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Commentary

Mode of delivery and pondering potential sources of the neonatal microbiome

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The 'Developmental Origins of Health and Disease [DOHaD] Hypothesis' [1] encompasses a substantial body of evidence which temporally and functionally link maternal exposures to adverse outcomes in her offspring (largely obesity, metabolic disorders, cardiovascular disease, and behavioral outcomes) via meaningful and persistent modulations in postnatal gene expression resulting from epigenomic changes [2]. More recently, many similar observations led to the genesis of the 'Hygiene Hypothesis', which alternately suggests that it is a lack of exposure to microbes early in life which primarily predisposes offspring to developing not only these same adverse outcomes, but atopic and allergic diseases later-in-life [3]. The convergence of thought encompassing both the DOHaD and Hygiene Hypotheses has led to several teams of investigators query whether there is (or is not) a mechanistic link between a maternal exposure, resultant perturbations of the offspring microbiome, and risk of later-in-life disease [4].

One area of focused exploration has been whether birth by Cesarean imparts an independent risk of later-in-life disease. However, it is yet unclear if the risk is truly attributable to the Cesarean surgery itself, the underlying maternal or fetal medical indication that led to the surgery, or other co-linear and co-morbid factors which accompany either the surgery or its underlying medical cause. Cesarean delivery is one of the most common and safest abdominal surgeries performed, and ready availability to medically indicated Cesarean with surgically competent providers is absolutely crucial in the reduction of maternal and neonatal mortality and decreasing social disparities worldwide [https://www.who.int/reproductivehealth/publications/maternal_perinatal_health/cs-statement/en/].

However, with a rising worldwide trend in the number of Cesarean deliveries, there is increasing scrutiny on whether or not there is potential real and meaningful risk of harm to the infant over its lifetime. For example, Yuan et al. [5] found that among siblings born to the same mother, those delivered via Cesarean had a 64% higher odds of developing obesity than did their siblings born via vaginal delivery, albeit the overall cohort adjusted risk ratio was only 1.15 (95% CI 1.09–1.54, $p = 0.002$). What this means is that Cesarean birth was associated with a 15% increase in the risk of obesity from childhood into young adulthood after adjusting for identified confounders, which is actually a rather small attribution given the current population prevalence of obesity. Among 2 million Danish births from 1977 to 2012, there was a significant ($p<0.05$) but similarly modest increase in risk of asthma, arthritis or mixed connective tissue disorders, and inflammatory bowel disease (aIRRs 1.10–1.46) in children born via Cesarean [6]. In contrast, meta-analyses or large and prospective studies which aim to carefully control for confounders have failed to observe such associations, including any association with unlabored (planned) Cesarean and obesity at age 3 [7]. Ultimately, prospective acquired data from economically, ethnically, and racially diverse populations which code for the underlying medical indication prompting the Cesarean delivery (such as poorly controlled gestational diabetes resulting in a large fetus, with arrest of descent in labor) and key mitigating factors (such as neonatal hypoglycemia, infant feeding, and maternal diet) are necessary to determine which offspring outcomes, if any, can truly be attributed to a Cesarean surgery *per se*.

Nevertheless, if the postulate that Cesarean born infants go onto develop obesity, metabolic or atopic diseases, or developmental disorders as a result of the failure to be colonized in their gut (or skin or mouth) by vaginally derived microbes is true, then the gut (or skin or mouth) microbiome ought be readily and predictably distinguishable among Cesarean and vaginally born offspring. Herein lies the crux of the controversy. While many studies have reported such differential relative abundance by generally course and crude taxonomic analyses of infants [8], several lines of evidence are contrary or present conflicting results. First, one of the key tenets of the Human Microbiome Project (HMP) was the observation of unique body niche speciation. In the vagina, the ecology is dominated by *Lactobacillus* spp. which are highly adept at living at low pH; these same microbes do not dominate the neonatal gut nor other body niches [9]. Second, one of the more consistent relatively "missing beneficial microbes" among Cesarean delivered infants is reported to be *Bacteroides*, which have also been shown to be lacking in 20% or more of vaginally

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Abbreviations: DOHaD, Developmental Origins of Health and Disease; NICU, Neonatal Intensive Care Unit
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delivered infants [8,9]. Interestingly, we and others have shown that infant feeding with formula increases the *Bacteroides* count [8]. Since breastfeeding is recommended due to its association with long-term mitigation of nearly every late-in-life outcome presumptively associated with Cesarean birth, this observation would appear to be contrary. Third, any relative lack or increased abundance of microbes does not appear to persist beyond the immediate period after birth or early infancy [8,9], raising into question whether relative differences in the abundance of a very few taxa in the first hours or days of life can have a profound enough influence on disease trajectory years or decades later. Fourth, only a limited few studies have examined single or multiple body sites with shotgun metagenomics, which enables functional analysis of metabolic genes encoded by these and other taxa [9]. This is important, since a guiding principle of microbial ecology is 'form follows function', meaning that since multiple taxa have overlapping metabolic function, subtle variations in which microbiota occupy a niche may not contribute to physiologically meaningful differences in the host. Fifth, studies which simultaneously and systematically rigorously address the potential contribution of the maternal body niches (vagina, rectum, oral), the intrauterine environment (placenta, amniotic fluid, fetal amniotic membranes), and environmental contaminants of both space and time, have heretofore been lacking. In a recent article in *EBioMedicine*, Liu and colleagues [10] catalogue both bacteria and archaea in 550 samples from 78 maternal-neonatal dyads (38 vaginally delivered, 42 Cesarean delivered) representative of precisely these presumptive sources of the neonatal meconium over a 2 week span of time in a single delivery hospital. Because of the volume of deliveries occurring in this hospital, the vaginal deliveries occur on a separate floor than the operative Cesarean births.

The findings of Liu et al. regarding 16S rRNA gene based detection of microorganisms are notable. Both by count estimate and taxonomic classification, the microbiomes and mycobiomes of the placenta, amniotic fluid, and membranes are distinguishable from not only robust environmental and contaminant controls representing each step of the delivery, sampling, extraction, and sequencing process, but are more similar to each other than to the mode or hospital subsite of delivery. Of note, all maternal samples were uniformly collected 1 h prior to delivery and placental and amnion samples immediately at birth; strict "sterile" precautions were observed with each collection. Of samples collected, only amniotic fluid was distinguishable between Cesarean and vaginal births. This is to be expected, as the amniotic fluid was collected at or immediately after delivery, and would be anticipated to have vaginal cellular aspirate in the case of vaginal deliveries. In contrast, each body niche site of the dyad was more similar to its body site counterpart in another individual than to a distinct site in the same individual (or its maternal-neonate counterpart). A more subtle finding of Liu et al. was that the taxonomic structure of maternal body sites at the time of delivery (vaginal, oral and rectal) similarly did not vary by mode of delivery. This may result from either the low parity, low rate of co-morbidities, or the shared decision-making practices of the women and their providers allowing for maternal choice regarding mode of delivery in the Yan'an Affiliated Hospital of Kunming Medical University in Yunnan Province, China. This remains speculative, as the authors do not comment extensively on the underlying indications nor rates of medical comorbidities in these women.

The contributions of Liu et al. do not resolve the ongoing controversy. However, by experimentally controlling for multiple potential sources of contamination and confounding, they do lend further evidence to the mounting notion that the placenta (and amniotic fluid and membranes) do in fact harbor a unique low biomass, low abundance microbiome and mycobiome. In their current study, Liu et al. are not only able to distinguish these taxa from environmental and maternal body site (*e.g.,* vaginal) contamination controls in the exact same woman and delivery room where the birth occurred, but also from contamination which might have resulted from extraction and sequencing. Moreover, since they fail to observe significant variation in any given body niche sample (except for amniotic fluid) by mode of delivery in the entirety of their study cohort, their findings are further consistent with vertical transmission of the microbiome and mycobiome from mother to fetus. While Liu et al. lends to a substantial and growing evidence suggesting that Cesarean delivery *per se* is not associated with any appreciable differences in the neonatal microbiome, it does not address whether maternal co-morbidites or pregnancy exposures might result in the difference observed reported by others [8].

One interpretation of this [8–10] is that the moment of delivery is not the inception not first initiation point in the development nor establishment of the infant microbiome. There are other lines of evidence and multiple publications which support the alternate hypothesis that the infant microbiome and mycobiome originate earlier in fetal life. First, the uterus, upper reproductive tract, and preterm placenta has long been known to harbor sparse but cultivatable and histologically and metagenomically detected microbial communities. In fact, early microbial colonization is crucial for metabolic, gut, and immune health: neonatal mice raised in germ-free conditions have deranged immune repertoires and altered innate immunity, metabolic and behavioral disturbances, and disrupted gastrointestinal physiology. That said, it is important to reiterate that microbiome science and metagenomic studies cannot discern whether the placenta, fetus, or intrauterine environment is colonized by live commensal microbes. There is a need to fund and publish research aimed at determining whether the low biomass microbial communities of early infant development (be they the placenta, breastmilk, or intrauterine environment) are, in fact, established *in utero* by coordinated process of immune tolerance, pathogen colonization resistance, microbial community pruning, and/or true fetal colonization. Given the implications of this work for this next and future generations in the face of a rapidly changing world laden with environmental exposures affecting pregnancy, this is perhaps one of the most important scientific and public health issues of our time.

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Declaration of Competing Interest

None.

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