Review

The use of multi-omic approaches to study the microbiome during disease states paves the road towards comprehensive understanding of disease processes

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The human microbiome encompasses the microorganisms (also known as microbiota), their genomes[1] and the surrounding environmental conditions of where they are found throughout the body.[2] The microbiome is highly varied in the population,[3] affected by genetics, dietary changes and mode of delivery at birth.[4,5]

The integrative Human Microbiome Project (iHMP) is the second phase of the Human Microbiome Project (HMP). The HMP sought to examine taxonomic and metagenomic elements present in the microbiome of healthy individuals and those in specific disease states. It concluded that more nuanced studies looking beyond microbiome composition are needed to fully appreciate the host–microbiome interplay during good health and diseased states due to significant inter-individual compositional variation.[3] The iHMP sought to use multi-omics, the study of analytes such as metatranscriptomes, metabolomes, microbiome composition and cytokine profiles, longitudinally to study host–microbiome interactions during three specific states: inflammatory bowel disease (IBD), preterm birth (PTB) and prediabetes. This article aims to explore and summarise the findings and conclusions of the three flagship studies conducted as a part of the iHMP.

Multi-omics in IBD

Lloyd-Price and colleagues looked at identifying potential multi-omic changes in participants with and without IBD.[6] They found cross-sectional differences, such as decreased metabolome diversity and the lack of vitamin B5 and B3 in the guts of individuals with IBD compared with those without. Analyte variation corresponds more with dysbiotic periods than with IBD phenotype, making it difficult to determine whether dysbiotic changes were a cause or a result of IBD. Extensive taxonomical shifts were observed in both groups, but IBD cases had a slightly higher rate of shifts and the species undergoing the greatest change in relative abundance differed between groups. The study chose to forgo collecting samples from patients selected for active disease and instead focused on subsets of inactive and relatively more active IBD. This may have caused the lack of significant difference in metagenomic species (species genomes recovered in samples) between participants, a result that contradicts existing studies.[7-9] This is because these subsets of IBD cases may be metagenomically closer to non-IBD controls than to active IBD. Therefore, future studies must compare metagenomes of all four subsets (active IBD, inactive IBD, relatively more active IBD and non-IBD patients) and establish a consensus. The multi-omic approach enabled the analysis of how each analyte varied in IBD, allowing for closer study of cross-sectional changes during the disease course. Studies with homogenous methodology and larger cohorts are necessary to understand the aetiology of IBD, leading to development of management and therapeutic strategies.

Preterm births and the vaginal microbiome

Building on previous studies to find population-specific effects of the vaginal microbiome composition on the risk of PTB, Fettweis and colleagues[10] compared the vaginal microbiome between two groups of mothers experiencing either term birth (TB) or PTB delivery. Throughout longitudinal analysis, taxa abundance and transcription activity varied differentially and, in some cases, similarly between PTB and TB women throughout pregnancy. For example, women who had PTBs experienced large decreases in *Sneathia amnii* during pregnancy as compared with those who had TBs, but both groups experienced decreases of *Gardnerella vaginalis*. Interestingly, the

changes differed by women's ancestry, suggesting that ancestry possibly influences pregnancy taxa dynamics. PTB-associated taxa decrease throughout pregnancy, therefore, samples from early pregnancy are more useful for predicting adverse outcomes. A model based on four taxa in samples taken at 24 weeks' gestation was produced to predict the risk of PTB delivery using vaginal microbiome profiles. It had 5–7% greater sensitivity and specificity but a slightly lower area under receiver operator characteristic (AUROC) curve (0.723 vs 0.764) than a purely clinical model, meaning that the clinical model is slightly better at predicting risk of PTB. With some improvements and refinement, this microbiome model's remit can extend to all populations, showcasing the model's impressive potential. The formulation of a sensitive and specific model in the study demonstrated that the vaginal microbiome has an impact on the risk of PTB. Improving our understanding of the underlying mechanisms will help to improve health outcomes in the estimated 15 million PTB deliveries that occur worldwide[11] through preparation and, potentially in the future, therapeutic intervention.

Prediabetes

Zhou et al examined the microbiomes of healthy individuals and those with prediabetes or diabetes aiming to better understand associations of biological processes at the earliest stage of these conditions.[12] Individuals were categorised by measuring HbA_{1c}, fasting glucose and annual oral glucose tolerance. Sixty-six of the 106 participants underwent insulin suppression tests and were classified as either insulin sensitive or insulin resistant using steady-state plasma glucose (SSPG) measurements. Following adjustment, multi-omic measures strongly correlated with SSPG. Known associations of SSPG, such as with inflammation, were also confirmed. Both insulin-resistant and insulin-sensitive groups had unique and significant associations between analytes and gut microorganisms. Correlations seen in insulin-sensitive individuals were absent in insulin-resistant individuals; the findings suggested that insulin resistance affects the interaction between gut microorganisms and host cytokines. Respiratory viral infections (RVIs) were found to dysregulate molecular pathways, including insulin receptor signalling and neuroinflammatory signalling, which were not previously shown to be affected by RVIs. The differences between the insulin-resistant and insulin-sensitive groups (e.g. the affected pathways) were attributed to the elevated baseline inflammation and impaired immune response seen with insulin resistance. Surprisingly, immunisations inversely correlated with type 1 diabetes and type 2 diabetes signalling and downregulated insulin receptor signalling. The fact that strong interpersonal differences exist and that variations in profiles can be contained within 'healthy' reference ranges in the disease states was highlighted by the study,[12] and demonstrates the need for large population studies in this field. Further studies are required to identify patterns present in healthy individuals and in those at risk of type 2 diabetes on a population level that would help to predict outcomes despite the significant interpersonal differences and variation. The impact of insulin resistance on the microbiome and how that culminates in the dynamics between RVIs, immunisations and the risk of developing metabolic disorders in insulin-resistant individuals was explored by this study, undoubtedly contributing to improving the treatment of insulin-resistant individuals.

Conclusion

The microbiome's far-reaching influence on health outcomes is just being appreciated through the findings of pioneering studies, like iHMP. Future studies must establish causal links between host–microbiome interplay and disease processes to open up new avenues for diagnosis and clinical interventions. Multi-omic studies will have a key role in this process because they facilitate the comprehensive study of the microbiome, as seen in the three studies discussed in this article. It is clear that the coming years will be exciting for the study of the microbiome.

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