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Research Article

Gut Microbiome Composition and Metabolic Changes Are Correlated with Sleep Efficiency and Disease Phenotype in Pediatric Inflammatory Bowel Disease

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Abstract

Background and aims: Sleep disturbances are common in patients with Inflammatory Bowel Disease (IBD), defined by alterations in the quality, timing, and amount of sleep. We hypothesized that disruption of the gut microbiota in pediatric IBD patients may induce sleep disturbances and worsen patient outcomes.

Methods: Children < 17 years with IBD were assessed for sleep disturbances using a sleep diary. Clinical disease activity indices were calculated. Patient stool was collected for calprotectin measurement, and to examine microbiota (metagenomics; Kraken2-R), Short Chain Fatty Acid (SCFA; gas chromatography), and metabolite (metagenomics; Maaslin2) profiles. Regression analyses assessed association of Fecal Calprotectin (FCal), clinical activity indices, sleep efficiency/length, and time in bed.

Results: Fifteen of 80 (18.6%) children had clinically active disease; 23 (28.8%) had FCAL > 100 ug/g. 24 of 80 (30%) children had abnormal sleep efficiency < 90% and 22 (27.5%) had subnormal sleep duration. High FCAL was associated with altered sleep efficiency ($P = 0.03$). Disease phenotype (L3, B2) demonstrated numerous significant correlations with microbiota (e.g., Veillonella, Akkermansia, Allistipes) and metabolites (e.g., H-transport, NADH-dehydrogenase, 6-phospphofruktokinase). Average length of sleep was associated with significant changes in microbiota diversity (e.g., Bacteroides, Enterococcus, Bifidobacterium, Alistipes, Streptococcus, Ruminococcus) and vast metabolic changes, primarily related to energy production. Finally, stool propionate negatively correlated with sleep efficiency and total time in bed ($P < 0.05$).

Conclusions: Our findings suggest that gut microbiota diversity, abundance, and functions (metabolites) are associated with altered sleep patterns in pediatric IBD and more aggressive disease phenotypes. These data support the need to further investigate causal relationships between gut microbiota, sleep efficiency, and disease outcomes in children with IBD.

ABBREVIATIONS

Inflammatory Bowel Disease (IBD); Crohn Disease (CD); Ulcerative Colitis (UC); Fecal Calprotectin (Fcal); Pediatric Crohn's Disease Activity Index (PCDAI); Pediatric Ulcerative Colitis Activity Index (PUCAI); Short Chain Fatty Acid (SCFA)

INTRODUCTION

Inflammatory Bowel Diseases (IBD), including Crohn Disease (CD) and Ulcerative Colitis (UC), are chronic conditions, manifested by remissions and relapses that may lead to disabling inflammation and ulceration of the gastrointestinal tract [1]. Although a clear etiology remains unknown, several environmental factors including diet, genetics, gut microbiota changes and abnormal immune response may be pathogenetically important [2,3]. Pro-inflammatory cytokines in the gut (IL-1β and TNF-α) are thought to regulate sleep [4], while sleep disturbances are in-turn thought to trigger IBD, highlighting

the need to better understand how sleep disturbances may fuel IBD, and vice versa.5 Sleep disturbances are common in IBD and sleep efficiency is significantly reduced in active CD compared to those in remission [5]. Over 50% of adults with IBD in disease remission report poor sleep quality, which increases even further for those with active disease [6,7]. Over 55% of children with IBD experience moderate to severe sleep disturbances including fatigue (75%) and sleep fragmentation (54%) [7].

Common sleep measures include length of sleep, time in bed, and sleep efficiency (total sleep time/total time in bed) [8]. Sleep can be assessed using polysomnography, actigraphy, sleep logs and diaries, or self-report questionnaires. Of these, sleep logs and diaries are considered to be the gold standard method of assessment of insomnia due to their prospective nature, which avoids recall bias and recency effects. Further, polysomnography is cost-prohibitive, influenced by an unnatural sleep environment, and does not assess subjective sleep quality. Actigraphy may be invalid in adolescent populations due to gender and pubertal differences. For example, teenage males exhibit more nocturnal movement than teenage females, which can be incorrectly classified as awake time in actigraphic sleep scoring systems [9]. As pubertal maturity increases, there is an increase in light stage sleep and thus more movement, which leads to further incorrect classifications. As a result, the American Academy of Sleep Medicine recommends use of a sleep log or diary to confirm the presence of insomnia for adults [10]. Although not perfectly correlated, sleep diary ratings have been shown to correlate significantly with results obtained using polysomnographic monitoring [11,12]. Sleep diaries tend to provide overestimates of SOL and WASO, and underestimates of TST, relative to PSG. While there are several adolescent self-report measures, very few measures have been validated for children ages 8-12 years [13].

Altered gut microbiome is a hallmark of IBD and can even predict therapy failure in pediatric IBD [14], however, the correlation between gut dysbiosis, sleep efficiency and IBD remains poorly understood. Several pathogenic microbes affect sleep patterns, typically resulting in an increase in slow wave sleep following clearance of the infection [15]. While these studies support a potential link between the gut microbiome, immunoregulation, and sleep, only a handful of studies to date have examined the links between these factors in health and IBD. Animal and human studies support the ability of sleep fragmentation to alter diversity of the gut microbiome [16,17], while other human studies demonstrate that partial sleep deprivation over only 48 hours can alter the gut microbiome composition [18]. More recently, positive correlations between sleep efficiency and total sleep time with microbiome diversity have been identified, along with correlating microbiome diversity with IL-6, a cytokine known for its effects on sleep.19 Specifically Bacteroidetes and Firmicutes are positively correlated with IL-6 and sleep efficiency while taxa including *Lachnospiraceae, Corynebacterium, Verrucomicrobia and Lentisphaerae* and *Blautia* are negatively correlated with sleep measures [19]. Despite associations identified in these studies, the relationship between sleep disruptions, gut microbiome, and clinical outcomes in pediatric IBD remains poorly understood. Here we hypothesized that gut dysbiosis in pediatric IBD patients is associated with sleep disturbances, and worse patient outcomes.

MATERIAL AND METHODS

Consent and Ethics Approval

Consent/assent was obtained from patients/guardians; approved by the University of Manitoba Health Research Ethics Board and Manitoba Shared Health Impact (Study ID HS20099), Winnipeg, MB, Canada. The study is publicly accessible at the U.S. National Institute of Health database (clinicaltrials.gov identification number NCT02970149).

Patient Criteria

In a single center prospective study, we approached all children aged 8-17 years under the care of the Pediatric IBD Program at the Children's Hospital, Winnipeg and with an established diagnosis of IBD according to the North American Society of Pediatric Gastroenterology (NASPGHAN) [20]. We excluded all children with IBD and known cognitive dysfunction or global developmental delay and also children with IBD and concurrent gastrointestinal infection.

Data and Sample Collection

Consented participants completed a standardized validated daily sleep diary to assess sleep for one week prior to the clinic visit. The prospective Consensus Sleep Diary [8] was used to collect information on sleep (e.g., Total Sleep Time [TST], Sleep Onset Latency [SOL], Sleep Efficiency [SE], number of nocturnal awakenings, wake after sleep onset, and sleep quality). Sleep diary measures were completed daily, scored, and averaged across the recording period. The diary was circulated to patients during clinic visits or by mail/email to be completed for the week before the clinic visit to avoid recall bias. Patients were reminded via phone calls to completer the diary 8-10 days before the clinic visit. The sleep diary provided information about sleep duration, time in bed (bed-time duration), and sleep efficiency (sleep duration/time in bed X 100) and responses were averaged over the 7-day recording period [13]. Patients were also asked to complete a dietary evaluation questionnaire Version 3.17A.NB (2015-01-23).

Stool containers were sent to participants to collect fecal samples one day before the clinic visit for Fecal Calprotectin (FCAL) measurement as a surrogate marker for mucosal inflammation and for fecal microbiota analysis. Stool samples were further used to determine if participants had diarrhea, and if any infection was present to assess and exclude those with gastrointestinal infection. Disease relapse was measured using FCAL (stool) and the Pediatric Crohn's Disease Activity Index (PCDAI) or Pediatric Ulcerative Colitis Activity Index (PUCAI) examined clinically [21,22]. The quantum blue single point of care test (ALPCO) was used to measure stools FCAL. A disease relapse

was defined as FCAL measurement greater than 100 or PUCAI/PCDAI measurement greater than 10 [21,22]. Disease phenotype, duration, baseline characteristics, patient demographics, smoking habits, medications including antibiotics and probiotics, and initiation of new medications or other treatments for IBD during the course of the study were obtained by chart review using an information form [23].

As parent and youth reports may differ, both were obtained in assessing for symptoms of anxiety and depression [24]. The Spence Children's Anxiety Scales were used to assess self- and parent reports of anxiety symptoms using 6 sub-scales (e.g., panic attacks, separation anxiety, physical injury fears, social phobia, obsessive compulsive, and generalized anxiety) [22]. With response options ranging from 0 (never) to 3 (always), the 45-item self-report and the 39- item parent-report scales were administered. Total scores across subscales were used, and higher scores reflected more anxiety. The scale has demonstrated high internal consistency and good test–retest reliability over a six-month period. Evidence of convergent and divergent validity has been reported [23].

The Child Depression Inventory-II (short-form) measured self- and parent reports of depressive pediatric symptoms in youth aged 8-17 years [24]. The CDI-II self-report is a 12-item scale with 3 response options per item [e.g., 0 ("I am sad once in a while"), 1 ("I am sad many times"), 2 (" I am sad all the time")]. The CDI-II parent-report is a 17-item Likert scale, with responses ranging from 0 (not at all) to 3 (much or most of the time). For both scales, responses are summed to produce a total score, and higher scores represent more depressive symptomatology. It represents one of the most widely used measure of depression in this age group, has excellent reliability, concurrent, construct, and predictive validity [25]. Blood samples were collected at the clinic visit to measure serum hemoglobin, serum albumin, and inflammatory markers (C-reactive protein and Erythrocyte Sedimentation Rate).

Gas Chromatography for Volatile Fatty Acids

The concentrations of SCFA in stool samples were determined by gas chromatography performed by Department of Agriculture, chromatography core at the University of Alberta, as reported previously [26]. Briefly, phosphoric acid was added to samples and 1 mL of supernatant was combined with 200 µL of the internal standard in a GC vial for analysis. Isocaproic acid was used as internal standard. Samples were run on a Varian 430-GC with FID (Varian, Inc., USA) using a Stabilwax-DA fused silica column (Restek Corp., 30m, 0.53mm ID, 0.5um film). The carrier gas was helium at 10ml/min. The injector and detector temperatures were maintained at 250°C, and the injection split was 5:1. The injection volume was 1uL. The oven was held for 0min at 80°C, then increased to 180°C at 20°C/min and held for 3 min for a total run time of 8 min. Retention times and concentrations were determined using standard compounds and the internal standard.

NGS Library Construction and Shotgun Metagenomics

Library Construction and Sequencing: Genomic DNA from stool samples was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Mississauga, ON, Canada) with additional steps to assure bacterial cell lysis of Gram-positive bacteria, as previous [26]. Briefly, samples were homogenized with 1 mL ASL lysis buffer (Qiagen, Mississauga, ON, Canada) and the cells were disrupted by vortexing with zirconium beads (diameter 0.1 mm; 300 mg), followed by a 15 min incubation at 95˚C and mechanical lysis in a FastPrep-24 (MP Biomedicals, Solon, OH) in 3 cycles of 50-second bead-beating step at 6 m/s speed followed by cooling on ice for 5 min each. The cell lysate was then subjected to a protein precipitation with 1/10 volume 3M Sodium Acetate, followed by a nucleic acid precipitation with 1 volume isopropanol. The nucleic acid pellet as washed with 70% Ethanol and resuspended in TE Buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH 9). The DNA was further incubated with proteinase K and Qiagen Buffer AL at 70˚C for 10 min and column purified as per the QIAamp Fast DNA Stool Mini Kit protocol. DNA was then quantified using a Qubit 2.0 Fluorometer with the dsDNA High Sensitivity Assay quantification kit (Thermo Fisher). Libraries were constructed from 1 ng of DNA using the NextEra XT DNA Preparation Kit (Illumina Inc.). Briefly, the genomic DNA was incubated with the transposome to perform a fragmentation reaction, which fragmented the DNA and added adapter sequences at the 5' and 3' ends of each amplicon. The products were then amplified by 12 cycles of PCR using specific index adapters for Illumina sequencing (NextEra XT Index Kit v2, Illumina). The resulting libraries were cleaned up using AMPure beads (bead: library at 1.8:1 ratio), eluted in 50 µl of kit resuspension buffer, and quantified on Qubit 2.0 Fluorometer. The cleaned libraries were then re-amplified using the same index adapters as some of the samples yielded low concentration (< 1 ng/µL). All libraries were then assayed on QIAxcel Fragment Analyzer System (Qiagen) to identify average library size and further quantified using Qubit Fluorometer prior to sequencing to calculate molarity. Multiplexed libraries were sequenced on a NovaSeq 6000 system (Illumina Inc.) using a S2 flow cell at an average depth of 100 million reads per sample.

Bioinformatics Analysis

Sequences were inspected with Fastqc (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and bases at the end or reads with quality scores (Qscore) smaller than 30 were trimmed with mcf-fastq (https://github.com/ExpressionAnalysis/ea-utils/blob/wiki/FastqMcf.md), allowing a minimal length of trimmed reads of 120 base pairs. Taxonomic classification was conducted with Kraken2 and a customized database was compiled using the standard Kraken2 database, supplemented with bacterial genomes in the Genome Taxonomy Database (GTDB; gdtb.ecogenomic.org). The GTDB hosts 145,512 bacterial accessions and 2,392 archaeal accession. Kraken2 hits accumulating less than 10% of K-mers matching the reference sequence were discarded and a hit was considered true only if at least 50 reads were aligned against the reference. For metabolic

profiling, HUMAnN2. Output tables were labelled with UniRef90 names using the script human2_rename_table, and gene family abundance was renormalized with script humann2_renorm_table, from RPK to compositional units (counts per million) to enable between-sample comparisons. Genes were regrouped to functional categories with script humann2_regroup_table, to Enzyme Commission (EC) categories level 4. Analyses were conducted using R Studio 2022.02.2. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Statistical Analysis

Descriptive analyses of the variables will be performed comparing disease type (CD/UC) and disease status (inactive (F Cal < 100 microgram/gram stools /active (F Cal > 100 microgram/gram of stool)). Comparisons of disease subtypes and active/inactive subgroups were performed using two-tailed t-tests for continuous variables, and chi-square/Fisher exact tests of association for categorical variables. As the hypothesis of the study is an association between sleep disturbance and disease relapse, the exploratory variables are three sleep scores (sleep duration, bed-time duration, sleep efficiency) measured at consecutive seven time points and taken an average of each of them. Besides, possible confounding variables (age, sex, disease type and duration, and anxiety and depression scores for children and parents) were included in our multivariable regression model. For any missing data (less than 10% missing) we did mean imputation for continuous variables and most occurring value imputation for categorical variables. One of our outcome variables, FCAL has about 38% missing data; hence, we did multiple imputation for FCAL. As our outcome of interest is dichotomous, standardized logistic regression analysis was conducted with the imputed dataset to determine the effect of sleep abnormalities on disease relapse. In addition, and as our interest is the relationship between sleep parameters (as independent variable) and disease relapse (as a dependent variable), we did univariable analysis with 3 sleep predictors and 2 disease relapses separately and then included age and sex one at a time.

For the microbiome analysis, row sequencing reads were deposited at the Short Reads Archive (SRA) database of NCBI and are publicly available under accession number PRJNA690735.

RESULTS

Sleep Disturbances Were Associated with Fecal Calprotectin and Disease Activity

Eighty children with IBD (67.5% with CD, and 60% boys) completed the study, with diary completion rate of over 90%. Baseline characteristics are summarized in (Table 1). Fifteen of 80 (18.6%) children had clinically active disease (clinical activity index > 10), all of which had elevated FCAL. In addition, 8 children had elevated FCAL with normal clinical activity indices i.e. 23 (28.8%) had FCAL > 100 ug/g. Twenty-four (30%) children had abnormal sleep efficiency < 90%. 22 (27.5%) had subnormal sleep duration for their age and sex. Six (7.5%) children and 13 (16.3) parents reported elevated anxiety scores, and 2 (2.5%) children and 4 (5%) parents reported elevated depression scores. In univariable regression analysis, we found significant positive association between high FCAL altered sleep efficiency (P = 0.03) but not in multivariable analysis in which bedtime duration become significant (P = 0.04) factor affecting FCAL while adjusting for age. There was also an association between clinically active disease (PUCAI/PCDAI > 10) and both sleep duration and sleep efficiency (P = 0.02 for both). There was no association between disease activity and other variables including depression and anxiety score.

Table 1: Baseline characteristics and outcomes in children with inflammatory bowel disease.

Variable	Median	Q1	Q3
Age (y)	13.14	11.75	15
Disease duration (y)	1.54	0.8	2.71
Disease activity index	0	0	5
Calprotectin(ug/g)	99	100	984
Albumin (g/L)	38.38	36.75	40
C reactive protein (mg/L)	0	0	3
Sleep duration (h)	8.96	8.32	9.45
Bedtime duration (h)	9.98	9.33	10.45
Sleep efficiency (%)	91.29	88.14	99.25
Disease Phenotype (n)*			
Crohn	54		
L1	4		
L2	12		
L3	33		
L4	9		
Ulcerative colitis	26		
E1	1		
E2	4		
E3	3		
E4	19		
Medications(n)*			
Biologics	29		
Immunomodulators	30		
5-ASA	15		
Corticosteroids	6		
EEN	5		

*Numbers do not add to a total of 80 as several patients had L3+L4 disease and were on more than one medication. 5-ASA 5 aminosalicylates, EEN exclusive enteral nutrition

Altered Microbiota Was Associated with Disease Diagnosis and Severity

Only 28 (18 boys, mean age at diagnosis = 13.8y) children with IBD (18 CD, 10 UC) provided their stool samples for microbiome analysis. Of these, 10 (36%) patients had clinically active disease with PCDAI/PUCAI ≥ 10, and 12 (43%) patients had FCal >100ug/g. Stool samples were collected from patients and shotgun metagenomics sequencing was used to determine metabolic and taxonomic profiles. Diagnosis of pediatric CD differed from diagnosis of UC in microbiota abundance and metabolite levels (e.g., *Clostridium, Akkermansia, Blautia*, serine-type carboxypeptidase and β-fructofuranosidase; (Figure 1).

Furthermore, disease phenotype (Montreal classification L3,B2 vs all other phenotypes) demonstrated numerous significant correlations with microbiota (e.g., *Veillonella, Akkermansia, Allistipes*) and metabolites (e.g., H-transport, NADH-dehydrogenase, 6-phospphofruktokinase).

Sleep Patterns Were Associated with Changes in Microbiota Taxa and Metabolites

Average length of sleep was associated with significant changes in microbiota diversity (e.g., *Bacteroides, Enterococcus, Bifidobacterium, Alistipes, Streptococcus, Ruminococcus*) and vast metabolic changes, primarily related to energy production (Figure 1).

The SCFA Propionate Negatively Correlated with Sleep Efficiency and Total Time in Bed

Stool SCFA concentration were measured in patient stool samples by gas chromatography volatile fatty acid analysis. SCFA were correlated again microbiota taxonomic and metabolic changes along with disease phenotype (L3, B2) and sleep patterns. No difference in any of the SCFA measured was found to correlate with disease diagnosis, disease activity, age, FCal or treatments. The SCFA propionate negatively correlated with sleep efficiency (total sleep time/total time in bed; $P < 0.05$) and total time in bed ($P < 0.05$; Figure 2).

CONCLUSIONS

Approximately 27-30% of the children in our study had sleep problems (PSQI), in line with prior studies which demonstrated that sleep disturbances are common in children with IBD [27]. Similar studies showed that children with IBD have impaired objective sleep patterns such as more awakenings and longer sleep latency, with correlations uncovered between sleep dysfunction and inflammatory markers [28]. We found that short sleep duration and poorer sleep efficiency positively correlated

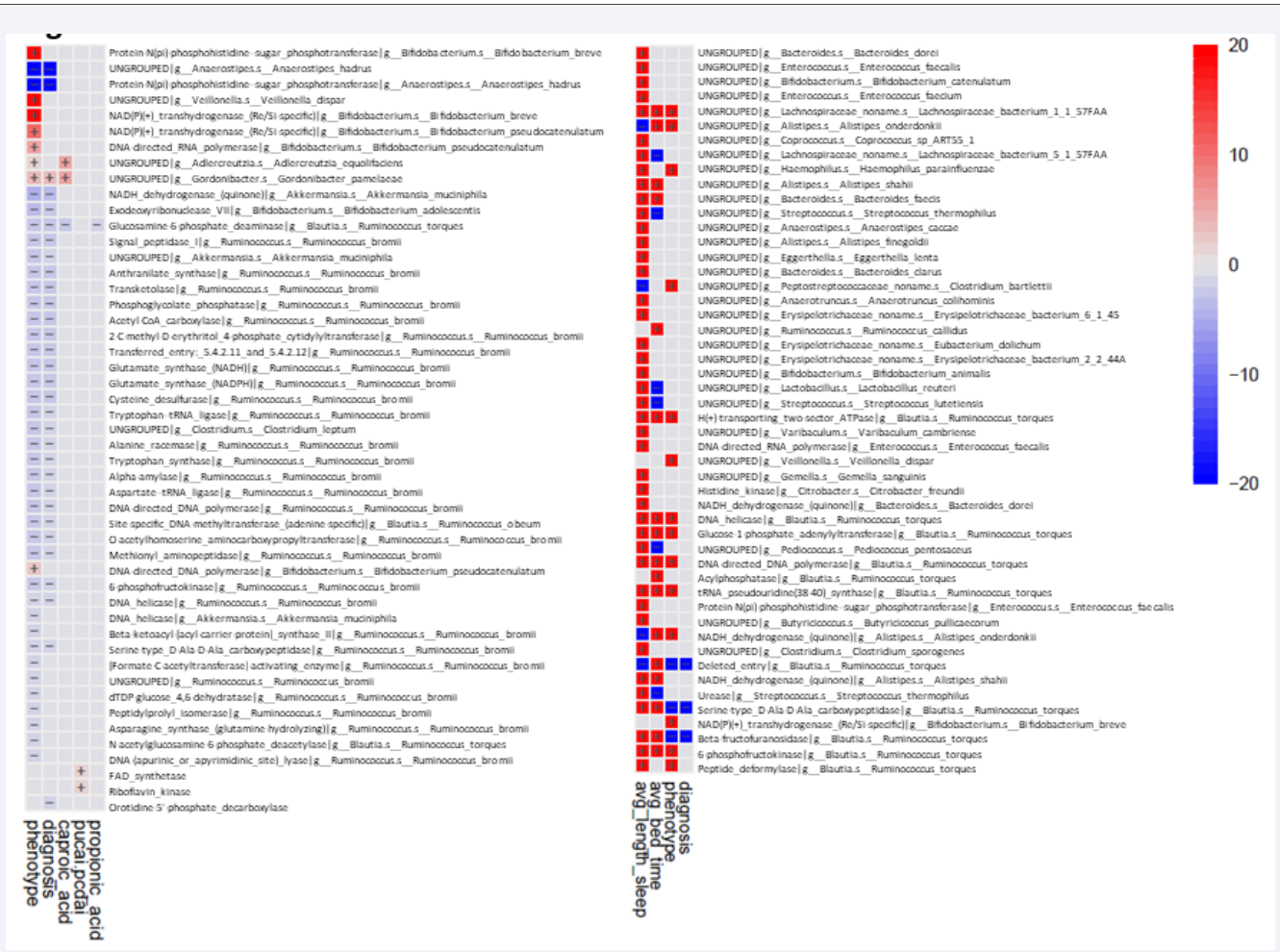

Figure 1 Microbiome taxonomic and metabolic profiles correlated with disease diagnosis, phenotype, sleep patterns and SCFA. Microbiota were collected from patient stool samples and sequenced by shotgun metagenomics. Microbiota taxonomic changes (Kraken2) and metabolic (Maaslin2) were associated with disease diagnosis (CD *vs* UC), disease phenotype (Montreal classification L3, B2 *vs* all other phenotypes), sleep patterns, and SCFA. Red (positive correlation), blue (negative correlation).

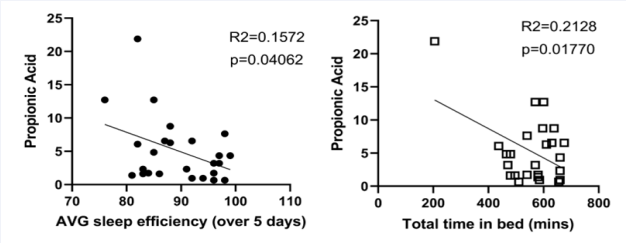

Figure 2 Propionic acid correlated with average sleep efficiency and time in bed. SCFA were measured in patient stool by volatile fatty acid analysis and correlated with sleep data.

with active IBD [29]. While studies suggest over-reporting by parents compared to self-reporting by the patients themselves, those with severe IBD symptoms and their parents reported significantly more frequent sleep abnormalities compared to healthy control [30]. Our group previously investigated sleep abnormalities in adults with IBD using PSQI and found an indirect relationship between sleep quality and the number of sleep hours and IBD-related quality of life [31]. Direct correlation between the level of fatigue and poor sleep quality was reported [32].

In a subset of participants who provided stool samples for microbiome analysis we also found sleep dysfunction correlated with microbiome abnormalities especially in children whose IBD was active. At the family level CD is associated with higher *Lachnospiraceae* while UC is associated with higher *Clostridiaceae,* and *Ruminococcaceae* [33]. At the species level CD patients display greater *R. lactaris* while UC patients display greater *C. catus, R. callidus*, and *F. prausnitzii* [33]. In our study we confirmed differences in microbial abundance between pediatric CD and UC (e.g., *Clostridium, Akkermansia, Blautia*). We further identified abundance of serine-type carboxypeptidase and *β*-fructofuranosidase differed between pediatric CD and UC. Serine proteases such as carboxypeptidase have been positively correlated with disease activity in IBD [34]. Carboxypeptidases are involved in protein digestion and are typically found in higher abundance in the small intestine, suggesting one reason why CD, which impacts the small intestine, displays altered levels of these enzymes compared to UC [34]. *β*-fructofuranosidase are involved in hydrolysis of sugar molecules from fructooligosaccharides fibers and catalyzes the synthesis of these fiber structures suggesting potential differences between CD and UC in regards to fiber fermentation of SCFA production [35].

Elsewhere, disease severity has been associated with altered microbiome in pediatric IBD [36]. Microbes involved in fiber fermentation and production of SCFA (*Lachnospiraceae, Blautia, Roseburia, Ruminococcus, Clostridium*, and *Faecalibacterium*) are negatively associated with disease severity suggesting production of SCFA is reduced in CD patients with more severe disease [37]. Microbes positively associated with disease severity include those involved in production of toxic gases such as hydrogen sulphate (*Atopobium, Fusobacterium, Veillonella, Prevotella, Streptoccocus*, and *Leptotrichia*) suggesting microbially-driven gut damage is associated with worsened disease scores [37,38]. Our findings corroborate that pediatric IBD disease phenotype (L3,B2) correlated with microbiota abundance (e.g., *Veillonella, Akkermansia, Allistipes*) and metabolites (e.g., H-transport, NADH-dehydrogenase, 6-phospphofruktokinase). Higher NADH-dehydrogenase (mitochondrial dysfunction) is associated with IBD severity [39]. Furthermore, these pathways are also associated with sleep quality supporting the hypothesis that sleep quality and gut dysbiosis are interrelated in IBD [40].

Sleep fragmentation, sleep efficiency, total sleep time, and disease severity have been shown to be negatively correlated with microbiome diversity and composition (*e.g., Lachnospiraceae, Corynebacterium, Verrucomicrobia, Lentisphaerae* and *Blautia*) [19]. Our findings support a significant association between average length of sleep with changes in microbiota diversity (e.g., *Bacteroides, Enterococcus, Bifidobacterium, Alistipes, Streptococcus, Ruminococcus*) and metabolic changes (e.g., energy production and mitochondrial function), suggesting a decrease in dietary fiber fermentation and production of beneficial SCFA. In our study, the SCFA propionate negatively correlated with sleep efficiency ($P < 0.05$) and total time in bed ($P < 0.05$).

Our study has several strengths within this novel study including use of validated scales and robust design. However, our results are limited by the lack of a control group or sample size calculation (our study power = 0.67; goal was 0.8), lack of stool samples for many participants as stool sample collection can be very challenging in pediatric population, lack of endoscopic data, examination of stool microbiome versus site-specific or mucosal microbiomes, and examinations of correlative rather than causative or mechanistic data. It is well known that sleep deprivation can alter composition of the gut microbiome in under 48 hours [19] and gut microbiota themselves in turn influence sleep patterns suggesting a vicious cycle in diseases such as IBD that are hallmarked by altered microbiota [41]. Yet the study of the microbiota can be complicated as there are key differences in function and stability of the luminal microbiota (stool; studied here) and the mucosal microbiota (not studied here) [42]. It is however, recognized that both the luminal and mucosal microbiota display an IBD footprint with a core set of microbial taxa similarly represented in both communities [42]. Furthermore, our understanding of the directionality of the links between sleep, microbiome, and disease activity in IBD remains limited, as each factor influences the other, making it difficult to define an initiating factor [43]. Changes in the gut microbiota in IBD involve a reduced capacity to ferment dietary fibers to produce SCFA [26]. Interestingly, decreased SCFA are also associated with worsened sleep efficiency, further supporting the role of the gut microbiota in sleep quality and IBD disease outcomes [44]. Lastly, altered microbial functions associated with energy production and mitochondrial functions are influenced by SCFA in the gut [45]. Propionate can alleviate mitochondrial dysfunction and oxidative stress potentially promoting improved sleep quality [40]. Taken together, our findings support a dynamic interaction that occurs between the gut microbiome, sleep efficiency, and disease outcomes in pediatric IBD patients. These data support the need to further investigate causal relationships between gut microbiota, sleep efficiency, and disease outcomes in pediatric IBD.

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Author Contributions

WEM conceived, developed, acquired funding and coordinated the project. NV and CNB provided help in planning the study and interpreting the results. HKA, MBJ, and SV processed stool samples and performed and analyzed experiments. SS and MT helped with statistical analysis. JJ and HKA performed bioinformatics and statistical analyses. HKA was responsible for the figure preparation. SH and MT provided statistical help, EW provided additional supervision and oversight. HKA & WEM drafted the manuscript. All authors contributed to manuscript edits and approved the final version of the manuscript.

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Data Transparency Statement

Study data, analytic methods, and materials have been made available to other researchers in the main text; there are no restrictions on data availability. Raw sequences of libraries described in this article are publicly available at NCBI Short Sequences Archive (SRA) under the accession number PRJNA922068.

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