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Full-length Article

Efficacy of a synbiotic in the management of adults with Attention-Deficit and Hyperactivity Disorder and/or Borderline Personality Disorder and high levels of irritability: Results from a multicenter, randomized, placebo-controlled, "basket" trial

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A B S T R A C T

Irritability worsens prognosis and increases mortality in individuals with Attention-Deficit and Hyperactivity Disorder (ADHD) and/or Borderline Personality Disorder (BPD). However, treatment options are still insufficient. The aim of this randomized, double blind, placebo-controlled study was to investigate the superiority of a synbiotic over placebo in the management of adults with ADHD and/or BPD and high levels of irritability. The study was conducted between February 2019 and October 2020 at three European clinical centers located in Hungary, Spain and Germany. Included were patients aged 18–65 years old diagnosed with ADHD and/or BPD and high levels of irritability (i.e., an Affectivity Reactivity Index (ARI-S) $\geq$ 5, *plus* a Clinical Global Impression-Severity Scale (CGI-S) score $\geq$ 4). Subjects were randomized 1(synbiotic):1(placebo); the agent was administered each day, for 10 consecutive weeks. The primary outcome measure was *end-of-treatment response* (i.e., a reduction $\geq$ 30 % in the ARI-S total score compared to baseline, *plus* a Clinical Global Impression-Improvement (CGI-I) total score of $<$ 3 (very much, or much improved) at week 10). Between-treatment differences in secondary outcomes, as well as safety were also investigated. Of the 231 included participants, 180 (90:90) were randomized and included in the intention-to-treat-analyses. Of these, 117 (65 %) were females, the mean age was 38 years, ADHD was diagnosed in 113 (63 %), BPD in 44 (24 %), both in 23 (13 %). The synbiotic was well tolerated. At week 10, patients allocated to the synbiotic experienced a significantly higher response rate compared to those allocated to placebo (OR: 0.2, 95 % CI:0.1 to 0.7; $P = 0.01$). These findings suggest that that (add-on) treatment with a synbiotic may be associated with a *clinically meaningful* improvement in irritability in, at least, a subgroup of adults with ADHD and/or BPD. A superiority of the synbiotic over placebo in the management of *emotional dysregulation* (−3.6, 95 % CI:-6.8 to −0.3; $P = 0.03$), *emotional symptoms* (−0.6, 95 % CI:-1.2 to −0.05; $P = 0.03$), *inattention* (−1.8, 95 % CI: −3.2 to −0.4; $P = 0.01$), *functioning* (−2.7, 95 % CI: −5.2 to −0.2; $P = 0.03$) and *perceived stress levels* (−0.6, 95 % CI: −1.2 to −0.05; $P = 0.03$) was also suggested. Higher baseline RANK-L protein levels were associated with a significantly lower response rate, but only in the synbiotic group (OR: 0.1, 95 % CI: −4.3 to – 0.3, $P =$ 0.02). In the placebo group, higher IL-17A levels at baseline were significantly associated with a higher improvement in in particular, emotional dysregulation ($P =$ 0.04), opening a door for new (targeted) drug intervention. However, larger prospective studies are warranted to confirm the findings.
Trial Registration: ClinicalTrials.gov Identifier: NCT03495375.

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1. Introduction

Attention-deficit and hyperactivity disorder (ADHD) and borderline personality disorder (BPD) are two different (but highly comorbid) psychiatric conditions, with a particularly high symptomatic overlap (Weiner et al., 2019). Irritability, which can be defined as an increased proneness to anger relative to peers at the same developmental level (Vidal-Ribas et al., 2016; Stringaris et al., 2018) is particularly overrepresented.

Irritability is often associated with the presence of other symptoms, such as emotional symptoms, emotion regulation abnormalities and/or impulsivity (Blair et al., 2020; Keluskar et al., 2021), contributing to not only disease persistence but also, to mortality (Stringaris and Vidal-Ribas, 2019). Moreover, it has also been described as an independent factor for suicidal behavior (Towbin et al., 2020; Jha et al., 2021). Despite this, testing of possible treatments for the management of this symptom in individuals diagnosed with ADHD and/or BPD is still at a nascent state and treatment options (which include antidepressants, antipsychotics, psychostimulants and/or cognitive behavioral therapy) are insufficient and/or often associated with side effects (van Schalkwyk et al., 2017; Bell et al., 2021).

During the last decade, researchers have hypothesized about the existence of a shared biological background underlying ADHD and BPD (Weiner et al., 2019). In line with this idea, accumulating evidence suggests that intestinal dysbiosis (i.e., an inappropriate diversity in gut microbiota) may be involved in the pathophysiology of both conditions. In support of such view, an increased Bacteroidetes-to-Firmicutes ratio and bacterial gut microbiome abnormalities have been repeatedly reported in individuals diagnosed with both conditions (Richarte et al., 2021; Rössler et al., 2022; Wang et al., 2022a,b). Moreover, an association between early modulation of gut microbiota and a reduced risk of developing neuropsychiatric conditions (such as ADHD) in the future has also been described (Pärtty et al., 2015).

Accordingly, modulation of gut microbiota has been recently suggested as a viable treatment option for the management of both conditions (Kalenik et al., 2021). Treatment options may include fecal microbiota transplantation (Hooi et al., 2022) and/or (add-on) treatment with probiotics, prebiotics, or synbiotics. A probiotic is considered a live, non-pathological microorganism that, when ingested in adequate amounts, exerts a health benefit (Dynan and Quigley, 2011; Dynan et al., 2013). Prebiotics were originally defined as dietary ingredients selectively inducing the growth or activity of gastrointestinal bacteria thereby improving the microbial balance in the gut (Gibson, 2022). A synbiotic contains both prebiotics and probiotics. These agents are in general well tolerated (Cai et al., 2018; Wallace and Milev, 2021) and do not require invasive techniques to be administered.

Unfortunately, literature on the use of probiotics and/or synbiotics for the management of irritability is, to the best of our knowledge, non-existent. Interestingly, in a previous report performed in adults with ADHD, a beneficial effect of a synbiotic (i.e., Synbiotic 2000) on emotional dysregulation was suggested (Skott et al., 2020). Given the described association between emotion regulation abnormalities and irritability (Nigg et al., 2020; Salazar de Pablo et al., 2023), it can be hypothesized that (add-on) treatment with a similar synbiotic than the one used in this previous study would also improve irritability in adults with ADHD. Since ADHD and BPD may share a common biological background, a benefit of this agent in adults with BPD is expected.

The aim of this study was therefore to explore, for the first time, the superiority of a synbiotic over placebo for the management of adults diagnosed with two different (but symptomatic overlapped) psychiatric conditions (i.e., ADHD and/or BPD) and high levels of irritability. The efficacy of this agent in the management of emotional dysregulation, impulsivity, inattention, functioning and/or perceived stress levels, as well as safety, was also investigated.

2. Methods

2.1. Trial design and study setting

This randomized, parallel group, double-blind, placebo-controlled "basket" trial was carried out at three European clinical centers: the Semmelweis University (Budapest, Hungary), the Vall d'Hebron University Hospital (Barcelona, Spain) and the University Hospital Frankfurt Goethe University (Frankfurt, Germany). The term basket trial refers to studies where a targeted therapy is evaluated on multiple diseases that may have common molecular alterations (Park et al., 2020; Fountzilas et al., 2022). In other words, these studies allow to assess the improvement in specific (but transdiagnostic) symptoms in different (but with a presumable common biological background) diseases.

The study protocol was reviewed and approved by the local Ethic Committees at the three study centers (Budapest:44101-1/2018/EKU; Barcelona:311/2018; Frankurt:269/18) and registered at ClinicalTrials.gov (NCT03495375). Research procedures adhered to the relevant national and institutional Good Clinical Practice (GCP) guidelines on human experimentation, as well as to the Declaration of Helsinki of 1975, as reviewed in 2008.

Written informed consent was obtained from all study participants. The trial followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

2.2. Study participants

Eligible participants were recruited between February 2019 and October 2020 via advertising and hospital outpatient clinics. Included were adults aged 18–65 years with high levels of chronic irritability (i.e., an Affective Reactivity Index Self-Report (ARI-S) $\geq$ 5 *plus* a Clinical Global Impression-Severity (CGI-S) total score $\geq$ 4 (i.e., moderately ill)).

Patients had to meet Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) (American Psychiatric Association, 2013) criteria for ADHD and/or BPD. After clinical evaluation, diagnoses were confirmed by structured diagnostic interviews that were administered to all eligible participants, irrespective of the initially suspected diagnosis; ADHD was confirmed by the Diagnostic Interview for Adult ADHD (DIVA 2.0) (Kooij and Francken, 2010) and BPD by the Structured Clinical Interview for DSM-IV (SCID-II) (First and Gibbon, 2004). For the assessment of comorbid psychiatric conditions, the MINI-International Neuropsychiatric Interview-Plus (MINI-Plus) (Van Vliet and de Beurs, 2007) was administered.

Excluded were patients with past and/or current psychotic symptoms, as well as those with any major neurological, cardiovascular, endocrine, pulmonary, immunological and/or gastrointestinal disorder at screening. Patients under anti-inflammatory and/or psychiatric medication were included only in case the type of agent and dosage taken were stable for at least 30 days prior to study entry. On the contrary, patients undergoing immunosuppression or under antibiotics or probiotics within the last 30 days prior to study entry were also excluded. For further details see **Table S1**.

2.3. Study intervention

The active treatment (i.e., Synbiotic 2000 Forte, purchased from Synbiotics AB, Sweden; https://en.supersynbiotics.se) was a lyophilized composition of 400 billion lactic acid bacteria/dose (i.e., *Pediococcus pentosaceus* 5–33:3/16:1, *Lactobacillus casei ssp paracasei* F19, *Lactobacillus plantarum* 2362 and *Leuconostoc mesenteroides* 77:1) and 2.5 g/dose of each of the following fermentable fibers: β-glucan, inulin, pectin, and resistant starch. The placebo (also purchased from Synbiotics AB, Sweden) was composed of the oligosaccharide maltodextrin. Both were designed to be identical regarding packaging, volume, weight, content

color, texture, and flavor; both were without smell. Participants were instructed to mix the powder in drinks or foods (<40 °C) and take it once daily; sachets had to be stored at a refrigerated temperature (i.e., 4–6 °C) during the 10 weeks of study.

2.4. Randomization, allocation concealment, and blinding

Randomization was completed by a research assistant not otherwise involved in the study by the use of an independent randomization service. Random block sizes were applied to control the number of participants assigned to each group, stratified by site and in a 1:1 ratio. Individual treatment kits were pre-packaged for the 10 weeks of intervention by the same research assistant; kits were sequentially numbered and allocated to the synbiotic or placebo based on the randomization list. Study participants, investigators and clinicians were unaware of participant́s treatment allocation until the database was locked and data analysis was completed.

2.5. Study assessments

2.5.1. Primary outcome measure

End-of-treatment response was considered as the primary outcome measure; it was assessed 5 weeks after the intervention started and at the end of the intervention at week 10. *End-of-treatment response* was defined by a ≥ 30 % decrease in the ARI-S total score compared to baseline, *plus* a Clinical Global Impression-Improvement Scale (CGI-I) score of either 1 or 2 at week 10.

The ARI-S is a 7-item self-reported questionnaire aimed at assessing irritability over the past six months, that has demonstrated validity in children, adolescents and adults (Stringaris et al., 2012; Mulraney et al., 2014). For our study, the ARI-S was rated relative to the past seven days (including the day of the visit up to and through the visit). The ARI-S consists of six symptom items plus one impairment item. Each item is composed of a three-level response category: *not true*, *somewhat true, certainly true* scored as 0,1, 2 respectively, giving a range of possible scores of 0–12. The total score is the sum of the first six items, with higher scores representing higher levels of irritability. The CGI-I is a clinician-rated scale that has been widely utilized as an efficacy measure in clinical drug trials, informing about how much a patient́s *overall* clinical condition has improved or worsened relative to the baseline visit (Guy, 1976; Kadouri et al., 2007). In the CGI-I, the following one query is rated on a 7-point scale: *"Compared to the patient́s condition at admission, this patient has: 1 = very much improved, 2 = much improved, 3 = minimally improved, 4 = no change from baseline, 5 = minimally worse, 6 = much worse, 7 = very much worse"*.

The rational for combining an at least 30 % symptom reduction in the ARI-S total score (i.e., a self-reported assessment) with a CGI-I score of < 3 (i.e., a clinician-rated assessment) to a single outcome was to better capture *clinically meaningful* improvements in irritability by, for example, reducing a potential overestimation of patient́s improvement by the clinician (Cumming, 2014). If used as a single outcome, rating scales may also be confounded by factors (e.g., medication side effects) that may be incorrectly scored as new symptoms (e.g., irritability, tiredness) by either patients or unexperienced raters. Therefore, and as a way of gaining further insight into cliniciańs relation to patient́s perspective, it has been recommended to incorporate self- and/or clinician-rated questionnaires in addition to the CGI-I, for the evaluation of treatment efficacy in clinical trials (Forkmann et al., 2011; Cumming, 2014). The cut-off point of a 30 % decrease was chosen by research team consensus, but supported by previous existing literature (Sprich et al., 2016; Rucklidge et al., 2019). Nevertheless, cut-off points of 10 %, 50 % and 70 % decrease were additionally *post-hoc* applied for the definition of *end-of-treatment* response.

2.5.2. Secondary outcome measures

Secondary outcome measures were selected to assess the efficacy of the synbiotic in the management of emotional dysregulation, emotional symptoms, impulsivity, compulsivity and inattention, via a series of selected scales or questionnaires. The efficacy in functioning and in perceived stress levels was also investigated (**Table S2**).

Also, and with the aim to identify putative major alterations in body mass index (BMI), nutrient intake and/or in physical activity between baseline and week 10 that may have influenced the findings (Rudcklinge et al., 2021), the changes in these parameters were also assessed. Participants were asked to complete at least three 24-h dietary recalls (i.e., baseline, between baseline and week 5, between week 5 and 10), including two weekdays, and one weekend day, on non-consecutive days, starting with the first intake after waking up in the morning. Physical activity was assessed by the International Physical Activity Questionnaire (IPAQ) (Hagströmer et al., 2006). The IPAQ is a 27-item self-reported measure of physical activity in adults (i.e., high: ≥3000 Metabolic Equivalent of Task (MET)-min/week, moderate: ≥ 600 MET-min/week, low: not meeting criteria).

2.6. Safety and tolerability

Adverse events were structurally assessed at week 5 and week 10 by a Body System Questionnaire configurated by the research team; it was composed of a checklist of 12 possible adverse event groups: heat, gastrointestinal, musculoskeletal, dermatological, cardiovascular, pulmonal, genitourinary, drug allergy, immunologic, hematologic, psychiatric, others.

2.7. Biological parameters

Blood samples were obtained under fasting conditions and between 7:30–15:30 at baseline at the respective Psychiatry Departments of all centers involved. Blood was then collected into BD Vacutainer® K2-EDTA tubes (ref. BD 267525) and centrifuged to obtain plasma that was aliquoted into cryotubes and stored at −80 ºC until further analysis. The levels of 72 inflammation-related protein markers were then assessed in n = 126 of the 180 individuals included via the Olink Target 96 Inflammation panel (Olink Proteomics, Uppsala, Sweden), and final levels were recorded as normalized log2 scaled protein expression (NPX) values, as previously described in (Schnorr et al., 2024). The inflammation-related protein markers assessed included EN-RAGE, CCL23, CD8A, NT-3, SCF, DNER, uPA, TWEAK, CST5, OPG, CCL28, MMP-10, TNFB, LIF-R, Fit3L, CX3CL1, FGF-19, FGF-23, IL-18R1, HGF, OSM, TGF-α, IL-6, FGF-21, CCL19, CCL25, CDCP1, IL18, MCP-1, CCL11, TRAIL, IL-10RB, CD5, VEGFA, CSF-1, TNF, IL-15RA, SLAMF1, CD6, TNFRSF9, RANK-L, CXCL10, CXCL9, IFN-y, IL-12B, ADA, CD40, SIRT2, AXIN1, STAMBP, CD244, PD-L1, EIF4EBP1, CASP8, TNFSF14, ST1A1, CCL3, CCL4, MCP-2, LAPTGF β1, CXCL6, MCP-4, CXCL11, CXCL5, CXCL1, IL-8, IL-7, MMP1, IL-17A, IL-17C, IL-10 and CCL20. For further details see Schnorr et al. (2024).

2.8. Sample size calculation and statistical analysis

The primary outcome was categorical, i.e., *end-of-treatment response.* Accordingly, a sample size of 150–160 was needed to allow for detection of an odds ratio (OR) = 2.55 with ≥ 80 % power, assuming a response rate of 20 % for the placebo group, when alpha was set at 0.05. Assuming an attrition rate of about 10 %, the final sample size was set at 180 study participants.

Baseline comparisons were performed using Mann-Whitney U or chi-square tests, depending on variable scale and distribution. The results of these comparisons (*P*-values) were not reported to meet CONSORT recommendations of reporting of baseline characteristics in randomized clinical trials.

The primary outcome measure was assessed by a binary logistic regression model with *end-of-treatment response* (no/yes) as the dependent variable, and treatment allocation group (synbiotic/placebo), study center (Hungary/Spain/Germany), age, sex (male/female), medication naïve (yes/no) and primary psychiatric diagnosis (ADHD/BPD/both) as covariates.

Following recommendations for randomized, placebo-controlled trials (van Breukelen, 2006; Wang et al., 2019), secondary outcome measures were tested by a general linear mixed analysis of covariance (ANCOVA) model, with mean questionnaire score at week 10 as the dependent variable, treatment allocation group as fixed effect variable, and study center, age, sex, medication status, primary psychiatric diagnosis, and the corresponding mean questionnaire score at baseline, as covariates. Raw questionnaire scores were used in the analyses. Estimates of treatment efficacy were calculated on the intention-to-treat (ITT) population (i.e., patients randomized who received at least one dose of study medication and had a baseline assessment). Categorical outcomes were summarized as OR and 95 % confidence intervals (95 % CI), continuous outcomes as between-group mean differences at post-test and 95 % CI. Partial eta squared (η_p^2) effect size estimates were calculated. Missing outcome data were handled by multiple imputation (i.e., Predictive Mean Matching (PMM)), as it has been described as the recommended method for randomized trials (Twisk et al., 2020). Analyses on the per-protocol (PP) population (i.e., participants who were randomized and took their allocated product for the full intervention period and did not violate the study protocol) were additionally performed, leading to similar conclusions.

Associations of immune-related protein levels at baseline with *end-of-treatment response* were analyzed using Spearmańs rank correlation coefficient (r_s). Since the dependent variable is discrete, logistic regression (controlling for age, sex, bmi, medication status and study center) was also applied to predict treatment response. Associations between baseline levels of immune-related proteins and the change in secondary measures (i.e., questionnaire score at baseline – questionnaire score at week 10) were assessed by linear regression models. Again, analyses were controlled for age, sex, bmi, medication status and study center. All tests were two-tailed, with *P*-values ≤ 0.05 being considered as significant. SPSS, version 28 (IBM, Armonk, N.Y.) was used for the analyses and graphs.

3. Results

3.1. Patieńts characteristics

Out of 231 patients screened for eligibility, 180 satisfied all eligibility criteria and underwent randomization, 90 were assigned to the synbiotic and 90 to placebo. A total of 46 (26 %) individuals discontinued the study before the end of the intervention. All reasons for discontinuation are listed in **Figure S1**.

Table 1 summarizes the baseline characteristics of the 180 study participants included in the ITT analyses. Age ranged from 20 to 65 years; 117 (65 %) were females and 168 (93 %), white. Of the total of patients included, 113 (63 %) were diagnosed with ADHD, 44 (24 %) with BPD and 23 (13 %) with both conditions. A comorbid psychiatric condition was diagnosed in 94 (52 %) of the included patients. 46 (26 %) of the subjects included were free of any psychiatric medication at baseline; 78 (43 %) were under ADHD medication (47 (26 %) under methylphenidate, 21 (12 %) under lisdexamfetamine, 5 (3 %) under atomoxetine, 2 (1 %) under guanfancine and 3 (2 %) under a combination). For a description of the psychotropic agents taken at baseline, see Table 1.

Significant differences were not found between treatment groups for any of their baseline socio-demographic and clinical characteristics (Table 1, **Table S3**). Significant differences were also not found in relation to their baseline nutritional intake and/or physical activity/week (**Table S4**). Sample characteristics of the PP sample are summarized in Tables S5-7.

3.2. Primary outcome measure: End-of-treatment response

According to our a priori established definition of *end-of treatment response*, 19/180 (11 %) of the patients were classified as responders, and 161/180 (89 %) as non-responders. Of the 19 patients who responded to treatment, 15 (79 %) were allocated to the synbiotic and 4 (21 %) to placebo ($\chi^2 = 6.2$, $P = 0.01$). A logistic regression analysis was performed to determine how treatment group (synbiotic vs. placebo) affected patieńts probability to achieve a *clinically meaningful* response at week 10. The model explained 14 % of the variation in response rate, and correctly classified 70 % of cases; the odds of a patient allocated to placebo to respond was 0.2 of the odds of a patient allocated to the synbiotic to respond (95 % CI: 0.1 to 0.7; $P = 0.01$) (Table 2). If the cut-off point for the decrease in the ARI-S total score was set at 10 %, 50 %, or 70 %, similar findings were found (Fig. 1). Analyses on the PP sample led to similar conclusions (**Table S8**).

3.3. Secondary outcome measures: Estimates of treatment effect in emotion regulation, emotional symptoms, impulsivity, compulsivity, inattention, functioning, and perceived stress levels

At the end of the intervention, significant between-group differences (i.e., synbiotic superior to placebo) were found for emotion regulation abnormalities (DERS-16, −3.6, 95 % CI: −6.8 to −0.3; $\eta_p^2 = 0.03$; $P = 0.03$), emotional symptoms (SDQ, −0.6, 95 % CI: −1.2 to −0.05; $\eta_p^2 = 0.03$; $P = 0.03$), and inattention (ADHD-RS, −1.8, 95 % CI: −3.2 to −0.4;; $\eta_p^2 = 0.04$; $P = 0.01$) (Table 3, Fig. 2**A-C**). Also, a superiority of the synbiotic over placebo was found for the improvement in functioning (FAST, −2.7, 95 % CI: −5.2 to −0.2; $\eta_p^2 = 0.03$; $P = 0.03$) (Table 3, Fig. 2**D**) and in perceived stress levels (PSS, −0.6, 95 % CI: −1.2 to −0.05, $\eta_p^2 = 0.03$; $P = 0.03$) (Table 3, Fig. 2**F**). Patients allocated to the synbiotic also experienced a significantly higher improvement in peer relationship problems compared to those allocated to placebo (Table 3, Fig. 2**E**). A synbiotic-specific improvement in several impulsive facets (in particular, in the tendency to act rashly in response to extreme positive emotions, i.e., positive urgency), was only suggested (Table 3). Again, analyses on the PP sample led to similar conclusions (**Table S9**). Study center, medication status, primary psychiatric diagnosis, age and/or sex did not moderate the findings (data not shown); nor did sensitivity analyses evaluating the impact of the change in BMI, nutritional intake, and physical activity (**Table S10**).

3.4. Safety and tolerability

Adverse events included headache/migraine, gastrointestinal (e.g., diarrhea, constipation, abdominal pain), musculoskeletal (e.g., muscle pain, arthralgias), dermatological (e.g., rash), psychiatric (e.g., sleep disturbances, anxiety, depressive symptoms), fever and/or others (e.g., sore throat, herpes virus reactivation, fatigue). Significant between-group differences were not found between both treatment groups in relation to the type and/or severity of side effects. No severe adverse events were observed (**Table S11**).

3.5. Baseline levels of immune activity proteins and end-of-treatment response

Data about baseline immune activity protein levels were available in a subgroup of n = 126 patients with ADHD. Of those, 61 (48 %) were allocated to the synbiotic and 65 (49 %) to placebo. Patients allocated to the synbiotic and those allocated to placebo did not statistically significantly differ in any of their socio-demographic and clinical baseline characteristics (data not shown). Patients did also not statistically significantly differ in plasma levels of the immune activity proteins assessed except for CST5 and IL-15RA (**Table S12**).

After treatment, a total of 14 (11 %) of the patients were classified as responders; 11/14 (79 %) belonged to the synbiotic group vs 3/14 (21

Table 1
Baseline Demographic and Clinical Characteristics by Treatment Group.

Characteristic	Total sample n = 180	Synbiotic n = 90	Placebo n = 90
Age (years), mean (SD)	37.9 (11.6)	36.5 (11.5)	39.3 (11.7)
BMI (kg/m^2), mean (SD)	26.0 (5.9)	25.8 (5.3)	26.2 (6.5)
Sex (females), n (%)	117 (65 %)	53 (59 %)	64 (71 %)
Ethnicity[a], n (%)			
− Asian	7 (4 %)	6 (7 %)	1(1 %)
− Black	1 (1 %)	1 (1 %)	0 (0 %)
− Hispanic/Latino	4 (2 %)	2 (2 %)	2 (2 %)
− White	168 (93 %)	81 (90 %)	87 (97 %)
Primary Psychiatric Disorder, n (%)			
− ADHD	113 (63 %)	56 (62 %)	57 (63 %)
− BPD	44 (24 %)	23 (26 %)	21 (23 %)
− ADHD + BPD	23 (13 %)	11 (12 %)	12 (13 %)
Comorbid Psychiatric Disorders (yes), n (%)	94 (52 %)	50 (56 %)	44 (49 %)
− Major Depressive Episode (current)	18 (10 %)	12 (13 %)	6 (7 %)
− Bipolar Disorder	29 (16 %)	18 (20 %)	11 (12 %)
− Dysthimia	22 (12 %)	11 (12 %)	11 (12 %)
− Agoraphobia	16 (9 %)	9 (10 %)	7 (8 %)
− Social Anxiety Disorder	17 (9 %)	10 (11 %)	7 (8 %)
− Generalized Anxiety Disorder	28 (16 %)	13 (14 %)	15 (17 %)
− Panic Disorder	17 (9 %)	9 (10 %)	8 (9 %)
− Obsessive-Compulsive Disorder	10 (6 %)	8 (9 %)	2 (2 %)
− Post-traumatic Stress Disorder	7 (4 %)	4 (4 %)	3 (3 %)
− Anorexia nervosa	2 (1 %)	1 (1 %)	1 (1 %)
− Bulimia	3 (2 %)	1 (1 %)	2 (2 %)
− Antisocial Personality Disorder	6 (3 %)	4 (4 %)	2 (2 %)
− Alcohol Dependence	4 (2 %)	4 (4 %)	0 (0 %)
− Non-Alcohol Substance Dependence	8 (4 %)	4 (4 %)	4 (4 %)
Suicidality risk, n (%)			
None-Low (yes)	159 (88 %)	74 (82 %)	85 (94 %)
Medium-High (yes)	21 (12 %)	16 (18 %)	5 (6 %)
Psychiatric medication (yes), n (%)	119 (66 %)	57 (63 %)	62 (69 %)
− Methylphenidate	50 (28 %)	27 (30 %)	23 (26 %)
− LDX	24 (13 %)	15 (17 %)	9 (10 %)
Atomoxetine	5 (3 %)	2 (2 %)	3 (3 %)
− Guanfacine	2 (1 %)	1 (1 %)	1 (1 %)
− SSRIs	35 (19 %)	12 (13 %)	23 (26 %)
− SNRIs	14 (8 %)	7 (8 %)	7 (8 %)
− Tricyclics	2 (1 %)	1 (1 %)	1 (1 %)
− Bupropion	6 (3 %)	3 (3 %)	3 (3 %)
− Agomelatine	1 (1 %)	1 (1 %)	0 (0 %)
− Mirtazapine	5 (3 %)	2 (2 %)	3 (3 %)
− Trazodone	4 (2 %)	3 (3 %)	1 (1 %)
− Typical Antipsychotics	4 (2 %)	2 (2 %)	2 (2 %)
− Atypical Antipsychotics	22 (12 %)	12 (13 %)	11 (12 %)
− BZD/Analogues	16 (9 %)	4 (4 %)	12 (13 %)
− Lamotrigine	5 (3 %)	1 (1 %)	4 (4 %)
− Lithium	1 (1 %)	0 (0 %)	1 (1 %)
− Valproic acid	1 (1 %)	1 (1 %)	0 (0 %)
− Pregabalin	3 (2 %)	2 (2 %)	1 (1 %)
− Gabapentin	2 (1 %)	0 (0 %)	2 (2 %)
Topiramate	1 (1 %)	0 (0 %)	1 (1 %)
Tobacco use, n (%)			
Non-smoker	89 (49 %)	45 (50 %)	44 (49 %)
Smoker	70 (39 %)	33 (37 %)	37 (41 %)
Occasional- smoker	21 (12 %)	12 (13 %)	9 (10 %)
ARI-S total score, mean (SD)	7.3 (2.1)	7.4 (2.1)	7.2 (2.0)
CGI-S total score, n (%)	4.6 (0.7)	4.5 (0.7)	4.7 (0.7)
− 4	94 (52 %)	50 (56 %)	44 (49 %)
− 5	66 (37 %)	33 (37 %)	33 (37 %)
− 6	18 (10 %)	6 (7 %)	12 (13 %)
− 7	2 (1 %)	1 (1 %)	1 (1 %)

Abbreviations: ADHD: Attention Deficit and Hyperactivity Disorder; ARI-S: Affectivity Reactivity Index-Self Report, BMI: body mass index; BPD: Borderline Personality Disorder; CGI-S: Clinical Global Impression-Severity Scale; LDX: Lisdexamfetamine; SSRIs: Selective serotonin reuptake inhibitors; SNRIs: Serotonin-norepinephrine reuptake inhibitors. [a] Race and Ethnicity data were collected through self-report by choosing one of the following categories: Asian (Chinese, Filipino, Asian Indian, Vietnamese, Korean, Japanese, Other Asian), Black (African American, Jamaican, Haitian, Nigerian, Ethiopian, Somali, Ghanaian, South African, Barbadian, Kenyan, Liberian, Bahamian), Hispanic/Latino (any), White (Caucasian), some other race (any).

%) who belonged to the placebo group ($\chi^2 = 5.74$, $P = 0.02$). In the synbiotic group, higher baseline levels of IL-17A and lower baseline levels of RANK-L and LIF-R were significantly associated with *end-of-treatment-response* at week 10 (IL-17A, $r_s = 0.28$, $P = 0.03$; RANK-L, $r_s =$ -0,35, $P = 0.01$; LIF-R, $r_s =$ -0.26, $P = 0.04$). None of the immune activity proteins assessed were statistically significantly associated with *end-of-treatment-response* in the placebo group.

Table 2
Prediction of Treatment Response in the Intention-to-Treat Sample.

Variable		Coef (B)	SE	OR (Exp B)	95 % CI	*P*-value
Treatment Group	Synbiotic			1		
	Placebo	−1.52	0.61	0.22	0.07 to 0.72	**0.01**
Study Center	Hungary			1		0.32
	Spain	1.14	0.78	3.12	0.68 to 14.40	0.14
	Germany	0.97	0.75	2.64	0.61 to 11.42	0.19
Age	Age in years	0.02	0.02	1.01	0.98 to 1.07	0.31
Sex	Male			1		
	Female	0.29	0.59	1.34	0.42 to 4.31	0.62
Primary Psychiatric Diagnosis	ADHD			1		0.73
	BPD	0.3	0.69	1.34	0.35 to 5.24	0.66
	Both	0.58	0.77	1.79	0.39 to 8.08	0.45
Medication naïve	Yes			1		
	No	−0.24	0.59	0.79	0.25 to 2.52	0.69

Abbreviations: ADHD: Attention Deficit and Hyperactivity Disorder; BPD: Borderline Personality Disorder. Analyses were based on a binary logistic regression model. Significant *P*-values are highlighted in bold.

We thus included baseline levels of IL-17A, RANK-L and LIF-R as single predictors in a logistic regression model of synbiotic response (controlled for age, sex, bmi, medication status and study center). With a total of 86 % correct predictions of non-response/response, the regression model with RANK-L levels was significantly superior in predicting response by chance, with 56 % correct results (Table 4). Alternative models with IL-17A and LIF-R showed less prediction accuracy compared with the model with RANK-L levels only (data not shown).

3.6. Baseline levels of IL-17A, RANK-L and LIF-R and their association with symptomatic and functional improvements

Subsequently, we investigated if baseline levels of IL-17A, RANK-L and LIF-R were also associated with a higher improvement in secondary outcome measures. For this purpose, we included these parameters as covariables in a regression model of the change in secondary outcome measures (i.e., inattention, emotional symptoms, emotional dysregulation, functioning and perceived stress levels) at week 10. Again, analyses were controlled for age, sex, bmi, medication status and study center (**Table S13**). By doing so, we found that higher baseline IL-17A levels were associated with a significantly higher improvement in emotion regulation abnormalities (DERS-16, B = -7.16, SE = 3.38, 95 % CI: −14.02 to −0.31, $P = 0.04$), but only in the placebo group. No associations between baseline RANK-L and/or LIF-R levels and the change in secondary outcome measures were found, neither in the synbiotic nor in the placebo groups (data not shown).

4. Discussion

To the best of our knowledge, this is the first randomized, placebo-controlled clinical trial with the primary research question being the superiority of a synbiotic over placebo in the management of adults diagnosed with two different (but symptomatic overlapped) psychiatric conditions (i.e., ADHD and/or BPD) and high levels of multidimensional irritability.

In our study, responders represented the subgroup of patients who experienced a *clinically meaningful* improvement in irritability levels at week 10. This was assessed by the improvement in both a self-reported (i.e., ARI-S) and a clinician-rated (i.e., CGI-I) questionnaire. The combination of a self-reported with a clinician-rated questionnaire, enabled us to: (1) capture the range of results expected at an individual level, (2) gain further insight into clinicians relation to patients perspective (Cumming, 2014; Dvir, 2015; Mark et al., 2016). Overall, a total of 19/180 (11 %) of the patients included were considered as responders. Among responders, a statistically significantly higher proportion of patients were initially allocated to the synbiotic (i.e., 15/19 (79 %) vs. 4/19 (21 %) who were initially allocated to placebo). This suggests a superiority of this agent over placebo for the management of irritability in individuals with ADHD and/or BPD. Findings were robust and not moderated by study center, age, sex, medication status and/or primary psychiatric diagnosis.

We also investigated if the levels of a set of immune activity proteins at baseline were associated with *end-of-treatment response* in both the synbiotic and placebo groups. In our study, lower RANK-L levels were

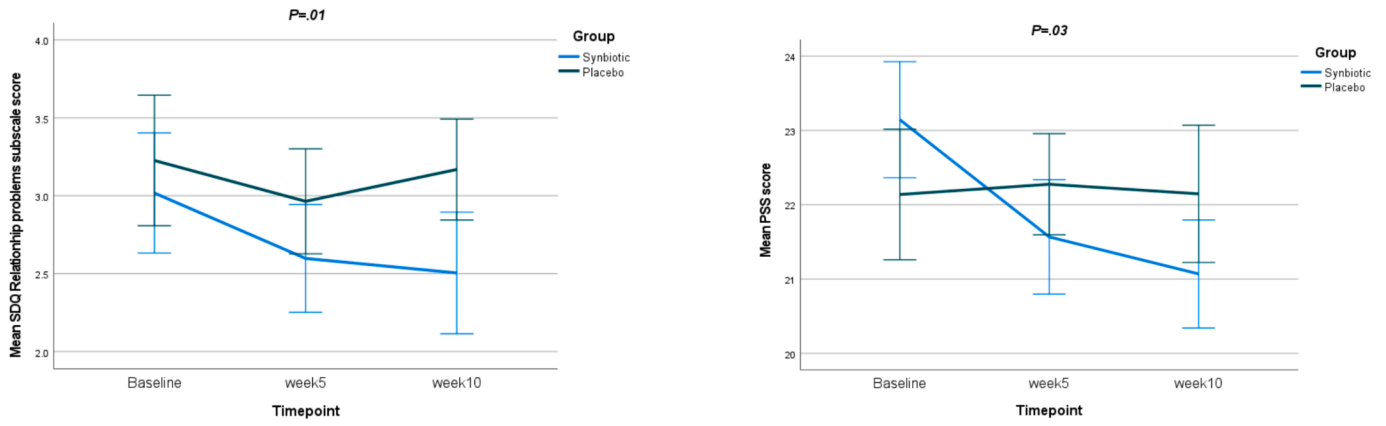

Fig. 1. *End-of-treatment response* was defined by ≥ 30 % decrease in the ARI-S total score at baseline, *plus* a Clinical Global Impression-Improvement Scale (CGI-I) score of either 1 or 2 at week 10. A higher proportion of responders was found in the synbiotic group, compared to the placebo group. Sensitivity subgroup analyses showed that, if the ARI-S score decrease *cut-off* point was set at 10 %, 50 %, or 70 %, again, a statistically significantly higher proportion of responders was found in the synbiotic group, compared to placebo (i.e., 10 %: $\chi^2 = 7.1$, $P = 0.01$; 50 %: $\chi^2 = 6.2$, $P = 0.01$; 70 %: $\chi^2 = 4.1$, $P = 0.04$).

Table 3
Estimates of Treatment Effect on symptoms and functioning in the Intention-to-Treat Sample.

	Synbiotic				Placebo					
	n = 90				n = 90					
Questionnaire score	Baseline	Week 5	Week 10	Change from baseline MD (95 % CI)	Baseline	Week 5	Week 10	Change from baseline MD (95 % CI)	Treatment effect Estimate (95 % CI)	*P*
UPPS-P	139.0 (15.8)	142.1 (14.7)	145.3 (15.7)	**6.2 (3.2 to 9.3)** ***	137.9 (16.7)	141.1 (15.9)	141.7 (14.1)	**3.8 (0.5 to 7.1)** *	3.4 (−0.5 to 7.3)	0.09
– Positive Urgency	35.1 (9.1)	36.9 (9.1)	38.9 (9.1)	**3.8 (2.1 to 5.5)** ***	33.2 (19.3)	35.5 (8.7)	36.0 (7.5)	**2.9 (0.9 to 4.8)** **	**2.2 (0.0 to 4.3)**	**0.05**
– Negative Urgency	25.1 (5.6)	26.9 (5.7)	28.0 (5.9)	**2.9 (1.9 to 3.9)** ***	24.9 (5.8)	26.8 (5.7)	27.2 (4.7)	**2.3 (1.1 to 3.4)** ***	0.6 (−0.9 to 2.1)	0.45
– (Lack of) premeditation	26.1 (5.7)	24.9 (5.2)	24.9 (5.6)	**−1.2 (−2.1 to −0.3)** *	27.2 (6.1)	25.6 (5.1)	25.6 (5.7)	**−1.6 (−2.6 to −0.6)** **	0.0 (−1.2 to 1.3)	0.99
– (Lack of) perseverance	25.1 (4.0)	23.9 (3.0)	23.5 (2.9)	**−1.6 (−2.4 to −0.9)** ***	25.3 (3.9)	24.4 (3.5)	24.2 (3.4)	**−1.1 (−1.8 to −0.4)** **	−0.8 (−1.6 to 0.0)	0.07
– Sensation seeking	27.6 (8.4)	29.3 (7.9)	29.9 (7.8)	**2.4 (1.1 to 3.6)** ***	27.4 (7.6)	28.8 (7.7)	28.7 (6.9)	1.3 (−0.03 to 2.7)	1.2 (−0.4 to 2.9)	0.13
DERS-16	51.5 (12.9)	47.5 (13.8)	44.0 (12.8)	**−7.5 (−10.1 to −4.9)** ***	50.3 (13.7)	47.3 (13.4)	46. (13.0)	**−3.6 (−6.3 to −0.8)** *	**−3.6 (−6.8 to −0.3)**	**0.03**
– (Lack of) emotional clarity	5.6 (2.4)	5.4 (2.2)	4.88 (1.9)	**−0.8 (−1.3 to −0.3)** ***	5.1 (2.1)	4.8 (1.9)	4.8 (1.9)	−0.3 (−0.7 to 0.1)	−0.3 (−0.7 to 0.2)	0.23
– (Lack of) goal-directed behavior	11.6 (2.5)	10.5 (2.9)	10.0 (2.9)	**−1.6 (−2.1 to −0.9)** ***	11.0 (3.2)	10.7 (3.2)	10.4 (3.1)	−0.6 (−1.2 to 0.0)	**−0.8 (−1.6 to 0.0)**	**0.05**
– (Lack of) impulse control	9.0 (2.8)	8.5 (3.1)	7.9 (2.9)	**−1.1 (−1.7 to −0.5)** ***	9.2 (3.5)	9.0 (3.3)	8.2 (3.0)	**−1.0 (−1.8 to −0.3)** **	−0.3 (−1.1 to 0.5)	0.46
– (Lack of) emotional strategies	16.0 (5.3)	14.5 (4.7)	13.7 (4.9)	**−2.3 (−3.2 to −1.3)** ***	15.6 (5.2)	14.3 (4.3)	14.5 (4.6)	**−1.1 (−2.2 to −0.1)** *	−0.96 (−2.1 to 0.2)	0.11
– Non-acceptance	9.3 (3.4)	8.7 (3.1)	8.1 (3.1)	**−1.3 (−1.9 to −0.6)** ***	9.3 (3.1)	8.7 (3.4)	8.8 (3.3)	−0.5 (−1.2 to 0.2)	−0.7 (−1.6 to 0.1)	0.09
ADHD-RS	28.8 (9.9)	24.9 (9.7)	21.6 (9.7)	**−7.2 (9.2 to −5.1)** ***	30.0 (10.3)	27.6 (8.7)	25.4 (8.4)	**−4.5 (−6.6 to −2.5)** ***	**−3.5 (−5.9 to −1.1)**	**0.005**
– Inattention	14.5 (5.5)	12.9 (5.5)	11.1 (5.4)	**−3.4 (−4.5 to −2.3)** ***	15.3 (5.8)	14.11 (4.9)	13.2 (5.2)	**−2.1 (−3.3 to −0.9)** ***	**−1.8 (−3.2 to −0.4)**	**0.01**
SDQ	18.6 (4.6)	15.98 (4.9)	14.7 (5.4)	**−3.9 (−4.8 to −2.9)** ***	18.6 (5.3)	16.9 (4.9)	16.4 (5.1)	**−2.2 (−3.0 to −1.3)** ***	**−1.8 (−3.1 to −0.6)**	**0.004**
– Emotional symptoms	5.9 (2.5)	4.7 (2.3)	4.3 (2.5)	**−1.7 (−2.2 to −1.2)** ***	5.5 (2.8)	5.1 (2.3)	4.7 (2.3)	**−0.8 (−1.3 to −0.3)** **	**−0.6 (−1.2 to −0.0)**	**0.03**
– Conduct problems	3.0 (1.3)	2.8 (1.4)	2.6 (1.3)	**−0.4 (−0.7 to −0.1)** *	3.2 (1.3)	2.6 (1.3)	2.7 (1.1)	**−0.5 (−0.7 to −0.2)** ***	−0.1 (−0.4 to 0.3)	0.69
– Peer relationship problems	3.0 (1.8)	2.6 (1.6)	2.5 (1.9)	**−0.5 (−0.8 to −0.2)** **	3.2 (2.0)	2.96 (1.6)	3.2 (1.5)	−0.1 (−0.4 to 0.3)	**−0.5 (−0.96 to −0.1)**	**0.01**
– Prosocial behavior	8.0 (2.0)	5.8 (2.0)	8.0 (2.1)	−0.01 (−0.5 to 0.4)	8.3 (1.9)	6.2 (1.9)	8.1 (2.1)	−0.2 (−0.7 to 0.3)	0.0 (−0.6 to 0.6)	0.99
YBOCS	6.0 (8.7)	5.7 (7.2)	5.1 (7.3)	−0.9 (−2.5 to 0.6)	5.7 (7.2)	6.5 (7.8)	6.2 (7.6)	0.5 (−0.8 to 1.8)	−1.4 (−3.2 to 0.3)	0.11
FAST	25.9 (11.6)	23.3 (11.6)	21.4 (10.9)	**−4.4 (−6.3 to −2.6)** ***	26.2 (10.9)	25.5 (12.1)	24.4 (10.6)	−1.8 (−3.9 to 0.4)	**−2.7 (−5.2 to −0.2)**	**0.03**
– Autonomy	2.9 (2.5)	2.6 (2.4)	2.1 (2.1)	**−0.8 (−1.2 to −0.3)** ***	3.1 (2.8)	2.8 (2.4)	2.7 (2.3)	−0.4 (−0.9 to 0.1)	−0.4 (−0.99 to 0.2)	0.16
– Occupational	5.4 (3.8)	4.9 (3.6)	4.7 (3.5)	−0.7 (−1.5 to 0.1)	5.7 (4.1)	5.8 (3.7)	5.6 (3.7)	−0.1 (−1.0 to 0.7)	−0.7 (−1.7 to 0.2)	0.14
– Cognitive	6.8 (3.1)	6.4 (3.1)	5.9 (2.8)	**−0.9 (−1.4 to −0.4)** ***	7.1 (3.1)	6.9 (2.9)	6.6 (2.8)	−05 (−1.0 to 0.0)	−0.5 (−1.1 to 0.1)	0.09
– Financial issues	1.98 (1.9)	1.7 (1.8)	1.5 (1.6)	**−0.5 (−0.8 to −0.2)** **	1.9 (1.8)	1.7 (1.7)	1.7 (1.6)	−0.3 (−0.7 to 0.1)	−0.2 (−0.6 to 0.2)	0.3
– Interpersonal relationships	6.4 (4.2)	5.8 (3.7)	5.2 (3.6)	**−1.3 (−2.0 to −0.5)** ***	6.3 (4.4)	6.1 (4.0)	5.5 (3.5)	**−0.8 (−1.6 to −0.0)** *	−0.3 (−1.2 to 0.5)	0.44
– Leisure time	2.4 (1.8)	2.0 (1.7)	2.1 (1.5)	−0.3 (−0.7 to 0.1)	1.9 (1.7)	2.4 (1.7)	2.3 (1.6)	**0.3 (0.0 to 0.7)** *	−0.4 (−0.8 to 0.0)	0.08
PSS	23.1 (3.7)	21.6 (3.7)	21.1 (3.5)	**−2.1 (−2.9 to −1.2)** ***	22.1 (4.2)	22.3 (3.2)	22.1 (4.4)	0.01 (−1.2 to 1.3)	**−0.6 (−1.2 to −0.0)**	**0.03**

Abbreviations: ADHD-RS: Attention-Deficit and Hyperactivity Disorder Rating Scale; DERS-16: Difficulties in Emotion Regulation Scale; FAST: Functioning Assessment Short Test; MD: PSS; Perceived Stress Scale; SDQ: Strengths and Difficulties Questionnaire; UPPS-P Impulsive Behavior Scale; YBOCS: Yale-Brown Obsessive Compulsive Scale. Significant values are highlighted in bold. Negative values represent an improvement of symptoms and/or functioning by the synbiotic compared to placebo; in the case of the *positive urgency*, *negative urgency*, and *sensation seeking* subscales, positive values represent an improvement of the synbiotic compared to the placebo.

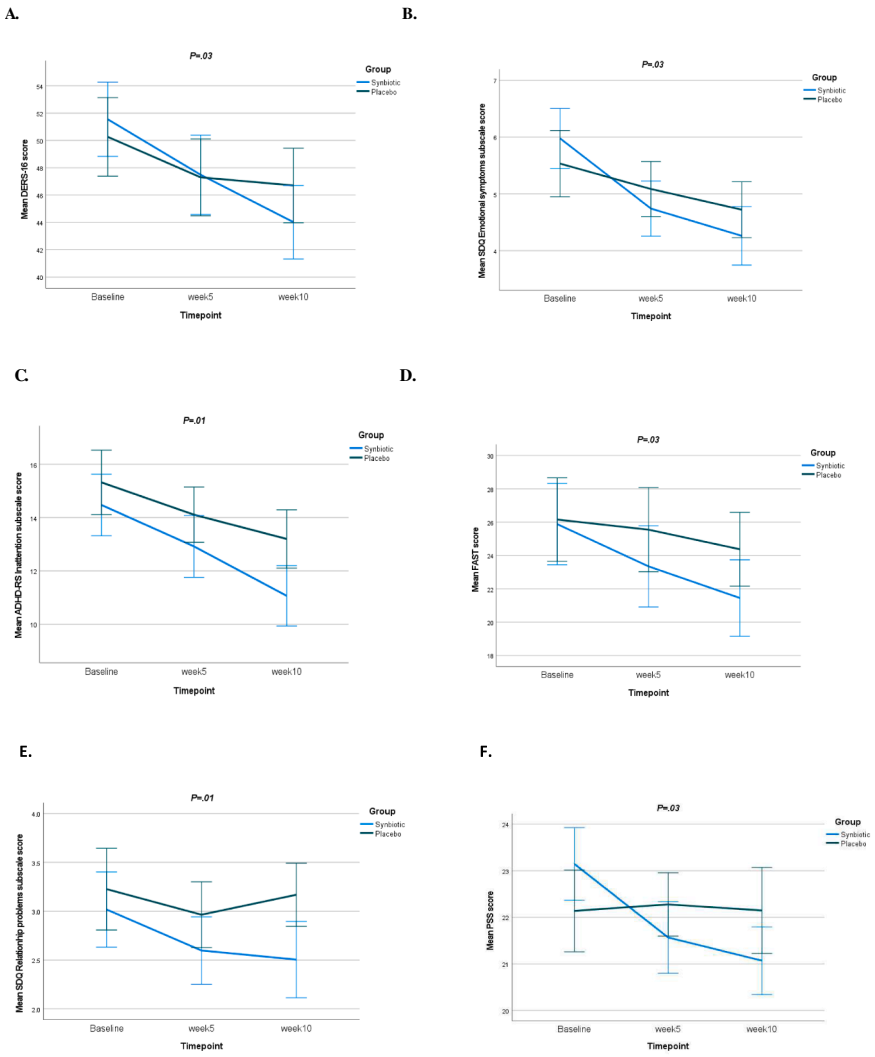

Fig. 2. Mean trajectory of scores on the different questionnaires from baseline to week 5 and 10 in the ITT sample. Error bars represent 95 % CI.

Table 4
Predicting synbiotic response by RANK-L levels before treatment.

Variable	Coef (B)	SE	OR (Exp B)	95 % CI	*P*-value
Age (years)	0.07	0.05	1.08	−0.02 to 0.17	0.13
Sex (male/female)	−1.13	1.01	0.32	−3.10 to 0.85	0.26
bmi (kg/m^2)	0.01	0.09	1.01	−0.18 to 0.20	0.92
Medication status (no/yes)	1.62	1.37	5.07	−1.06 to 4.31	0.24
Study center (Hungary/Spain/Germany)	2.21	1.07	1.01	0.12 to 4.32	**0.04**
RANK-L	−2.33	1.01	0.10	−4.33 to −0.33	**0.02**

Analyses were based on a binary logistic regression model. Significant P-values are highlighted in bold.

associated with a significantly higher response rate, but only in the synbiotic group. RANK-L is a member of the tumor necrosis factor (TNF) family that acts as a key regulator of the immune system. Recent reports have suggested that RANK/RANK-L signaling may be neuroprotective, decreasing microglial activation by, among other mechanisms, attenuating toll-like-receptor (TLR)3/TLR4 signaling in the brain (Kichey et al., 2017; Glasnovic et al., 2020). This is in line with previous research, where the existence of (chronic) low levels of several cytokines, such as TNF-alpha has been also associated with neurodegeneration and microglial/macrophage activation (De Lella Ezcurra et al., 2010). An association between (chronically) activated microglia and several neurodevelopmental conditions, such as ADHD, has been repeatedly described (Dunn et al., 2019). Moreover, accumulating evidence suggests an association between microglia activation and irritability in, for example, individuals with neurodegenerative conditions, such as Alzheimer's disease (Schaffer Aguzzoli et al., 2023). Therefore, we hypothesize that synbiotics may exert their action on irritability by, among other mechanisms, the modulation of RANK/RANK-L signaling (Kageyama et al., 2022), contributing to decrease microglial activation (Chunchai et al., 2018).

Irritability is often associated with the presence of other symptoms, such as emotional dysregulation, depression and/or anxiety and several lines of evidence have indeed suggested a shared genetic background (Vidal-Ribas and Stringaris, 2021). In our study, a synbiotic-specific

improvement (i.e., synbiotic superior to placebo) in emotional symptoms and in emotion regulation abnormalities (and in particular, in difficulties in engaging in goal-directed behaviors) was interestingly found. These findings are supported by a previous randomized, placebo-controlled trial, where a superiority of a synbiotic in the management of emotion regulation abnormalities in adults with ADHD was also suggested (Skott et al., 2020). In the mentioned study, patients were followed over a 9-week period and, as in our study, the synbiotic was particularly beneficial in improving patient's difficulties in engaging in goal directed behaviors. Interestingly, the synbiotic agent given (i.e., Synbiotic 2000) was of a very similar composition than the one used in our study. More specific, it consisted of a lyophilized composition of 4 x 10^{11} CFU per dose of three lactic acid bacteria (i.e., *Pediococcus pentosaceus* 5–33:3/16:1, *Lactobacillus casei ssp paracasei* F19, *Lactobacillus plantarum* 2362) and 2.5 of each of the fermentable fibers: β-glucan, inulin, pectin and resistant starch. Our findings are also supported by another randomized, placebo-controlled study where the efficacy of a probiotic in the management of depressive symptoms in individuals diagnosed with major depressive disorder was investigated (Nikolova et al., 2023). In this trial, a significantly greater improvement in depressive and anxious symptoms was found in the subgroup of adults allocated to the probiotic agent, compared to those allocated to placebo (Nikolova et al., 2023). In this case, patients were followed over an 8-week period, and the probiotic was composed of 14 bacteria strains (2 of them: *Lactobacillus casei* and *Lactobacilluss plantarum*) (Nikolova et al., 2023). Moreover, in another randomized, 12-week, placebo-controlled study performed in individuals diagnosed with Parkinson's disease, synbiotic supplementation resulted in a significantly higher improvement in depressive symptoms compared to placebo (Mehrabani et al., 2023).

An increasing body of evidence suggests that emotional dysregulation, depression and/or anxiety may be associated with an altered functioning of, in particular, the IL-23/IL-17 axis (Grosse et al., 2016; Blizniewska-Kowalska, 2023). IL-23 is a heterodimeric cytokine composed of two subunits, p19 and p40, and is principally produced by dendritic cells and macrophages. The p40 subunit mainly functions in conjunction with either p35 (to form IL-12), or p19 (to form IL-23) to support differentiation and maintenance of Th1 or Th17 cells, respectively. While IL-12 promotes Th1 differentiation and IFN-gamma production, IL-23 contributes to Th17 development and IL-17 production. Both cytokines are crucial for mucosal immune system, for the defense against pathogens and, as RANK-L, act as important immune system regulators. Accordingly, an abnormal function of this axis has been also associated with the development of different immune-mediated conditions, such as psoriasis and/or crohn's disease. The mechanisms by which IL-23/IL-23 may impact brain development and function are not fully understood, but may be related to their effects on the central nervous system (CNS), which include formation of reactive oxygen species by binding of IL-17 to its receptor on endothelial cells of the blood brain barrier (BBB), infiltration of peripheral immune cells with potential neuronal damage and/or increased production of pro-inflammatory cytokines by microglia, impacting dopaminergic and glutamatergic neurotransmission (Arteaga-Henríquez et al., 2021). In line with this idea, in a previous report performed in individuals with depression, elevated levels of IL-17 were associated with a greater reduction in depression severity with the primarily norepinephrine/dopamine-reuptake inhibitor agent, bupropion (Jha et al., 2017). On the contrary, patients with low levels of IL-17 at baseline were characterized by a poorer response to bupropion as compared with other treatments with a predominantly non-dopaminergic action (Jha et al., 2017). Interestingly, we found that higher IL-17A levels at baseline were statistically significantly associated with a greater improvement in emotional dysregulation, but only in the subgroup of subjects allocated to placebo. In this subgroup of patients, a total of 34/65 (52 %) of the patients were under psychostimulants, which (as bupropion), have been shown to increase dopamine and norepinephrine availability in the synaptic cleft. This supports a modulating role of dopaminergic agents on IL-17 levels as a potential mechanism to reduce emotional symptoms and/or emotional dysregulation in individuals with psychiatric conditions. Findings for the synbiotic groups deserve further discussion. In a previous report where the effect of a synbiotic on plasma levels of a set of immune activity markers was evaluated, it was found that the synbiotic agent given significantly reduced IL-12/IL-23p40 in the population under study (Yang et al., 2023) and accumulating recent research in both animal and human studies has repeatedly suggested that several bacterial strains, such as *Lactobacillus casei*, *Lactobacillus plantarum* may exert their action by acting as modulators of the IL-23/IL-17 axis, decreasing IL-17 levels (Noto Llana et al., 2013; Lenoir et al., 2016; Guo et al., 2023). However, other reports have also described how several bacterial strains, such as *Lactobacillus rhamnosus* may also increase, in a dose-dependent manner, IL-17 levels (Zhu et al., 2014). We thus hypothesize that the effect of synbiotics/probiotics on this axis may thus depend, among other factors, on the type of bacterial strains and/or dosage given, deserving further research. Nevertheless, and since *irritability* and *emotional dysregulation* predispose to aggressive behaviors (Slattery and Young, 2019; Zik et al., 2022), substance abuse (Estévez et al., 2017) and also, suicidality (Gvion and Apter, 2011; Colmenero-Navarrete et al., 2022), we believe our findings may have important potential clinical and therapeutic implications, opening a door for new (targeted) drug interventions for the management of these symptoms in at least, a subgroup of adults with ADHD and/or BPD.

Not only irritability but also *inattention* is frequently overrepresented in individuals diagnosed with ADHD and/or BPD (Ditrich et al., 2021; Weiner et al., 2019). In our study, a superiority of the synbiotic over placebo for the management of this symptom was also suggested. Findings were again not moderated by study center, age, sex, medication status and/or primary psychiatric diagnosis. Findings are also supported by previous research conducted on children, adolescents and adults without or with different psychiatric conditions (Liu et al., 2019; Wu et al., 2021; Wang et al., 2022a,b). However, findings are however not supported by the study of Skott et al. (2020). In this trial, about 72 % of the adults included in this trial were under the consumption of diet supplements (e.g., vitamins and also, probiotics) at baseline, something which may have contributed to the divergent findings.

Finally, our study also suggested a superiority of the synbiotic over placebo for the improvement in global and/or psychological (i.e., peer relationship problems) functioning in the patients under study. In addition, patients allocated to the synbiotic experienced, compared to those allocated to placebo, a significantly higher reduction in their perceived stress levels at week 10. In line with this, previous research has suggested that prebiotics and/or probiotics may reduce cortisol (i.e., a stress marker) blood levels in individuals with or without psychiatric and/or somatic conditions (Andersson et al., 2016; Chong et al., 2019; Venkataraman et al., 2021). Subjects with ADHD and/or BPD often suffer from functional impairments and from chronic stress (Adamou et al., 2013; Thadani et al., 2022), implying significant costs at the personal and societal level. In addition, an association between chronic stress (and/or chronically elevated cortisol levels) and the development and/or persistence of numerous somatic and psychiatric conditions (e.g., diabetes, cancer, cardiovascular disease, major depressive disorder) (Salleh, 2008; Musazzi et al., 2017; Druzhkova et al., 2022) has been repeatedly described, further highlighting the potential implications of the present findings.

Although encouraging, our findings should be considered in light of important limitations. Unfortunately, the trial was conducted during some of the hardest month of the covid-19 pandemic in Europe, and a national lockdown was implemented in Hungary, Spain, and Germany, resulting a in a loss of follow-up, particularly in the placebo group. Despite this, baseline clinical characteristics between study completers and drop-outs were similar, suggesting that this drop-out did not add an important bias to the findings (**Table S14**). The reasons for the higher drop-out rate in the placebo group can only be speculated on.

Interestingly, four patients in the placebo group (vs. one in the synbiotic group) didńt want to come to the hospital anymore because of being afraid of getting infected with covid-19, and fourteen patients in the placebo group experienced a protocol violation (vs. four in the synbiotic group). Antibiotic prescription was the reason for the protocol violation in 9/14 (i.e., 64 %) of the patients in the placebo group, vs 1/4 (i.e., 25 %) of the patients in the synbiotic group. We hypothesize that, as suggested by our findings, patients belonging to the placebo group may have been under higher stress (fear) levels during the performance of the trial. This may have made them more reluctant to go to the hospital to continue participating in the study. Patients belonging to the placebo group may also have consulted their doctors more frequently (i.e., at the slightest symptom), resulting in an inappropriate (i.e., excessive) antibiotic prescription in this group, as supported by previous existing literature (Armitage and Nellums, 2021; Nandi et al., 2023). Another limitation was the relatively short follow-up period of 10 weeks, which may not reveal the long-term benefits and side-effects of the synbiotic under study. Unfortunately, blinding success was not assessed, and a healthy control group was not included. In addition, intervention effects on anti-inflammatory, and/or immune parameters have not been analyzed, and the effect of the synbiotic on gut microbiota was not confirmed. Finally, multiple measures were performed, and the study was underpowered for secondary outcomes, increasing the chance of a type 1 error. However, results were all in the same direction (favoring synbiotic over placebo), and supported by previous existing literature, making it unlikely that our findings were compromised by false positives. Nevertheless, and given that many of the observed effect size estimates were small to medium, it is important to emphasize that findings should be considered as encouraging, but of preliminary nature.

5. Conclusions

Our study offers, for the first time, preliminary evidence suggesting (add-on) treatment with a synbiotic, as a reliable and safe therapeutic option in the management of adults diagnosed with ADHD and/or BPD and high levels of irritability. After 10 weeks of continuous treatment, a statistically significantly higher proportion of patients in the synbiotic group achieved a *clinically meaningful* improvement in irritability, compared to placebo. A synbiotic-specific improvement in emotional dysregulation, emotional symptoms, inattention, several facets of functioning and perceived stress levels was also suggested. Lower baseline levels of RANK-L were associated with end-of-treatment response in the synbiotic group, while higher baseline levels of IL-17A were associated with a significantly higher improvement in emotional dysregulation in the placebo group. As there are currently only a few treatments available for the management of the above-mentioned symptoms, we believe our findings may have important potential clinical and therapeutic implications. However, our preliminary suggestive findings must be confirmed in larger prospective cohorts. In addition, the effect of synbiotics on inflammatory/immune and/or microbiome parameters should be investigated.

CRediT authorship contribution statement

Gara Arteaga-Henríquez: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Carolina Ramos-Sayalero:** Writing – review & editing, Data curation. **Pol Ibañez-Jimenez:** Writing – review & editing, Software, Project administration. **Silvia Karina Rosales-Ortiz:** Writing – review & editing, Data curation. **Tünde Kilencz:** Writing – review & editing, Data curation. **Carmen Schiweck:** Writing – review & editing, Data curation. **Isabel Schnorr:** Visualization, Data curation. **Anne Siegl:** Writing – review & editing, Data curation. **Alejandro Arias-Vasquez:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **István Bitter:** Writing – review & editing, Methodology, Conceptualization. **Christian Fadeuilhe:** Writing – review & editing. **Marc Ferrer:** Writing – review & editing. **Catharina Lavebratt:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Silke Matura:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Andreas Reif:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **János M. Réthelyi:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Vanesa Richarte:** Writing – review & editing. **Nanda Rommelse:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Josep Antoni Ramos-Quiroga:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Arteaga-Henríquez reported receiving personal fees from Janssen outside the submitted work. Dr. Bitter reported receiving consulting fees from Gedeon Richter and Janssen/Janssen-Cilag; speaker's honoraria from Gedeon Richter, Hikma Pharmaceuticals, Janssen/Janssen-Cilag, KRKA, Lundbeck, and Medichem Pharmaceuticals Inc. by Unilab; research grant from Gedeon Richter; royalties from Oxford University Press. Dr. Reif reported receiving personal fees from Medice, Shire/Takeda, SAGE/Biogen, Boehringen Ingelheimm, Janssen, and Cyclerion outside the submitted work. Dr. Ramos-Quiroga reported being on the speakers' bureau and/or having acted as a consultant for Janssen Cilag, Novartis, Shire, Takeda, Bial, Shionogi, Sincrolab, Novartis, BMS, Medice, Rubió, Uriach, Technofarma and Rafo, received travel awards (air tickets + hotel) from Janssen-Cilag, Rubió, Shire, Takeda, Shionogi, Bial and Medice for taking part in psychiatric meetings, outside the submitted work. No other disclosures were reported.

Data availability

Data will be made available on request.

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Previous Presentations:

This study was partly previously presented as an oral presentation at the Networks in Child and Adolescent Psychiatry Congress (ESCAP) (June 19-21; Maastricht, The Netherlands), and at the 33rd ECNP Congress (September 12-15; virtual).

Author contributions:

Ramos-Sayalero had full access to all of the data in the study and takes responsibility for the integrity of the data. Dr Arteaga-Henríquez takes responsibility of the accuracy of the data analysis. *Concept and design:* Arias-Vasquez, Lavebratt, Reif, Rommelse, Ramos-Quiroga.

Analysis, interpretation, acquisition of data: All authors.

Drafting of the manuscript: Arteaga-Henríquez.

Critical revision of the manuscript for important intellectual content: Ramos-Sayalero, Schiweck, Arias-Vasquez, Bitter, Lavebratt, Matura, Reif, Réthelyi, Rommelse, Ramos-Quiroga.

Statistical analysis: Arteaga-Henríquez.

Obtained funding: Arias-Vasquez, Reif, Rommelse, Ramos-Quiroga.

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Appendix A. Supplementary data

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