Eat2beNICE

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Gut microbiome in adults with ADHD and its relation to functional activation of the emotion recognition network (IMPACT study)

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Report Abstract

For this deliverable, initial plans involved using the MATRICS cohort. However, due limited availability of microbiome samples in this cohort, the current project was performed on the IMPACT sample collected at the Radboud University Medical Centre, as described in the deviations section of our previous progress report. Within this sample, we were able to relate microbiome data and functional brain activation with clinical ADHD data.

Gut Microbiome composition has been shown to be associated with both ADHD symptoms and the functioning of the emotion recognition network. The current study aimed to investigate microbiome composition in adults with ADHD, and its interaction with emotion recognition network activation in an fMRI paradigm.

We demonstrate that microbiome abundance in five bacterial genera is altered in adults with ADHD. However, we find no association between microbiome abundance and neural activation in any of the ROI's of the emotion recognition network.

Our results indicate that although both microbiome abundance and neural activation during emotion recognition are altered in adult ADHD, these two factors are not likely part of the same causal network underlying the neurobiology of ADHD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, characterized by symptoms of inattention and/or impulsivity and hyperactivity. Issues with emotion regulation are also often associated with ADHD, and are considered a major factor in determining disease burden and treatment efficacy (Shaw et al., 2014). Recent studies (e.g. Viering et al., 2021) have indicated that emotion regulation is also associated with altered functional brain activation in subjects with ADHD, suggesting that emotion regulation influences the neurobiological profile of ADHD.

Recent studies have shown that dietary interventions for patients with ADHD may lead to a significant reduction in ADHD symptoms, although there is heterogeneity across studies (Nigg et al., 2012; Sonuga-Barke et al., 2013; Pelsser et al., 2011). Conceivably, diet might influence behavior and ADHD symptoms by affecting gut microorganisms (i.e. the gut microbiome) (David et al., 2014). The gut microbiome has an increasingly recognized impact on brain functioning and behavior (Cryan et al., 2012). One proposed mechanism for the effects of gut microbiota on brain and behavior is through their ability to synthesize neurochemicals and their precursors that are analogous in structure to those of the host nervous system (Lyte, 2013). Precursors of monoamines involved in ADHD (i.e. dopamine, noradrenaline, serotonin; see above) are produced by several members of the gut microbiota (Desbonnet et al., 2008; Clayton et al., 2012; Gertsman et al., 2015). These precursors (i.e. phenylalanine, tyrosine, tryptophan) might be absorbed through the intestinal epithelium, enter the portal circulation (Lyte et al., 2013), and cross the blood-brain barrier; in this way, they could potentially influence host monoamine synthesis. Consequently, differences in abundance and/or metabolic activity of monoamine precursor-producing inhabitants of the gastrointestinal tract may affect monoamine-related brain functioning and behavior relevant to ADHD, such as emotion regulation. Indeed, a lowered abundance of Bifidobacterium in infancy has been associated with increased risk of developing ADHD and Asperger syndrome in childhood in a study focusing on particular microbiota (Partty et al., 2015). Several recent studies have indicated altered microbiome constitutions in ADHD may be related to emotion regulation factors (e.g. Osadchiy et al., 2019; Mathee et al., 2020).

A study by our group demonstrated altered neural activation during emotional face processing in relation to traits of reactive aggression adults with ADHD (Jakobi et al., under review). The current report is a follow up of our previous work by Jakobi et al., (under review), and aims to expand and unite these previous brain imaging findings with microbiome data in a large sample of adults with ADHD. This allows us to investigate the role of the microbiome in determining the association between emotion regulation, reactive aggression and neural alterations in ADHD.

Methods

A total number of 83 adults with a confirmed diagnosis of ADHD and 79 healthy control subjects participated in this fMRI experiment. Participants were included in the ADHD group if they had been diagnosed with ADHD by a clinician. To confirm the diagnosis and assess previous and current symptoms in all participants, we conducted the Diagnostic Interview for Adult ADHD (DIVA 2.0; (Kooij and Francken 2010)).. Exclusion criteria for all participants comprised (1) an age younger than 18 or older than 60 years, (2) neurological disorders, (3) psychosis or substance abuse in the last 6 months, (4) current major depression, (5) psycho-pharmaceutical therapy other than stimulants, (6) impairments of hearing, seeing and sensorimotor abilities, (7) problems with understanding Dutch (to ensure that all of the participants understood the study protocol and the task instructions) as well as (8) non-Caucasian ethnicity. The 40 participants with ADHD that received regular pharmacological treatment with stimulants, were asked to pause their medication intake 24 hours prior to participation. Missing data of the reactive proactive aggression questionnaire of five participants from the ADHD and failed fMRI data preprocessing of one control subject resulted in a total sample of 78 participants with and 78 participants without ADHD. Both groups had comparable distributions of age, sex, IQ and educational background (see Table 1).

Measure	Control group, n=78	ADHD group, n=78	Difference, p-value
Sex, percentage male participants	48.7%	43.6%	*0.596*
Age in years (SD)	34.2 (13.1)	34.1 (10.54)	*0.656*
Education (SD)	4.6 (1.6)	4.1 (1.6)	*0.019*
IQ (SD)	106.1 (13.6)*	108.7 (13.8)	*0.479*
DIVA, mean number of symptoms			
Attention symptoms in Adulthood (or current) (SD)	0.79 (1.27)	7.32 (1.99)	$p < 0.001$ ***
Attention symptoms in childhood (SD)	0.49 (0.84)	7.23 (1.83)	$p < 0.001$ ***
Hyperactivity/Impulsivity Adult (SD)	0.83 (1.37)	5.59 (2.24)	$p < 0.001$ ***
Hyperactivity/Impulsivity Child (SD)	0.83 (1.36)	5.57 (2.65)	$p < 0.001$ ***
DIVA, percentage of adults reporting impairment			
Occupation	0%	74.3%	$p < 0.001$ ***
Relationship and Family	0%	65.4%	$p < 0.001$ ***
Social Contacts	0%	39.7%	$p < 0.001$ ***
Hobby	1.2%	53.8%	$p < 0.001$ ***
Self-image	0%	64.1%	$p < 0.001$ ***

*Table 1. Demographic description of the sample, reporting mean scores and standard deviations of age, highest achieved educational degree (measured on a scale of 1 to 8 in the Dutch education system), BMI, IQ score, number of present symptoms of inattention and hyperactivity/Impulsivity in childhood and adulthood and the percentage of subjects reporting impairments in occupation, relationship and family, social contacts, hobbies and self-image. *The IQ estimate of one control subject was missing. Statistical testing was performed using the Mann Whitney test as well as the Chi-squared test for distribution free comparisons of independent samples with a significance level of p = .001, marked by ***.*

Experimental Procedure

Participation was structured in two parts. The first part included the diagnostic screening for ADHD using the DIVA and a short screening for comorbid psychiatric disorders following the Structured Clinical Interview for DSM, SCID-5. Demographic information was collected and IQ testing was performed using block-design and vocabulary subtests of the Wechsler Adult Intelligence Scale (WAIS; (Wechsler 1955)). In the second part, structural and functional MRI scans were performed. Participants were asked to withhold the use of stimulant medication for 24 hours prior to the scanning appointment. After the visit, participants were asked to fill in questionnaires via an online platform, among others the Reactive-Proactive Aggression Questionnaire (RPQ; (Raine, Dodge et al. 2006)). The RPQ is a 23-item self-report questionnaire inquiring 11 items of reactive and 12 of proactive aggression scores. As reactive, but not proactive aggression is implicated in ADHD as well as emotion regulation issues, only the reactive subscale was used in the subsequent analyses.

Analysis of Reactive Aggressive Behavior

To investigate the association between ADHD and the reactive aggression score of the RPQ, we used a linear regression analysis, modeling the Reactive Aggression scores by the binomial factor 'diagnosis' (1 = ADHD group, 0 = control group). We included age and sex as covariates in the model. The analysis was conducted in R version 3.6.1.

fMRI Task

To investigate subliminal emotion processing during MRI, an adapted dynamic facial expression task was applied, showing faces morphing from a neutral face to an angry, fearful or happy facial expression in short clips of four frames (Hermans, Putman et al. 2006). The stimuli were taken from a standardized set and consisted of 10 grey-scale clips per emotion with a duration of approximately 450 milliseconds, each represented by a different actor of male and female gender in equal distribution. During the experimental session, we presented 6 blocks per emotion in counterbalanced order interleaved with 9 blocks showing a fixation cross. In one trial of each block, a red dot was displayed on the forehead of the actor (see figure SF1 in the section Supplementary Materials). Participants were asked to press the button on a response box fixated on their leg as soon as the red dot appeared on the actor's face. The scanning time for this task was approximately 10 minutes.

Image Acquisition

MRI scans were conducted using a 32-channel 3 Tesla Siemens Magnetom Prisma scanner (Siemens Trio, Erlangen, Germany). A T1-weighted MPRAGE sequence (TI = 1100 ms, flip angle = 8°, TE = 3.03 ms, TR = 2300.0 ms, bandwidth = 130 Hz/Px) with 192 sagittal slices (slice thickness = 1.0 mm) was used for the structural scanning, providing whole-brain coverage. Functional blood oxygen level–dependent (BOLD) images were collected using a T2*-weighted echo-planar imaging (EPI) sequence (TR = 1000 ms, TE = 34.0, flip angle = 60°, FOV = 210 mm, voxel size = 2x2x2 mm^3, 66 slices, interleaved acquisition, slice thickness = 2.00 mm). Preprocessing was performed in FSL FEAT. The first 5 images were discarded from further analysis. After grand mean scaling and boundary based registration to the structural image and realignment as motion correction, a Gaussian filter of 5 mm kernel was applied to the images. Motion correction was performed using a dedicated independent component analysis based selection algorithm (ICA-AROMA; (Pruim, Mennes et al. 2015)). Additionally, the average signal of white-matter and corticospinal fluid were subtracted from the data.

fMRI task activation

Single subject fMRI analysis were performed in FSL FEAT (version 6.0.3) using a general linear model (GLM) with three regressors of interest modeling the onsets of happy, angry and fearful face blocks with a duration of 22.5 seconds as well two regressors of no interest modeling the trials with a red

dot and the timing of the response as event markers with a duration of 0 seconds. All events were convolved with the canonical hemodynamic response function (HRF).

Three group level contrasts were defined by contrasting each emotional condition against the implicit baseline of the fixation blocks resulting in Happy > Fixation, Angry > Fixation and Fear > Fixation images. We included the factors group and emotion in the three levels angry, fear and happy in a mixed effects model and investigated the group effects for each emotion separately as well as for all conditions together (Emotion > Fixation). Results are reported at a family wise error (FWE) corrected significance level of $p < 0.05$.

Analysis of fMRI Task Activation and Reactive Aggression

To analyze the relationship between reactive aggressive scores with implicit emotion regulation and emotional reactivity, we conducted a covariate analysis. Hereby, we included the contrast of the sum of all emotions versus the implicit baseline (Emotion > Fixation) as one summary measure per subject as well as the individual reactive aggression scores as a mean centered continuous covariate in the second level GLM. Since only participants with ADHD showed significantly elevated reactive aggression scores compared to control subjects in the linear regression analysis, we were interested in the group specific correlates of reactive aggressive behavior during emotion processing. Therefore we investigated the interaction of group with reactive aggression and further looked at the effect in each group individually. To correct for multiple comparisons in this analysis, we applied a Monte-Carlo-simulation of 1000 iterations for cluster extent correction, resulting in a cluster extent of 11 resampled voxels at a significance level of $p = 0.001$.

Microbiome

The human fecal samples were collected at home by the participant and sequenced using the V1-V2 region of the 16S rRNA gene. The sequenced data was analyzed through NG-Tax 16S rRNA pipeline and classified using the SILVA reference database (version 128). For more information about the collection and pre-processing of the microbiome, see Szopinska-Tokov et al. (2020).

A prevalence cut-off of 20% for genera and samples was applied before taxonomic analyses. Three meta-analyses were performed to associate bacterial relative abundance between NeuroIMAGE and COMPULS with 1) participants with and without ADHD, 2) age, only in participants with ADHD, and 3) age, only in participants without ADHD. The package MMUPHin was used with total sum scoring and an arcsine square-root transformation (Ma 2019, Ma et al. 2021). As covariates age, gender and BMI was taken into account.

Statistics

Subsequently, effects of the significant microbiome genera were investigated using linear mixed effects models, using the beta-values from each ROI which showed significant effects in the main fMRI group analysis as predictors (using the formula displayed in Figure 1). Here, bacteria strain was entered as the independent variable. Diagnosis, age, BMI and Sex were covariates.

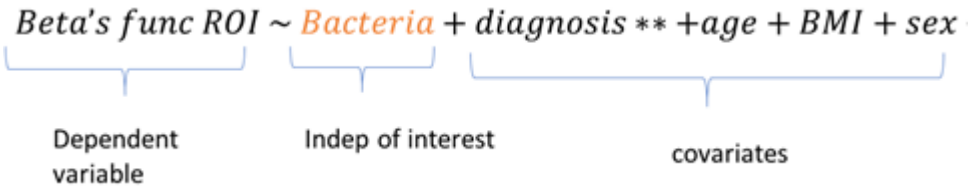

Figure 1. formula for comparison of microbiome effects within each ROI

Results

Microbiome analysis between groups

Between group analysis for cases vs controls was performed for all bacterial genera performed using the linear models depicted in Figure 1. Significant effects from this analysis indicate five genera which show significant abundance differences between cases and controls (see Table 2).

The observed abundance of these genera was subsequently entered as predictors in the fMRI ROI analysis.

	baseMean	log2FoldChange	lfcSE	stat	p-value	p-adjusted
Megamonas	31.11	11.39	2.63	4.33	0.0001	0.0034
Erysipelatoclostridium	277.05	1.24	0.33	3.71	0.0002	0.0232
Colidextribacter	1232.31	-0.52	0.15	-3.42	0.0006	0.0348
Fournierella	136.57	-1.84	0.55	-3.36	0.0008	0.0348
Eisenbergiella	66.66	1.75	0.52	3.39	0.0007	0.0348

Table 2. Candidate microbiota with significant differences between adults with ADHD and controls.

fMRI task activation

For the whole-brain analysis of viewing all emotions compared to the implicit baseline, ADHD cases and controls did not show any differential activation patterns. Both groups had higher activity to emotional faces in areas including temporoparietal, inferior and superior frontal cortical as well as subcortical areas such as parts of the limbic system. No significant differences were found when analyzing the emotions separately. Comparisons were made at the FWE-corrected threshold of p = 0.05.

fMRI between group effects

The analysis of reactive aggression in the ADHD group showed significantly elevated activation levels of clusters including the precentral gyrus, cortical frontal and temporal areas as well as subcortical structures such as the hippocampus, corrected for cluster extent threshold. Furthermore two clusters within the right Amygdala (p = 0.00043, xyz = 22,-8,-8 and p = 0.000375, xyz = 28,-10,-1) were activated, but did not exceed the threshold of 11 voxels each. All clusters were implicated in higher reactive aggression. In accordance with generally high ratings of reactive aggression within the ADHD group, there was no significant effect of low reactive aggression, see Figure 2 and Table 4 for more information on the significant clusters.

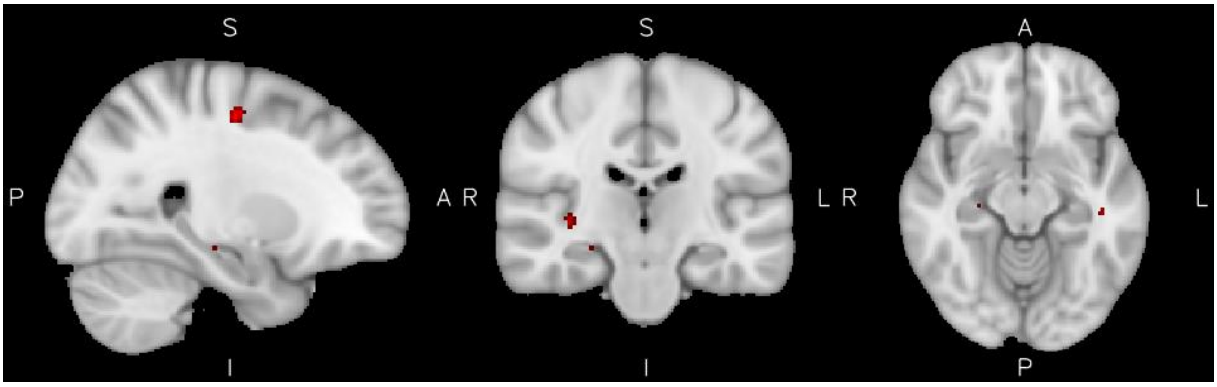

Figure 1. Results from the reactive aggression analysis in the ADHD group at the maximum of the cluster in the Hippocampus, highlited by a black square in the left figure (slices x= 26 left, y= -22 middle and z = -12 right), at a cluster extent correction of 11 voxels for p = 0.001.

Cluster label peak	Voxels	P	Z-MAX	X (mm)	Y (mm)	Z (mm)
ADHD						
R Precentral gyrus	83	2.47e-08***	5.45	24	-12	50
L Middle frontal gyrus	42	9.27e-05***	3.74	32	18	56
R Superior frontal gyrus	33	7.7e-05***	3.78	6	32	62
R Inferior/middle temporal gyrus	33	2.96e-07***	4.99	64	-32	-18
R Insula	20	5.85e-06***	4.38	36	-22	2
R Lingual gyrus	18	0.000236***	3.5	16	-70	-6
R Hippocampus	14	0.000304***	3.43	26	-22	-12

Table 3. Results of the whole-brain analysis for the analysis of reactive aggression within the ADHD group, cluster extent correction of 11 voxel for p = .001.

fMRI ROI analysis of microbiome effects.

The seven clusters which showed a significant activation difference as a function of reactive aggression were subsequently used as ROIs. The extracted beta-values from these areas were entered as outcome variables in a linear model, with the five microbiome genera abundance measures as predictors. Results are shown in Table 4., depicted p-values are uncorrected for multiple comparisons. Only one uncorrected significant effect is observed, that of the genera Fourierella on Left Middle Temporal Gyrus activation, but this effect does not survive multiple comparisons corrections.

	Hippocampus		R_SFG		R_Precentral		R_MFG		R_Lingual		R_Insula		L_MTG	
Genera	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Erysipelatoclostridium	0.988	0.325	0.732	0.465	-0.577	0.565	-0.259	0.796	-0.324	0.747	0.921	0.359	0.778	0.438
Eisenbergiella	1.012	0.313	1.165	0.246	0.690	0.491	-0.076	0.940	-0.545	0.586	0.414	0.680	-0.304	0.762
Colidextribacter	-0.556	0.579	-0.506	0.614	-0.313	0.755	-1.028	0.306	0.218	0.828	-0.251	0.802	-0.370	0.712
Fournierella	1.119	0.265	-0.213	0.832	1.114	0.267	1.107	0.270	-1.932	0.055	2.291	0.023	1.988	0.049
Megamonas	1.327	0.187	0.966	0.336	0.417	0.677	0.288	0.774	-0.303	0.762	0.032	0.974	0.134	0.893

Table 4. Results of the linear regression models, testing the effect of each of the microbiome genera on fMRI activation in seven individual ROI's.

Discussion

Our results indicate that there are differences in the microbiome between cases with ADHD and controls, specifically in the abundance of the genera Megamonas, Erysipelatoclostridium, Colidextribacter, Fournierella and Eisenbergiella. Subsequent fMRI analyses of the association between these bacterial abundances and neural activation as a function of reactive aggression show a small effect of the Fournierella bacteria on Left Middle Temporal Gyrus activation, but this effect did not survive correction for multiple comparisons.

Our results show that both microbiome and neural activation differences in the emotion recognition network can be observed in this sample of adults with ADHD, but these two types of factors seem to influence the ADHD phenotype independently.

It must be noted however, that this was a relatively small sample of adults with and without ADHD, and the statistical power to detect subtle effects of microbiome on brain and behavior was likely limited. A more extensive sample might be warranted for future research, as might a more powerful behavioral paradigm to measure reactive aggression in a laboratory setting. An additional further avenue of investigation can be indicated into the larger biological pathway connecting microbiome, brain activation and behavior. Factors like stress, inflammation markers and structural brain alterations may need to be incorporated to obtain a fuller view of the causal pathways underlying microbiome effects in ADHD.

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