Dysbiosis in Snoring Children: an Interlink to Comorbidities?

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Funding Source: No external funding for this manuscript.

Financial Disclosure: The authors have no financial relationships relevant to this article to disclose.

Conflict of Interest: The authors have no conflicts of interest relevant to this article to disclose.

Word count of the manuscript body: 2016 words

Number of figures: 3

Number of tables: 1

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Background and Objectives: Early microbial colonization has a key impact on infant health through nutritional, immunological, and metabolic programming. The origin of child snoring is multifactorial and complex, and may thereby also generate long-term health problems. The link between child snoring and gut microbes remains unclear, although indirect evidence exists regarding this relationship. This study aimed to characterize the connection between gut microbiota and child snoring.

Methods: In a prospective, observational CHILD-SLEEP birth cohort study, gut microbiota in a subcohort of 43 of these children at 2 years of life was profiled with 16S ribosomal RNA gene amplicon sequencing.

Results: A higher abundance of the Proteobacteria phylum, the Enterobacteriaceae family, and Erysipelotrichaceae family, as well as a higher ratio of Firmicutes to Bacteroidetes were detected in snorers as compared to controls. Furthermore, snorers showed significantly lower microbial diversity and richness than non-snorers.

Conclusions: The snoring children manifest different gut microbiota as compared with healthy children. Considering that snoring and sleep disorders can be a source of long-term consequences, including cardiovascular, metabolic, immunological, neurocognitive and behavioural consequences, our results proposes early microbiota as a new treatment target.

What is Known:

- Child snoring is a multifactorial disorder that may have other health connections such as cardiovascular, metabolic, immunological, neurocognitive, and behavioural outcomes.
- Early microbiota has been related to risk of disease although the link between snoring and gut microbes remains to be uncovered.

What is new:

- Snoring children harbor different microbiota when compared with healthy children presenting lower microbial diversity and higher abundance of pro-inflammatory bacteria and higher ratio of Firmicutes to Bacteroidetes.
- Our results suggest the impact of early microbiota as a new treatment target.

Key Words: snoring, microbiota, health

The sleeping patterns of healthy infants vary, but sleep-disordered breathing, affecting one infant in ten (1-8) is not harmless. This disorder encompasses a group of conditions that range in severity and prognosis, with snoring being the most common symptom. Despite extensive research into the origins of infant snoring, the pathomechanisms involved remain unclear (9). Regardless of the potential causes, a child's snoring can be a source of long-term consequences, such as cardiovascular, metabolic, immunological, neurocognitive, and behavioural outcomes. Reversing this process requires identifying and characterizing the related mechanisms (10,11).

Snoring in otherwise healthy children coincides with several maturational processes that take place in the gastrointestinal tract, the largest portal for early environmental exposures, particularly microbial colonization and food intake. Indeed, breastfeeding has been suggested as a potential protective element against childhood snoring (12,13). Interestingly, breastfeeding holds a key position with regard to promoting healthy, age-appropriate gut colonization (14). In point of fact, early exposures that interfere with the compositional development of the intestinal microbiota have been associated with the development of diseases such as obesity, diabetes, allergies, and even neurodevelopmental disorders (15,16) which are also common consequences of child snoring. To uncover the impact of the earliest and the most massive environmental exposure, the present study was undertaken to characterize the interconnection between the gut microbiota and child snoring.

MATERIAL AND METHODS

Study Design

The original sample was composed of 1,673 neonates and their parents from the CHILD-SLEEP birth cohort. The children were born between April 2011 and February

2013. The details of the protocol and recruitment procedure have been reported previously (17). The recruitment and baseline measurement occurred prenatally, and the follow-up evaluation included the birth characteristics and assessments at 3 months, 8 months, and 2 years of age, when questionnaires were provided to both the mothers and fathers.

The present study involves families who responded to the 8 month and 24-month questionnaires concerning the child. The questionnaire was sent only to Finnish speakers. Thus, the majority of the families were Finnish. The CHILD-SLEEP study protocol was approved by the Ethics Committee of Pirkanmaa Hospital District (9 March 2011, ethical research permission code R11032).

Study subjects and sampling

Families reporting snoring in their children were contacted by phone. Snoring frequency was confirmed in a brief interview. The inclusion criterion was that the child was snoring 3 or more times per week. Children who snored less than three times per week were defined as controls. At 8 months, 22 snorers and 13 non-snorers (controls) were enrolled with parental consent, and these participants were re-evaluated at 24 months of age. Then, based on the questionnaire filled out at 24 months of age, new snorers were identified and recruited. Altogether, 32 snoring children and 20 controls completed the study protocol at 24 months. The protocol included an accurate physical examination by a pediatrician, together with anthropometric measurements. Fecal samples, collected at 24 months of age, were available from 27 snorers and 16 non-snorers, who comprised the final study population. Samples were frozen and stored at -80°C for later analysis.

DNA extraction and 16S gene amplicon sequencing

Total DNA was isolated from the fecal pellets as previously described.¹⁸ Briefly, 100–125 mg of feces was weighed and homogenized in the presence of lysis buffer via bead-beating with FastPrep-24 (MP Biomedicals, Irvine, California, USA). DNA was extracted following the commercial kit InviMag Stool DNA kit (Stratec Molecular, Berlin, Germany) by use the automated KingFisher DNA system (Thermo Fisher Scientific Oy, Vantaa, Finland). The KingFisher's system protocol steps included nucleic acid binding on magnetic beads, five-step washing, and elution.

Isolated DNA concentrations were measured using a Qubit® 2.0 Fluorometer (Life Technology, Carlsbad, CA, USA). The 16S rRNA gene (V3-V4 region) was amplified following Illumina protocols, and the libraries were sequenced using a 2x300pb paired-end run (MiSeq Reagent kit v3) on a MiSeq-Illumina platform (FISABIO sequencing service, Valencia, Spain) according to the manufacturer's instructions. To rule out and control for potential reagent contamination, the reagents for DNA extraction and PCR amplification were also sequenced as controls.

Bioinformatics and statistical analysis

Data processing was performed using the QIIME pipeline (Version 1.9.0, default parameters) (19). Chimeric sequences and sequences that could not be aligned were ~~also~~ removed from the dataset. An open reference Operational Taxonomic Unit (OTU) picking method with 97% identity to the Greengenes 13.8 database was selected. OTUs with a relative frequency below 0.01 and those that could not be classified at the domain level were removed from each sample. Sequences classified as cyanobacteria and chloroplasts were also removed from the dataset because they likely represent ingested plant material. Alpha diversity indices (Chao1 and Shannon), beta diversity based on unweighted UNIFRAC (phylogenetic) and Bray Curtis distance (non-phylogenetic)

among the samples were obtained. PERMANOVA test was used to test for significance between group. The analysis of composition of microbiomes (ANCOM) test for comparing the taxa abundance was obtained (20). The DESeq2 method was also used to determine whether snoring status was associated with altered taxa abundance at the family, genus, and OTU levels. *P* -values after False Discovery Rate (FDR) correction ≤ 0.05 were regarded as statistically significant. Calypso software Version 8.24 (<http://cgenome.net/calypso/>) was used, along with total sum normalization (TSS), for the statistical analysis and data mining. The bacterial profiles were further compared between snorers and controls via linear discriminant analysis (LDA), as well as effective size (LEfSe) pairwise analysis in a Galaxy environment using an alpha cut-off of 0.05 and an effect size cut-off of 2.0 (21). In order to assess the associations between microbiota and clinical variables (such as weight, BMI, and others). Spearman rank test correlations were calculated.

RESULTS

Gut microbiota composition in snorers and non-snorers

In the study population (n=52), a total of 43 children with available fecal samples were included (n=27 snorers and n=16 controls). There were no significant differences in the clinical characteristics between the groups as shown in **Table 1**.

Significant differences in the gut microbiota between snorers and controls were found, as shown in the multivariate-method PERMANOVA with 999 permutations of the UNIFRAC distance ($R^2=0.0316$, $P=0.038$ for unweighted UNIFRAC distance) and Bray Curtis distance (non-phylogenetic distance, $R^2=0.0420$, $P=0.037$). Furthermore, multivariate redundant discriminant analysis (RDA) showed statistically significant differences in microbial composition between snorers and controls (Variance=12.2; F

model= 1.30, $P = 0.048$) (Figure 1A,B). Snorers showed lower microbial diversity ($P = 0.019$, Shannon index) and richness ($P = 0.00634$, Chao1 index) than non-snorers (Figure 1 C,D).

Specifically, the distinction was due to a higher abundance of members of the Proteobacteria phylum in snorers as compared to controls (1.06% vs 0.38%, $P = 0.01$, FDR=0.06) (Figure 2A). A significantly higher ratio of Firmicutes to Bacteroidetes ($P = 0.036$) and a lower ratio of Actinobacteria to Proteobacteria ($P = 0.006$) was detected in snorers than in controls (Figure 2 C,D). ANCOM test identified significant higher presence of Enterobacteriaceae family and at genus level, higher unclassified Enterobacteriaceae and *Eubacterium* in snorers as compared to controls. DESeq2 test was also used to identify the differential abundances of specific bacteria, snoring children showed significantly higher abundances of Enterobacteriaceae ($P < 0.0001$, FDR=0.0013) and Erysipelotrichaceae ($P < 0.0001$, FDR= 0.0088), compared to controls.

LefSe analysis shows a statistically significant enrichment of the Enterobacteriaceae and Erysipelotrichaceae families in snorers as compared to non-snorers (LDA score>3.90), while the unclassified Clostridiales and Ruminococcaceae families were enriched in controls (Figure 3). At the OTU level, the LefSe test showed the higher enrichment of specific OTUs, especially in those belonging to Bacteroidetes phylum, in snorers as compared to controls.

DISCUSSION

The redundancy of the Proteobacteria phylum in snorers, documented for the first time here, suggests a proinflammatory tone in the gastrointestinal microenvironment. The shift in bacterial profile culminates in a reduced Actinobacteria to Proteobacteria ratio in

snorers as compared to children who are free of sleep-disordered breathing symptoms. Concomitantly, an increased ratio of Firmicutes to Bacteroidetes was documented in snorers. Analogous dysbiosis has previously been reported in obesity (22,23), with a heightened risk of cardiovascular, metabolic, and immunological disadvantages at later ages (23,24). Snoring has been reported to have identical long-term consequences (10, 25-28). Interestingly, in the present study, a snoring-associated shift in microbiota was observed in metabolically healthy, non-obese children. Taken previous demonstrations on the interlink between microbiota and human health together (29, 30), we hypothesize here that aberrant gut microbiota, as a common denominator, might initiate immune and metabolic disturbances locally and, consequent to impaired gut barrier function, systemic chronic inflammation, might increase the risk of non-communicable diseases, such as allergy and asthma, as well as overweight and cardiovascular and neuropsychiatric disorders. Furthermore, snoring would also affect the physiological and behavioural outcomes affecting gut microbiota. Associations cannot be linked to causation and it is needed studies focused on the validation of causation and also, to identify other factors involved in the development of snoring problem and short and long-term health outcomes.

Specifically, the distinctive microbiota profile of snorers showed consistently higher Enterobacteriaceae and Erysipelotrichaceae at both the phylum level and the genus level, and these findings were confirmed via the LEfSe analysis. The Enterobacteriaceae are a large family of gram-negative bacteria that along with many harmless symbionts, also harbors many common pathogens (e.g., *Salmonella*, *E. coli*). This family is the only representative of the *Enterobacteriales* order in the *Gammaproteobacteria* class and the Proteobacteria phylum, which is often associated with inflammation. The *Erysipelotrichia* are a class of bacteria in the Firmicutes

phylum. Species of this class are known to predominate among the gut microbiome. Their predominance has been reported in diet-induced obesity (31) and in pediatric inflammatory bowel disease (32).

Our results are in agreement with previous data on subjects suffering from partial sleep deprivation (33). The study in question reported higher abundances of Coriobacteriaceae and Erysipelotrichaceae and lower abundance of Tenericutes (33). Interestingly, the risk of insulin resistance, type 2 diabetes and obesity – metabolic pathologies that have been linked to deviant gut microbiota – have also been reported to result from chronic sleep deprivation (34). It has been reported that frequent snoring in an adult population (35) is associated with higher levels of insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), triglycerides, leptin, and inflammatory markers (such as C-reactive protein, interleukin-6 and -18 and lipopolysaccharide binding protein (LBP)) and lower concentrations of high-density lipoprotein (HDL) and adiponectin (35). In agreement with these results, it has been reported that chronic sleep disruption in mice alters the gut microbiota and induces systemic and adipose tissue inflammation and insulin resistance (36). Lipopolysaccharide binding protein has been suggested to be a marker of underlying low-grade endotoxemia in the gut. Lipopolysaccharide (LPS)-induced metabolic endotoxemia is the first step in the development of insulin resistance and diabetes (37, 38). Gram-negative bacteria contain LPS as the major constituent of the outer leaflet of the outer cell membrane. Thus, the increased presence of gram-negative bacteria Proteobacteria would be related to increased LPS levels and inflammation. Dietary fats have been associated with the increased absorption of LPS, which in turn, may be related with specific gut microbiota changes, such as a reduction in the *Eubacterium rectale*–*Blautia coccooides* group, *Bacteroides*, and *Bifidobacterium* (39). These results are in agreement with our findings

regarding snorers as compared to controls. In support of our theory of dysbiosis as a causative mechanism in snoring and its debilitating consequences, children with obstructive sleep apnea have been shown to exhibit significantly higher LPS-binding protein in their plasma as compared with controls (28).

More recently, obstructive sleep apnea (40) and sleep deprivation (41) have been characterized as a necessary initial step in oxidative stress and ATP depletion, or vice versa (42, 43) The activation of the oxidative pathways in gut cells can be initiated by circulating inflammatory mediators or reactive oxygen or nitrogen species (42, 43), which in the neonatal gut, are correlated with an abundance of the Enterobacteriaceae family (the *Escherichia* and *Enterobacter* genera) and a reduction of the Actinobacteria phylum (mainly *Bifidobacterium* genus) and Bacteroidetes (mainly *Bacteroides* genus) (44).

Recent advances in microbiome research have inextricably linked gut bacteria to the development and function of the immune system, the metabolism and diseases as phenotypically different as inflammatory bowel disease, obesity, allergic diseases, and neurodevelopmental disorders. The present findings extend this list of conditions to include sleep-disordered breathing. Moreover, our data may furnish one explanation for the heightened risk of metabolic, immunological, neurocognitive, and behavioural consequences associated with snoring in infancy, with the common denominator being the aberrant compositional development of gut microbiota, or dysbiosis.

Snoring children manifest deviated microbiota as compared with healthy children. This result, if verified in other populations, invites the modification of the gut microbiota, or bringing the gut microbiota back into balance, as a new treatment target in children with sleep-disordered breathing.

REFERENCES

1. Kuehni CE, Strippoli MP, Chauliac ES, et al. Snoring in preschool children: Prevalence, severity and risk factors. *Eur Respir J* 2008; 31(2):326-333.
2. Gislason T, Benediktsdottir B. Snoring, apneic episodes, and nocturnal hypoxemia among children 6 months to 6 years old. An epidemiologic study of lower limit of prevalence. *Chest* 1995; 107(4):963-966.
3. Montgomery-Downs HE, Gozal D. Sleep habits and risk factors for sleep-disordered breathing in infants and young toddlers in louisville, kentucky. *Sleep Med* 2006; 7(3):211-219.
4. Kelmanson IA. Snoring, noisy breathing in sleep and daytime behaviour in 2-4-month-old infants. *Eur J Pediatr* 2000; 159(10):734-739.
5. Bonuck KA, Chervin RD, Cole TJ, et al. Prevalence and persistence of sleep disordered breathing symptoms in young children: A 6-year population-based cohort study. *Sleep* 2011; 34(7):875-884.
6. Piteo AM, Lushington K, Roberts RM, et al. Prevalence of snoring and associated factors in infancy. *Sleep Med* 2011; 12(8):787-792.
7. Li AM, Sadeh A, Au CT, et al. Prevalence of habitual snoring and its correlates in young children across the asia pacific. *J Paediatr Child Health* 2013; 49(2):E153-9.
8. Biggs SN, Nixon GM, Horne RS. The conundrum of primary snoring in children: what are we missing in regards to cognitive and behavioural morbidity? *Sleep Med Rev* 2014; (18)463-475.
9. Cielo C, Marcus C. Obstructive sleep apnea in children with craniofacial syndromes. *Paediatr Respir Rev* 2015; 16(3):189-96.
10. Nisbet LC, Yiallourou SR, Walter LM, et al. Blood pressure regulation, autonomic control and sleep disordered breathing in children. *Sleep Med Rev* 2014;(18):179-189.
11. Brew BK, Marks GB, Almqvist C, et al. Breastfeeding and snoring: A birth cohort study. *PLoS One* 2014; 9(1):e84956.

12. Galbally M, Lewis AJ, McEgan K, et al. Breastfeeding and infant sleep patterns: An Australian population study. *J Paediatr Child Health* 2013; 49(2):E147-52.
13. Beebe DW, Rausch J, Byars KC, et al. Persistent snoring in preschool children: Predictors and behavioral and developmental correlates. *Pediatrics* 2012; 130(3):382-389.
14. Rautava S. Early microbial contact, the breast milk microbiome and child health. *J Dev Orig Health Dis* 2016; 7(1):5-14.
15. Rautava S, Luoto R, Salminen S, et al. Microbial contact during pregnancy, intestinal colonization and human disease. *Nat Rev Gastroenterol Hepatol* 2012;9(10):565-76
16. Sharon G, Sampson TR, Geschwind DH, et al. The Central Nervous System and the Gut Microbiome. *Cell* 2016; 167(4):915-932.
17. Paavonen EJ, Saarenpää-Heikkilä O, Pölkki P, et al.. Maternal and paternal sleep during pregnancy in the child-sleep birth cohort. *Sleep Med* 2017; 29:47-56.
18. Nylund L, Heilig HG, Salminen S, et al. Semi-automated extraction of microbial DNA from feces for qPCR and phylogenetic microarray analysis. *J Microbiol Methods* 2010; 83(2):231-235.
19. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; 7(5):335-336.
20. Mandal S, Van Treuren W, White RA, et al. Analysis of composition of microbiomes: A novel method for studying microbial composition. *Microb Ecol Health Dis* 2015; 26: 27663.
21. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011; 12(6):R60.
22. Luoto R, Collado MC, Salminen S, et al. Reshaping the gut microbiota at an early age: functional impact on obesity risk? *Ann Nutr Metab* 2013; 63(2):17-26.
23. Collado MC, Rautava S, Isolauri E, et al. Gut microbiota: a source of novel tools to reduce the risk of human disease? *Pediatr Res* 2015; 77(1-2):182-8.
24. Battson ML, Lee DM, Weir TL, et al. The gut microbiota as a novel regulator of cardiovascular function and disease. *J Nutr Biochem* 2017; 56:1-15.

25. Arens R, Muzumdar H. Childhood obesity and obstructive sleep apnea syndrome. *J Appl Physiol* 2010;108:436–444.
26. Gozal D, Serpero LD, Sans Capdevila O, et al. Systemic inflammation in non-obese children with obstructive sleep apnea. *Sleep Med* 2008; (9):254-259.
27. Gozal D, Kheirandish-Gozal L. Childhood obesity and sleep: relatives, partners or both? – a critical perspective on the evidence. *Ann N Y Acad Sci* 2012; 1264:135-141.
28. Kheirandish-Gozal L, Peris E, Wang Y, et al. Lipopolysaccharide-binding protein plasma levels in children: effects of obstructive sleep apnea and obesity. *J Clin Endocrinol Metab* 2014 ;99(2):656-63.
29. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. *Science* 2012; 336(6086):1262-7.
30. Sommer F & Bäckhed F. The gut microbiota--masters of host development and physiology. *Nat Rev Microbiol* 2013;11(4):227-38.
31. Turnbaugh PJ, Ridaura VK, Faith JJ, et al. The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 2009; 1(6):6ra14.
32. Rajilić-Stojanović M, Biagi E, Heilig HG, et al. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* 2011; 141(5):1792-801.
33. Benedict C, Vogel H, Jonas W, et al. Gut microbiota and glucometabolic alterations in response to recurrent partial sleep deprivation in normal-weight young individuals. *Mol Metab* 2016; 5(12):1175-1186.
34. Cedernaes HB, Schiøth C. Benedict Determinants of shortened, disrupted, and mistimed sleep and associated metabolic health consequences in healthy humans. *Diabetes* 2015; 64 (4):1073–1080.
35. Sun L, Pan A, Yu Z, et al. Snoring, inflammatory markers, adipokines and metabolic syndrome in apparently healthy Chinese. *PLoS One* 2011;6(11):e27515.

36. Poroyko VA, Carreras A, Khalyfa A, et al. Chronic Sleep Disruption Alters Gut Microbiota, Induces Systemic and Adipose Tissue Inflammation and Insulin Resistance in Mice. *Sci Rep* 2016; 6:35405.
37. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; 56: 1761-1772.
38. Cani PD, Neyrinck AM, Fava F, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* 2007; 50: 2374-2383.
39. Caricilli AM, Saad MJ. The role of gut microbiota on insulin resistance. *Nutrients* 2013; 5: 829-851.
40. Fernandez Alvarez R, Rubinos Cuadrado G, Alonso Arias R, et al. Snoring as a Determinant Factor of Oxidative Stress in the Airway of Patients with Obstructive Sleep Apnea. *Lung* 2016; 194(3):469-73.
41. Trivedi MS, Holger D, Bui AT, et al. Short-term sleep deprivation leads to decreased systemic redox metabolites and altered epigenetic status. *PLoS One* 2017; 12(7):e0181978.
42. Jones RM, Neish AS. Redox signaling mediated by the gut microbiota. *Free Radic Biol Med* 2017;105:41-47
43. Aviello G, Knaus UG. ROS in gastrointestinal inflammation: Rescue Or Sabotage? *Br J Pharmacol* 2017; 174(12):1704-1718.
44. Cernada M, Bäuerl C, Serna E, et al. Sepsis in preterm infants causes alterations in mucosal gene expression and microbiota profiles compared to non-septic twins. *Sci Rep* 2016; 6:25497.

FIGURE LEGENDS

Figure 1. Gut microbial diversity in snorers and control infants.

(Panel A) Beta diversity by Principal Coordinate Analysis (PCoA) ordination plots based on unweighted UNIFRAC distance (PERMANOVA $R^2=0.0316$, $P=0.038$) (Panel B) Multivariate Redundancy analysis (RDA) plot to identify significant associations between microbial communities according to the infant classification (snorer and control, Variance=12.2; F-model=1.30, $P=0.048$). Alpha-diversity measures of gut microbiome using diversity Shannon index (Panel C) and Chao1 richness estimations (Panel D).

Figure 2. Gut microbial profile in snorers and control infants.

Relative abundances (%) at the phylum (Panel A) and family levels (Panel B) and, the ratios of Firmicutes to Bacteroidetes (Panel C) and Actinobacteria to Proteobacteria (Panel D) in each group.

Figure 3. Differential microbial composition between snorers and control infants.

Linear Discriminant Analysis (LDA) Effect Size (LEfSe) plot of microbial biomarkers identified at the family (Panel A) and OTU levels (Panel B) (red=snorers and blue=controls).