tsavkelova, E. A., Klimova, S. Y., Cherdyntseva, T. A. & Netrusov, A. I. Hormones and hormone-like substances of microorganisms: a review [Article in Russian]. *Prikl. Biokhim. Mikrobiol.* **42**, 261–268 (2006).
40. Lyte, M. & Cryan, J. F. (eds) *Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health* (Springer, New York, 2014).
41. Mazzoli, R. & Pessione, E. The neuro-endocrinological role of microbial glutamate and GABA signaling. *Front. Microbiol.* **7**, 1934 (2016).
42. Feehily, C., O'Byrne, C. P. & Karatzas, K. A. G. Functional γ-aminobutyrate shunt in *Listeria monocytogenes*: role in acid tolerance and succinate biosynthesis. *Appl. Environ. Microbiol.* **79**, 74–80 (2013).
43. Nogueira, T. et al. Horizontal gene transfer of the secretome drives the evolution of bacterial cooperation and virulence. *Curr. Biol.* **19**, 1683–1691 (2009).
44. Gao, K. et al. Of the major phenolic acids formed during human microbial fermentation of tea, citrus, and soy flavonoid supplements, only 3,4-dihydroxyphenylacetic acid has antiproliferative activity. *J. Nutr.* **136**, 52–57 (2006).
45. Goldstein, D. S., Holmes, C., Lopez, G. J., Wu, T. & Sharabi, Y. Cerebrospinal fluid biomarkers of central dopamine deficiency predict Parkinson's disease. *Parkinsonism Relat. Disord.* **50**, 108–112 (2018).
46. Bienenstock, J., Forsythe, P., Karimi, K. & Kunze, W. Neuroimmune aspects of food intake. *Int. Dairy J.* **20**, 253–258 (2010).
47. Petty, F. Plasma concentrations of gamma-aminobutyric acid (GABA) and mood disorders: a blood test for manic depressive disease? *Clin. Chem.* **40**, 296–302 (1994).
48. Inoshita, M. et al. Elevated peripheral blood glutamate levels in major depressive disorder. *Neuropsychiatr. Dis. Treat.* **14**, 945–953 (2018).
49. Chow, S., Shao, J. & Wang, H. *Sample Size Calculations in Clinical Trial Research* (Chapman and Hall, Boca Raton, 2008).
50. Aaronson, N. K. et al. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. *J. Clin. Epidemiol.* **51**, 1055–1068 (1998).
51. WHO Collaborating Centre for Drug Statistics Methodology *ATC Classification Index with DDDs* (WHO, 2017).
52. American Psychiatric Association *Diagnostic and Statistical Manual of Mental Disorders: Text Revision* 4th edn (American Psychiatric Association, Washington, 2002).
53. Mujagic, Z. et al. A novel biomarker panel for irritable bowel syndrome and the application in the general population. *Sci. Rep.* **6**, 26420 (2016).
54. Tito, R. Y. et al. Brief report: *Dialister* as a microbial marker of disease activity in spondyloarthritis. *Arthritis Rheumatol.* **69**, 114–121 (2017).
55. Hildebrand, F., Tadeo, R., Voigt, A. Y., Bork, P. & Raes, J. LotuS: an efficient and user-friendly OTU processing pipeline. *Microbiome* **2**, 30 (2014).
56. Callahan, B. J. et al. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583 (2016).
57. Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73**, 5261–5267 (2007).
58. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
59. Schmieder, R. & Edwards, R. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. *PLoS ONE* **6**, e17288 (2011).
60. Cock, P. J. A. et al. Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics* **25**, 1422–1423 (2009).
61. Li, J. et al. An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841 (2014).
62. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
63. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923–930 (2014).
64. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, 2015).
65. Oksanen, J. et al. vegan: Community Ecology. R package version 2.4-2 http://CRAN.R-project.org/package=vegan (2017).
66. McMurdie, P. J. & Holmes, S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**, e61217 (2013).
67. Gloor, G. B. & Reid, G. Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data. *Can. J. Microbiol.* **62**, 692–703 (2016).
68. Fletcher, T. D. QuantPsyc: Quantitative Psychology Tools. R package version 1.5 http://cran.r-project.org/package=QuantPsyc (2012).

69. Morgan, M. DirichletMultinomial: Dirichlet-Multinomial Mixture Model Machine Learning for Microbiome Data. Bioconductor version 1.20.0 http://bioconductor.org/packages/release/bioc/html/DirichletMultinomial.html (2017).
70. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis* (Springer, New York, 2009).
71. Revell, L. J. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).
72. Wei, T. et al. corrplot: Visualization of a Correlation Matrix. R package version 0.77 http://CRAN.R-project.org/package=corrplot (2016).
73. Caspi, R. et al. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Res.* **42**, 459–471 (2014).
74. Kanehisa, M. et al. Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Res.* **42**, D199–D205 (2014).
75. Vieira-Silva, S. et al. Species–function relationships shape ecological properties of the human gut microbiome. *Nat. Microbiol.* **1**, 16088 (2016).
76. Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res.* **44**, D457–D462 (2016).
77. Haft, D. H. et al. TIGRFAMs and genome properties in 2013. *Nucleic Acids Res.* **41**, D387–D395 (2013).
78. Powell, S. et al. eggNOG v3.0: orthologous groups covering 1133 organisms at 41 different taxonomic ranges. *Nucleic Acids Res.* **40**, D284–D289 (2012).
79. Cheng, F. et al. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J. Chem. Inf. Model.* **52**, 3099–3105 (2012).
80. Federhen, S. The NCBI Taxonomy database. *Nucleic Acids Res.* **40**, D136–D143 (2012).
81. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, 590–596 (2013).
82. Asnicar, F., Weingart, G., Tickle, T. L., Huttenhower, C. & Segata, N. Compact graphical representation of phylogenetic data and metadata with GraPhlAn. *PeerJ* **3**, e1029 (2015).

Acknowledgements

We would like to thank the FGFP, TR-MDD and LLD participants and staff for their collaboration, and all members of the Raes Laboratory for participating in scientific discussions regarding the manuscript. This study is partially funded by JPND grant JPCOFUND_FP-829-047. The FGFP was funded with support from the Flemish government (grant number IWT130359), Research Fund–Flanders (FWO) Odysseus program (grant number G.0924.09), King Baudouin Foundation (grant number 2012-J80000-004), VIB, Rega Institute for Medical Research and KU Leuven. The LLD study was funded by the Top Institute Food and Nutrition, Cardiovasculair Onderzoek Nederland, Netherlands Organization for Scientific Research (NWO-VIDI, grant number 864.13.013) and an EU FP7 European Research Council (ERC) Advanced Grant (to C.W.; grant number 322698). M.V.-C., J.W., M.J. and S.V.-S. are funded by (post-) doctoral fellowships from Research Foundation-Flanders. A.Z. is funded by an ERC starting grant (grant number 715772) and by a NWO-VIDI grant (grant number 016-178-056). C.W. has an NWO Spinoza prize (number NWO SPI 92-266).

Author contributions

M.V.-C., G.F., C.W., A.Z., S.V.-S. and J.R. conceived and designed the study. M.V.-C., G.F., E.F.T., R.Y.T., C.S., A.K., M.J., C.W., S.C., A.Z., S.V.-S. and J.R. acquired the data and participated in cohort recruitment. M.V.-C., Y.D., J.W. and R.Y.T. performed data preprocessing. M.V.-C., G.F. and S.V.-S. performed the data analysis. M.V.-C., G.F., L.V.O., S.V.-S. and J.R. interpreted the data. M.V.-C., G.F., S.V.-S. and J.R. wrote the manuscript with all authors providing critical revision of the manuscript. All authors approved the final version for publication.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41564-018-0337-x.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.R.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

nature research

Corresponding author(s): Jeroen Raes

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒ Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection
Data analysis	16S data pre-processing was performed using LotuS (version 1.565, used for demultiplexing sequencing reads) and the DADA2 pipeline (version 1.6.0), with the RDP classifier (version 2.1254) for taxonomy assignment. For analysis of shotgun sequencing data, paired-end reads were quality trimmed with Trimmomatic (version 0.32), decontaminated from phiX and human sequences using DeconSeq (version 0.4.3), and broken pairs were fixed using a custom Biopython script (available at https://github.com/raeslab/raeslab-utils/). Reads were mapped on the integrated gene catalog using BWA (version 0.7.8), and the mapping was summarized to functional profiles with featureCounts (version 1.5.3). The code used to compute GBM abundances from a KO abundance table is shared on GitHub (https://github.com/raeslab/omixer-rpm). The GBM input file and references used to assemble each GBM can be downloaded from http://raeslab.org/software/gbms.html. Data analysis and graphical representations were performed using R, a free software environment for statistical computing.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

FGFP 16S sequencing data and metadata on the microbiota covariates used in this study are available at the European genome-phenome archive (EGA, https://www.ebi.ac.uk/ega/) - accession nr EGAS00001003296. The LLD sequence data and age and sex information per sample are also available at EGA with accession number EGAS00001001704, while the rest of microbiota covariates can be requested from the LifeLines cohort study (https://lifelines.nl/lifelines-research/access-to-lifelines) following the standard protocol for data access. FGFP and TR-MDD shotgun sequencing data and metadata are available at EGA - accession nr EGAS00001003298.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. All samples from the already published FGFP and LLD datasets after excluding any subject without GP report of depression but taking medication labeled as "antidepressant" by the Anatomical Therapeutic Chemical classification system (ATC codes N06A) were included in the analysis (N=1054 and N=1063 respectively). For the FGFP shotgun dataset, 150 samples (N=80 cases and 70 controls) were selected for shotgun sequencing based on recent literature indicating that groups with N>20 are sufficient for depression vs controls group comparison analysis (PMID: 24888394, 25882912, 27067014, 27491067).
Data exclusions	No data were excluded from the analyses.
Replication	Extensive validation was performed: Microbial taxa associations with QoL scores were validated in the LLD dataset (N=6/28 associations replicated). Taxa associations with depression were validated in the FGFP non-medicated subset (N=2/4), in the LLD dataset (N=2/4), in the LLD non-medicated subset (N=2/4), and compared to published literature (N=2/4). GBM associations with QoL were replicated in the LLD (N=1/9 associations replicated). GBM associations with depression were tested in the LLD dataset and TR-MDD dataset.
Randomization	Samples were only classified according to the original datasets. To partial out the effects of covariates of microbiota composition (age, sex, BMI, bowel transit time, and gastrointestinal disease) in associations between bacterial genera or gut-brain modules (GBMs) and metadata variables, we fitted generalized linear models (GLMs).
Blinding	Investigators were not blinded during data collection and analyses.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	FGFP cohort: 576 female/478 male. Mean age: 50.9; BMI: 24.9; BSS: 4. LLD cohort: 616 female/447 male. Mean age: 57.9; BMI: 25.3; BSS: 3.8.
Recruitment	Samples from the already published FGFP (PMID:27126039) and LLD (PMID:27126040) datasets were included in the analysis (N=1054 and N=1063 respectively). Participants in the newly collected TR-MDD dataset (N=7) were balanced on age, sex, and BSS to the FGFP healthy subset selected for shotgun sequencing (N=70), and to the FGFP-depression shotgun subset (N=80).