Microbiota and Flatography in Pediatric Gastrointestinal Disease

de Meij, T.G.J.

2017

document version
Publisher's PDF, also known as Version of record

CHAPTER 6

Disturbance of microbial core species in new onset Juvenile Idiopathic Arthritis

Petra C.E. Hissink Muller
Tim G.J. de Meij
P. Michiel Westedt
Evelien F.J. de Groot
Cornelia F. Allaart
Danielle M.C. Brinkman
Dieneke Schonenberg-Meinema
J. Merlijn van den Berg
Lisette W.A. van Suijlekom-Smit
Marion A.J. van Rossum
A.E. Budding
R. ten Cate

J Ped Inf Dis. 2017;12:131-5

ABSTRACT

Over the past decades, the intestinal microbiota has increasingly gained attention in studies addressing pathophysiology of (paediatric) autoimmune diseases, like inflammatory joint diseases, inflammatory bowel disease (IBD) and type 1 diabetes. In this study, we have analysed composition of gut microbiota of newly diagnosed juvenile idiopathic arthritis (JIA) patients, prior to initiation of disease-modifying antirheumatic drugs (DMARD). Faecal microbiota profiles of 8 JIA patients (median age 11,1 years, 6 girls) were compared to 22 healthy age-matched controls using IS-pro, a 16S-23S interspacer (IS) region-based, eubacterial molecular detection technique. By partial least squares discriminant analysis (PLS-DA), microbiota profiles of JIA and controls could significantly be discriminated based on a limited set of species belonging to the phylum *Bacteroidetes* (Figure 6.2), with sensitivity of 88% and specificity of 73% (Figure 6.3), Area Under the Curve (AUC) 0.87 (95% CI: 0.73-0.87), but not within other phyla. These discriminative species have been considered to be part of the microbial core in healthy children.

Conclusion

Our findings add to the increasing notion that the gut microbiota may be involved in the pathophysiology of JIA. Involved species in the discrimination between JIA and controls are members of the microbial core in health state. Expanding knowledge on JIA-specific microbial signatures and host-interactions may open avenues to explore options to develop individualized, microbiota-based preventive and therapeutic interventions in JIA.

INTRODUCTION

In the pathogenesis of Juvenile Idiopathic Arthritis (JIA) environmental triggers are considered to provoke the onset of symptoms in genetically susceptible hosts.[1] Auto-reactive T-cell responses to yet unrecognized antigens have been described to trigger an inflammatory response. In adults the role of the intestinal microbiota in the aetiology of rheumatoid arthritis and other auto-immune diseases has been increasingly recognized.[2] Exemplary are Reactive arthritis after a gastro-intestinal infection and the association between arthritis and jejuno-ileal-bypass surgery.[3,4] In the latter, disruption of gastrointestinal anatomy provokes microbial disturbance by small intestinal bacterial overgrowth, leading to antibody production and synovial inflammation. Multiple animal studies have established a link between the intestinal microbiota in the onset or reduction of arthritis. Illustrative is the occurrence of adjuvant-induced arthritis exclusively in rats grown in germ-free conditions. The potential immunomodulatory effect of the microbiome is also reflected in the protective role of some enterobacteria in arthritis-susceptible rats.[2] In humans with new-onset rheumatoid arthritis (RA) one recently described example is the higher abundance of *Prevotella copri* in combination with less *Bacteroides* compared to controls.[2]

In RA, disturbance of bacterial homeostasis (dysbiosis) is considered to provoke an increased mucosal permeability, loss of immune tolerance to microbial components and trafficking of immune cells and antigenic material to joints, provoking an inflammatory cascade leading to RA.[5]

Analogue to RA, an aetiological role for the gut microbiota has been suggested in JIA.[6-9] Comparable observations on alterations in microbiota are currently rapidly accumulating for other paediatric auto immune diseases, like type 1 diabetes[10] and IBD.[11] However, despite recognition of disease-specific microbial signatures for different diseases, it remains largely unclear whether microbial changes precede or are rather a consequence of these diseases.

Aim of this prospective, case-control study was to describe composition and diversity of intestinal gut microbiota of children with new-onset, DMARD-naive JIA, compared to age-matched, healthy controls.

MATERIALS AND METHODS

JIA patients, enrolled in the BeSt for Kids study (Dutch trial register 1574), were eligible to participate in this prospective pilot-study. The BeSt for Kids study is a multi-center clinical trial which included patients with newly diagnosed JIA and aimed to investigate treatment strategies. (1) initial methotrexate or sulphasalazine monotherapy, (2) initial therapy with methotrexate and prednisolone, (3) initial therapy with methotrexate and etanercept in certain categories (rheumatoid factor negative oligo- or polyarticular JIA, JIA with psoriasis) of DMARD-naive JIA patients.[12] From September 2011 to May 2012, eight consecutive patients were included in this add-on study and instructed to collect and store a faecal sample in regular freezers until centrally stored at -20°C. Controls were 22 age-matched children, selected from a cohort consisting of 61 healthy children, aged 2-18 years, who participated in a previous study on microbiota composition and microbial dynamics in healthy state.[13] Similar exclusion criteria were applied to both groups: an episode of infectious gastroenteritis within 3 months prior to inclusion, use of antibiotics or immune-modulating agents both within 3 months prior to inclusion, history of major surgery of gastrointestinal tract and an established diagnosis of chronic gastro-intestinal disease (celiac disease, short bowel syndrome, IBD).

Ethical considerations

This study was conducted according to the principles expressed in the Declaration of Helsinki. The protocol was approved by the Medical Ethical Committee from the Leiden University Medical Center. JIA patients and parents signed a written informed consent.

IS-pro

We used IS-pro, an eubacterial molecular detection technique to characterize the microbiota.[13,14] IS-pro makes use of phylum-specific fluorescently labelled PCR primers and differentiates bacterial species by the length of the 16S–23S rDNA IS region. For a detailed description of the used protocol on DNA isolation and sample preparation we refer to previous report.[13,14] The procedure consists of two separate multiplex PCRs: the first PCR contains two different fluorescently labeled primers. One amplifying the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* (FAFV) and the other labeled primer for the phylum *Bacteroidetes*. A separate PCR with a third labeled primer is performed for the phylum *Proteobacteria*. The resulting polymerase chain reaction (PCR) products were subsequently amplified by means of IS-pro.[10]

Data analysis

DNA fragment analysis was performed on an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems Carlsbad, California, USA). Data were further analyzed with the BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) and Spotfire (TIBCO, Palo Alto, CA, USA) software packages. Correlation between profiles were calculated with Pearson's product-moment correlation coefficient on log2 transformed data. Fragments were assigned a taxonomic classification based on fragment length. Lengths were compared to a database consisting of IS lengths of known bacterial species. This classification was confirmed by separating fragments on an agarose gel and excising and sequencing these fragments. Sequences were compared to the GenBank database with the BLAST algorithm. Taxonomic classification to species level was based on > 97% sequence identity.[14]

Within-sample microbial diversity was calculated as the Shannon diversity index based on the resulting profiles using the R 2.15.2 software package. Diversity was calculated both per phylum and for overall microbial composition (by pooling the phyla FAFV, *Bacteroidetes* and *Proteobacteria* together). A p-value of < 0.05 was considered statistically significant.

Partial least squares discriminant analysis (PLS-DA) regression, a supervised classification technique, was used for the prediction of the clinical status of faecal samples, JIA or healthy.[15] This statistical model was performed for each phylum and for all phyla together to predict case-control classification. Validation of this PLS-DA model was carried out by a 10-fold cross-validation procedure.[16] In practice, the dataset was split into 90% of samples for model construction (i.e., training set), with the aim to predict the other 10% (i.e., test set). This procedure was repeated for ten iterations, where each sample served as a test sample exactly once. Accuracy rates, specificity, and sensitivity were computed for the samples that were used as a test set in every iteration, and the model predictive power was further assessed using a receiver operating characteristic (ROC) curve with calculation of the Area Under the Curve (AUC) and 95% CI values.

RESULTS

All included patients suffered from polyarticular rheumatoid factor negative JIA. Other patient characteristics are shown in Table 6.1. No significant differences in microbial diversity were observed between JIA and controls; median Shannon diversity index for all phyla together was 3.92 and 3.88, for *Bacteroidetes* 2.95 and 2.87, for *Proteobacteria* 2.75

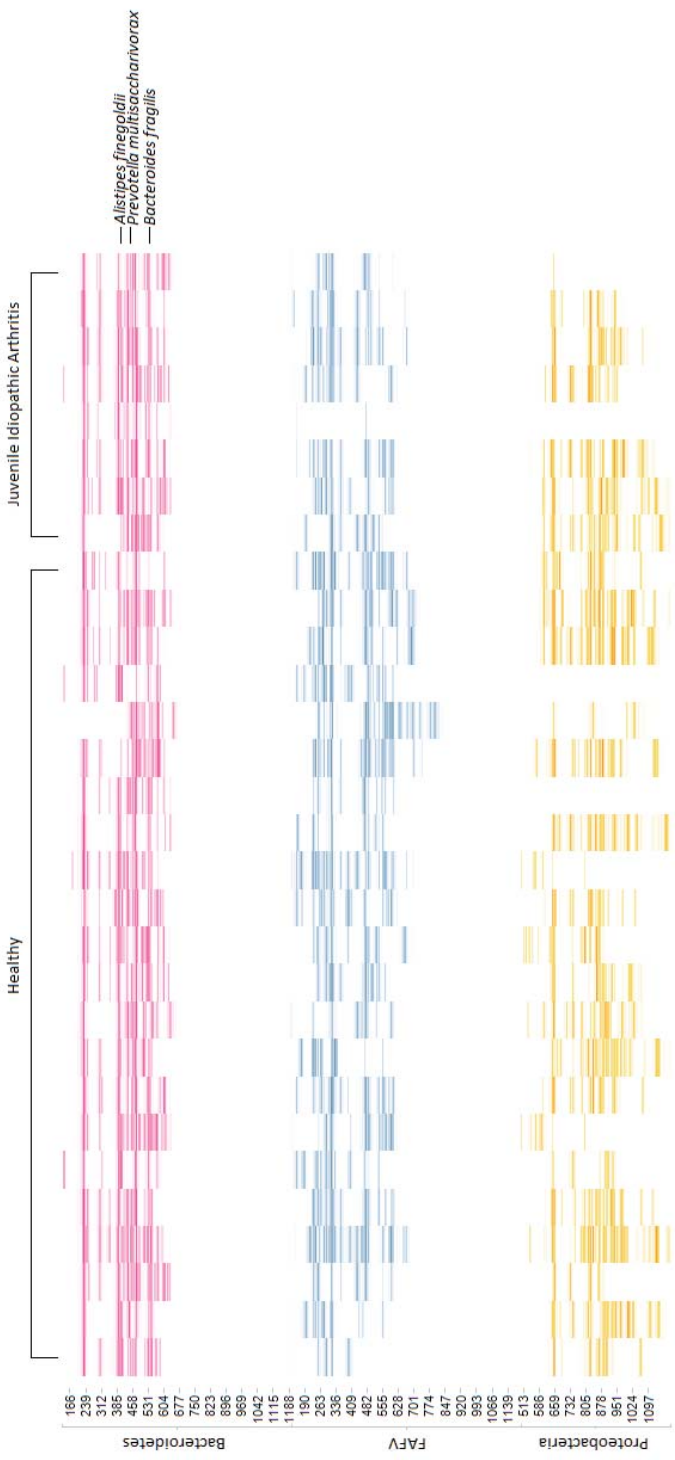

Figure 6.1 Heat map displaying IS profiles of 8 children with JIA and 22 healthy controls.
Individual subjects are shown on the X axis; children with JIA in red, healthy controls in green. On the Y axis, IS fragment lengths are expressed in number of nucleotides, corresponding with bacterial strain type (OTU). Blue peaks represent OUT belonging to the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* (FAFV), red peaks represent *Bacteroidetes* and yellow peaks represent *Proteobacteria*. Intensity of the colors reflect the relative abundance of each indicated OTU, grey signals represent less prevalent IS fragment lengths. The most discriminative OTUs between both study groups, as calculated by PLS-DA, were *Alistipes finegoldii* (peaks 231/396/400/406), *Prevotella multisaccharivorax* (peak 437) and *Bacteroides fragilis* (peak 537), all within the phylum *Bacteriodetes*.

Table 6.1 Patient and control characteristics

	JIA patients n = 8	Controls n = 22
Female (%)	6 (75)	11 (50)
Median age (years)	11.1 (7.3-13.1)	8.7 (7.5-11.5) (p = 0.33)
ANA positive (%)	3 (38)	NA
Median clinical symptoms (months)	7.1 (4.4-13.2)	NA
VAS physician (mm)	47 (32-58)	NA
VAS patient well-being (mm)	32 (27-52)	NA
ESR (mm)	8 (2-9)	NA
Active Joint Count	10 (7-14)	NA
Limited Joint Count	2 (0-4)	NA
CHAQ score (0-3)	1.2 (0.4-1.7)	NA

ANA = Antinuclear Antibodies; VAS = Visual Analogue Scale; ESR = erythrocyte sedimentation rate; CHAQ = Child Health Assessment Questionnaire; NA = not applicable.
Characteristics are presented in medians (Interquartile Range).

and 2.75 and for FAFV 2.41 and 2.75, respectively. A heatmap consisting of all microbial data from all JIA patients and healthy controls is shown in Figure 6.1. By partial least squares discriminant analysis (PLS-DA), profiles of JIA and controls could significantly be discriminated on the level of the phylum *Bacteroidetes* (Figure 6.2), with sensitivity of 88% and specificity of 73%, AUC 0.87 (95% CI 0.73-0.87) but not within other phyla (Figure 6.3). Most discriminative species between the two subgroups were *Alistipes finegoldii* and *Prevotella multisacharivorax* (decreased in JIA) and *Bacteroides fragilis* (increased in JIA).

DISCUSSION

In this study we have shown that gut microbiota in JIA can be discriminated from matched controls with high accuracy, based on a limited set of species belonging to of the phylum *Bacteroidetes*. In a recent study by Tejesvi et al.,[6] faecal microbiota analysis of 30 patients with newly onset JIA revealed an increased abundance of species belonging to the phylum *Bacteroidetes*, in particular *Bacteroides spp.*, and a low abundance of bacteria within the phylum of *Firmicutes* compared to controls6. Secondly, Stoll et al.[7] observed a reduced abundance of faecal *F. prausnitzii* in juvenile enthesitis related arthritis (ERA) patients compared to controls. Additionally, a non-statistically significant increased abundance in *Bacteroides*

Figure 6.2 Scatterplot for the discrimination of patients with ulcerative colitis at presentation (full triangles) and healthy controls (empty circles), by electronic nose.
Axes depict two orthogonal linear recombination of the original 32 sensor data by means of principal component analysis.

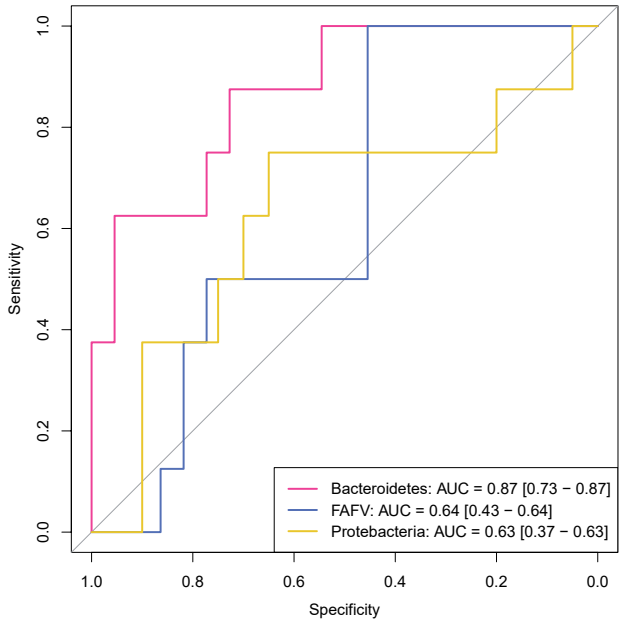

Figure 6.3 Receiver operating characteristic (ROC) curves summarizing the predictive power of the PLS-DA model for clinical status (JIA or control) per phylum, including 95% confidence intervals.

spp. and *Akkermansia municiphila* was observed in subsets of ERA patients, suggesting a role for a humoral response to these specific species in the pathophysiology.[7] An increased abundance of *Bacteroides spp.* was observed in paediatric ERA patients, with decreased abundance of *Prevotella spp*.[8] Di Paola et al. described different microbial profiles between JIA-ERA patients and non-ERA patients versus healthy controls and, notably, also between active disease and remission state.[9] Similar alterations in microbiome were previously reported in paediatric type 1 diabetes and IBD.[10,11] This study adds as novelty the increased knowledge on the composition of the microbiota of a large control group of healthy children.[13] In that study, species within the phylum *Bacteroidetes* were described to be dominant members of a shared microbial core in healthy state.[13] Notably, we found in the present study that JIA could be differentiated from controls based on alterations in the abundance of species belonging to this healthy core. This suggests that onset of JIA may be associated with disruption of this microbial core and that JIA-related dysbiosis is rather reflected by a loss of healthy microbial state than by the introduction of pathogens. The high accuracy to discriminate new onset JIA from healthy state based on a limited number of bacterial species, as observed in this study, may have several implications. Firstly, a microbiota-based test with high sensitivity may have the potential to serve as a diagnostic tool in clinical practice. However, to reliable assess the potential of microbiota analysis as a diagnostic instrument, comparison with an intention-to-diagnose cohort, including children with suspected JIA, is preferable, and has to be tested in future studies. Secondly, the presence of a JIA-specific microbial signature, which can robustly discriminate diseased state from controls could possibly allow for the development of microbiota targeted therapeutic or even preventive interventions in JIA treatment.

Future studies are needed to externally validate our findings, to address the significance of the observed disturbance of core species and to assess whether clinical remission of JIA merges with restoration of this core. In a recent review, an overview was given of current knowledge on the role of gut microbiota in JIA aetiology, including factors possibly predisposing to dysbiosis, and mechanisms by which altered microbiota might predispose to arthritis.[17] Important factors influencing the composition of microbiota are mode of delivery,[18] feeding habits in early life[19] and exposure to (multiple) medication, in particular antibiotics.[17] Sex related differences and body morphometrics have also been described to affect microbiota composition, however, in the control group of this study, microbial communities did not differ between both sexes.[20-23] Limitation of this study is that the cohort was too small to take different environmental factors possibly affecting microbiota composition into account, increasing the risk for type I error.

In conclusion, our observation of intestinal dysbiosis in new onset JIA confirms the increasing notion that aberrant microbiota composition may play a role in the aetiology of JIA. In particular, we found compositional alterations in species within the phylum *Bacteriodetes*, which have been described dominant members of a microbial core in healthy state.

Expanding knowledge on JIA-specific microbial signatures and host-interactions may open opportunities to explore the options to develop individualized, microbiota-based preventive and therapeutic interventions in JIA.

REFERENCES

1. Prakken BJ, Albani S. Using biology of disease to understand and guide therapy of JIA. Best. Pract. Res. Clin. Rheumatol. 2009;23(5):599-608.
2. Scher JU, Littman DR, Abramson SB. Microbiome in Inflammatory Arthritis and Human Rheumatic Diseases. Arthritis Rheumatol. 2016;68(1):35-45.
3. Carubbi F, Ruscitti P, Pantano I, et al. Jejunoileal bypass as the main procedure in the onset of immune-related conditions: the model of BADAS. Expert Rev. Clin. Immunol. 2013;9(5):441-52.
4. Del Val Del Amo N, Ibanez Bosch R. Post intestinal bypass arthritis-dermatitis syndrome. Clin. Exp. Rheumatol. 2008;26(2):386.
5. Yeoh N, Burton JP, Suppiah P, Reid G, Stebbings S. The role of the microbiome in rheumatic diseases. Curr. Rheumatol. Rep. 2013;15(3):314.
6. Tejesvi MV, Arvonen M, Kangas SM, et al. Faecal microbiome in new-onset juvenile idiopathic arthritis. Eur. J. Clin. Microbiol. Infect. Dis. 2016;35(3):363-70.
7. Stoll ML, Kumar R, Morrow CD, et al. Altered microbiota associated with abnormal humoral immune responses to commensal organisms in enthesitis-related arthritis. Arthritis Res. Ther. 2014;16(6):486.
8. Aggarwal A, Sarangi AN, Gaur P, Shukla A, Aggarwal R. Gut microbiome in children with enthesitis-related arthritis in a developing country, and the effect of probiotic administration. Clin. Exp. Immunol. 2016.
9. Di Paola M, Cavalieri D, Albanese D, et al. Alteration of Fecal Microbiota Profiles in Juvenile Idiopathic Arthritis. Associations with HLA-B27 Allele and Disease Status. Front. Microbiol. 2016;7:1703.
10. Paun A, Danska JS. Modulation of type 1 and type 2 diabetes risk by the intestinal microbiome. Pediatr. Diabetes. 2016.
11. Maukonen J, Kolho KL, Paasela M, et al. Altered Fecal Microbiota in Paediatric Inflammatory Bowel Disease. J Crohns Colitis. 2015;9(12):1088-95.

12. Hissink Muller P. A comparison of three treatment strategies in recent onset DMARD naïve juvenile idiopathic arthritis: 3-months results of the BeSt for Kids-Study. Late breaking abstracts presented at the ACR Annual Meeting 2014 [online]. 2014;http://acrabstracts.org/abstracts/a-comparison-of-three-treatment-strategies-in-recent-onset-dmard-naive-juvenile-idiopathic-arthritis-3-months-results-of-the-best-for-kids-study (2014).
13. de Meij TG, Budding AE, de Groot EF, et al. Composition and stability of intestinal microbiota of healthy children within a Dutch population. FASEB J. 2016;30(4):1512-22.
14. Budding AE, Grasman ME, Lin F, et al. IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota. FASEB J. 2010;24(11):4556-64.
15. Rajilic-Stojanovic 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.
16. Daniels L, Budding AE, de KN, et al. Fecal microbiome analysis as a diagnostic test for diverticulitis. Eur. J. Clin. Microbiol. Infect. Dis. 2014;33(11):1927-36.
17. Arvonen M, Berntson L, Pokka T, Karttunen TJ, Vahasalo P, Stoll ML. Gut microbiota-host interactions and juvenile idiopathic arthritis. Pediatr. Rheumatol. Online J. 2016;14(1):44.
18. Rutayisire E, Huang K, Liu Y, Tao F. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. BMC Gastroenterol. 2016;16(1):86.
19. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. Front. Microbiol. 2014;5:494.
20. Markle JG, Frank DN, Mortin-Toth S, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science. 2013;339(6123):1084-8.
21. Kalliomaki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. Am. J. Clin. Nutr. 2008;87(3):534-8.
22. Bervoets L, Van Hoorenbeeck K, Kortleven I, et al. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. Gut Pathog. 2013;5(1):10.
23. Santacruz A, Marcos A, Warnberg J, et al. Interplay between weight loss and gut microbiota composition in overweight adolescents. Obesity (Silver Spring). 2009;17(10):1906-15.