1 **Early fecal microbiota composition in children who later develop celiac**

2 **disease and associated autoimmunity**

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Abstract

Objectives: Several studies have reported that the intestinal microbiota composition of celiac disease (CD) patients differs from healthy individuals. The possible role of gut microbiota in the pathogenesis of the disease is, however, not known. Here we aimed to assess the possible differences in early fecal microbiota composition between children that later developed CD and healthy controls matched for age, sex, and HLA risk genotype.

Materials and methods: We used 16S rRNA gene sequencing to examine the fecal microbiota of 27 children with high genetic risk of developing CD. Nine of these children developed the disease by the age of 4 years. Stool samples were collected at the age of 9 and 12 months, before any of the children had developed CD. The fecal microbiota composition of children who later developed the disease was compared with the microbiota of the children who did not have CD or associated autoantibodies at the age of 4 years. Delivery mode, early nutrition, and use of antibiotics were taken into account in the analyses.

Results: No statistically significant differences in the fecal microbiota composition were found between children who later developed CD (n = 9) and the control children without disease or associated autoantibodies (n = 18).

Conclusions: Based on our results, the fecal microbiota composition at the age of 9 and 12 months is not associated with the development of CD. Our results, however, do not exclude the possibility of duodenal microbiota changes or a later microbiota-related trigger for the disease.

Keywords: celiac disease, HLA risk haplotype, fecal microbiota, next-generation sequencing, 16S rRNA gene sequencing

62 ## Introduction

63 Celiac disease (CD) is an immune-mediated disorder of the small intestine where gluten induces
64 a mucosal inflammatory reaction. In Finnish population, the prevalence of CD has doubled
65 during the last 30 years [1]. 90% of patients carry HLA-DQ2.5 molecule encoded by *HLA-*
66 *DQA1*05* and *–DQB1*02* genes, and most of the remaining patients carry HLA-DQ8 molecule
67 encoded by *HLA-DQA1*03* and *–DQB1*03:02* genes. Around 30-40% of people of Northern
68 European descent are positive for HLA-DQ2.5 and/or DQ8, demonstrating the role of other
69 etiological factors in the disease development in addition to these major genetic components [2].
70
71 Several studies have shown a relation between altered intestinal microbiota composition and CD
72 [3-5]. However, the observed differences may arise from disease process itself, or be dependent
73 on genetic differences. For example, the HLA-DQ2.5 haplotype has been reported to influence
74 the gut microbiota composition of infants [6]. The possible changes and differences in the
75 intestinal microbiota before the onset of CD are unclear.
76
77 The aim of our study was to screen the fecal microbiota composition of Finnish children
78 genetically at the risk for CD before the onset of the disease. Our objective was to detect the
79 possible differences in the microbiota between children who later developed CD and those who
80 did not. Through this study, we hope to broaden the understanding on the impact of early
81 microbial colonization in the pathogenesis of CD, and to reveal whether early fecal microbiota
82 composition could reflect to the disease risk. To our knowledge, this is the first study so far
83 where the samples have been collected before the onset of CD.

Materials and Methods

Study subjects

Stool samples were obtained from 27 Finnish children (recruited at the Kuopio University Hospital and the Kätilöopisto Maternity Hospital in Helsinki) with a high genetic risk for CD participating in a follow-up study [7]. Newborn children were screened for the presence of HLA-DQB1*02 and HLA-DQA1*05 alleles [8] and followed until 3 or 4 years of age by screening for tissue transglutaminase autoantibodies (tTGA) [9]. The diagnosis of CD was based on typical histological findings, villous atrophy and crypt hyperplasia, in the duodenal biopsy of small intestine [10]. Nine children (all girls) were diagnosed with duodenal biopsy at the median age of 3.5 years (range 2.6 - 4.2 years) after the development of tTGA autoantibodies at the median age of 3 years (18 months to 3 years). They were selected for the study together with 18 control infants, matched by gender and date of birth, remaining negative for tTGA during the follow-up.

Stool samples were collected at the age of 9 and 12 months (9mo and 12mo samples, respectively). Samples were stored in home freezers (-20°C) for a maximum period of two months, after which they were delivered to the study centers in cool boxes filled with ice bags and stored at -80°C until processing. The 9mo sample from one case infant and 12mo stool sample from one control infant had not been delivered. Data on mode of delivery were obtained from the participating families or obstetric records, and data on nutrition and antibiotics were collected using dietary questionnaires and phone calls at the age of 1-2 weeks, 1.5 months, 3.5 months, 6 months and 9 months. After the age of 9 months, the health-related information was collected by general follow-up questionnaires. Written informed consent was obtained from all

106 families. The study plan was accepted by the ethics committee of the Kuopio University
107 Hospital.
108

109 **DNA extraction**

110 Bacterial DNA from ~100 mg of the frozen stool specimens was extracted with a semi-automatic
111 GXT Stool Extraction Kit VER 2.0 (Hain Lifescience GmbH, Nehren, Germany) combined with
112 an additional homogenization by bead-beating in 0.1 mm Glass Bead Tubes (MO BIO
113 Laboratories, Inc., Carlsbad, CA, USA) at 1000 rpm for 3 minutes with MO BIO PowerLyzer™
114 24 Bench Top Bead-Based Homogenizer (MO BIO Laboratories, Inc., Carlsbad, CA, USA) to
115 enhance the cell lysis. The DNA concentrations of the DNA extracts were measured with Qubit
116 dsDNA HS assay kit and Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). The
117 DNA extracts were stored at -80 °C.
118

119 **Fecal microbiota composition analysis**

120 Bacterial profiles of the stool specimens were analyzed with 16S rRNA gene sequencing. The
121 16S rRNA gene libraries were generated in a single PCR with custom-designed dual-indexed
122 primers. The approach is described in Supplemental Materials and Methods.
123

124 **Data analysis**

125 The raw sequence quality was checked with FastQC
126 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and data analysis was performed
127 with Qiime (v. 1.9) pipeline as described previously [11-13]. Sequence reads were filtered with a
128 quality score acceptance rate of 20 or better. Chimeric sequences were filtered using usearch (v.

129 6.1), and operational taxonomic units (OTUs) were picked using uclust algorithm with 97%
130 sequence similarity. OTUs representing less than 0.05% of the total sequence count were
131 excluded. To minimize the effect of inter-sample variation in the sequencing efficiency, samples
132 were subsampled (rarefied) by random sampling without replacement to the lowest common
133 sequencing depth (134 414 reads). Annotations for the resulting OTUs were derived from
134 GreenGenes database [14].
135
136 All analyses of the 16S rRNA data were made from the randomly subsampled OTU tables. To
137 study the bacterial diversity of the samples, α-diversity metrics were computed and α-rarefaction
138 plots were generated with Qiime. Differences in the Shannon diversity indices were then
139 assessed with JMP Pro 12 (SAS Institute, Inc., Cary, NC, USA), applying non-parametric
140 methods and considering $P < 0.05$ as statistically significant. Outliers were excluded before the
141 analyses. Taxonomic summary produced by Qiime was visualized as bar charts and statistical
142 differences in the taxonomic richness, i.e. in the OTU abundances, were assessed with non-
143 parametric Kruskal-Wallis test. Taxonomic levels phylum and genus were studied, and False
144 Discovery Rate (FDR) adjusted *P*-value < 0.05 was considered as statistically significant. OTUs
145 existing in less than 25% of the samples were excluded before statistical testing. To analyze the
146 differences in the overall bacterial diversity across the samples, weighted UniFrac distance
147 matrices were generated from the randomly subsampled OTU tables and principal coordinate
148 analysis (PCoA) plots were produced. The PCoA plots were visualized with EMPeror. To
149 confirm the visual observations, adonis analyses were performed. Adonis returns an R^2 value
150 showing the amount of variation explained by the grouping variable, and a *P*-value for statistical
151 significance [15].

152

153 Differences in the nutritional variables (duration of breastfeeding, introduction of solid food, and
154 introduction of gluten), and in the number of received antibiotic courses were analyzed with JMP
155 Pro 12 (SAS Institute, Inc.), using non-parametric methods and considering $P < 0.05$ as
156 statistically significant. To eliminate the mode of birth as a confounding factor, all statistical
157 analyses were first performed for the whole data set (n = 27), and then repeated for the vaginally
158 born infants (n = 24).

159

160 ## Results

161 The characteristics of the participating children are presented in Table 1. Of the 27 infants in this
162 study, 24 were born by vaginal delivery while three were born by cesarean section (CS). All
163 infants were breastfed at the hospital, but the continuation of breastfeeding varied from seven
164 days to over 18 months (Table 1). The median duration of the breastfeeding was 11 months, yet
165 breastmilk was often supplemented with formula. The median age for solid food introduction
166 was 4.1 months (range 2.0 - 6.2 months), and for gluten introduction 5.7 months (range 4.4 - 7.7
167 months). Infants that were earlier introduced to solid foods were also introduced to gluten earlier
168 (Spearman's correlation 0.679, $P < 0.001$). No differences in the average breastfeeding duration
169 or in the age of solid food or gluten introduction were observed between the case and control
170 infants (Mann-Whitney U $P = 0.5395$, $P = 0.4712$ and $P = 0.5539$, respectively; Table 2).
171 Control children seemed to be breastfed at the age of gluten introduction more often than the
172 case infants, but the difference was not statistically significant (Pearson's chi-squared test $P =$
173 0.2113; Table 2). Furthermore, no differences were observed in the prescription of antibiotics
174 between the case and control infants (Table 2). In total, 80.8 % of the study subjects had received
175 at least one antibiotic course by the age of 4 years. Exclusion of the infants born by CS did not
176 significantly affect the results regarding the nutritional information or the antibiotics (Table 2).

177

178 The 16S rRNA gene sequencing of the 52 infant stool specimens resulted in 134k – 330k OTUs
179 per sample (mean 191k, SD 40k), the overall sequence count being 9.9×10^6 OTUs. The average
180 bacterial diversity of the stool samples, represented as median Shannon index values, was 3.32
181 (range 2.66 - 4.32) for 9mo samples and 4.10 (range 3.27 - 4.54) for 12mo samples, showing
182 significant increase in the bacterial diversity between 9 and 12 months ($FDR = 0.0002$). The

183 difference remained significant after the exclusion of the CS infants (*P = 0.0014*). When all
184 study subjects were included in the analysis, the Shannon indices did not differ between the case
185 and control infants (*P = 0.1113* for 9mo samples and *P = 0.686* for 12mo samples). The
186 "observed species" metric of Qiime confirmed these findings, representing little difference
187 between the case and control infants (Fig. 1a and 1b) but a clear difference between the 9mo and
188 12mo samples (Fig. 1c). However, when only the vaginally born infants were studied, the
189 Shannon indices in the 9mo samples tended to be higher in the cases than in the controls (*P =*
190 *0.049*). In the 12mo samples, the difference between the case and control infants remained
191 insignificant after exclusion of the infants born by CS (*P = 0.302*).
192
193 The average phylum and genus level bacterial composition of the case and control samples, i.e.
194 the average relative bacterial OTU abundances, are represented as bar charts in Fig. 2. Minor
195 differences can be visually seen in the bacterial taxonomy between the case and control infants in
196 both 9mo and 12mo samples (Fig. 2a and 2b). However, Qiime reported no statistically
197 significant differences in the bacterial composition between the case and control infants when the
198 microbial abundances at phylum and genus levels were assessed. Further, no differences were
199 observed in *Bacteroides-Prevotella* or *Bacteroides-Bifidobacterium* ratios between the case and
200 control infants in neither 9mo nor 12mo samples (*P = 0.201* and *P = 0.388* for *Bacteroides-*
201 *Prevotella* and *P = 0.955* and *P = 0.572* for *Bacteroides-Bifidobacterium*, respectively). In
202 principal coordinate analysis (PCoA) plot, where individual samples with similar microbiota
203 composition cluster together, no differences could be observed between the case and control
204 infants (Fig 3a and b). Further, adonis analysis confirmed that no significant differences between
205 the cases and controls occurred in neither 9mo nor 12mo samples (*P = 0.808* and *P = 0.696*,

206 respectively). One 9mo control sample had a significantly deviating bacterial profile (Fig. 3a)
207 with more than 50% of the bacteria being *Enterobacteriaceae*, but as this had no effect on the
208 results of the statistical analyses and did not significantly affect the bar chart figures, the sample
209 was not excluded from the final analyses. The results regarding the fecal microbiota composition
210 between the case and control infants remained unaltered when excluding the infants born by
211 cesarean section, with only minor differences in the *P* values (results not shown).
212
213 The results concerning the effect of age, delivery mode and nutritional variables on the fecal
214 microbiota composition are presented in Supplemental Results. Briefly, the bacterial composition
215 differed significantly between the 9mo and 12mo samples (Fig. 2b and 3c) and between the
216 infants born vaginally or by CS. Further, the duration of breastfeeding correlated with several
217 bacterial genera. However, the fecal microbiota composition did not significantly differ between
218 the children that had been breastfed during gluten introduction and the ones that had not ($FDR >$
219 0.1 for all bacterial phyla and genera in both 9mo and 12mo samples).

220 ## Discussion

221 Previous studies have reported that the gut microbiota composition of CD patients differs from
222 healthy individuals [3,16]. It is, however, unclear whether the altered gut microbiota has a role in
223 the pathogenesis of CD or merely is a consequence of the disease [17]. The HLA-DQ2.5
224 haplotype has been reported to influence the intestinal bacterial community of infants [6], but
225 most of these children do not develop CD. In the Finnish population, less than 10% of the DQ2.5
226 positive subjects develop the disease. We compared the early fecal microbiota composition
227 between children with the risk genotype who later developed CD and genetically susceptible
228 children who did not develop the disease or associated autoantibodies during the follow-up.
229 Based on the 10% lifetime risk and identification of already 6.5% of children with tTGA (cases
230 included) in the whole follow-up group, we don´t expect many new cases among the selected
231 tTGA negative controls. Follow-up studies also suggest that the appearance of new CD
232 associated autoantibodies is leveling off after 3 to 4 years of age [18].
233
234 Stool samples were collected at the age of 9 and 12 months and analyzed with 16S rRNA gene
235 sequencing enabling the detection and relative quantification of the bacterial taxa present in the
236 samples. Previous studies have reported differences in the levels of *Bacteroides*, *Clostridium* and
237 *Staphylococcus* genera, alongside with the *Bacteroides-Prevotella* ratio between the CD patients
238 and healthy controls [16]. In addition, one certain *Bacteroides* species, *Bacteroides dorei,* has
239 been associated with active CD [19]. In this present study, the abundance of *Bacteroides dorei*
240 was not assessed, as the species level identification by 16S rRNA gene sequencing is uncertain
241 [11,20]. At the genus level, however, neither *Bacteroides* abundance nor the *Bacteroides-*
242 *Prevotella* ratio differed between the case and control infants. Further, the abundance of

243 *Clostridium* genera did not differ between the case and control infants, and *Staphylococcus* genus
244 was completely undetectable in this sample cohort. Altogether, in this study, no statistically
245 significant differences in the fecal microbial diversity or composition were found between the
246 children who later developed CD and the ones who did not. Thereby, our results suggest that
247 early fecal microbiota composition would not be associated with the pathogenesis of CD, while
248 the previous findings concerning the differences in the gut microbiota between CD patients and
249 healthy individuals might have risen for example from dietary changes after the disease onset or
250 from the disrupted gut homeostasis due to altered mucosal immune responses [21,22]. To some
251 extent, the findings regarding the gut microbiota composition in CD may also be methodology
252 dependent. Further, as the 16S rRNA sequencing only reveals the microbial composition of the
253 stool samples, functional profiles of the microbiota remain uncovered. In order to study the
254 functionality of the gut microbes, metaproteomic or metabolomic analyses should be performed
255 [23,24]. It has been previously shown that the metabolic activity of the gut microbiota in CD
256 children differs from healthy individuals both before and after the implementation of gluten-free
257 diet [25,26].
258
259 Even though the results of this study suggest that the early fecal microbiota composition may not
260 predict the development of CD, this does not exclude the possibility of a microbiota-related
261 trigger for the disease. As e.g. dietary changes, antibiotics and infections can disrupt the
262 intestinal homeostasis [27], it remains possible that some external trigger could later disastrously
263 disturb the gut microbiota balance, leading to the onset of the disease [28]. In fact, one
264 hypothesis for the CD onset is that intestinal infections could interfere the intestinal homeostasis
265 and lead to increased intestinal permeability, i.e. leaky gut (for review, see [29]), which could

266 then allow the absorption of undigested gliadin molecules that initiate the immune processes
267 leading to the disease [30]. However, few studies have investigated the role of specific infectious
268 agents in the development of the disease, yet in some studies an increased prevalence of CD has
269 been associated with repeated early infections [31,32]. In this study, use of antibiotics was taken
270 into account in the analyses, but the possible impact of early viral infections was not assessed.
271
272 In addition to sudden external triggers, gradual unfavorable progression of the gut microbiota
273 may also lead to leaky gut and the activation of the inflammatory pathways [29]. For example,
274 epidemiological studies have reported that children born by CS have an increased risk for CD
275 compared to the vaginally delivered infants [33-35]. In this study cohort, the mode of delivery
276 had a significant effect on the fecal microbiota composition, but due to the limited number of CS
277 children, no conclusions regarding the role of delivery mode on CD risk could be drawn.
278 However, based on the results of this study, the individuals who develop CD do not already in
279 the early infancy have a distinct fecal microbiota composition compared to other infants with
280 risk-HLA-haplotype, suggesting that the onset of CD is more likely a consequence of a strong
281 external trigger rather than gradual development due to a peculiarly vulnerable gut microbiota.
282 However, feces may not be the most optimal sample material for CD studies, as CD primarily
283 affects the small intestine and fecal microbiota inadequately reflects the duodenal microbiota
284 [4,36]. Thus, this study does not rule out the possibility of small intestinal microbiota changes
285 prior to the onset of the disease. On the other hand, the duodenal mucosal homeostasis was not
286 assessed, as studies on the mucosal host-microbial crosstalk and the possibility of unfavorable
287 immune functions would have required the collection of duodenal biopsies. Previous studies

288 regarding the role of duodenal microbiota in CD-linked mucosal immune responses remain
289 rather inconclusive [22,37,38], making this an extremely interesting area for future research.
290
291 The possible protective role of breastfeeding against CD onset has been analyzed in several
292 studies, and the gradual gluten introduction during ongoing breastfeeding has been suggested to
293 protect against the disease [39,40]. Some recent studies, however, have not been able to confirm
294 these results [39-42]. In this study, the average duration of breastfeeding did not differ between
295 the case and control children, but the controls tended to be more likely breastfed during the
296 gluten introduction. The duration of breastfeeding correlated with several bacterial genera, of
297 which especially *Lactobacillus* and *Bifidobacterium* have been previously linked to positive
298 health outcomes (for review, see [43]). However, no difference in the abundance of
299 *Lactobacillus* or *Bifidobacterium* could be observed between the infants who later developed CD
300 and the infants who did not. Further, no differences in the fecal microbiota composition were
301 seen between the infants that were breastfed during gluten introduction and the ones who did not,
302 suggesting that even though breastfeeding might induce changes in the gut microbiota, the
303 possible protective role of breastfeeding against CD might not be related to gut microbiota.
304 However, due to the limited number of infants not breastfed during the gluten introduction in this
305 study, these results are merely indicative.
306

307 **Conclusions**

308 Our results indicate that the fecal microbiota composition at the age of 9 and 12 months is not
309 associated with the development of CD. Our results do not exclude the possibility of duodenal
310 microbiota differences or a later microbiota-related trigger for the disease, but suggest that the

311 infants developing CD do not originally have distinct fecal microbiota composition compared to
312 individuals that possess the risk-HLA-haplotype but do not develop the disease.
313

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318

319 Declaration of interest

320 The authors declare no conflicts of interest.

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420

421

422 ## Appendices

423 Supplemental Materials and Methods

424 Supplemental Results

425 # Tables

426 **Table 1: Background information of the study subjects**

ID	Case/control	Mode of delivery	Solid food introduction (months)	Gluten introduction (months)	Duration of breastfeeding (months)
1	case	vaginal	6.24	6.24	> 18.0
2	control	vaginal	4.27	7.66	6.0
3	control	vaginal	3.02	4.50	0.23
4	case	vaginal	3.98	4.40	1.0
5	control	vaginal	4.63	5.55	N/A
6	control	vaginal	3.88	5.75	11.0
7	case	vaginal	4.01	5.03	2.5
8	control	vaginal	4.60	5.13	13.0
9	control	vaginal	4.04	5.52	6.0
10	case	vaginal	4.04	5.62	N/A
11	control	vaginal	4.17	5.85	13.0
12	control	vaginal	3.84	5.36	12.0
13	case	vaginal	2.00	4.50	0.33
14	control	vaginal	4.07	5.26	6.0
15	control	vaginal	4.50	5.26	10.0
16	case	vaginal	5.55	6.31	12.0
17	control	cesarean section	3.94	6.05	7.0
18	control	vaginal	5.16	5.85	12.0
19	case	vaginal	5.36	6.67	14.0
20	control	vaginal	5.59	5.95	18.0
21	control	vaginal	5.55	6.44	7.5
22	case	vaginal	5.42	6.08	16.0
23	control	cesarean section	3.81	5.22	7.5
24	control	vaginal	4.14	5.65	16.0
25	case	cesarean section	4.07	6.28	> 18.0
26	control	vaginal	4.01	5.65	11.0
27	control	vaginal	3.48	5.00	5.0

427

428 **Table 2: Nutritional information and antibiotic courses of the study subjects**

	All infants in this study (n = 27)			Vaginally born infants (n = 24)		
	Cases (n = 9)	**Controls (n = 18)**	***P***	**Cases (n = 8)**	**Controls (n = 16)**	***P***
Average duration of breastfeeding (months)	10.2 (SD 7.69)	9.48 (SD 4.43)	0.5395[M]	9.12 (SD 7.59)	9.78 (SD 4.64)	0.9155[M]
Average age for solid food introduction (months)	4.52 (SD 1.27)	4.26 (SD 0.66)	0.4712[M]	4.57 (SD 1.34)	4.31 (SD 0.69)	0.8301[M]
Average age for gluten introduction (months)	5.68 (SD 0.84)	5.65 (SD 0.67)	0.5539[M]	5.61 (SD 0.86)	5.65 (SD 0.69)	0.6025[M]
Breast feeding during gluten introduction (% of subjects)	66,7 %	87.5 %	0.2113[P]	62.5 %	85.7 %	0.2113[P]
Antibiotic courses before 9 months (% of subjects)	37.5 %	35.3 %	0.9146[P]	28.6 %	26.67 %	0.9256[P]
Antibiotic courses before 12 months (% of subjects)	42.9 %	47.1 %	0.8511[P]	33.3 %	40.0 %	0.7763[P]
Antibiotic courses before 4 years (% of subjects)	87.5 %	77.8 %	0.5615[P]	85.7 %	75.0 %	0.5665[P]

[M]Mann-Whitney U test, [P]Pearson's chi-squared test

429

430 ## Figure Legends

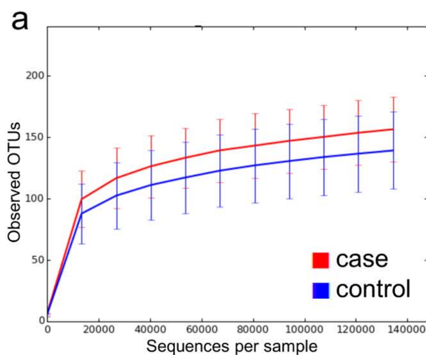
431 **Figure 1: Bacterial diversity of the study samples, represented as observed different OTUs**
432 **per sequences.** At the age of nine months, case infants tend to have a slightly more diverse fecal
433 microbiota than the controls (a), but this difference is statistically significant only when the
434 infants born by cesarean section are excluded from the analysis. At the age of 12 months, no
435 differences can be seen (b). By contrast, a clear difference can be observed in the bacterial
436 diversity between the 9mo and 12mo samples (c). The rarefaction level is 134 414 reads per
437 sample.

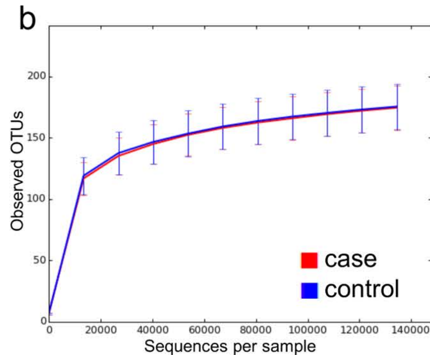
438

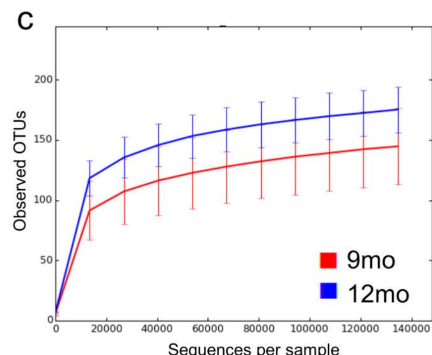
439 **Figure 2: The average bacterial composition of the study samples.** No significant differences
440 can be visually observed in the phylum level bacterial composition neither between the case and
441 control infants nor between the 9mo and 12mo samples (a). Differences in the genus level
442 bacterial composition are more prominent; 9mo and 12mo samples differ clearly from each
443 other, and in the 9mo samples, minor differences can be observed between the case and control
444 infants (b). The rarefaction level is 134 414 reads per sample.

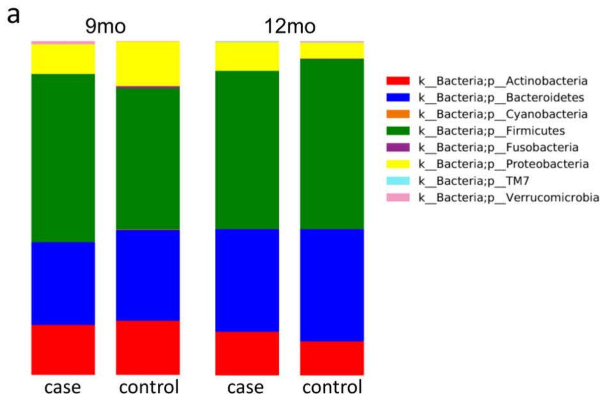
445

446 **Figure 3: Principal Coordinate Analysis (PCoA) of the study samples.** Case and control
447 infants do not cluster separately in the PCoA in either 9mo (a) or 12mo (b) samples.
448 Furthermore, 9mo and 12mo samples do not form clearly separate clusters in the PCoA (c). The
449 rarefaction level is 134 414 reads per sample.


a
Observed OTUs
Sequences per sample
case
control
0
50
100
150
200
0
20000
40000
60000
80000
100000
120000
140000


b
Observed OTUs
Sequences per sample
case
control
0
50
100
150
200
0
20000
40000
60000
80000
100000
120000
140000


c
Observed OTUs
Sequences per sample
9mo
12mo
0
50
100
150
200
0
20000
40000
60000
80000
100000
120000
140000



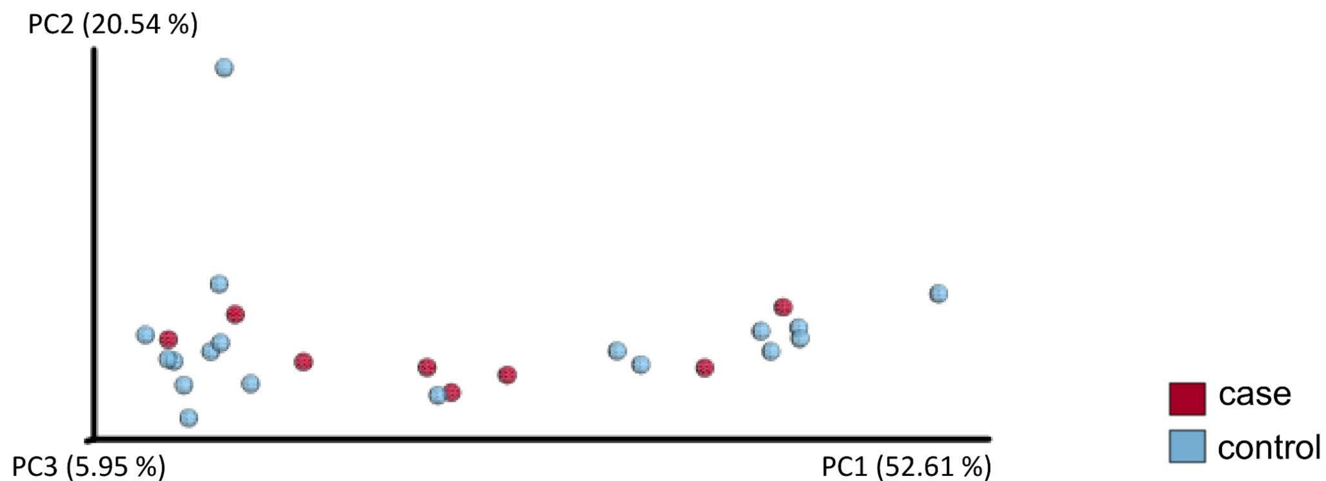
b

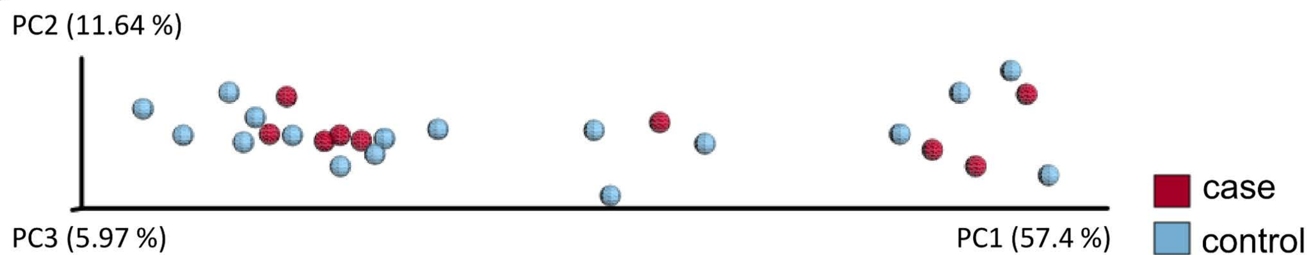
9mo

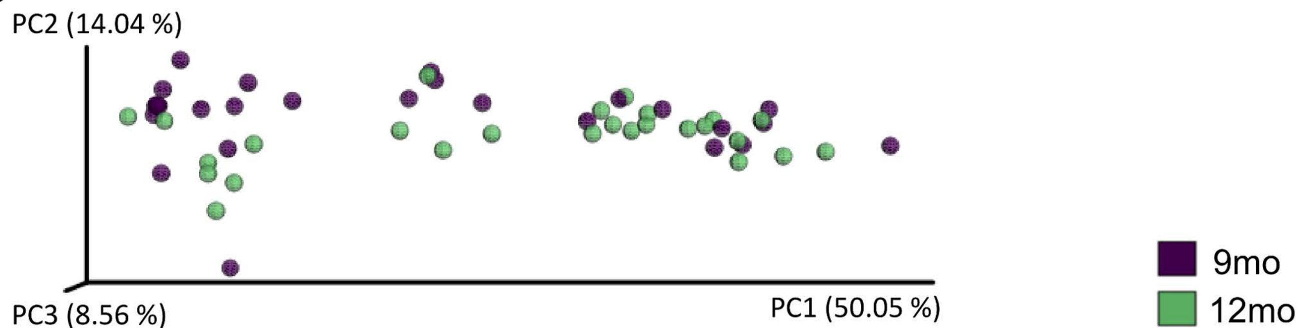
12mo

case control case control

f__Actinomycetaceae;g__Actinomyces
f__Micrococcaceae;g__Rothia
f__Bifidobacteriaceae;g__Bifidobacterium
f__Coriobacteriaceae;g__
f__Coriobacteriaceae;g__Collinsella
f__Coriobacteriaceae;g__Eggerthella
f__Bacteroidaceae;g__Bacteroides
f__Porphyromonadaceae;g__Parabacteroides
f__Prevotellaceae;g__Prevotella
f__Rikenellaceae;g__
f__S24-7;g__
p__Cyanobacteria;c__4C0d-2;o__YS2;f__;g__
p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__;g__
p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae;g__
p__Firmicutes;c__Bacilli;o__Lactobacillales;Other;Other
f__Enterococcaceae;g__Enterococcus
f__Lactobacillaceae;g__Lactobacillus
f__Streptococcaceae;g__Lactococcus
f__Streptococcaceae;g__Streptococcus
f__Turicibacteraceae;g__Turicibacter
o__Clostridiales;Other;Other
o__Clostridiales;f__;g__
o__Clostridiales;f__Clostridiaceae;Other
f__Clostridiaceae;g__
f__Clostridiaceae;g__Clostridium
f__Lachnospiraceae;Other
f__Lachnospiraceae;g__
f__Lachnospiraceae;g__Blautia
f__Lachnospiraceae;g__Clostridium
f__Lachnospiraceae;g__Coprococcus
f__Lachnospiraceae;g__Dorea
f__Lachnospiraceae;g__Epulopiscium
f__Lachnospiraceae;g__Lachnospira
f__Lachnospiraceae;g__Roseburia
f__Lachnospiraceae;g__[Ruminococcus]
f__Peptococcaceae;g__rc4-4
f__Peptostreptococcaceae;g__
f__Ruminococcaceae;Other
f__Ruminococcaceae;g__
f__Ruminococcaceae;g__Anaerotruncus
f__Ruminococcaceae;g__Faecalibacterium
f__Ruminococcaceae;g__Oscillospira
f__Ruminococcaceae;g__Ruminococcus
f__Veillonellaceae;g__Dialister
f__Veillonellaceae;g__Megamonas
f__Veillonellaceae;g__Megasphaera
f__Veillonellaceae;g__Phascolarctobacterium
f__Veillonellaceae;g__Veillonella
f__Erysipelotrichaceae;g__
f__Erysipelotrichaceae;g__[Eubacterium]
f__Fusobacteriaceae;g__Fusobacterium
c__Alphaproteobacteria;o__RF32;f__;g__
f__Alcaligenaceae;g__Sutterella
f__Comamonadaceae;g__
f__Neisseriaceae;g__Neisseria
f__Desulfovibrionaceae;g__Bilophila
f__Campylobacteraceae;g__Campylobacter
f__Enterobacteriaceae;g__
f__Enterobacteriaceae;g__Citrobacter
f__Enterobacteriaceae;g__Trabulsiella
f__Pasteurellaceae;g__
f__Pasteurellaceae;g__Actinobacillus
f__Pasteurellaceae;g__Aggregatibacter
f__Pasteurellaceae;g__Haemophilus
c__TM7-3;o__;f__;g__
c__TM7-3;o__CW040;f__;g__
f__Verrucomicrobiaceae;g__Akkermansia


a
PC2 (20.54 %)
PC3 (5.95 %)
PC1 (52.61 %)
case
control


b
PC2 (11.64 %)
PC3 (5.97 %)
PC1 (57.4 %)
case
control


c
PC2 (14.04 %)
PC3 (8.56 %)
PC1 (50.05 %)
9mo
12mo