Diversity and Composition of the Adult Fecal Microbiome Associated with History of Cesarean Birth or Appendectomy: Analysis of the American Gut Project

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ABSTRACT

Background: Cesarean birth is associated with altered composition of the neonate’s microbiota and with increased risk for obesity and other diseases later in life. The mechanisms of these associations, and whether cesarean birth is associated with an altered adult microbiota, are unknown.

Methods: In 1097 adult volunteers without diabetes, inflammatory bowel disease, or recent antibiotic use, fecal microbiome metrics were compared by history of cesarean birth (N = 92) or appendectomy (N = 115). Associations with potential confounders, microbiome alpha diversity, and individual microbial taxa were estimated by logistic regression. Permutation tests assessed differences in microbial composition (beta diversity) based on Jensen–Shannon divergence.

Findings: Cesarean birth history was associated with younger age; appendectomy with older age and higher body mass index. Neither was associated with fecal microbiome alpha diversity. Microbial composition at all taxonomic levels differed significantly with cesarean birth (P ≤ 0.008) but not with appendectomy (P ≥ 0.29). Relative abundance differed nominally for 17 taxa with cesarean birth and for 22 taxa with appendectomy, none of which was significant with adjustment for multiple comparisons.

Interpretation: Adults born by cesarean section appear to have a distinctly different composition of their fecal microbial population. Whether this distinction was acquired during birth, and whether it affects risk of disease during adulthood, are unknown.

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1. Introduction

Prenatal and early postnatal exposures and events can affect the entire life course. As one example, cesarean birth has been associated with an increased likelihood of asthma and cardiovascular disease in children (Renz-Polster et al., 2005; Thavagnanam et al., 2008; Friedemann et al., 2012), hypertension in young adults (Horta et al., 2013), and obesity in both children and adults (Pei et al., 2014; Darmasseelane et al., 2014; Blustein et al., 2013; Mueller et al., 2014). While these associations are certainly multifactorial, differences in the gut microbiota could contribute. As well summarized by Arrieta and colleagues, several studies have noted differences in the neonatal fecal microbiota by route of delivery (Arrieta et al., 2014). Using aerobic and anaerobic cultures, Adlerberth et al. found higher abundance of Escherichia coli and lower abundance of enterobacteria in 99 vaginally delivered compared 17 cesarean delivered newborns (Adlerberth et al., 2006). Biasucci et al. used PCR amplification of Bifidobacterium species as well as PCR-denaturing gradient gel electrophoresis to find that 23 cesarean-delivered newborns had lower bacterial diversity and an absence of Bifidobacteria compared to 23 vaginally delivered newborns (Biasucci et al., 2008). Among 1032 infants studied at age 1 month, Penders and colleagues used polymerase chain reaction to quantify total bacteria and five bacterial taxa, finding that cesarean birth was associated with higher carriage of Clostridium difficile and lower abundance of Bacteroides and Bifidobacteria (Penders et al., 2006), thereby confirming Biasucci et al. (2008). More recently, with comprehensive analysis based on next generation sequencing of 16S rRNA genes, Dominguez-Bello and colleagues reported that route of delivery was associated with differences in the composition of the microbial populations that initially colonized the offspring. Notably, neonates who were born vaginally were colonized by vagina-associated bacteria, whereas those born by cesarean section were initially colonized by skin-associated bacteria (Dominguez-Bello et al., 2010).

Early life alteration of the gut microbiota may have a lasting effect. Trasande et al. observed that exposure to antibiotics up to age 6 months was associated with elevated body mass index (BMI) up to age 7 years (Trasande et al., 2013). In a recently reported murine model, Cox and colleagues observed that prenatal and postnatal exposures to

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subtherapeutic doses of penicillin resulted in an alteration of the gut microbiota that was transient (Cox et al., 2014). However, the early life exposure to penicillin also caused prolonged metabolic alterations including exacerbated diet-induced obesity (Cox et al., 2014). These observations are consistent with studies in humans showing that the distal gut microbial population may have a major effect on the risks for obesity and malnutrition. Among American adults, the composition of the microbial population in feces is generally altered with obesity, with enrichment by taxa in the phylum Firmicutes (Turnbaugh et al., 2009). Conversely, Malawian infants and young children with Kwashiorkor also have an altered population of fecal microbes, without a clear taxonomic signature but with a disease phenotype that could be transmitted by transplantation of Kwashiorkor feces to gnotobiotic mice (Smith et al., 2013). Likewise, the penicillin-induced obesity phenotype in the mouse could be transferred by fecal transplantation (Cox et al., 2014).

If cesarean delivery has a prolonged effect on the microbiota, this could contribute to the risk for metabolic diseases later in life. Herein, we explored whether the fecal microbiota differs between adults who reported that they were born by cesarean versus vaginal delivery. For comparison, in the same population we looked for differences in the reported that they were born by cesarean versus vaginal delivery. For we explored whether the fecal microbiota differs between adults who could contribute to the risk for metabolic diseases later in life. Herein, Randal Bollinger et al., 2007). The appendix, particularly its microbial-
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strongly associated with cesarean birth at all taxonomic levels, from phylum (permutation $P = 0.0076$) to species ($P = 0.0041$, Table 2). In contrast, age was unrelated to average J–S divergence ($R = 0.02$). As shown in Fig. 1, of four genus-level J–S hierarchical clusters, the prevalence of cesarean birth was 9% in cluster A and 8% in cluster D, whereas cesarean prevalence was high in cluster C (13%) and low in cluster B (0 of 30, $P = 0.03$). Appendectomy history had no association with beta diversity defined by average J–S divergence (Table 2).

Table 2 presents 14 taxa with increased relative abundance and 3 taxa with decreased relative abundance with Cesarean birth history. Cesarean-delivered adults had nominally reduced abundance of *Coprobacillus* and *Holdemania* (phylum Firmicutes) and of *Neisseria* (Proteobacteria), as well as nominally increased abundance of five genera in the Lachnospiraceae, Peptococcaceae, and Ruminococcaceae (Proteobacteria), as well as nominally increased abundance of three Bacteroidales and two Firmicutes, all increased, with appendectomy history. These are nominally significant associations, none of which survived adjustment for multiple comparisons. Mean

| Variable (N; N$^a$) | Cesarean birth history (N = 92) | Appendectomy history (N = 115) |
|----------------------|--------------------------------|-------------------------------|
|                      | Estimate | Std. error | z value | P-value$^b$ | Estimate | Std. error | z value | P-value$^b$ |
| Age, mean 46 years (SE 16 years) | −0.04 | 0.01 | −6.11 | 9.7E−10 | 0.05 | 0.01 | 6.51 | 7.5E−11 |
| Body mass index, mean 24 (SE 5) kg/m$^2$ | 0.01 | 0.02 | 0.22 | 0.81 | 0.01 | 0.02 | 4.26 | 2.1E−05 |
| Sex, male (461; 486) | 0.01 | 0.22 | 0.23 | 0.96 | −0.28 | 0.20 | −1.47 | 0.17 |
| Glutent, yes (200; 204) | −0.05 | 0.28 | −0.19 | 0.85 | −0.22 | 0.27 | −0.80 | 0.42 |
| Lactose, yes (183; 187) | −0.13 | 0.29 | −0.43 | 0.67 | −0.20 | 0.28 | −0.74 | 0.46 |
| Asthma, yes (86; 89) | 0.07 | 0.39 | 0.19 | 0.85 | 0.08 | 0.35 | 0.22 | 0.83 |

$^a$ Number of participants, cesarean birth analysis; appendectomy analysis.

$^b$ P-values by Wald test.
relative abundances for all 1949 taxa and Wald P-value comparisons are provided in Supplemental Table 2.

4. Discussion

This analysis was primarily motivated by the observation that the composition of the microbiome of neonates differed significantly between those born vaginally and those born by cesarean section (Arrieta et al., 2014; Dominguez-Bello et al., 2010). With vaginal delivery, the neonatal microbiome resembled the vaginal microbiome, with high relative abundance of Prevotella and especially Lactobacillus taxa. In contrast, cesarean-delivered neonates had a diverse array of taxa resembling the skin microbial community, including Staphylococcus, Streptococcus, Propionibacterineae, Haemophilus, and Acinetobacter (Dominguez-Bello et al., 2010). Cesarean-delivered neonates and infants typically have a paucity of Bifidobacterium and Bacteroides species (Arrieta et al., 2014). In the current analysis, we observed that the fecal microbiome composition differed in adults who reported that they had been delivered by cesarean section. This suggests that a difference by route of delivery may persist into adulthood. Of the taxa noted to be increased in cesarean-delivered neonates and infants (Arrieta et al., 2014; Penders et al., 2006; Dominguez-Bello et al., 2010), only Haemophilus and certain Clostridia genera had elevated abundance in the fecal microbiome of cesarean-delivered adults (Table 3).

In the placenta, microbiome composition (beta diversity) was reported to generally resemble the healthy oral microbiome. Placenta microbial composition differed for two types of women — those who reported a first-trimester urinary tract infection, with enrichment of several genera including Streptococcus and Acinetobacter; and those who had a pre-term delivery, with enrichment of Burkholderia and other genera (Aagaard et al., 2014). No overlap was observed between these taxa and the taxa that we found to differ, with nominal statistical significance, in the fecal microbiome of adults who had been born by cesarean section or who reported removal of their appendix (Table 3). The placenta microbiome was not found to differ between cesarean and vaginal birth (Aagaard et al., 2014).

Our study had several strengths, including relatively large size; high quality, unbiased profiles of the microbiome; restriction to individual adults; careful exclusion of recent antibiotic use and medical conditions that might have altered the microbiota; state-of-the-art statistical methods; and comparison of two conditions postulated to alter the gut microbiota. Although widely dispersed across the USA, the participants were not representative of the American adult population, being overwhelmingly non-Hispanic Caucasian (93%) and non-smokers (96%). Nonetheless, the prevalence of cesarean birth in our population, which was born on average in 1967, was 8.8%, comparable to the estimated cesarean rates of 5.5% and 10.4% for births throughout the USA in 1970 and 1975, respectively (Anon., 1995). The prevalence of appendectomy in our study, 14%, was similar to the 11%–14% prevalence reported by Dutch and British general population controls (Russel et al., 1997; Gent et al., 1994).

Our study also had important limitations, of which our lack of data on clinical indication for cesarean section may be the most important, given the fetal distress and antibiotic exposures associated with emergency cesarean. All of the covariate data (metadata) that we did have were self-reported, including birth and appendectomy history. We lacked data to confirm the Human Microbiome Project’s finding that fecal microbial composition differed with self-reported history of having been breastfed (Ding and Schloss, 2014). The validity of some self-reported early life events is modest at best. Among Swedish adults, self-reported birth weight compared to birth records had a Spearman correlation of 0.76, and 53% of the self-reported birth weights were off by 250 g or more (Andersson et al., 2000). Among ethnically diverse, middle aged women in New York, self-report for pre-eclampsia in their mothers had a sensitivity of 36% but a specificity of 97% (Terry et al., 2009). Accuracy of self-report of cesarean birth is unknown. To reduce misclassification in our study, we excluded participants with uncertain history or missing data. We also excluded for self-reported use of an antibiotic within one month of participation. Nonetheless, altered taxonomy of the bacterial communities of both feces and saliva has been seen at 2 months to as long as 4 years after treatment with an antibiotic (Fouhy et al., 2012; Jakobsson et al., 2010; Dethlefsen and Relman, 2011). Despite this, both antibiotic use during infancy and cesarean birth, with or without antibiotic exposure, appear to increase the risk of obesity in childhood (Bluestein et al., 2013; Trasande et al., 2013). In our study of adults, antibiotic use more than one month earlier was not associated with cesarean or appendectomy history (Table 1) and thus would not have substantially confounded our findings. Residual antibiotic effects or misclassification of birth history would likely bias toward the null. Finally, although we excluded duplicate participants, data were lacking to assess whether any twin pairs were included. This is an important limitation, because the composition and
diversity of the gut microbiota within twin pairs is significantly more alike than expected by chance alone (Turnbaugh et al., 2009; Cozen et al., 2013).

In summary, we found a distinct difference in the composition of the fecal microbiota of adult volunteers who were born by cesarean section. History of appendectomy had no such distinction. The cesarean-birth
distinction was independent of age and robust across all levels of bacterial taxonomy, but insufficient to define a cesarean-associated microbiome signature. The weaker association with hierarchical clusters (Fig. 1), suggests that many rare taxa contribute to the distinction. The individual taxa that were associated with cesarean birth or appendectomy (Table 3) were not statistically significant when adjusted for multiple comparisons, but they do provide hypotheses that can be examined in future studies. It remains to be seen whether neonatal differences in the gut microbiota directly or indirectly affect the risk of immunologic and metabolic diseases in adult life.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2014.11.004.

Conflict of Interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Author contributions

JJG conceived and designed the study, interpreted the data, and drafted the manuscript. XH developed the analytic pipeline, led the analysis, and generated the tables and figure. GY processed the raw data, generated the relative abundance and alpha diversity estimates through QIIME, and drafted critical parts of the manuscript. JS supervised the analysis and drafted critical parts of the manuscript. All authors contributed to and approved the final version of the manuscript.

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