Eat2beNICE

Effects of Nutrition and Lifestyle on Impulsive, Compulsive, and Externalizing Behaviours

H2020 - 728018

D5.5: Manuscript on the association between the gut microbiota and host genetics with psychiatric symptoms

Dissemination level	Public
Contractual date of delivery	28.02.2023
Actual date of delivery	09.03.2023
Type	Report
Version	1.0
Workpackage	WP5 - Intestinal microbiota as a mechanistic link between nutrition/lifestyle and impulsivity and compulsivity
Workpackage leader	Alejandro Arias Vasquez, RadboudUmc

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 728018.

Author list

Organisation	Name	Contact information
RUMC	Mirjam Bloemendaal	Mirjam.Bloemendaal@radboudumc.nl
RUMC	Danique Mulder	Danique.Mulder@radboudumc.nl
RUMC	Alejandro Arias Vasquez	Alejandro.AriasVasquez@radboudumc.nl

1. Deliverable report

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Introduction

The gut microbiome has gained attention in recent years for its potential role in the development of psychiatric disorders, and evidence for alterations in the gut microbiota has accumulated for multiple psychiatric disorders, including Attention Deficit/Hyperactivity Disorder (ADHD), Autism Spectrum Disorder (ASD), mood disorders and anxiety disorders. Such alterations appear to occur across disorders, rather than in a disease-specific manner, possibly indicating a common risk factor or mechanism (Nikolova et al., 2021). Similarly, recent research suggests that there is a significant overlap in the genetic risk for several psychiatric disorders (Wu et al., 2020). Although the composition of the gut microbiome itself shows only weak heritability, there are examples where the interplay between host genetics and the gut microbiome contribute to disease risk, for example in inflammatory bowel disease (Knights et al., 2014). It is not yet knownwhether genetic risk also modulates the microbiome-symptom association in the context of psychiatric disorders or symptomatology.

In this project, we used data from a richly phenotyped and highly comorbid psychiatric cohort – the Measuring Integrated Novel Dimensions in Neurodevelopment and Stress-related Mental Disorders (MIND-Set) (Eijndhoven et al., 2022), which was designed to capture the complex multifactorial nature of stress-related and neurodevelopmental disorders, in accordance with the Research Domain Criteria (RDoC) framework. The RDoC framework provides a transdiagnostic dimensional approach to investigating the mechanisms underlying psychiatric disorders by focusing on six domains of functioning (i.e., negative and positive valence, social processes, cognitive systems, arousal/regulatory systems and sensorimotor systems), each of which covers cross-disorder processes related to different aspects of brain functioning and behavior (Cuthbert & Insel, 2013). Using this approach, this project aims to 1) identify cross-disorder associations between the gut microbiome and genetic risk for psychiatric disorders and RDoC domains of functioning, and 2) assess the interplay between the microbiome and genetic risk in the in this association.

Methods

Participants

We included 293 participants (239 patients/54 unaffected individuals) from the MIND-Set cohort (Eijndhoven et al., 2022) for whom psychiatric symptom, microbiota, and genetic data were available. Participants fulfilled the diagnostic criteria for a mood disorder, anxiety disorder, ADHD, ASD and/or substance use disorder (SUD). There was a high degree of comorbidity in the sample, with a median of two psychiatric diagnoses.

Measures

Domains of Functioning: Participants were asked to complete eight questionnaires (31 subscales) assessing disorder-specific symptoms, personality traits and several psychological constructs (alexithymia, repetitive thoughts and executive functioning). To identify and extract the cross-disorder domains of functioning, a principal component analysis (PCA) with promax rotation was performed on the 31 questionnaire (sub)scale scores, yielding four principal components. The components replicated the findings from previous reports in a different subset of the same cohort (Mulders et al., 2022; Shi et al., 2022) and overlapped with the known Research Domain Criteria domains of

functioning (Cuthbert & Insel, 2013): 1) negative valence, 2) social processes, 3) cognitive systems, 4) arousal/regulatory systems (**Fig. 1**).

Microbiome: The V4 region of the 16s rRNA gene was amplified and sequenced, and the sequenced data was filtered, aligned and classified using the QIIME2 processing pipeline, resulting in an Amplicon Sequence Variant (ASV) table. Chao1, Inverse Simpson Diversity and Faith's Phylogenetic Diversity (PD) were used as measures of alpha-diversity (within sample diversity). Aitchison distance was used as a measure of beta-diversity (between sample diversity). For taxonomic analyses, we used Randomized Lasso stability selection with a selection threshold of 0.25 (Machlab et al., 2022; Meinshausen & Bühlmann, 2010) on CLR-transformed relative abundances to identify genera that consistently contributed to the outcome variable (i.e., domains of functioning) across different subsamples of the data. Statistical analyses were only performed on the selected genera.

Polygenic Risk Score (PRS): A PRS for broad depression was computed using PRSice 2.3.3 (Choi & O'Reilly, 2019), using the most recent and well-powered GWAS for depressive symptoms (Howard et al., 2019). We chose to use this PRS because a previous report in the same cohort showed associations between this PRS and mood disorder, SUD and ADHD disease status, as well as with comorbidity status and the negative valence domain of functioning (Shi et al., 2022).

Analyses

Microbiome-only regression models: To examine the association between the alpha-diversity or genus abundance and the domains of functioning, multiple linear regression was used with the domains of functioning as the outcome variable. The association between beta-diversity (Aitchison distance) and the domains of functioning was assessed using permanova (vegan::adonis2 in R, 999 permutations) with Aitchison distance as the outcome variable.

PRS-only regression models: The associations between the PRS for broad depression and the domains of functioning were assessed using multiple linear regression. Either an FDR-correction (linear regression models) or Bonferroni correction (permanova models) was applied to the obtained p-values, and a corrected p-value ≤ 0.05 was considered statistically significant. All models were adjusted for age, sex and number of psychiatric diagnoses.

Joint microbiome-PRS regression models: To test whether the microbial and PRS factors explained common of additive variance, we compared a joint microbiome–PRS model with individual microbial and PRS models. First, we subtracted the R^2 from a null-model, which only included the covariates (i.e., age, sex, number of diagnoses), from the microbiome-only, PRS-only and joint microbiome-PRS models, to obtain the variance explained by the microbiome and/or PRS factors (ΔR^2). Next, we created an additive ΔR^2 score by adding the ΔR^2 from the microbiome-only and PRS-only models, and compared this value to the ΔR^2 of the joint microbiome–PRS model. If the microbial and PRS factors explained common variance, the joint model was expected to have a lower ΔR^2 compared to the additive ΔR^2 of the microbiome-only and PRS-only models. The third step was performed only for domains of functioning that showed a significant association with both the PRS for broad depression, and at least one microbial factor (i.e., alpha-diversity/beta-diversity/genus abundance)

Results

Microbiome-only regression models

None of the alpha-diversity and beta-diversity indices were significantly associated with any of the four domains of functioning after correction for multiple testing (**Table 1-2**). *Sellimonas*, *CHKCI001*, and *Oscillibacter* show a positive, and *Clostridium Sensu Stricto1* shows a negative association with the negative valence domain. *Flavonifractor (*also selected by Randomized lasso) was not significantly

associated with the negative valence domain. *Sellimonas* was positively associated with the social processes domain. *Hungatella* and *Allisonella* were positively, and *Sporobacter* was negatively associated with the cognitive systems domain. *Ruminococcus Torques group* (selected by Randomized lasso) was not significantly associated with the arousal/regulatory systems domain (**Table 3**).

PRS-only regression models

The PRS for broad depression was positively associated with the negative valence domain, but not with the other domains of functioning (**Table 4**).

Joint microbiome-PRS regression models

We constructed joint microbiome-PRS regression models for the negative valence domain, including the PRS for broad depression and *Sellimonas*, *CHKCI001*, *Clostridium sensu stricto* or *Oscillibacter* and compared them with the microbiome-only and PRS-only regression models (**Table 1**, **Table 4**). The ΔR^2 of the joint microbiome-PRS models was always lower than the additive ΔR^2 of the microbiome-only and PRS-only models. However, the ΔR^2 of the joint microbiome-PRS models was still higher than what would be expected if the microbiome and PRS factors only explained shared variance, as it is still higher than the microbiome-only and PRS-only ΔR^2 (**Fig. 2**).

Discussion

In a highly comorbid psychiatric cohort, we identified cross-disorder associations between the gut microbiome and domains of functioning. In addition, the polygenic risk score for broad depression, as reported in a previous study (Shi et al., 2022), was associated with the negative valence domain, but not with the other domains of functioning. The effects of microbiome and genetic factors appear to be largely additive, suggesting that the microbiome and genetic risk explain different portions parts of the variance.

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2. Tables and other supporting documents where applicable and necessary

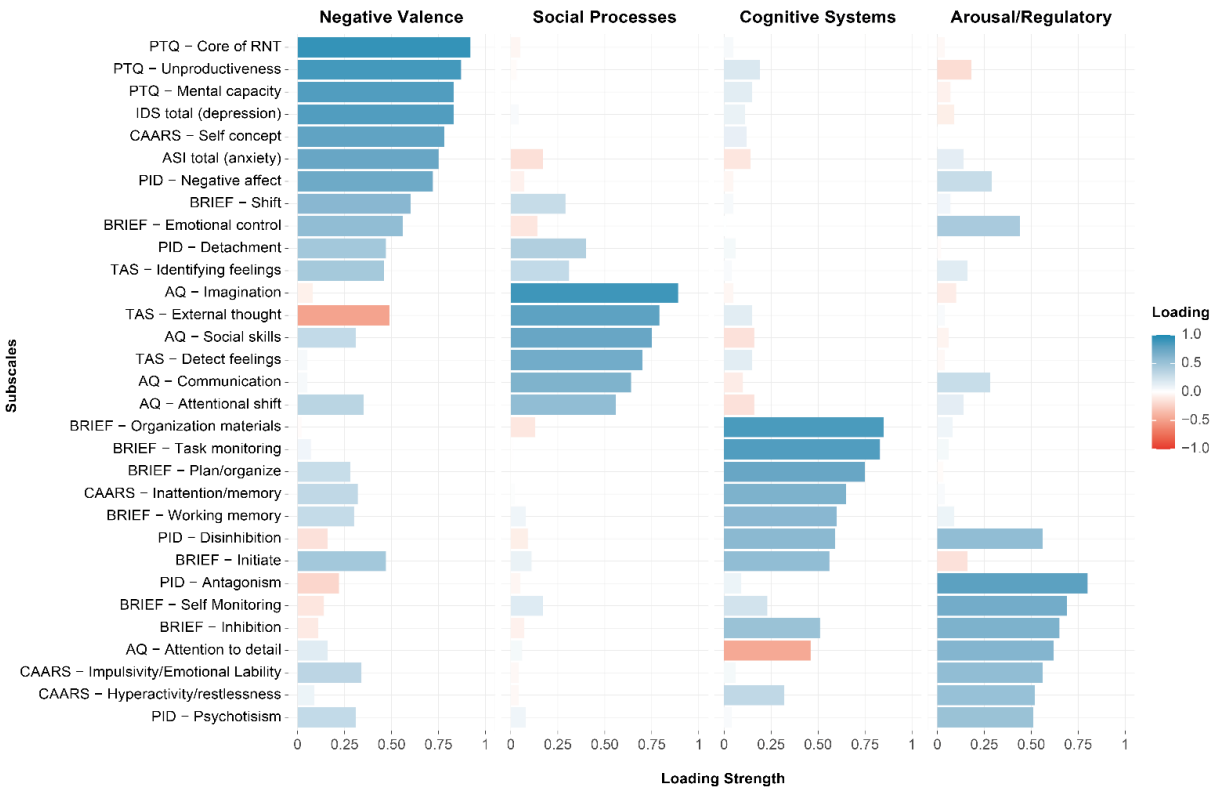

Figure 1. Component matrix of Principal Component Analysis, showing the loading of each of the 31 subscales on the four principal components/domains of functioning.

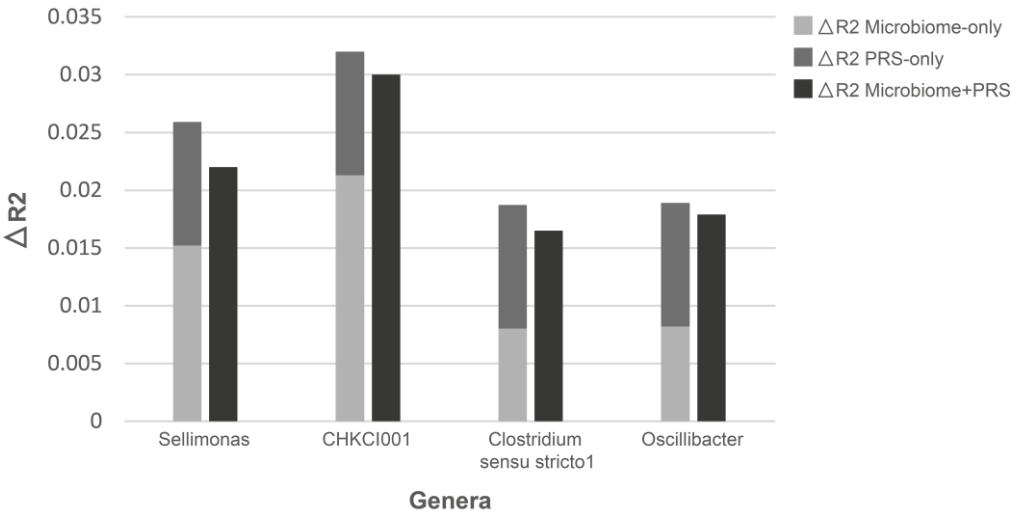

Figure 2. The ΔR^2 for the individual microbiome and PRS regression models (grey) and the ΔR^2 for the joint microbiome-PRS regression models (black). Abbreviations: PRS: polygenic risk score

Table 1. Associations between alpha diversity and the domains of functioning, based on multiple regression analyses

Domain of Functioning	Alpha-Diversity Index	Est.*	t	p
Negative Valence	Chao	-0.076	-1.67	0.096
	Inverse Simpson	-0.009	-0.21	0.835
	Faith's PD	-0.073	-1.60	0.111
Social Processes	Chao	0.024	0.43	0.665
	Inverse Simpson	0.067	1.23	0.220
	Faith's PD	0.018	0.33	0.739
Cognitive systems	Chao	-0.130	-0.24	0.812
	Inverse Simpson	-0.034	-0.63	0.532
	Faith's PD	-0.027	-0.50	0.616
Arousal/Regulatory systems	Chao	-0.050	-1.00	0.321
	Inverse Simpson	-0.079	1.59	0.114
	Faith's PD	-0.049	-0.98	0.330

Findings are based on a model including only a microbiome factor. We did not construct a joint microbiome-PRS model for alpha diversity measures, as none of the alpha-diversity indices exhibited a significant association with the domains of functioning. All models were adjusted for age, sex and number of diagnoses. Abbreviations: est.: estimate, PD: phylogenetic diverity

*Estimates are mean-centered and scaled by 1SD

Table 2. Beta diversity findings based on permanova analyses

Domain of Functioning	R^2	F	p
Negative Valence	0.004	1.32	0.021
Social Processes	0.004	1.08	0.214
Cognitive Systems	0.003	0.95	0.631
Arousal/Regulatory Systems	0.004	1.06	0.231

Findings are based on a model including only a microbiome factor. All models were adjusted for age, sex and number of diagnoses.

Table 3. Associations between genus abundance and the domains of functioning, based on multiple regression analyses

				Microbiome only			Microbiome-PRS		
Domain of Functioning	Genus	Sel. Prob.	Prev.	Est.*	t	p (fdr)	Est.	t	p (fdr)
Negative Valence	*Sellimonas*	0.57	36.4%	0.046	2.95	0.017	0.113	2.59	0.020
	CHKCl001	0.52	19.0%	0.154	3.44	0.007	0.147	3.30	0.004
	Clostridium sensu stricto	0.32	94.9%	-0.101	-2.24	0.037	-0.089	-1.98	0.049
	Oscillibacter	0.31	99.2%	0.104	2.26	0.037	-0.098	2.15	0.044
	Flavonifractor	0.27	98.1%	0.087	1.88	0.068	-	-	-
Social Processes	*Sellimonas*	0.62	36.4%	0.126	2.27	0.037	-	-	-
Cognitive Systems	*Hungatella*	0.44	46.9%	0.112	2.07	0.049	-	-	-
	Allisonella	0.39	26.0%	0.134	2.54	0.037	-	-	-
	Sporobacter	0.26	22.5%	-0.122	-2.29	0.037	-	-	-
Arousal/Regulatory Systems	*Ruminococcus torques group*	0.44	99.7%	0.047	0.929	0.358	-	-	-

Findings refer to two different models: a microbiome-only model or a joint microbiome-PRS model (including both a microbiome factor and the PRS for broad depression). All models were adjusted for age, sex and number of diagnoses. Abbreviations: fdr: false discovery rate, prev: prevalence, PRS: polygenic risk score, sel.prob: selection probability, est: estimate

*Estimates are mean-centered and scaled by 1SD

Table 4. Associations between the PRS for broad depression and the domains of functioning, based on multiple regression analyses

Domain of Functioning	Est.*	t	p (fdr)
Negative Valence	0.114	2.51	0.049
Social Processes	0.049	0.89	0.433
Cognitive Systems	-0.042	-0.78	0.433
Arousal/Regulatory Systems	0.059	1.17	0.433

Findings are based on a model including only a polygenic risk score (PRS) factor. All models were adjusted for age, sex and number of diagnoses. Abbreviations: est.: estimate, fdr: false discovery, rate

*Estimates are mean-centered and scaled by 1SD

3. Acknowledgement and Disclaimer

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 728018.