Eat2beNICE

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

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D5.6 Manuscript on the predictive power of selected diet/exercise/inflammation biomarkers for impulsive/compulsive behaviour

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Executive Summary

We used the PROBIA-dataset with all ADHD cases to assess the relationship between a wide range of inflammatory proteins and symptoms of impulsivity and stress. To generate an unbiased dataset, we assessed 96 inflammation-related proteins in serum of 126 fasted patients with ADHD collected in WP2. We used network analysis to visualize the proteins and pathways. Next, we distinguished two cluster of patients: those showing higher levels of inflammatory proteins compared to those with lower levels of proteins. We then compared the association of these clusters with symptoms of impulsivity and compulsivity, as well as stress. We used linear regression for association analysis including various covariates (age, sex, BMI, study site, anti-depressant medication and smoking status). We found significant associations of the inflammatory cluster with perceived stress scores and ADHD severity (in particular inattention and impulsivity), but the associations were reduced to a trend after correction for multiple testing.

Abbreviations

ADHD	Attention deficit / hyperactivity disorder
RCT	Randomized Controlled Trial
OLINK	Olink Target 96 Inflammation panel
BMI	Body Mass Index
ADHD RS	ADHD Rating Scale
FDR	False Discovery Rate

1. Deliverable report

Introduction

Attention deficit hyperactivity disorder(ADHD), is a heterogeneous neurodevelopmental syndrome characterized by behavioural manifestations such as impulsivity, inattention, and hyperactivity. ADHD typically starts during childhood, but persists into adulthood in up to 60% of patients, although a shift in symptoms can be observed. External symptoms attenuate, while internal symptoms (e.g., particularly restlessness, impulsivity, and inattention) remain or even aggravate. To help improve symptoms, pharmacological treatment of ADHD is highly efficient in approximately 82%. Yet, the patho-mechanistic effects leading to ADHD are still incompletely understood. Next to genetic and environmental etiopathological factors, imbalance of neurotransmitters, and dopamine and noradrenaline deficiency, recent literature suggests (neuro-)inflammation and defective immune regulation as potential contributing factor to ADHD.

However, it is unclear, if inflammation relates to ADHD severity and in particular, impulsivity. Therefore, in a large population with aADHD who have been stable on medication for at least 4 weeks, we aim to compare inflammatory protein levels and explore associations of symptoms and inflammation.

Participants

Data for this deliverable was derived from the PROBIA cohort, collected between 2016 and 2021. The first cohort includes patients with ADHD which have participated in the PROBIA study and have extensive information on medication intake available. For the purpose of this study, only baseline data of patients with ADHD was used. Participants had to be between 18 and 65 years of age, with no major neurological, cardiovascular, endocrine, pulmonary, or gastrointestinal illness nor any major psychiatric disorders with psychotic symptoms present in history or at screening. Importantly, successful inclusion required stable medication for ADHD (e.g., type and stable dosage for at least 30 days, or alternatively, no medication for at least 30 days). Subjects were excluded if antibiotics, probiotics, or immunosuppressants (e.g., glucocorticoids, but not aspirin) were currently prescribed or taken within the last 30 days. Patients with ADHD had to have received a diagnosis by a clinician, or the suspicion of diagnosis had to be mentioned in the patient files. Participants were excluded if ADHD diagnosis was not confirmed, or the participant did not show at least moderate symptoms of impulsivity and/or clinician-judged illness.

Table 1. Demographics of study population; 1 Mean (SD); Median (IQR); n (%); 2 in years, estimated by the calculation of (current year - birth year)

Demographics	***N***	***126***
ADHD Rating Scale	123	33 (9)
Age2	126	40 (28, 48)
Sex	126	
Female	73	73 (58%)
ADHD-Diagnose	126	
Combined type		97 (77%)
Predominantly inattentive type		24 (19%)
Predominantly hyperactive-impulsive type		5 (4.0%)

Demographics	***N***	***126***
Ethnicity	126	
White (Caucasian)		97 (77%)
Other ethnic group		24 (19%)
Asian		3 (2.4%)
American Indian		1 (0.8%)
Hispanic or Latino		1 (0.8%)
BMI (kg/m^2)	125	25.2 (22.0, 28.9)
Tobacco use	126	
Non-Smoker		66 (52%)
Smoker		55 (44%)
Occasional-Smokers		5 (4.0%)
ISCE	125	
Tertiary education		95 (76%)
Secondary education		19 (15%)
not else classified		6 (4.8%)
Primary education		5 (4.0%)
Salary	116	
average salary		47 (41%)
< mean salary		45 (39%)
> mean salary		24 (21%)
ADHD in family	126	
No		51 (40%)
Unknown		40 (32%)
Yes		35 (28%)

Data Acquisition

Approximately one week after the screening appointment, a blood sample was collected by venipuncture, between 7:30 -15:30 during a study visit at the respective Departments of Psychiatry at Goethe University Clinic Frankfurt, Semmelweis University Budapest, Hungary, and Universitari Vall d'Hebron, Barcelona; Spain. Participants were asked to remain sober for at least 8 hours prior to blood sampling. All testings were completed in the period between March 2019 and March 2021. On the testing day, participants were asked to rate their current ADHD symptoms severity via the self-reported ADHD Rating scale (ADHD-RS). The 18 items (9 in inattention, 5 in hyperactivity, and 4 in impulsivity) refer to the last six months before assessment and include a total score ranging from 0 to 54. Moreover, participants were asked to indicate their perceived stress level within the last month on the well-established self-reporting Perceived Stress Scale (PSS). Ten question items are to be answered, referring to how often felt or thought. The total score ranges from 0 to 40.

Protein Analysis

Proteomic analysis was performed via Olink Target 96 Inflammation panel. Collected plasma samples were used to detect levels of 92 inflammation-related protein biomarkers simultaneously. A high-throughput, multiplex inflammation panel by Olink (Olink Proteomics, Uppsala, Sweden) was used for proteomics analyses.

Statistical Analysis

A cluster analysis of protein level means from ADHD participants was performed. Missing values were computed with a random forest imputation. Eucludian distance was used. The ward.2 algorithm was applied, where its minimum variance criterion minimizes the total within-cluster variance. With the use of the R packages NbClust, cluster and factoextra, optimal number of clusters was found, and data was plotted. Means of each cluster's observation were compared and a silhouette analysis for internal cluster validation was performed. Clusters were then compared based on demographics and psychological characteristics. Age, BMI, inattention, impulsivity and hyperactivity were not normally distributed and transformed with the Tukey's Ladder of Powers. The Shapiro-Wilk test was used to check normal distribution again and characteristics were displayed as median ± standard deviation (SD) (if normally distributed) or median ± interquartile ranges (IQR) for continuous variables.

Results and Discussion

Cluster Analysis and signaling pathways

Two patient clusters were identified based on the inflammatory protein panels measured: cluster 1 with higher levels of protein and cluster two with relatively lower expression of proteins (**Figure 1**.). Patients differed particularly in the protein expression for 18 inflammatory proteins, nine of which (CCL3, CCL4, CXCL1, CXCL11, CXCL5, CXCL6, IL7, MMP1, TFGB1) are highly connected in their role as pro-inflammatory mediators in NF-kappa-B and prostaglandin activation and are involved in cytokine to cytokine receptor interactions based on KEGG pathways.

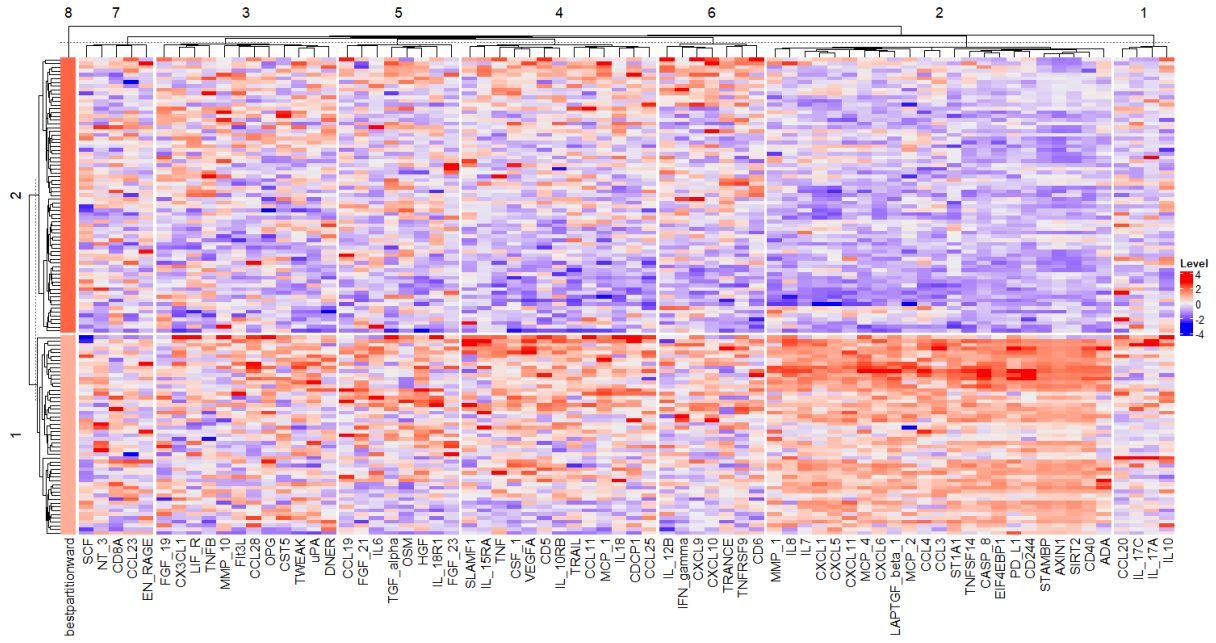

Figure 1. Heatmap illustrating the identified clusters. Patient clusters 1 and 2 differ in the expression levels of proteins particularly in the protein cluster 2 (ranging from MMP-1 to ADA). Red illustrates higher protein levels and blue lower protein levels.

Association of clusters with symptoms of impulsivity, inattention, hyperactivity and stress

Patient clinical characteristics were explored based on cluster assignment. Characteristics that were explored include stress (based on the sum of the Perceived Stress Scale), overall ADHD Rating Scale and hyperactivity, impulsivity, and inattention (as subcategory scores form ADHD Rating Score) and various covariates (age, sex, BMI, smoking status, ADHD medication and antidepressant medication.) The outcome revealed participants in cluster 1 (i.e., with higher levels of inflammatory proteins) had higher stress levels (p-value = 0.015, q-value = 0.065) and higher ADHD symptoms, as assessed with the overall ADHD RS score (p-value = 0.026, q-value = 0.065). Exploration of ADHD RS sub-scores revealed that particularly the inattention (p-value = 0.045 q-value = 0.067) and impulsivity score (p-value = 0.053, q-value = 0.067) were most associated with the cluster of elevated inflammatory protein levels (**Table 2**). However, all p values were no longer significant after correction for FDR. In conclusion, we did find a difference in protein expression between patients, which could be described as clusters. However, only a weak association of higher inflammatory proteins with stress, as well as ADHD severity was found and the association did not withstand correction for FDR.

Table 2. Psychological characteristics between Cluster 1 and Cluster 2. Inattention, Hyperactivity, and Impulsivity as subscores from ADHD Rating Scale (ADHD-RS) and transformed with the Tukey's Ladder of Powers. Perceived Stress Scale: sum of Perceived Stress Scale (PSS). 1 Mean (SD); Median (IQR); 2 Wilcoxon rank sum test; 3 Benjamini & Hochberg correction for multiple testing

Psychological Characteristics	***N***	***Overall[1] N = 126***	***1[1], N = 53***	***2[1], N = 73***	***p-value[2]***	***p.adjust[3]***
Perceived Stress Scale	123	22 (7)	24 (6)	21 (7)	0.015	0.065
ADHD Rating Scale	123	33 (9)	35 (8)	31 (9)	0.026	0.065
Inattention Score (ADHD-RS)	125	64 (28)	70 (25)	60 (29)	0.045	0.067
Hyperactivity Score (ADHD-RS)	125	15 (9, 19)	17 (9, 19)	15 (7, 17)	0.14	0.14
Impulsivity Score (ADHD-RS)	124	8.1 (6.6, 11.9)	9.4 (6.9, 13.2)	8.1 (5.6, 10.6)	0.053	0.067

2. Acknowledgement and Disclaimer

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A manuscript with the data is currently in preparation