Advances in Gut Microbiome Research and Relevance to Pediatric Diseases

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The human microbiota is the collection of microorganisms that reside within and on the surface of humans, and the term "human microbiome" refers to the genes that these microorganisms harbor. Although the human bacterial microbiome is the most commonly described, all 3 kingdoms of life, *Archaea*, *Bacteria*, and *Eukarya* are represented within this dense community. There are distinctive microbial communities among body sites, but by far, the vastest population of microorganisms resides within our gastrointestinal tracts. An estimated 10^{13} individual bacteria belonging to over 1000 species reside in the mammalian gastrointestinal tract, making it the most densely populated microbial community on Earth.[1] Indeed, the collective genome of the human gut microbiome is predicted to be 100-fold greater than that of its human host.[2] Although there are over 50 bacterial phyla on Earth, the majority of the bacteria in the human gut belong to 1 of 4 phyla, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes.

As humans, we have evolved to live in a mutualistic relationship with our microbiota. In fact, humans should be viewed as a biologic "supraorganism" where we provide an essential niche for our microbiota and in turn, our microbiota carry out critical physiological functions. For example, within the gut, bacteria participate in fermentation of indigestible carbohydrates to produce short chain fatty acids that are used by the host, biotransformation of conjugated bile acids, synthesis of certain vitamins, degradation of dietary oxalates, hydrolysis of urea by urease activity that participates in host nitrogen balance, and education of the mucosal immune system.[3] Indeed, it was not very long ago when the term "gut flora" denoted an obscure and relatively unexplored space. However, advances in genome sequencing technologies and metagenomic analysis methods have led to numerous discoveries regarding community membership and the relationship between the gut microbiota and human health. Subsequently, it has become apparent that aberrations in the composition and functions of the gut microbiota may potentially be fundamental to the development of certain diseases, many of which are very relevant to pediatrics.

CD	Crohn's disease
CF	Cystic fibrosis
FMT	Fecal microbiota transplantation
IBD	Inflammatory bowel diseases
IgE	Immunoglobulin E
IL	Interleukin
iTregs	Induced Tregs
UC	Ulcerative colitis

Technological Advances in Gut Microbiome Research

Most species of gut-residing organisms are obligate anaerobes, many of which are fastidious and difficult to grow in vitro making traditional culture techniques of limited value in characterizing the composition of the gut microbiota. The development of culture-independent methods, mainly through the use of high-throughput DNA sequencing, has provided a novel means to evaluate the gut microbiota and its relationship to disease. There are 2 primary methods that use deep-sequencing technologies to characterize the microbiome. The first approach, uses small-subunit ribosomal RNA (16S ribosomal RNA) gene sequences (for Archaea and Bacteria), or internal transcribed spacer gene sequences (for Eukaryotes) as stable phylogenetic markers to define the lineages present in a sample.[4] Another approach uses shotgun metagenomic sequencing in which the total community DNA is sequenced, thereby allowing for the microbial community structure and genomic representation of the community to be evaluated. The genomic community evaluation provides an understanding of the functions encoded by the genomes of the gut microbiota.[5] Metatranscriptomics and metaproteomics provide a deeper understanding of microbial function through direct evaluation of gene expression.[6] These advances in sequencing technologies have allowed investigators to characterize the bacterial composition of the gut throughout different stages of life, a critical step in the study of health and disease.

Development of the Infant Microbiome and the Effect of Early Life Exposures

It was once thought that the healthy human fetus develops in an environment that is completely sterile.[7] However, there is an evolving body of literature suggesting that even the human placenta harbors a low-abundance commensal (nonpathologic) microbiota with potential biological relevance.[8] For example, there may be an association between the composition of the placental microbiota and birth weight in full-term neonates.[9] However, this needs to be further explored. Still, it is generally accepted that significant colonization of the infant does

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not occur until the time of delivery when infants are rapidly exposed to an abundance of organisms.[10] There is increasing knowledge regarding the colonization succession of the infant gut and its relevance to health and disease. However, the initial colonization of other body sites remains relatively unexplored. In a study of 10 infants who underwent sampling across multiple body sites within 24 hours of delivery, the microbiota seemed to be homogenously distributed across skin, oral, and gut habitats.[10] In contrast, their mothers harbored distinctly different communities at various body sites.[10]

The initial intestinal colonization pattern depends upon mode of delivery.[7] Infants born vaginally are initially colonized by bacterial taxa found in the vagina, such as *Lactobacillus* and *Prevotella*, whereas infants who are born by cesarean delivery are initially colonized by bacteria found in the skin microbiota.[10] After this primary inoculation, infants are regularly exposed to microbes, and diversity increases rapidly.[11] Diversity is a term used to describe the microbial community in terms of richness, or number of microbial species present and also evenness, the proportions in which each species is represented. High diversity has been associated with health and low diversity has been associated with various disease states.[12] This highlights the importance of this process of attaining diversity early in life as it likely has important implications for future health. It is also important to recognize that studies of humans around the world have consistently demonstrated that inhabitants of developed countries harbor microbial communities with reduced diversity. This has led to the hypothesis that loss of diversity is secondary to "Westernization" and may be involved in the pathogenesis of various chronic diseases, which have been rapidly increasing in incidence in developing countries.[12]

In infants, the initial colonization pattern is thought to be chaotic, and a growing body of literature has shown that environmental exposures early in life, including diet, are responsible for these fluctuations. Characterization of the intestinal microbiota in a single infant, over a period of 2.5 years, showed how the bacterial taxa changed with life events, such as illnesses, dietary changes, and antibiotic treatment. Interestingly, the greatest change in the composition of the infant's intestinal microbiota occurred with the introduction of solid foods. There was also a shift toward a more stable, adult-like microbiota with weaning.[11] This particular finding was replicated in a subsequent analysis of fecal samples from 98 full-term infants where cessation of breastfeeding seemed to drive the transition to a more mature gut microbiome.[13] Ultimately, the intestinal microbiota of the young resembles that of the adult by approximately the age of 3 years.[14]

Within the first year of life, there are significant interindividual differences in the composition of the intestinal microbiota, yet some similarities exist. Similarities among individual infants can be attributed to the major taxonomic groups associated with the infant diet. Multiple studies have established differences in the composition of the intestinal microbiota based on whether infants are breastfed or formula fed.[15-17] Indeed, this introduces the concept of a potential association between infant diets, the composition of the intestinal microbiota, and health.

Delivery mode and dietary factors are clearly not the only determinants of early gut colonization pattern. Genetics[18] and other environmental exposures, such as antibiotic usage,[19] also play a role. It is clear that appropriate colonization of the infant gut is important for education of the mucosal immune system and optimal gut function.[20] Thus, it is logical to think that abnormal microbial exposures leading to dysbiosis, or an abnormal composition of the gut microbiota, play a role in the development of pediatric diseases.

The Relationship between the Gut Microbiome and Pediatric Diseases

Pediatric Obesity

The prevalence of pediatric obesity has increased in recent decades and represents a serious public health concern. In 2011-2012, approximately 12.7 million children and adolescents in the US were considered obese.[21] Many obese children go on to become obese adults[22] who have significant comorbidities such as diabetes, hypertension, atherosclerosis, and certain types of cancer, such as colorectal cancer. There are also, of course, more immediate health consequences for obese children and teens, such as hypertension and dyslipidemia.[23] Therefore, it is important to understand underlying biological factors that may be related to the development of obesity, including factors that may be important early in life. For example, rapid infant weight gain in the first year of life has been associated with the development of obesity, and many factors related to weight gain in the first year of life have been identified including maternal obesity, mode of delivery, and early exposure to antibiotics.[24] Dietary factors are also thought to be important.[25] This raises the question of whether these factors are influencing a single mechanism, namely the development of the infant gut microbiota.

The presence of an altered gut microbiota composition in obese individuals compared with lean individuals has been well established in both animal and human studies. This finding was first described by Ley et al[26] who reported a decreased abundance of Bacteriodetes and a proportional increased abundance of Firmicutes in the cecal contents of genetically obese mice relative to their lean counterparts. The difference was independent of kinship and sex and importantly, all animals were fed the same diet. A similar shift in the abundance of Bacteriodetes and Firmicutes in the intestinal tract was subsequently demonstrated in obese adults compared with lean, healthy controls.[27] Interestingly, these changes seemed modifiable as the relative abundance of Bacteriodetes increased as obese individuals lost weight on a low-calorie diet.[26]

One important function of the gut microbiota is degradation of otherwise indigestible components of our diet, thus, playing a role in energy balance. As mentioned previously, an example of this is fermentation of indigestible carbohydrates to produce short chain fatty acids. Thus, Turnbaugh et al[27] sought to examine whether the microbiota of obese mice were more efficient at extracting energy than the microbiota of lean mice. Indeed, when germ-free mice were inoculated with a microbiota