CANDY

Comorbid Analysis of Neurodevelopmental Disorders and epilepsY

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Report on the association between gut microbiome and NDCs

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1 Executive summary

CANDY focuses on the shared and distinct mechanisms that underpin Autism Spectrum Disorder (ASD), Attention-Deficit Hyperactivity Disorder (ADHD), Intellectual Disability (ID) and epilepsy. With on-going transfer of fecal samples from autism, ADHD, ID and epilepsy participants from various collection sites within the consortium, we took the opportunity to combine the analysis of microbiome data from CANDY participants and of adult ADHD samples to explore association between microbial alterations and neurodevelopmental conditions (NDCs), and between microbial alterations and quantitative ASD traits. To identify robust gut-microbiome alterations in people with NDCs, we harmonized bioinformatic pipelines and analyses of raw 16S rRNA sequencing data from CANDY participants (N_{ASD} = 191, $N_{No\ ASD}$ = 156) and four adult ADHD case-control studies (N_{ADHD}=312, N_{NoADHD}=305). We investigated diversity and differential abundance of selected genera (logistic regression), corrected for age and sex, and meta-analysed the study results. 3 bacterial genera were robustly associated with neurodevelopmental conditions, and one genus overlapped with ASD. Existing literature points towards a role of these genera in inflammatory processes. Irreproducible results in the field of gut-microbiota research, due to between study heterogeneity and small sample sizes, stress the need for meta-analytic approaches and large sample sizes. While we robustly identified genera associated with neurodevelopmental conditions, functional studies are needed to shed light on mechanistic pathways. These results contribute significantly to the understanding of whether the microbiome structure differs between patients with NDCs (specifically ADHD and ASD) and healthy controls, and how specific NDC microbiome affects (the risk of) developing NDCs altogether with the quantitative symptoms.

2 Introduction

Context: This work targets one of the project aims of characterizing the role gut microbiota in the risk and severity of NDCs with special focus on ASD, which is one of the main objectives of the CANDY project. The results of this work provide robust evidence for the association between gut microbiota variation across neurodevelopmental conditions.

The gut-microbiome is involved in early brain-development as well as every-day brain functioning; it can modulate the bioavailability of key-signaling molecules (e.g. neurotransmitters or nutrients relevant for energy homeostasis) by influencing the metabolism and the integrity of intestinal- and-blood-brain barriers (Warner, 2019). Through the regulation of the intestinal barrier, but also through the production of short-chain-fatty-acids (SCFA) and the release of cytokines, it further plays an important role in immune and inflammatory responses (for a review see (Warner, 2019)). These pathways might influence neurodevelopmental-related symptoms and pathophysiology (Bull-Larsen & Mohajeri, 2019; Dam et al., 2019). Studies showing associations between microbial composition and altered neurodevelopment (for a review, see (Liu et al., 2019)), psychiatric disorders (for a review, see (Nikolova et al., 2021)) and common (somatic) comorbidities of ASD (Bougeard et al., 2021) have fueled hypotheses about the potential role of the gut-microbiome alterations across neurodevelopmental disorders. The few published studies investigating gut-microbiome alterations in ADHD and ASD, however, report inconsistent results. Most authors reported no differences between individuals with and without the conditions or conflicting results for gut-microbiome diversity (Bundgaard-Nielsen et al., 2020; Checa-Ros et al., 2021; Gkougka et al., 2022; Shirvani-Rad et al., 2022; Sukmajaya et al., 2021). The three studies published on the gut microbiota in adults with ADHD showed no overlap in results. The scarcity of consistent results renders biological interpretations of gut-microbiome alterations difficult as well as consideration of the abundance of particular taxa as potential biomarkers or treatment targets for neurodevelopmental conditions complex.

The variability in gut-microbiome study results can be attributed to several factors. Most relevant may be an insufficient statistical power to detect the (expected) small effects due to the small sample sizes of gut-microbiome studies published to date (mean n_{ADHD} = 39, mean n_{noADHD} = 49 (Sukmajaya et al., 2021). Challenges in comparing study results further arise from differences in participant demographics like age, sex, and ethnicity, as well as methodological heterogeneity. The absence of standardized bioinformatic pipelines and statistical analyses exacerbates inconsistencies in (NDC-related) gut-microbiome research. Standardizing data analysis approaches,

like using logistic regression, could enhance result comparability and accuracy. Additionally, adopting consistent statistical methods across studies is crucial for obtaining reliable and comparable findings.

In this study, we aimed to investigate gut-microbiome alterations across neurodevelopmental conditions, identify genera associated with the conditions, and assess their specific link with autism symptom severity. To assure robustness of the results, we applied three strategies: 1) We harmonised the bioinformatic pipelines for sequencing data of five case-control cohorts of adults with and without NDCs (N=964); 2) we investigated diversity and differential abundance across tools and indices, correcting for common confounders per study; 3) we meta-analysed the results across studies; and 4) we performed association tests only on features relevant in ASD.

3 Main section

3.1 Methods

3.1.1 Cohort Description

We received clinical information and raw 16s rRNA fecal microbiota sequences from CANDY cohort and four adult cohorts (comprising the three articles including adults published to date: the NeuroIMAGE cohort (Aarts et al., 2017; Szopinska-Tokov et al., 2021) and the Mental-Cat cohort from the Vall d'Hebron Research Institute in Barcelona (VHIR) (Richarte et al., 2021); and unpublished data from the MIND-Set cohort (Eijndhoven et al., 2022) and our own cohort IMpACT2-NL (Jakobi et al., 2023) .

The CANDY cohort comprises children and adults with neurodevelopmental conditions, as well as unrelated unaffected controls. The NeuroIMAGE cohort comprises adolescents and young adults with ADHD, their family members, and unrelated healthy controls; only adult participants were included in this study. The Mental-Cat cohort consists of medication-naive adults with and without ADHD. The MIND-Set cohort includes adults with ADHD exhibiting a high level of psychiatric comorbidity and psychopharmacological treatment, as well as psychiatrically healthy individuals. **Table 1** provides a demographic overview of the included sample. At the time of this analysis, quantitative ASD traits were available only for a small subset of the CANDY cohort (n = 67).

Table 1: Demographic description and characteristics of the included studies.

Cohort Recruitment period Recruitment Country	**CANDY** 2020 – 2023 Multi-sites		**IMpACT2-NL** 2017-2020 The Netherlands		**NeuroIMAGE** 2009- 2012 The Netherlands		**MIND-Set** 2016-2021 The Netherlands		**Mental-Cat** 2016-2018 Spain	
Diagnostic group	Control	ASD	Control	ADHD	Control	ADHD	Control	ADHD	Control	ADHD
Number of subjects	156	191	78	81	39	29	100	104	100	100
Sex, proportion female participants	0.42	0.29	0.47	0.58	0.49	0.31	0.53	0.42	0.53	0.49
Age mean years (SD)	17.1 (15.9.)	16.7 (14)	34.4 (13.1)	33.9 (10.3)	21.8 (2.6)	22.2 (3.1)	38.9 (16.5)	37.4 (12.5)	30.3 (8.5)	33.2 (11.4)
Sequencing Region	V4		V4		V1/V2		V4		V3/V4	

Cohort Recruitment period Recruitment Country	**CANDY** 2020 – 2023 Multi-sites	**IMpACT2-NL** 2017-2020 The Netherlands	**NeuroIMAGE** 2009- 2012 The Netherlands	**MIND-Set** 2016-2021 The Netherlands	**Mental-Cat** 2016-2018 Spain
Sequencing platform	Illumina NovaSeq 6000	Illumina NovaSeq 6000	Illumina HiSeq PE300	Illumina NovaSeq 6000	Illumina MiSeq 300-nt
Psychiatric comorbidity	0.41	Exclusion criterium	Exclusion criterium	0.83	Exclusion criterium

3. 1.1. ASD quantitative

In the CANDY cohort, 202 (78 control & 124 ASD) participants have completed ASD symptom severity measurements using the Autism Diagnostic Observation Schedule second edition (ADOS-2) modules, among others (Hus & Lord, 2014). ADOS-2 module yielded two component scores and one cumulative score, namely social affect (SA), restricted and repetitive behaviors (RRB), and the total comparative severity scores (CSS). The total score is computed as the sum of both SA and RRB.

3.1.2 Microbiome processing

Preprocessing was harmonised and performed per study using QIIME2 pipeline defaults (Bolyen et al., 2019). In short, the raw forward and reverse reads were demultiplexed and then denoised using DADA2 (Callahan et al., 2016), where the primers were trimmed off, sequencing errors and erroneous read combinations were removed and clusters of representative sequences (amplicon sequence variants, ASVs) were identified. We assured high sequencing quality by truncating the reads displaying a median phred-score of 30 (marking 99.9% base-call accuracy) towards the end of the reads. Taxonomy was assigned using a naive bayes classifier, pre-trained on a SILVA reference database (version 138, 99% OTUs full-length sequences). Non-bacterial ASVs were removed. We then investigated potential differences in alpha and beta diversity as well as composition per study, correcting for age and sex.

3.1.3 Alpha Diversity

All further analyses were performed in R. We estimated three indices of alpha diversity, considering 1) number of observed ASVs, 2) abundance of ASVs (Shannon index), and 3) phylogenic relationships between ASVs (phylogenic distance) (*microbiome* package). We applied rank-based nonparametric regression analysis (*Rfit* package) for each study (alpha diversity ~ ADHD diagnosis + sex + age). Then, we extracted the standardized correlation coefficient (r) as effect size measure per study to meta

analyze across the five cohorts with a random-effects models, which estimates study heterogeneity (*metafor* package).

3.1.4 Beta Diversity

Aitchison distance was used as a measure of beta diversity. PERMANOVA (adonis2) with 999 permutations was applied per study and all study combined to estimate the association between beta diversity and NDC diagnosis. The models were adjusted for both age and gender.

3.1.5 Composition

To investigate microbial composition, we aggregated the data to the genus level, applied a prevalence threshold of 10% and transformed the abundances using a Center Log Ratio (CLR) transformation. We applied differential abundance analysis per study, using logistic regressions associating NDC diagnosis with the CLR-transformed abundance. To ensure that we are testing taxa that are relevant and shared across NDC, we only meta-analysed genera that are present in all cohorts with at least 10% prevalence. To determine taxa most relevant for ASD, we performed a separate meta-analysis excluding the CANDY cohort. Features that were not present in the first meta-analysis or present with smaller effect size were taken as ASD-relevant features. This approach allowed us sufficient statistical power to optimally retrieve information. ASD-relevant features were further tested for associations with comparative severity score total, social affect CSS, and RRB CSS. We applied a significance threshold of $p < 0.05$, where *p-values* were false discovery rate (FDR) corrected.

3.2 Results

3.2.1 Alpha Diversity

We found no significant differences of alpha diversity between adults with and without NDCs, neither on the individual study level nor in the meta-analysis, in terms of observed ASV, Shannon index, or Chao1 diversity.

3.2.2 Beta Diversity

At a combined study level, we found significant group effect in beta-diversity (table 2), meaning that people with NDC showed distinct gut microbial community composition. However, at the individual study level, only one out of five studies showed differences in beta diversity between people with and without NDC (IMpACT2-NL, $F = 1.36$, $p = 0.018$).

***Table 2* PERMANOVA results of all cohorts combined.**

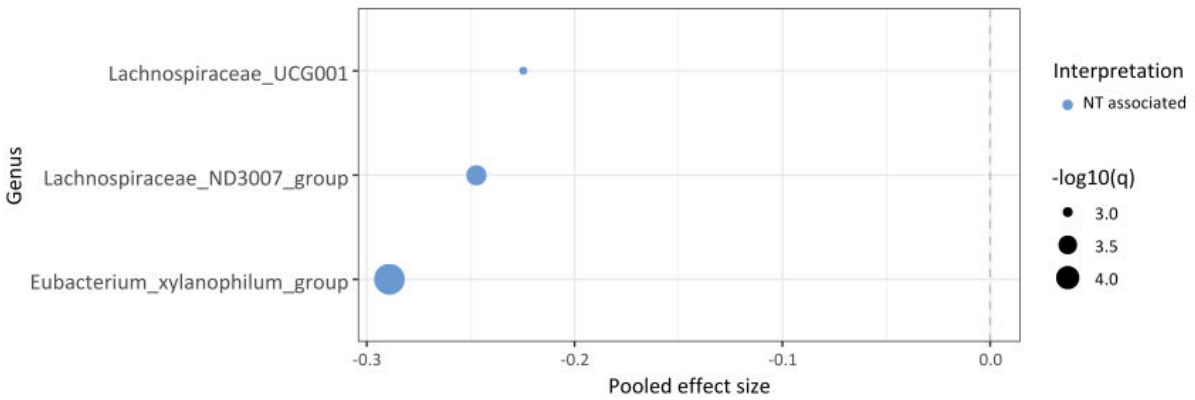
* significant at p < 0.001

	R2	F	P-value
Diagnosis (0/1)	0.00558	4.5135	0.001*
Age	0.02713	21.9243	0.001*
Sex	0.00431	3.4854	0.001*

3.2.3 Composition

A total of 93 genera were selected over all five studies based on 10% prevalence threshold in each cohort. Logistic regression-based meta-analysis of the 93 selected genera identified 3 significantly different genera between individuals with and without NDC (figure 1). All of them showed lower abundance in individuals with compared to individuals without NDC.

Figure 1 *Forest plot depicting pooled effect sizes of taxa showing significant associations with neurodevelopmental disorders. The effect sizes came from per-study logistic regression of one ASD cohort (CANDY) and four ADHD cohorts. The total sample sizes were 964. The size of each point represents the negative log10 of the FDR corrected p-values. NT = neurotypical*



To identify features especially relevant in ASD, we performed another meta-analysis excluding the CANDY cohort, resulting in one overlapping genus showing an increased effect size magnitude (figure 2). The two genera found uniquely in the full meta-analysis were further assessed for its correlation with quantitative ASD measurements in a subset of CANDY participants. We found no associations between these genera and any of the quantitative ASD traits (figure 3).

Figure 2 *Heatmap depicting effect sizes and FDR significance from meta-analysis with five cohorts and meta-analysis without the CANDY cohort. Genera that showed greater effect size magnitude on the first meta-analysis or only appeared on the first meta-analysis were taken as relevant genera for ASD. Values on the tiles represent* $p_{FDR.}$ ** significant at* $p_{FDR} < 0.05$.

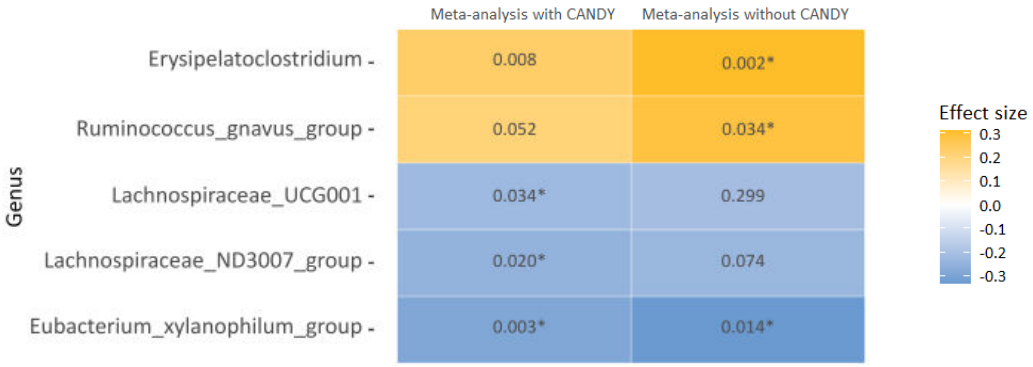
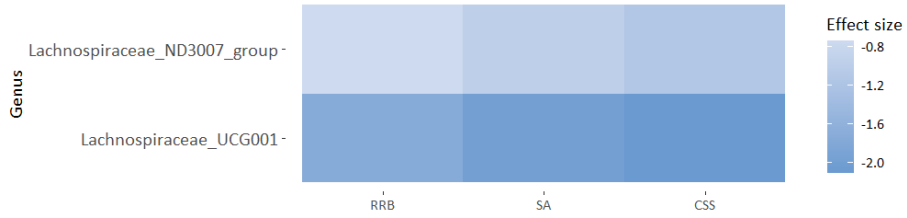


Figure 3 *Heatmap depicting standardized effect size of ASD-relevant genera. Positive effect sizes, depicted in shades of yellow, indicate positive associations while negative effect sizes, depicted in blue, indicate negative associations. None of the genera were linked to any ASD quantitative measurement from the ADOS-2. RRB: Repeated, Restricted Behaviour, SA: Social Affect, CSS: Comparative Severity Score.*



4 Conclusions

We found differences in beta diversity and identified robust microbiome-compositional alterations in individuals with NDC. Interestingly, some of these taxa seem to be more relevant for ADHD and some for ASD.

Two genera from the family Lachnospiraceae, genus *Lachnospiraceae ND3007 group* and *Lachnospiraceae UCG-001* were less abundant in individuals with NDC and especially relevant for ASD. The former was negatively associated with externalizing behavior in early developmental stage

(Ou et al., 2024). Our observation supports this finding, as we found the abundance of this genus to be lower in individuals with NDC diagnosis. Interestingly, this genus was found to also be associated with irritable bowel diseases (Vestergaard et al., 2023). Another study suggested its role in anti-inflammation as it is considered a producer of SCFAs (Nishiwaki et al., 2020). Metabolism of polyphenols into SCFAs by the gut microbiome is discussed as a potential mechanism through which polyphenols might unfold their anti-inflammatory properties (Diotallevi et al., 2020). Associations of this genus with beneficial effects on immune functioning might be relevant for NDC, potentially supporting favorable health outcomes, but these interpretations are speculative as functional studies in human have not been exhaustively studied yet.

The genus *Eubacterium_Xylanophylum_group* and *Rumminococcus_gnavus_group* were more abundant in individuals with ADHD. The former had been previously associated in ADHD from a meta-analysis of the same cohort (Jakobi et al., 2023) while the later had earlier been found to be enriched in individuals with gastrointestinal conditions such as irritable bowel syndrome (Zhai et al., 2023) and Crohn's disease (Feng et al., 2022). This genus has also been associated with both ASD and ADHD, although our analysis indicated that this feature may be more relevant for ADHD (Bundgaard-Nielsen et al., 2023; Lee et al., 2022). Studies reporting the relevance of this genus across NDC and gastrointestinal dysfunctions suggest a role for gut-barrier functioning and pro-inflammatory processes, which have previously been implicated in the etiology of psychiatric, neurodevelopmental, and neurological conditions (Bauer & Teixeira, 2019; Pellegrini et al., 2023). Additionally, the replicability across different cross-sectional studies for these genera suggests that the functional mechanism underlying its involvement in NDC is worth investigating in the future.

In conclusion, we identified alterations of the gut-microbiome across individuals with various NDC that were robust to study heterogeneity. The NDC-associated genera suggested potential relevance of inflammatory and immune processes for an NDC diagnosis. However, more human and functional studies are needed to support potential interpretations.

Impact: Our results suggest that alterations in the gut microbiota are associated with higher presence of neurodevelopmental conditions. These findings also imply that individuals with ASD and ADHD have shared gut microbiome profiles which are distinct to those of healthy individuals. These differences in gut microbiota composition may contribute to the development and severity of these conditions. While the exact mechanisms underlying the gut microbiota's influence on neurodevelopmental conditions are still being elucidated, the emerging research highlights the importance of gut microbiome health in brain development and function. In the future, this deliverable may be updated in case new data becomes available.

5 Glossary

Abbreviation / acronym	Description
ADHD	Attention Deficit/Hyperactivity Disorder
ADOS-2	Autism Diagnostic Observation Schedule 2nd Edition
ASD	Autism Spectrum Disorder
ASV	Amplicon Sequence Variant
CAP	Canonical Analysis of Principal Coordinates
CSS	Cumulative Severity Score
CLR	Center Log Ratio
FDR	False Discovery Rate
NDC	Neurodevelopmental Conditions
SCFA	Short Chain Fatty Acid

6 Bibliography

Aarts, E., Ederveen, T. H. A., Naaijen, J., Zwiers, M. P., Boekhorst, J., Timmerman, H. M., Smeekens, S. P., Netea, M. G., Buitelaar, J. K., Franke, B., van Hijum, S. A. F. T., & Arias Vasquez, A. (2017). Gut microbiome in ADHD and its relation to neural reward anticipation. *PloS One*, *12*(9), e0183509. https://doi.org/10.1371/journal.pone.0183509

Bauer, M. E., & Teixeira, A. L. (2019). Inflammation in psychiatric disorders: What comes first? *Annals of the New York Academy of Sciences*, *1437*(1), 57-67. https://doi.org/10.1111/nyas.13712

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9

Bougeard, C., Picarel-Blanchot, F., Schmid, R., Campbell, R., & Buitelaar, J. (2021). Prevalence of Autism Spectrum Disorder and Co-morbidities in Children and Adolescents: A Systematic Literature Review. *Frontiers in Psychiatry*, *12*, 744709. https://doi.org/10.3389/fpsyt.2021.744709

Bull-Larsen, S., & Mohajeri, M. H. (2019). The Potential Influence of the Bacterial Microbiome on the Development and Progression of ADHD. *Nutrients*, *11*(11), 2805. https://doi.org/10.3390/nu11112805

Bundgaard-Nielsen, C., Knudsen, J., Leutscher, P. D. C., Lauritsen, M. B., Nyegaard, M., Hagstrøm, S., & Sørensen, S. (2020). Gut microbiota profiles of autism spectrum disorder and attention deficit/hyperactivity disorder: A systematic literature review. *Gut Microbes*, *11*(5), 1172-1187. https://doi.org/10.1080/19490976.2020.1748258

Bundgaard-Nielsen, C., Lauritsen ,Marlene Briciet, Knudsen ,Julie Kristine, Rold ,Louise Søndergaard, Larsen ,Margit Hørup, Hindersson ,Peter, Villadsen ,Annemarie Brusen, Leutscher ,Peter D. C., Hagstrøm ,Søren, Nyegaard ,Mette, & and Sørensen, S. (2023). Children and adolescents with attention deficit hyperactivity disorder and autism spectrum disorder share distinct microbiota compositions. *Gut Microbes*, *15*(1), 2211923. https://doi.org/10.1080/19490976.2023.2211923

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), Article 7. https://doi.org/10.1038/nmeth.3869

Checa-Ros, A., Jeréz-Calero, A., Molina-Carballo, A., Campoy, C., & Muñoz-Hoyos, A. (2021). Current Evidence on the Role of the Gut Microbiome in ADHD Pathophysiology and Therapeutic Implications. *Nutrients*, *13*(1), 249. https://doi.org/10.3390/nu13010249

Dam, S. A., Mostert, J. C., Szopinska-Tokov, J. W., Bloemendaal, M., Amato, M., & Arias-Vasquez, A. (2019). The Role of the Gut-Brain Axis in Attention-Deficit/Hyperactivity Disorder. *Gastroenterology Clinics of North America*, *48*(3), 407-431. https://doi.org/10.1016/j.gtc.2019.05.001

Diotallevi, C., Fava, F., Gobbetti, M., & Tuohy, K. (2020). Healthy dietary patterns to reduce obesity-related metabolic disease: Polyphenol-microbiome interactions unifying health effects across geography. *Current Opinion in Clinical Nutrition & Metabolic Care*, *23*(6), 437. https://doi.org/10.1097/MCO.0000000000000697

Eijndhoven, P. van, Collard, R., Vrijsen, J., Geurts, D. E. M., Vasquez, A. A., Schellekens, A., Munckhof, E. van den, Brolsma, S., Duyser, F., Bergman, A., Oort, J. van, Tendolkar, I., & Schene, A. (2022). Measuring Integrated Novel Dimensions in Neurodevelopmental and Stress-Related Mental Disorders (MIND-SET): Protocol for a Cross-sectional Comorbidity Study From a

Research Domain Criteria Perspective. *JMIRx Med*, *3*(1), e31269. https://doi.org/10.2196/31269

Feng, L., Zhou, N., Li, Z., Fu, D., Guo, Y., Gao, X., & Liu, X. (2022). Co-occurrence of gut microbiota dysbiosis and bile acid metabolism alteration is associated with psychological disorders in Crohn's disease. *The FASEB Journal*, *36*(1), e22100. https://doi.org/10.1096/fj.202101088RRR

Gkougka, D., Mitropoulos, K., Tzanakaki, G., Panagouli, E., Psaltopoulou, T., Thomaidis, L., Tsolia, M., Sergentanis, T. N., & Tsitsika, A. (2022). Gut microbiome and attention deficit/hyperactivity disorder: A systematic review. *Pediatric Research*, *92*(6), 1507-1519. https://doi.org/10.1038/s41390-022-02027-6

Hus, V., & Lord, C. (2014). The Autism Diagnostic Observation Schedule, Module 4: Revised Algorithm and Standardized Severity Scores. *Journal of Autism and Developmental Disorders*, *44*(8), 1996-2012. https://doi.org/10.1007/s10803-014-2080-3

Jakobi, B., Vlaming, P., Mulder, D., Ribases, M., Richarte, V., Antoni Ramos-Quiroga, J., Tendolkar, I., Eijndhoven, P. van, Vrijsen, J. N., Buitelaar, J., Franke, B., Hoogman, M., Bloemendaal, M., & Arias Vasquez, A. (2023). *The gut-microbiome in adult attention-deficit/hyperactivity disorder: A meta-analysis*.

Lee, M.-J., Lai, H.-C., Kuo, Y.-L., & Chen, V. C.-H. (2022). Association between Gut Microbiota and Emotional-Behavioral Symptoms in Children with Attention-Deficit/Hyperactivity Disorder. *Journal of Personalized Medicine*, *12*(10), Article 10. https://doi.org/10.3390/jpm12101634

Liu, F., Li, J., Wu, F., Zheng, H., Peng, Q., & Zhou, H. (2019). Altered composition and function of intestinal microbiota in autism spectrum disorders: A systematic review. *Translational Psychiatry*, *9*(1), Article 1. https://doi.org/10.1038/s41398-019-0389-6

Nikolova, V. L., Smith, M. R. B., Hall, L. J., Cleare, A. J., Stone, J. M., & Young, A. H. (2021). Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. *JAMA Psychiatry*, *78*(12), 1343-1354. https://doi.org/10.1001/jamapsychiatry.2021.2573

Nishiwaki, H., Hamaguchi, T., Ito, M., Ishida, T., Maeda, T., Kashihara, K., Tsuboi, Y., Ueyama, J., Shimamura, T., Mori, H., Kurokawa, K., Katsuno, M., Hirayama, M., & Ohno, K. (2020). Short-Chain Fatty Acid-Producing Gut Microbiota Is Decreased in Parkinson's Disease but Not in Rapid-Eye-Movement Sleep Behavior Disorder. *mSystems*, *5*(6), e00797-20. https://doi.org/10.1128/mSystems.00797-20

Ou, Y., Belzer, C., Smidt, H., & De Weerth, C. (2024). Development of the gut microbiota in the first 14 years of life and its relations to internalizing and externalizing difficulties and social anxiety during puberty. *European Child & Adolescent Psychiatry*, *33*(3), 847-860. https://doi.org/10.1007/s00787-023-02205-9

Pellegrini, C., Fornai, M., D'Antongiovanni, V., Antonioli, L., Bernardini, N., & Derkinderen, P. (2023). The intestinal barrier in disorders of the central nervous system. *The Lancet Gastroenterology & Hepatology*, *8*(1), 66-80. https://doi.org/10.1016/S2468-1253(22)00241-2

Richarte, V., Sánchez-Mora, C., Corrales, M., Fadeuilhe, C., Vilar-Ribó, L., Arribas, L., Garcia, E., Rosales-Ortiz, S. K., Arias-Vasquez, A., Soler-Artigas, M., Ribasés, M., & Ramos-Quiroga, J. A. (2021). Gut microbiota signature in treatment-naïve attention-deficit/hyperactivity disorder. *Translational Psychiatry*, *11*(1), Article 1. https://doi.org/10.1038/s41398-021-01504-6

Shirvani-Rad, S., Ejtahed, H.-S., Ettehad Marvasti, F., Taghavi, M., Sharifi, F., Arzaghi, S. M., & Larijani, B. (2022). The Role of Gut Microbiota-Brain Axis in Pathophysiology of ADHD: A Systematic Review. *Journal of Attention Disorders*, *26*(13), 1698-1710. https://doi.org/10.1177/10870547211073474

Sukmajaya, A. C., Lusida, M. I., Soetjipto, & Setiawati, Y. (2021). Systematic review of gut microbiota and attention-deficit hyperactivity disorder (ADHD). *Annals of General Psychiatry*, *20*(1), 12. https://doi.org/10.1186/s12991-021-00330-w

Szopinska-Tokov, J., Dam, S., Naaijen, J., Konstanti, P., Rommelse, N., Belzer, C., Buitelaar, J., Franke, B., Bloemendaal, M., Aarts, E., & Arias Vasquez, A. (2021). Correction: Szopinska-

Tokov et al. Investigating the Gut Microbiota Composition of Individuals with Attention-Deficit/Hyperactivity Disorder and Association with Symptoms. Microorganisms 2020, 8, 406. *Microorganisms*, *9*(7), Article 7. https://doi.org/10.3390/microorganisms9071358

Vestergaard, M. V., Allin, K. H., Eriksen, C., Zakerska-Banaszak, O., Arasaradnam, R. P., Alam, M. T., Kristiansen, K., Brix, S., & Jess, T. (2023). Gut microbiota signatures in inflammatory bowel disease. *United European Gastroenterology Journal*, *12*(1), 22-33. https://doi.org/10.1002/ueg2.12485

Warner, B. B. (2019). The contribution of the gut microbiome to neurodevelopment and neuropsychiatric disorders. *Pediatric Research*, *85*(2), Article 2. https://doi.org/10.1038/s41390-018-0191-9

Zhai, L., Huang, C., Ning, Z., Zhang, Y., Zhuang, M., Yang, W., Wang, X., Wang, J., Zhang, L., Xiao, H., Zhao, L., Asthana, P., Lam, Y. Y., Chow, C. F. W., Huang, J., Yuan, S., Chan, K. M., Yuan, C.-S., Lau, J. Y.-N., … Bian, Z. (2023). Ruminococcus gnavus plays a pathogenic role in diarrhea-predominant irritable bowel syndrome by increasing serotonin biosynthesis. *Cell Host & Microbe*, *31*(1), 33-44.e5. https://doi.org/10.1016/j.chom.2022.11.006