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JIA and the Gut Microbiome: More Clues, More Questions

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When we diagnose children with juvenile idiopathic arthritis, JIA's middle name serves as a reminder of our ignorance of its origins. Even the largest, most comprehensive genomic studies on JIA performed to date have explained only a minority of disease heritability. Families rightfully ask *why* their children have developed this condition, and many of us perform our own version of the “genetics and environment” hand-waving show, often (at least for this clinician) to an unsatisfied audience.

Researchers have sought answers to JIA's missing heritability problem in various corners of scientific inquiry, including immunology, epigenetics, and epidemiology. Studies in these areas have been conflicting and frustratingly inconclusive. Nonetheless, findings such as the potential protective effects of breastfeeding,(1) potential harmful effects of antibiotics,(2, 3), and circulating antibodies against bacterial proteins in children with JIA (4) have left clues pointing down another path: the microbiome.

The microbiome is the set of genomes within bacteria, archaeobacteria, fungi, and viruses that inhabit our gut, skin, mouth, and other body sites. Collectively, these microbial genes vastly outnumber human genes and perform critical functions that help maintain homeostasis not only in the microbes' respective niches but also more globally within the human host.(5) The list of these vital functions is extensive and includes dietary and bile acid metabolism, energy production, vitamin and neurotransmitter synthesis, and immune development and function(5)—products of millennia of unicellular/multicellular co-evolution. Given these and other important roles of microbiota and their sensitivity to various environmental influences—drugs (e.g., antibiotics), infections, toxins, diet, and many others—researchers across diverse clinical fields have studied whether incompletely understood diseases could result from imbalanced and dysregulated microbiota, termed *dysbiosis*. For example, disruptions of normal interactions between gut microbiota and immune cells early in life may impair immune maturation and tolerance.(6) Loss of anti-inflammatory commensals or expansion of potentially pathogenic microbiota (pathobionts) could promote pro-

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inflammatory cytokines and effector cells and ultimately contribute to the development of chronic inflammatory and autoimmune diseases.(6)

Data on the gut microbiome in children with JIA have emerged over recent years from case-control studies across 3 continents.(7–12) These studies have compared the bacteria in stool from children diagnosed with JIA and stool bacteria from unaffected children. These comparisons have generally focused on taxonomy—phylogenetic relationships of bacteria on the tree of life—by sequencing a portion of a key bacterial gene (16S rRNA) and then matching these sequences, where possible, to ever-expanding genetic libraries of named bacteria. This bar code-based approach allows researchers to detect differences between groups in diversity (for example, the total number and evenness of distribution of discrete types of bacteria within samples) and composition (the differences in taxonomic relationships of bacteria between groups, either globally or in the abundance of individual taxonomic groups). In short, all publications on JIA and the gut microbiome have reported differences in bacterial composition between cases and controls, globally and/or within taxa (e.g., families, genera, or species). One study also reported decreased microbial diversity among children with JIA.(8)

However, interpretations of these findings must be considered in light of certain common limitations in study design. Several prior studies focused on children previously diagnosed with and treated for JIA, a design that can make it difficult to distinguish the causes of disease from the effects of the disease, its treatment, or other factors (e.g., dietary changes). Only a few prior studies have focused on DMARD-naïve patients. In addition, most studies have used controls recruited from the same rheumatology clinics or from unstated locations, raising questions about whether those controls actually represented the greater population of children who *would* have been referred if they *had* developed JIA. Sample size has been another common limitation of previous studies, with the number of cases ranging from 8 to 33 and the number of controls, 13 to 27. Such small numbers are generally underpowered to detect differences or show equal abundance of individual taxa, particularly findings that are not anticipated *a priori*. Finally, most studies have evaluated children at a single point in time, which can limit causal inference.

In this month's *Arthritis & Rheumatology*, Van Dijkhuizen and colleagues have addressed many of these limitations with their well-designed case-control and cohort study on the gut microbiome in children with JIA across 3 large centers in Italy and the Netherlands.(13) Using a taxonomic bar code-based approach, these investigators analyzed stool samples from 99 children with newly diagnosed JIA who had not previously received glucocorticoids or disease-modifying drugs. Because country of origin was a key discriminating factor in bacterial composition, they stratified their analysis by country, essentially giving us two microbiome studies to consider side-by-side. They compared cases to 107 unaffected children recruited predominantly from schools and primary care clinics—locations generally considered to be more representative of the source population than specialty clinics. Furthermore, they performed a longitudinal cohort study, analyzing repeat samples from 44 cases to evaluate changes over time based on disease activity, either with the development of inactive disease or with flare or persistently active JIA.

Using this sound study design, Van Dijkhuizen et al showed that global microbial composition differed between cases and controls within each country, as analyzed using standard non-parametric and machine learning approaches. They also noted multiple taxa that were significantly enriched or depleted in the larger group of Italian cases. Many of these taxonomic differences persisted after excluding children with exposures that could have independently affected microbiota and confounded their results, such as antibiotics, pre- and probiotics, or gastrointestinal infections. Compared to controls, cases from Italy had lower bacterial diversity, a finding that persisted after adjustment for confounders. In exploratory analyses, the authors found either very modest or no significant relationships between microbiota composition and a variety of clinical factors, including JIA category, level of disease activity, and presence of uveitis.

Within the longitudinal cohort—one of the most novel aspects of the study—the investigators found only modest compositional differences over time between baseline and follow-up samples. The distinction in microbiota between states of inactive and active disease was slight. Interestingly, taxonomic diversity—already low for many at diagnosis—decreased further among many children who attained inactive disease. Given that inflammation and microbiota diversity declined in parallel, the authors speculate in their discussion (with appropriate caution) that inflammation may not have caused the low diversity seen in children with JIA. Perhaps anti-inflammatory or anti-rheumatic treatments contribute to reductions in both. We should also remember that clinically inactive JIA may not represent a state of true disease remission or inflammatory inactivity.⁽¹⁴⁾ Ultimately, overall changes in microbiota diversity may matter less than if deleterious strains are depleted or health-promoting microbiota flourish.⁽¹⁵⁾

With its laudable design, size, and international collaboration, this observational study has moved the bar higher for research on JIA and the gut microbiome. Yet how much has this study moved the needle of our understanding of the role of the microbiome in JIA? We are left with many unanswered questions.

As the largest study on the gut microbiome and JIA, was this study large enough? Each entity—the gut microbiome and JIA—is complex, heterogeneous, and incompletely understood. As a result, such studies may require even larger subgroups than in the current publication (e.g., uveitis, RF+ polyarticular JIA, inactive disease or remission) to see more meaningful differences in microbiota composition or diversity, if they exist at all. The smaller Dutch study population may also have been too small to appreciate the differences in diversity and composition seen in Italian participants.

How valid and generalizable are these findings? The current study used an older sequencing platform that produced sequencing depths orders of magnitude lower than possible with state-of-the-art methods. This low sequencing coverage and the exclusion of over 80% of taxa from analyses limited their ability to study the influence of less common taxa and may have biased the taxonomic comparisons. Like other investigators, Van Dijkhuizen et al reported global differences in the diversity and composition of gut microbiota between children with and without JIA. The similarities mostly end there. Among taxa that were most different in abundance between Italian cases and controls, a similar but smaller Finnish

study (children with newly-diagnosed oligo- or polyarticular JIA) either did not mention or showed opposite results for most of these taxa.(9) For instance, *Erysipelotrichaceae* and *Ruminococcaceae* were more abundant in Italian cases but more abundant in Finnish controls. Three notable taxa from the current publication— *Propionibacterium acnes* (a typical skin pathobiont), *Haemophilus parainfluenza* (an upper respiratory tract commensal), and *Allobaculum* (an intestinal commensal of rodents and canines)—were surprisingly abundant in Italian cases and controls; none has been mentioned in any prior study on JIA. Even the comparison of the Italian and Dutch populations in the current study was noteworthy: the most distinct taxa in each country were completely inconsistent, and the microbiota diversity of cases was lower than controls in one country but higher in the other. Several factors may explain these differences, including regional differences in genetics, diet, or the environment; differences in study design, such as the sources of controls and protocols for sample collection; and differences in the study population, including participants' demographics. Of note, Italian cases were mismatched in age with Italian controls and with the more balanced Dutch case and control groups; considerably more Italian cases were younger than 3, when dramatic changes in microbiota diversity and composition take place.(16) Given these concerns, replication in comparable, well-matched, adequately powered samples using current sequencing technology will be important.

What is the functional relevance of differences in diversity and composition among individuals and over time? The divergent results between countries highlight a key drawback of the standard taxonomic approach—that is, limited functional information and mechanistic interpretation. Standard taxonomic methods using 16S gene sequencing often fail to distinguish among closely related species or strains, which may differ considerably in their effects on inflammation and other processes. 16S gene sequencing can also misclassify distinct species as identical.(17) The NIH-funded Human Microbiome Project showed that, in the gut and most other body sites, taxonomic variability among healthy individuals was the rule rather than exception.(18) However, when more comprehensive sequencing was performed (whole genome shotgun metagenomics), little variability was observed in the metabolic functions of commensal microbiota within any given biologic niche.(18) In an experimental spondyloarthritis study, different patterns of dysbiosis were seen in different animal strains housed in different facilities even though the disease and inflammation-related changes in gene expression were very similar.(19) These and other studies have taught us that the names of microbiota do not necessarily connote their function.

Van Dijkhuizen and colleagues' study, like so much of microbiome research, raises critically important questions about causality: Is dysbiosis more than a mere biomarker? Does dysbiosis contribute to disease pathogenesis, and if so, how and in whom does it do so? If dysbiosis does play a causative role, can we treat or prevent the disease by changing or normalizing the microbiome?

We have started to see tantalizing hints of answers to these tough questions, including studies on the pathogenic role of *Akkermansia mucinophila* in juvenile spondyloarthritis(20) and the potential benefits of elemental formula in children with active JIA.(21) Still, these are early days of microbiome research in JIA, and causal interpretations of the published evidence would be premature.

To answer these critical questions about causality more definitively, we need other well-designed, well-powered, collaborative, longitudinal studies that provide the necessary temporal and functional data to draw clearer conclusions about causes and effects of JIA pathogenesis and course. We have much to learn about the roles of non-bacterial microbiota as well as non-gut microbiomes in JIA. Large collaborations and advanced analytics will be essential to understand how the microbiome interacts with other -omes (e.g., genomes, epigenomes, metabolomes, exposomes, etc.) and the immune system in the various diseases we call JIA. We need more mechanistic research testing how microbiota from children with biologically distinct forms of JIA affect the pathways that drive arthritis, uveitis, and other disease complications. Finally, we need more robust interventional studies that test whether strategies that modify microbiota actually change patients' disease course and response to treatment, or even the risk of developing JIA.

Patients and families that live with JIA are understandably dissatisfied with this condition's middle name and its imperfect treatments. Many are not waiting for scientific evidence to justify changes in their diet, use of pre- and probiotics, and potentially other microbiome-altering strategies. Greater understanding about the microbiome may help fill in the current gaps regarding the etiology of JIA. We owe our patients, their families, and ourselves more evidence to help distinguish the hope of the microbiome from the hype.

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