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Microbiomes in respiratory health and disease: An Asia-Pacific perspective

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Abbreviations: chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), faecal microbiota transplantation (FMT), gastrointestinal (GI), inflammatory bowel disease (IBD), short-chain fatty acids (SCFA)

ABSTRACT

There is currently enormous interest in studying the role of the microbiome and its role in health and disease. This is increasingly being applied to respiratory diseases, in particular chronic obstructive pulmonary disease, asthma, cystic fibrosis and bronchiectasis. The changes in respiratory microbiomes that occur in these diseases, and how they are modified by environmental challenges such as cigarette smoke, air pollution and infection are being elucidated. There is also emerging evidence that gut microbiomes play a role in lung diseases through the modulation of systemic immune responses and can be modified by diet and antibiotic treatment. There are issues that are particular to the Asia-Pacific region involving diet and prevalence of specific respiratory diseases. Each of these issues are further complicated by the effects of aging. The challenges now are to elucidate the cause and effect relationships between changes in microbiomes and respiratory diseases and how to translate these into new treatments and clinical care. Here we review the current understanding and progression in these areas.

Key words: Microbiome, gut, respiratory disease, COPD, asthma, cystic fibrosis, health, mouse models, prevention, treatment

Short title: Microbiomes in respiratory health

Introduction to the microbiome – respiratory and gut

In humans, the term 'microbiome' refers to the entire microbial constituents in a given organ or system, including the microorganisms, their genomes, surrounding environmental conditions and their interactions with the host.[1] The gastrointestinal (GI) microbiome is by far the largest in the body, containing more than 100 trillion microbes from over 1,000 species.[2, 3] Highly dynamic, the GI microbiome rapidly matures from an *Actinobacteria*–dominated composition soon after birth into the adult microbiome comprised largely of *Firmicutes* and *Bacteroidetes*, as well as *Proteobacteria* and *Actinobacteria* in lower proportions.[4-7] Given the enormous variety of microbial species and high variability in microbiome composition between individuals, it is likely that different taxa in different individuals can fulfil similar roles in their interactions and metabolism, resulting in a common 'functional microbiome'.[4]

The GI microbiome is the best studied, which results from the abundance of microorganisms and relative ease of sampling *via* faeces. In contrast, the respiratory microbiome has only recently been characterised through culture-independent techniques such as 16S rRNA sequencing. The respiratory microbiome is dominated by the same major phyla as the GI microbiome, including *Firmicutes*, *Bacteroidetes* and *Proteobacteria*, but with different relative abundances and a reduced total bacterial burden.[8-15] The lower respiratory microbiome is frequently compared to that of surrounding sites for microbial entry into the lungs, such as the upper respiratory tract, oral cavity and upper GI tract, and is most similar to oral microbiomes.[10, 11] Although translocation and aspiration of microorganisms from these sites are a major source of microbes in the lower respiratory microbiome,[9-12] the bi-directional movement of matter (inhalation/exhalation) coupled with robust and rapid immune responses has led some to hypothesise that the microorganisms of the respiratory microbiome are not resident and

growing populations, but are rather continuously recolonising the lower respiratory tract from surrounding sites in a dynamic and transient manner.[10, 16, 17] Nevertheless, the respiratory microbiome is distinct from that of surrounding sites, characterised by the enrichment of *Proteobacteria* and reduced abundance of *Prevotella* species.[8-13]

With greater understanding of the microbiome, its biological role at both local and distal sites has become increasingly well recognised. In this review, we explore the role of the GI and respiratory microbiomes in health and disease specifically in the Asia-Pacific region, as well as the future of microbiome research in clinical practice in this area.

Respiratory disease and the microbiome

Microbes and their host interact in an intricate and generally beneficial relationship. Conversely, the disturbance of the microbiome (i.e. dysbiosis) is commonly linked to the increased risk and severity of many diseases including of the respiratory tract. A critical role in normal development has been demonstrated in germ-free mice, which lack any resident microbes and have underdeveloped immune systems, anatomical and histological alterations, and significant digestive and metabolic disturbances. However, a healthy state can be restored in these mice by inoculation with resident GI microbes, such as through faecal transfer.[5, 18] While germ-free mice are an extreme case, factors such as age, diet and antibiotic use have been shown to maintain or impact the risk and outcomes of disease through alterations to microbiome composition.[19, 20] Conversely, the onset of disease may cause microbial dysbiosis at either local or distant body sites, which may in turn contribute to promoting the pathogenesis of disease.

Patients with chronic respiratory disease are two to three times more likely to have GI issues, while over half of inflammatory bowel disease (IBD) patients display pulmonary involvement.[21-23] These peripheral manifestations of disease highlight the

immunological crosstalk between these two mucosal sites, termed the 'gut-lung axis', whereby the immunological health of the gut impacts upon the health of the lung.[23] The GI and respiratory microbiomes are thought to play a major role in this axis as both are altered in chronic respiratory diseases, including chronic obstructive pulmonary disease (COPD),[8, 24, 25] asthma,[14, 15, 26] and cystic fibrosis (CF).[8, 27] This may involve the activation of inflammasomes, microRNAs and numerous other factors.[28-31] In disease, similar selective pressures caused by conditions in the local environment may occur at both sites, resulting in similar (but not identical) changes to microbiome composition. For example, a greater abundance of *Proteobacteria* was observed in both the faeces of smokers[24] and lung brushings of COPD patients[14], although variability between subjects confounds comparisons of separate studies. To date few, if any, investigations have simultaneously characterised both the GI and pulmonary microbiomes in respiratory disease, and so direct comparisons are difficult.

The GI microbiome affects the risk and/or outcomes of experimental respiratory infections and chronic respiratory disease through the modulation of pulmonary immune responses.[20, 32-35] It has also been implicated in GI pathology following pulmonary infection, with immune cells migrating from the lungs causing microbial dysbiosis and subsequently aberrant immune responses.[36] While the influence of the respiratory microbiome on immune responses is less well characterised, it may act as a reservoir for infections caused by *Proteobacteria* (e.g. *Haemophilus* species) in asthma and COPD,[14, 25, 37-39] or *Pseudomonas* colonisation in CF.[8] Modulation of the microbiome by prebiotic and probiotic treatment has significant benefits in the prevention and treatment of respiratory disease, although further studies are required for translation to clinical use.[20, 34, 35, 40, 41]

The Asia-Pacific region and the microbiome

The Asia-Pacific region is a large geographic area with substantial ethnic, cultural and genetic diversity.[42] Definitions of the region vary. We have used that of Jamrozik and Musk that divides countries based on income, since this has been extensively used in prior analysis of respiratory disease in this region.[43] The significant heterogeneity of the region is reflected in the growing number of Asia-Pacific microbiome studies that reveal microbial profiles shaped by ethnogeography, diet and host genetics. These studies show patterns and differences relative to Western and European studies are emerging (Figure 1). Diet and country of origin have strong influences on the microbiome and have been observed to correlate with gut microbiota composition in school children from several Asia-Pacific countries.[44] Furthermore, the dynamics of Mongolian microbiome signatures highlight reconfigurations associated with seasonal dietary changes.[45]

The Jamrozik and Musk definition includes Australia and New Zealand. These represent high-income countries with Western diets and microbiomes distinct to those found in other areas of the Asia-Pacific region. It is thought that Australia and New Zealand bear greater similarity to that of European rather than Asian populations. Additional heterogeneity in the Asia-Pacific comes from host genetics and evidence for its influence on the microbiome in the region is clearly emerging. This includes a recent Korean monozygotic twin study that shows the complex interaction between host and microbiome.[45] Other factors, such as nutritional status and cigarette smoking – a key causative agent for chronic respiratory disease – have been shown to directly influence the microbiome in India, China and Korea.[46-48] Socioeconomic status and urbanisation were also important predictors of microbiome composition in Malaysia, Thailand and Russia.[44, 49, 50] In an analysis of bacterial gut diversity in school-aged children from five Asia-Pacific countries, Nakayama *et al.*, noted a high abundance of *Bifidobacteria* (species belonging to

the genus *Bifidobacterium*); a trend previously identified in rural Russian and central Asian populations.[44, 50] Clear segregation into two distinct enterotype-like clusters was observed: one similar to the *Prevotella* enterotype described in European populations and a second representing a novel *Bifidobacteria*-rich microbiome ostensibly associated with carbohydrate-based Asian diets (Figure 1).[44] Other studies have linked fermented foods such as Japanese fermented milk products and Korean kimchi to specific alterations in the microbiota including increased abundance of such *Bifidobacteria*.[51, 52]

Commensal *Bifidobacteria* can induce altered immune responses and are abundant in early life among Western populations but decline with age.[53, 54] As the sustained prevalence of this bacterial group represents the most discernible difference between Asia-Pacific and Western microbiomes, it is interesting to speculate about its potential contribution to geographic variability in the rates of inflammatory diseases including those of the respiratory system. Regional data are also consistent with a negative correlation between gut *Bacteriodetes* to *Firmicutes* ratios with respect to latitude.[55] This trend likely reflects the environmental and dietary covariates of latitude that impact upon the microbiome. The trend is also evident in the Asia-Pacific region, although important exceptions exist. Mongolia has a northerly latitude and much colder average yearly temperatures yet the population exhibits a notably higher *Bacteriodetes to Firmicutes* ratio relative to other Asia-Pacific countries.[44, 45] Such contrasts suggest that further refinements of this concept are warranted. Despite this, the microbiome composition in the community is strongly context dependent and heavily influenced by geographic location.[54, 56]

Oral microbiome studies in the Asia-Pacific region have uncovered additional ethno-geographic differences among Japanese and Koreans that were correlated with susceptibility to periodontitis.[57] Furthermore, metagenomic analysis of Chinese subjects provided a clear demonstration of the influence of plaque microbiota on gingival

inflammation.[58] Intriguingly, oral, salivary and gut microbiomes of Chinese patients suffering from rheumatoid arthritis – an autoimmune condition with respiratory manifestations – were significantly altered compared to controls.[59-61]Thus changes in the gut microbiome related to local diets, geography or other environmental effects in the Asia-Pacific may induce changes in physiology at distal body sites or *vice-versa*, including the lung, and thus contribute to respiratory disease.[23, 62] As such, studies of the gut and oral microbiota performed in our region improve our understanding of the development and progression of respiratory diseases that afflict populations in this region, and contribute to global understanding. While direct metagenomic investigations of the airway microbiome in Asian populations are limited, several studies have emerged that have assessed diseases of increased regional prevalence such as non-CF bronchiectasis, *Mycobacterium tuberculosis* infection and lung cancer.[63-66] In the most comprehensive of these studies, Rogers and colleagues defined the airway microbiome of Australian non-CF bronchiectasis patients.[67] Importantly, lung microbiome composition correlated with lung function and permitted more refined stratification of patients into risk groups, with increasing risk of future exacerbations, compared to conventional diagnostic methodologies.[68] This remains the largest culture-independent molecular study of the lungs performed in the Asia-Pacific region to-date and provides a framework for future studies both within this area and globally.

Many diseases of particular relevance to the Asia-Pacific region including COPD, asthma, tuberculosis, and lung cancer have now begun to be assessed in metagenomic studies using high-throughput sequencing.[19, 21, 64, 65] These diseases have been directly linked to environmental exposures such as cigarette smoke, air pollution and occupational carcinogens. All these factors are of increasing importance in the Asia-Pacific region and have the potential to significantly alter the gut and lung microbiomes.[11, 43, 48, 65] In addition,

other diseases that are specific to the Asia-Pacific, such as diffuse pan-bronchiolitis, are also likely to involve an alteration in microbiomes, although this remains to be investigated from a metagenomic perspective.[69] Given the indication of macrolides as therapeutics in this disease, an analysis of the microbiota in macrolide-treated patients would be of interest and may reveal parallels with the remodelling of the microbiota seen in macrolide-treated bronchiectasis.[67, 70] The investigation of these Asia-Pacific respiratory health issues on a larger-scale particularly with the power of metagenomic studies will provide greater insight into the phenotype of respiratory disease observed in this region.

Past and present perspective on evolution of the microbiome

Respiratory and gut microbiomes in health

The microbiome has been implicated in several aspects of health, including acting as a direct competitor for pathogens, instructing the development of the immune system and the production of beneficial metabolites (e.g. vitamins, essential amino acids, short-chain fatty acids [SCFAs]). However, defining what constitutes a healthy microbiome in either the GI or respiratory tracts is a key concept that is still being satisfactorily clarified. While bacteria belonging to the phyla *Bacteriodetes* and *Firmicutes* make up the largest component of both GI and respiratory microbiota,[21, 71] there is increased variation at lower taxonomic ranks, to the extent that a bacterial species present in one healthy individual may not be detectable in another. This is more pronounced for the respiratory tract. Moreover, the microbiome is plastic, adapting to changes in its environment (e.g. diet). This leads to the concept of a 'functional microbiome', where the influence of the microbiome on health and disease is less dependent on any one species of bacteria, but rather on the presence of microbes that can facilitate a particular function, such as the fermentation of dietary fibre by *Bifidobacterium sp.* and *Faecalibacterium prausnitzii* to

make SCFAs which in turn serve as energy sources for colonic cells and as anti-inflammatory agents.[72]

The importance of the GI microbiota in digestion, intestinal homeostasis, and organ and immune system development are broadly recognised. How the microbiome contributes to normal immune system and organ development is not yet clear. Bacterial antigens such as lipopolysaccharide and peptidoglycan trigger immune responses through pattern recognition receptors such as toll-like receptors and drive the proliferation of B and T-cells in peripheral lymphoid tissue, contributing to the development of oral and systemic tolerance.[73-75] Microbial metabolites, such as vitamins and SCFAs, act in concert with host metabolism and can have additional direct effects on host immunity. Indole, a derivative of tryptophan produced by several microbial species has been linked to increased barrier function in colonic epithelial cells.[76] SCFAs, the most studied bacterial metabolites, have been linked to protection from infection, energy sources for colonic epithelial cells as well as modulation of inflammatory responses through free-fatty acid receptor 2.[77, 78]

While the microbiota of the GI tract has been extensively studied, few have investigated microbial communities of the respiratory tract, or how GI microbiota might affect extra-intestinal sites. Recent studies have shown that microorganisms are detectable in the healthy lung with culture-independent techniques. It is distinct from the microbiota found within the oral cavity, upper respiratory tract, or nasal cavity, the most likely sites of microbial entry to the lungs, and is most similar to oral microbiomes.[10, 11]

Respiratory and gut microbiomes in disease

Many studies now show that a change in composition of respiratory and gut microbiomes and dysbiosis is associated with disease. However, identifying the changes in microbiota that cause disease, rather than those that occur as a consequence of disease, remains

challenging. It is becoming increasingly clear that a dysregulated gut-lung axis plays a role in respiratory disease. This has been most widely linked to the development of asthma. Epidemiological studies demonstrate a steady increase in the incidence of allergic disease, including asthma, in developed countries, and together with recent experimental data suggest that a lack of proper exposure to varied, mostly gut-associated microbes, in early life may be a contributing factor.[35, 79, 80] Knowledge of the lung microbiota and how it influences lung health remains limited.[81] Manipulations of the gut microbiome in a bid to maintain or restore gut health is not a new concept. Probiotic formulations are sold over the counter with promises to maintain and restore digestive health and boost immunity while some yogurt is now similarly marketed. Whole community faecal microbiota transplantation (FMT), whereby faeces from a healthy donor is used to replenish or replace the microbiota of someone who is ill, had its first report in 4th century China, and was rediscovered by modern medicine in the 1950s.[82, 83] The best-documented change in microbiota causing disease is that of antibiotic-induced outgrowth of *C. difficile.* Human patients suffering from a *C. difficile* infection-induced colitis seek frequent healthcare and often take courses of antibiotics.[84] Antibiotics can disrupt both the overall microbial abundance and the community composition causing a change in the microbiome's functionality, particularly its ability to resist and compete with potential pathogenic microorganisms, such as *C. difficile*. FMT has been used with great success (90%) to restore the normal function of the microbiota to resist enteric pathogens in both the clinic and in animal models of antibiotic-induced pseudomembranous colitis.[84-86]

Antibiotics have also been implicated in the development of asthma. Children exposed to antibiotics *in utero* are at increased risk of developing asthma, as are those who are exposed to antibiotics within the first year of life, although to a lesser extent.[87] This is supported by data derived from animal models, which show that mice exposed as neonates

to vancomycin but not streptomycin developed more severe allergic airways disease when challenged with ovalbumin. Colonic CD4+ CD25+ Foxp3+ Tregs, which are known to be induced by several species of commensal bacteria,[75] were reduced in the vancomycin-treated animals.[19] This revealed that not only can antibiotic-induced changes in microbiota alter the immune response, but that these changes are antibiotic specific. Indeed we have recently shown that both the anti-inflammatory and antibiotic properties of macrolides are needed to effectively suppress severe allergic airway disease.[88] In other studies, faeces were transplanted from human infants with atopic wheeze into mice whose subsequent offspring developed more severe allergic airways. Four bacterial species were identified to be absent from the infants with atopic wheeze, which when supplemented into faeces, could protect mice from developing severe allergic airways disease.[35]

Environmental factors can also shape the microbiome and affect disease susceptibility. Cigarette smoking is the strongest risk factor for developing COPD as well as Crohn's Disease.[23] Indeed it is broadly accepted that COPD is associated with intestinal manifestations, such as decreased gut integrity and nutritional absorptivity.[23, 89] Although there are few studies, COPD patients have been shown to have altered lung microbiota compared to healthy smokers,[8] and one study has shown that cigarette smoking itself affects the composition of gut microbiota.[90] Air pollution also has effects on the respiratory system, and exposure is associated with lung cancer, COPD and asthma exacerbations.[91] While the association of air pollution to the microbiota has not been widely studied, ingested particulate matter has been shown to alter the faecal microbiota and increase intestinal inflammation in mice ,[92, 93] and a small study of sputum microbiota from never smokers residing in two regions within the same Chinese county revealed different microbial profiles associated with the two types of coal burned in those locations.[65] These strong associative links between smoke- and pollution-induced changes

to the gut and lung microbiome and respiratory disease warrant further investigation. Nevertheless, it will be difficult to demonstrate whether the microbiome changes are a cause or result of lung disease, or perhaps one of the contributing factors and further research in needed in this area.

Future perspectives of the microbiome

Antibiotic resistance and the microbiome

Recent large-scale studies have revealed unexpected links between microbiome structure, function and pathological disease. Many bacteria are harmless in a healthy system but can cause disease when the normal balance is compromised following exogenous infection, antibiotic treatment or immune modulation. What is also emerging is the role of commensal microbiota as a reservoir of antibiotic resistance genes. Antimicrobial resistance, while considered an emerging problem in modern medicine, is actually an ancient phenomenon deeply encoded in bacterial genomes from all ecological niches.[94] It should therefore be of no surprise that the human microbiome contains numerous and diverse sets of antibiotic resistance genes,[90] often organised in integrons that facilitate exchange between bacteria.[95] In addition to this natural phenomenon, escalating antibiotic selective pressure has increased the proportion of commensal bacteria carrying antibiotic resistance genes[96, 97] that may further be transmitted to opportunistic pathogens. In animal models, treatment of lung infections with high dose antibiotics rapidly selects for resistant microorganisms in the gut microbiome,[98] illustrating that treatment of a local infection impacts upon bacterial population structure and antimicrobial resistance at distal sites. Interestingly, decreasing the dose of antibiotics has a limited effect on the expansion of drug-resistant bacteria in the gut microbiota,[98] an observation that may hold important clinical relevance.

Transmission of antibiotic resistance genes between microbiota is facilitated by mobile elements such as integrons, transposons, bacteriophages[99] and even outer membrane vesicle release.[100] A high proportion of bacteria are infected by prophages that are induced and released under stress conditions, including antibiotic treatment,[101] further accelerating gene dissemination and antibiotic resistance among related bacteria. This issue is particularly well-illustrated in CF where patients receive frequent doses of antibiotics to treat chronic polymicrobial lung infections. Metagenomics analyses reveal a specific bacteriophage community in CF patients[102] that participate in the persistence and dissemination of multiple antimicrobial resistance genes.[103] This is correlated with the observation that the complex, pathologic, respiratory microbiota in CF is highly resistant and resilient to antibiotic treatment.[104, 105] Since the presence of *Pseudomonas aeruginosa* is associated with poor clinical outcome in these patients,[106-108] antibiotic treatment is typically designed to target this pathogen. Interestingly, a clinical study revealed that *P. aeruginosa* was not only largely resistant to antibiotic treatment, its relative abundance even increased significantly with treatment during episodes of pulmonary exacerbations, which may result from reduced competition with resident respiratory microbiota.[105]

The emergence and persistence of antibiotic resistance genes is a complex process that should be considered from an ecological perspective to be driven by complex interactions between the commensal, pathobiont and pathogenic bacteria composing our microbiome. This view advocates for the development of narrow-spectrum antibacterial agents that are less likely to disturb the ecology of the core microbiota or promote the transfer of resistance genes across bacterial species.

Aging and the microbiome

As we age, so do our microbiota, promoting change within it.[109, 110] In the gut, transition from a variable to stable microbiome occurs in the first three years of life.[54] During this period our immune system interacts with, and is likely influenced by commensal microbes.[111] Interactions with such commensals are essential for normal immune development and have key roles in the development of a healthy immune system in later life.[53] This is reflected in animal studies where disruption of gut microbiota leads to subsequent inflammatory airway disease.[112, 113] This translates well to human studies where reduced gut diversity in early life predicts subsequent development of asthma in schoolchildren.[114]

During aging the alterations in our microbiomes closely correlate with increases in pro-inflammatory markers, particularly in the elderly, including TNF-α, IL-6, IL-8 and C-reactive protein. Interestingly, increasing levels of IL-6 and IL-8 with age are associated with the enrichment of *Proteobacteria* and declines in butyrate producing bacteria.[109, 110]

Evidently, the gut microbiome has a significant bearing on respiratory disease and *vice-versa*. Further, its influence on the immune system likely underpins many facets of airway inflammation and infection that evolve with age and immunosenescence.[62, 115, 116] The emerging role of the microbiome in shaping our immune system and its loss of function with age may provide a context for understanding the impact of age-associated immunosenescence and "inflamm-ageing" on respiratory diseases such as COPD, late-onset asthma and pulmonary fibrosis.[62, 116, 117] The most extensive investigation of age-related effects on the pulmonary microbiome has been performed in CF patients revealing a decline in lung microbiome diversity with both age and lung function.[118] However, an authoritative metagenomic analysis of age as a predictor of the human airway microbiome in healthy subjects, such as that performed by Yatunenko and colleagues in the gut is yet to be undertaken.[54] Our understanding of the respiratory microbiome as a function of age thus

remains largely unexplored. This is coupled with the knowledge that the interplay between the microbiome and immune system is important and continues to reveal novel interactions. Respiratory microbiota could exist as a stable entity that is modified over time or is transient with continual re-seeding from the environment and cleared by immune responses and could be defined as a set of microbial genes rather than species. Nevertheless, defining respiratory microbiota throughout different stages of life is likely to advance our understanding of age-related lung disease such as COPD, asthma, pulmonary fibrosis, bronchiectasis and CF, offering opportunities for novel diagnostic aids and therapeutic approaches.[62]

Elucidating relationships between microbiomes and health and disease

Many studies of the roles of microbiomes in health and disease come from the cross-sectional assessment of clinical samples. This identifies associations but not causal relationships.[21] Mechanistic interactions can be elucidated in prospective human studies but these are long-term, expensive and affected by inherent genetic and environmental variabilities meaning that they need to be undertaken on a large scale.[35] Animal models are valuable in elucidating these relationships. They are genetically uniform, diets and environments can be tightly controlled, and are relatively quick and inexpensive experiments to perform. They can then be associated with changes in human disease and even if the precise taxa involved are not the same, but play similar functional roles, relationships can be determined. New animal models of COPD, severe asthma and their exacerbations as well as other diseases that closely resemble different human phenotypes have recently been developed.[88, 119-128] They are therefore likely to be valuable in facilitating the evaluation of relationships between microbiota, health and disease. These

can be combined with *ex vivo* studies using primary cells from humans,[119] including with microbial challenge.[129-131]

How to best translate findings into clinical care practice and bedside application?

A major goal in studying microbiomes is to translate findings into more targeted therapy and ultimately improved patient care. To this end, a promising area of application lies in the ability of microbiome status to predict disease severity and prognosis in respiratory diseases, including COPD, asthma, CF and non-CF bronchiectasis.[68, 114, 118, 132] Although we are only at the beginning of cataloguing our lung-associated microbiota, signals of disease are clearly emerging, some of which have already been exploited in stratifying patients according to clinical outcomes. Direct clinical application therefore hinges on whether information gained in measuring or quantifying microbiota can stratify patients with greater precision than conventional methods, or indeed recognise novel disease phenotypes that require special attention. Analysis of microbiota in non-CF bronchiectasis for example has shown that community profiling predicts disease, inflammation and disease-related outcomes more accurately than standard detection of known pathogens through conventional culture or even molecular methods.[68] Further and larger scale studies that specifically address respiratory disease states are clearly necessary and likely to reveal further information on disease onset, pathogenesis, progression, and underlying aetiology.

Knowledge of microbiome composition may be leveraged clinically to identify patients early with a poorer prognostic course and permit targeted and dedicated care.[133] Metagenomic analysis reveals clinical and environmental variables that perturb the microbiome (e.g. antimicrobial therapy or infection) and influence patients toward dysbiosis and associated disease states such as the unintended antibiotic-induced development of *Clostridium difficile* infection. Restoration of microbial diversity through

FMT has been successful in the gut;[134] however, how this may be applied to the lung remains unclear particularly in context of complex environments such as chronic lung infection and colonisation states. Thus, influencing respiratory disease through the gut-lung axis may be a more realistic and practical approach with early evidence emerging from pre-clinical studies.[135] Novel approaches such as probiotic therapy, more selective antimicrobial therapy and diet alterations also hold promise. Understanding resulting consequences for infection, immunity and inflammation in the lung using such approaches is an important first step and intervention during early life may therefore have greater impacts on respiratory disease outcomes and prevention as one ages.

Conclusions

It is now clear that changes in respiratory microbiomes accompany respiratory diseases and these changes are currently being catalogued. They are impacted by environmental exposures including cigarette and other forms of smoke [43], air pollution and infections, as well as antibiotic treatment. It is emerging that the GI microbiome may also impact the respiratory system, which, in turn are impacted by diet and antibiotics, although the effects are much less clear. The changes in respiratory and gut microbiomes could be a consequence of the development of disease or they could be a causal factor. Changes in inflammatory and immune responses in disease could be the cause of these changes. Conversely, alterations in microbiomes could alter the factors that they release including bacterial components and metabolites such as SCFAs that alter the pro- and anti-inflammatory balance. There are specific issues at play in the Asia-Pacific region with the prevalence of particular diets, respiratory diseases and latitudinal effects. Although to date numerous associational studies have been undertaken there have been few causal studies. These could involve long-term prospective clinical studies, and studies with animal models

that accurately reflect disease features and work with primary human cells *ex vivo*. There is the real potential for disease modification using microbiome alterations with FMT, diets or targeted antibiotics and as the field progresses these could be new therapeutic options for respiratory disease if they can be translated into the clinic.

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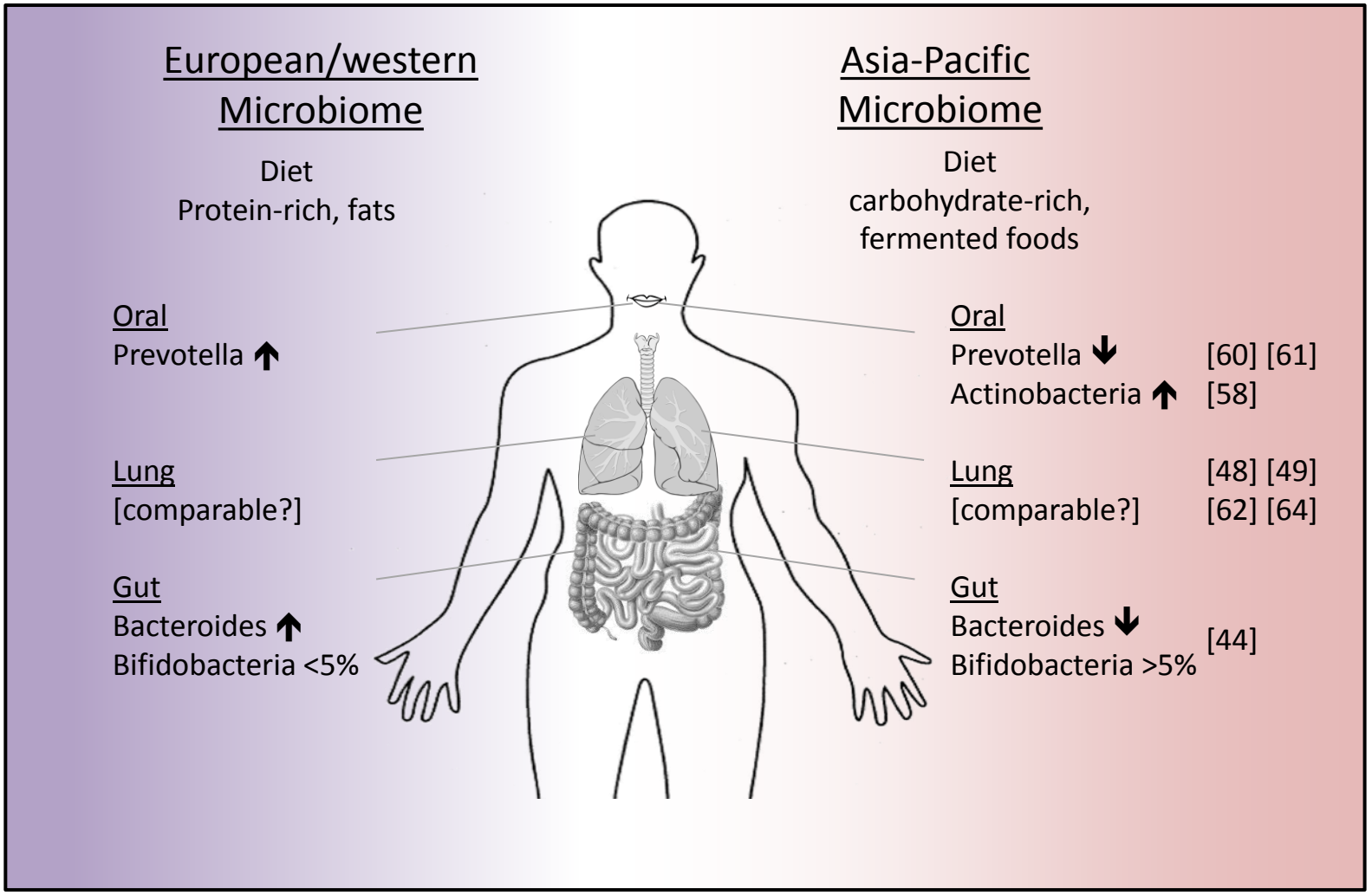
Figure legend

Figure 1. Summary of the established microbiome variations identified between Western and the Asia-Pacific and other countries shaped by differences in ethnogeography, diet and host genetics. While studies are limited, and further work required, a number of trends are emerging from early studies. Studies supporting these trends are cited in the figure.


European/western Microbiome
Diet
Protein-rich, fats
Oral
Prevotella ↑
Lung
[comparable?]
Gut
Bacteroides ↑
Bifidobacteria <5%
Asia-Pacific Microbiome
Diet
carbohydrate-rich, fermented foods
Oral
Prevotella ↓ [60] [61]
Actinobacteria ↑ [58]
Lung [48] [49]
[comparable?] [62] [64]
Gut
Bacteroides ↓
Bifidobacteria >5% [44]