Gut-lung axis microbiome: Towards precision medicine in respiratory disorders (Review)

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Abstract. The association of human respiratory diseases and the gut microbiota is a novel diagnostic area for the exploration of health research. The gut microflora is a naturally occurring symbiotic environment that is greatly required for various biological processes. It has been also now found that the alterations in the organisms is directly linked with the extent of invasion by disease. The present review article discusses the microbiome of the gut-lung axis in different respiratory disorders, associated beneficial microbes in reverting the disorders, and nutrient supplementation in the form of prebiotics and probiotics in order to incorporate the healthy gut microbiota. The present review also discusses the technologies including the concepts of omics, text mining and machine learning approaches in the field of precision medicine research.

Contents

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1. Introduction

Over the years, research on the gut microbiota and its influence on respiratory health has evolved into the concept of the gut-lung axis. Initially regarded as a simple connection, studies in the 1990s and early 2000s began exploring the role of probiotics in supporting immune function and reducing respiratory infections (1-7). The discoveries laid the foundation for understanding the intricate association between the microbiome and the immune system in maintaining lung health. A deeper understanding of the role of the microbiome in both healthy and diseased individuals has significantly contributed to identifying biomarker patterns, aiding in early disease detection and personalized treatment strategies. The integration of microbiome research into precision medicine has been particularly impactful, as specific beneficial bacterial strains have shown resilience to physiological stress, providing promising therapeutic potential. Patients with a well-defined microbial niche could benefit from next-generation treatments tailored to their microbiome composition (8).

Microbial communities within the human body play essential metabolic and immunological roles. The lung microbiome, primarily composed of *Pseudomonas, Streptococcus, Prevotella, Fusobacterium, Haemophilus, Veillonella* and *Porphyromonas* (9), contributes to respiratory health through the production of small-chain fatty acids (SCFAs), which help regulate immune responses. While precision medicine was initially centered on genetic and phenotypic variations, there is currently a paradigm shift towards the understanding the microbiome as a key factor influencing health and disease (10).

Investigating the crosstalk between gut and lung microbiomes in various diseases holds promise for the development of novel diagnostic markers and advancing personalized medicine (Fig. 1). This evolving perspective opens new avenues for integrating microbiome-targeted therapies into clinical practice.

2. Precision medicine and the human microbiome

Precision medicine is based on the deep characterization of the genome, proteome, metabolome and microbiome of an individual. Rapidity and technical advancements in genome studies (DNA-Seq, RNA-Seq, Exosome-Seq, etc.) decreased the cost of sequencing and genotyping that typically shifted the paradigm of precision medicine from academic exercise to clinical applications (11). The milestone projects, such as complete human genome sequencing, HapMap (Haplotype Map) project, Phase 1- NIH human microbiome project, and the phase 1- European Union MetaHit (Metagenomics of the Human Intestinal Tract) Project contributed to the understanding of the molecular basis of diseases and to the development of initiatives to propose new prototypes of personalized medicine (12,13). The most recent advancement in understanding human disease is to derive its association with the human microbiota. The human microbiome is referred to as the second genome, as trillions of microbial inhabitants are integrated ecologically and symbiotically with their host. The analyzed omics profile of an individual aids in the prevention, diagnosis and treatment of diseases. Knowledge of human genomic variants and phenotypic changes is critical for understanding the molecular basis of the disease (14). The human body colonizes diverse communities of microbes, and also resists the colonization of pathogenic organisms, such as *Clostridium difficile* (15), which clearly suggests the existence of symbiotic associations between the human body and its microbiota. A previous study confirmed the presence of bacterial peptides in blood serum and plasma of approximate range/of 0.1 nM to 1 μM (16). That study utilized the human microbiota protein sequences from NIH Human Microbiome Project which as useful for determining the microbiota composition in the intestinal regions. Well-structured databases are available to provide the genotypic and phenotypic related variants and related traits [ClinGen (https://clinicalgenome.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), dbVar (https://www.ncbi.nlm.nih.gov/dbvar/), The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/ccg/research/genome-sequencing/tcga), dbGENVOC (https://research.nibmg.ac.in/dbcares/dbgenvoc/home.php), HGMD (https://www.hgmd.cf.ac.uk/ac/index.php), OMIM (https://omim.org/), etc.]. The concept of 'One dose fits all' is ideologically replaced with personalized medicine to ensure a tailored treatment with effective and safe drug usage. For an accelerated research, the accessibility to Electronic Health Records (Electronic Medical Records and Genomics-Pharmacogenomics (eMERGE-PGx) project, GANI_MED project, SCAN-B initiative and Cancer 2015) provides an in-depth analysis of the medical history of an individual against various infections and higher predictive nature to derive the susceptibility of the patient to other comorbid diseases. Other than this, knowledge-based approaches derived from documented literature utilize sophisticated text-mining and natural language processing for literature curation and annotation to extract the vast amount of information buried about the genetic variants and their functional phenotypic associations (17).

3. Role of the gut microbiome in precision medicine: Gut-lung axis microbiome

In recent days, 'Gut Microbiota' is a buzz word and often termed as a forgotten organ/metabolic organ that plays a fine-tuned symbiotic association with the host. Hundreds of trillions of diverse microbial communities that include protozoa, fungi, archaea, viruses, protists and bacteria reside within the gastrointestinal tract (GIT) compared to the regions of skin, eye, urogenital and epithelial layers of the respiratory system. In general, the microbiome of an individual is more complex than the human genome and it imparts a specific immunity to the individual that characterizes the need to have personalized medicine. According to the studies of the Human Microbiome project (18), it was revealed that the ratio of commensal microbial genes to the total number of human genes was high and the adult human gut can harbor trillions of microbes; therefore, it exceeds the total number of somatic and germ cells by 10-fold (19).

The composition and pattern of the microbiota of an individual is very specific and hence, it enables the identification of disease-specific microbial signatures, providing personalized predictive biomarkers for various diseases (20,21). The insights from the gut-lung axis and the identification of microbial patterns aids in the early identification and diagnostics based on various immunological responses. With respect to conventional therapies, this also triggers microbiome-targeted interventions, such as probiotics, prebiotics and fecal microbiota transplantation, which are more promising and safe strategies (22,23). Given the complexity of microbiome data, analyzing it is a challenge. which is strategically now being done using various machine learning, deep learning and more recently quantum methods (24). The integration of microbiome-based diagnostics and therapies into clinical frameworks has the potential to optimize respiratory illness care, while maintaining microbial equilibrium, as precision medicine moves beyond genetic and phenotypic differences.

The reasons for studying the gut microbiome instead of the lung microbiome for addressing lung disorders. The lung was originally described to be a sterile organ; however, with technical advancements, the lung microbiome was then defined, which is composed of a complex and diverse bacterial community, with a low biomass. There is substantial variation between upper and lower respiratory tract microbiomes due to higher flux exhibited by microbes (25). Different environmental niches are created as a result of the differential availability of pH and nutritional factors, which plays a critical role during inflammation or through structural changes in chronic respiratory diseases (CRDs) (26). The respiratory microbiome is also combined with the upper respiratory tract and gut microbiota, which renders the assessment of its role difficult (27,28).

The gut-lung axis refers to the communication between the gut, lung and vice versa, where the gut microbial metabolites enter the lung via the blood stream and influence the

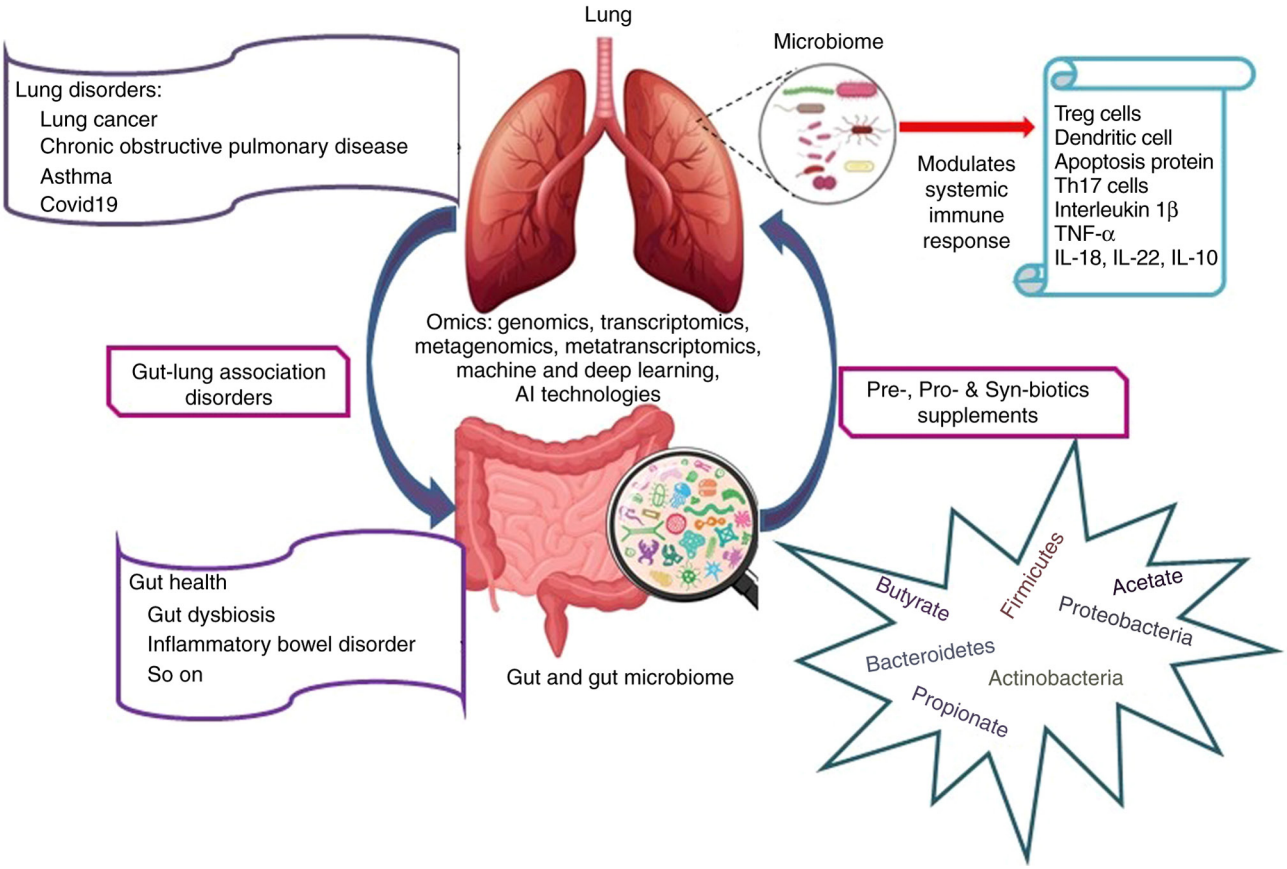

Figure 1. Integrating the gut microbiome dysbiosis to treat lung disorders through omics and machine learning technologies.

pulmonary microbiome. Emerging scientific reports demonstrate the modulation of systemic immune responses through altering the diet and antibiotic treatment. In addition, an altered lung microbiome in various chronic lung disorders indicates that the gut-lung axis is bidirectional (29). Immunological crosstalk between the respiratory and gut microbiome is reflected by the fact that patients with chronic respiratory disease are prone to GIT-related issues and vice-versa, where patients with inflammatory bowel disease display pulmonary involvement (30).

The gut and lung microbial communities overlap in their phylum, but differ in local compositions and total microbial biomass (31). This overlap may be responsible for microbial interference in different disease associations. The gut microbiota modulates immune responses differently in healthy individuals and in patients with lung disorders, which is dependent on diet and food intake. Diet-Microbiota-Immunity link supplemented by high fiber diet is well explained in different studies (30). The fiber intake influences the gut microbiota by increasing the production of bacterial metabolites, such as SCFAs (e.g., propionate, acetate and butyrate) (32); this in turn modulates different aspects of defense lines, such as promoting goblet cell differentiation and mucus production, stimulating the proliferation of T-regulatory cells which produces anti-inflammatory cytokine (e.g. IL-10), as well as the production of intestinal IgA by affecting plasma B-cell metabolism and modulating tight junction permeability to enhance intestinal epithelial barrier function in the gut of the host (33).

The so-called exposome that includes socio-economic factors, nutrition, stress, environmental factors (pollutants, antibiotics, etc.) and the individual's genetics, sex and age composes the microbial diversity of the GIT (34). Metabolites produced by the gut microbiota are capable of eliciting or activating the downstream cascades of reactions. The continuous interplay between the gut microbiota and intestinal epithelium sends signals for the regulation of immune responses to infectious diseases and disorders. In the case of infectious diseases, the microbial colonization of commensals and pathogenic organisms undergo crosstalk with host immune responses (35). The consumption of a healthy diet could modulate the local and systemic host physiology, metabolism, immune function and nutrition state.

In the GIT, the bacterial cell-cell communication takes place through the exchange of chemical messages using quorum sensing mechanisms that are capable of triggering changes in the gene expression. Extensive studies on quorum sensing between bacterial communities in a local niche are being carried out; the GIT is a complex system where bacteria respond to a milieu of chemical signals to synchronize bacterial behavior and convert them into reliable messages to coordinate the relative species, as well as with the host immune system. Hence, in the GIT, multidirectional communication pathways of quorum sensing occur and are referred generally as interspecies communications and interkingdom signaling (36). Gram-negative bacteria produce N-acyl-homoserine lactones (AHLs) that bind to LuxR receptors to communicate with each other and their accumulation to a threshold concentration

Table I. Formation of SCFA products in the GIT.

Name of SCFA	Formation/pathways	Microbes utilizes the pathway	Function	(Refs.)
Acetate	1. Decarboxylation of pyruvate followed by Acetyl-CoA is hydrolyzed to acetate by an acetyl-CoA hydrolase 2. Wood-Ljungdahl pathway	*Prevotella* spp., *Ruminococcus* spp., *Bifidobacterium* spp., *Bacteroides* spp., *Clostridium* spp., *Streptococcus* spp., *Akkermansia muciniphila*, *Blautia hydrogenotrophica*	1. Insulin level maintenance 2. Body weight loss 3. Energy production for other cells of host 4. Lipid synthesis 5. Protein acetylation	(49)
Propionate	1. Succinate pathway 2. Acrylate pathway 3. Propanediol pathway	Bacteroidetes and several Firmicutes, *Coprococcus catus*, *Salmonella enterica serovar Typhimurium*, *Roseburia inulinivorans*, *Akkermansia municiphilla*	Gluconeogenesis in the liver Inhibits histone deacetylase	(50)
Butyrate	1. Classical Pathway: phosphotransbutyrylase and butyrate kinase enzymes are involved 2. Butyryl-CoA: acetate CoA-transferase converts butyryl-CoA into butyrate and acetyl-CoA	*Ruminococcus bromii*, *Coprococcus* species, *Eubacterium rectale, Eubacterium rectale/Roseburia spp., Faecalibacterium prausnitzii*	Intestinal and extra-intestinal functions	(51)

activates the gene expression for toxin production and biofilm formation. Certainly, AHLs influence the host immune system through the secretion of IL-10, where their specific functions are to limit the host immune response towards pathogens; in addition, due to the lipid solubility nature of AHLs, they diffuse into the mammalian cells and induces apoptosis through cascades of reactions (37). A more systematic detail of the crosstalk of metabolites through quorum sensing that interrelates with the gut microbiota and host immune system has been previously discussed (36).

4. Microbial niches and their metabolites in the gut homeostasis

Gut microbes mostly constitute the phyla of Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and occasionally, Verrucomicrobia and Fusobacteria (38-40). Of these, Bateroidetes and Firmicutes constitute ~90% of intestinal microbiota. Firmicutes are abundant in populations consuming animal-based diets, whereas Bacteroidetes are largely found in populations consuming plant-based diets (41). The genes for the hydrolysis of plant-based polysaccharides are reported in the bacterial strains of *Prevotella* and *Xylanibacter;* therefore, the GIT possesses higher counts of these organisms in populations depending on plant-based food varieties. Among the Indian population, the genera of *Prevotella, Lactobacillus* and *Carnobacterium* are abundant, owing to vegetarian diets of the majority of the population (42). SCFAs are the most abundant form of saturated aliphatic organic acids where 10% of SCFAs are constituted by the daily diet and 70% are generated as a product synthesized through the microbial niche (43), found within the GIT (Table I). The concentration of SCFA plays a crucial role at local (colon) and systemic (blood) levels for immune regulations (44,45). SCFAs supplied from the diet are utilized by the host for its other metabolic process, whereas microbial-synthesized SCFAs are employed as energy sources for epithelial and endothelial cells of the colon (colonocytes). In a normal human gut, the ratio of the SCFA concentration comprises 60% of acetate, 25% of propionate and 15% of butyrate (46). Alterations in the microbiome may influence the susceptibility of an individual to infectious diseases. For example, the rate of ethanol-induced liver damage is reduced with the supplementation of butyrate than acetate (47). SCFAs regulate immune functions via several receptors and pathways; specifically, G-protein coupled receptors (GPCRs) namely GPCR43 and GPCR120 enhance reactive oxygen species-mediated killing, phagocytosis and cytokine production, leading to apoptosis (48).

5. Significance of pre- and probiotics

Prebiotics are non-digestible food ingredients that have a positive impact on the composition of the gut microbiome and their metabolic function at the level of the small intestine and colon. Alternating the nutrient supplements selectively stimulates the growth and/or activity of one or a bacterial community within the colon. Prebiotics and dietary fibers promote the growth of beneficial bacteria in the gut by acting as a substrate for fermentation (49-51). Prebiotic supplements maintain the homeostasis barrier and elevate mineral absorption, thereby modulating energy metabolism and satiety. The relevance of microbial diversity and its association with metabolizing

prebiotics and dietary fiber in the upper and lower GIT, as well as the colon has been previously discussed (52,53).

Probiotics are supplements with live bacterial feed; when supplied in adequate quantity, they provide health benefits to the host. The genera *Lactococcus* and *Bifidobacterium* strains are predominant in the gut. These microbes are considered significant for use as probiotics due to their exclusive properties, such as acid and bile tolerance, adhesion to mucosal and epithelial surfaces, antimicrobial activity against pathogenic bacteria and bile salt hydrolase activity. The rationale behind the supply of probiotics is to reshape and strengthen the commensal microbes to compete for nutritional and functional resources with the pathogens and to produce antimicrobial substances (54). However, factors such as safety, product compatibility, viability of the microorganism, pH, packaging, storage conditions and food processing should be considered in the case of probiotics supplementation. Therefore, probiotics should be improved for their viability and stability to achieve the 100% establishment of microbes in the GIT. Soft cheeses have an added advantage over yogurts and fermented products for their delivery system of viable products to the GIT. The selection of probiotics strains should meet up with the regulations according to the World Health Organization (WHO), the European Food Safety Authority (EFSA) and Food and Drug Administration (FDA) for their safety and functional criteria (55). Technologies, including microencapsulation, straw delivery system and viable spores of spore forming organisms are being improvised to overcome the delivery of live microorganisms into the GIT; however, advanced techniques to supply other beneficial microbes as probiotics need to be identified (56).

Dietary supplements can be a combination of prebiotics and probiotics, that are together represented as either conbiotics and as synbiotics. The use of synbiotics improves the survival rate of beneficial microbes and supplies the respective food or feed required for their proliferation. The challenge in synbiotics supplementation is to identify the right formulations of prebiotics and probiotics that are capable of modulating the intestinal microbiota (55).

6. Human microbiome and its association with diseases

Microbiome disease associations are very complex and various omics approaches are integrated to understand them. A major concern is the ongoing evolution of the microbiome and its direct effect on metabolite production and its susceptibility towards any disease, since it has the ability to modulate the immune system. The human gut is inhabited symbiotically by a complex and metabolically active microbial ecosystem, which systematically controls the metabolic activity of humans. In healthy individuals, the microbiota bacteria present in the lungs are *Pseudomonas*, *Streptococcus*, *Prevotella*, *Fusobacterium*, *Haemophilus*, *Veillonella* and *Porphyromonas* (57). The microbial metabolic products are of great use for maintaining gut homeostasis and their varied taxonomic associations provide detailed information on the diagnostic features of the individual for personalized medicine. Nutritional supplements mostly include dietary fibers, prebiotics and probiotics that play a critical role in the gut homeostasis, and have also been reported to ameliorate chronic disorders (58). The association between food intake, microbiome health and the occurrence of various diseases has been studied. CRDs includes a range of lung disorders and are major concerns among the global health burdens. CRDs affect the airways and lungs; among these, chronic obstructive pulmonary disease (COPD) is the third leading cause of mortality worldwide (59). Frequent exposure to polluted air, hazardous chemical substances such as carcinogens, poor lifestyle habits (smoking and alcohol consumption) are key factors for the increased prevalence of asthma and lung cancer (60,61). COVID-19, although an acute respiratory syndrome, increased risk of mortality of patients infected with COPD and asthma (62).

The microbial composition in diseased lower airways exhibits a decreased abundance of the phylum Bacteroidetes and increased *Gammaproteobacteria* in contrast to the healthy population. These Gram-negative pathogens of *Gammaproteobacteria* are capable of utilizing the inflammatory byproducts for their survival under anaerobic and lower oxygen conditions (63).

Cancer. The association between diet and cancer (colorectal, post-menopausal breast cancer) was recognized by the Dietary Guideline Advisory Committee (DGAC) in 2015 (64). It has been observed that the consumption of a high-fat ketogenic diet (HFD) affects the intestinal stem cell functions through the metabolite β-hydroxybutyrate-mediated Notch signaling through the expression of the gene, 3-hydroxy-3-methylglutaryl-CoA synthetase 2. A HFD along with glucose inhibits the 3-hydroxy-3-methylglutaryl-CoA synthase 2, an enzyme involved in the production of beneficial ketone bodies (65). Switching from glycogenic to ketogenic energy consumption benefits the growth of normal cells instead of cancer cells by limiting the glucose production (66). At present, the University of Iowa is investigating the potential effect of a ketogenic diet along with chemoradiation therapy for non-small cell lung cancer (NCT01419587).

Butyrate has pleiotropic functions and plays a role in the apoptosis of colon cancer cells, intestinal gluconeogenesis and in controlling gut dysbiosis. Butyrate indirectly regulates the health of the lungs through systemic inflammatory processes; treatment with butyrate attenuates inflammation and promotes mucus production in the lungs (67). Mucin production is promoted by the butyrate-producing bacteria, *Roseburia* spp. Although the mechanisms connecting the gut-lung axis have not yet been elucidated, there is some scientific evidence indicating that variant associations among species and their relative abundance can be used as markers for lung cancer prediction (68). The enhanced activation of glutamate metabolism requires the consumption of the majority of butyrate in the cells of lung cancer; therefore, the shortage of butyrate for mucin production leads to its destruction and causes inflammation (69). Consistently, dysbiosis has been found to occur in the gut microbiota among patients with lung cancer and the decrease in butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, *Clostridium leptum*, *Clostridium* cluster I, *Ruminococcus* spp., *Clostridium* cluster XIVa and *Roseburia* spp. are observed in NSCLC. Therefore, the mentioned butyrate-producing bacterial strains along with *Clostridium* cluster IV and *Eubacterium rectalis* can be considered as gut probiotics. In addition, these butyrate-producing

bacteria have attracted attention due to their ability to maintain the gut homeostasis (70). The three proposed theories for microbial dysbiosis for the onset of carcinogenesis include the disruption of the immune equilibrium, the initiation of chronic inflammation and the activation of cancer-causing pathways (71).

In lung cancer, cell proliferation is associated with various microbial genera. The microbiome number is altered in the normal and diseased condition. The most dominant phyla in the lungs of healthy individuals are *Actinobacteria*, *Bacteroides*, *Firmicutes* and *Proteobacteria* (9). Variations in these microorganisms are observed in different types of cancer. For example, in the patients affected with squamous cell carcinoma of the lung and adenocarcinoma, the genera *Capnocytophaga*, *Selenomonas* and *Veillonella* are abundant when compared with the genus *Neisseria* (72). These microbiota thrive due to their expression and their role in the activation of signaling and cell proliferation pathways. For example, the β-catenin signaling pathway, which promotes cell proliferation, is activated by *Fusobacterium nucleatum*. Estimating the occurrence of levels bacteria such as *Fusobacterium nucleatum* may be useful for predicting the progression of cancer. It has also been observed that patients with lung cancer, when compared with those with benign disease, have an abundant population of *Veillonella* and *Megasphaera*. Genetic variations in individuals are also helpful for developing biomarkers. Patients suffering from squamous cell carcinoma of the lung, lung cancer or benign lung disease with the TP53 mutation have been shown to have abundant levels of the genus *Acidovorax*. Microbiota dysbiosis can contribute to the pathogenesis of lung cancer, as it leads to the upregulation of IL6/8, IL17A, MAPK and inflammasome pathways (72).

In a previous study, when probiotic supplements with aqueous extracts of *Bifidobacterium* species were to NSCLC cell lines (A549, H1299 and HCC827) particularly reduced cell proliferation with the increased expression of cleaved caspase-3 and cleaved poly(ADP-ribose) polymerase (PARP) associated with the apoptotic pathway (73). In another study, the additional supplementation of probiotics containing *Bifidobacterium* and *Clostridium butyricum* in patients with NSCLC receiving anti-programmed cell death-1 therapy (nivolumab, or pembrolizumab monotherapy) increased their survival state (74). *Bifidobacterium* activates dendritic cell function and directs the T-cell mediated antitumor efficiency, whereas *Clostridium butyricum* arrests cancer cell invasion and migration by reducing Th17 cells (75). It has been shown that bacterial supplements with *Akkermansia muciniphila* along with butyrate inhibit tumor cell proliferation and migration by upregulating the expression of miRNA (interference RNA) (76). Treatment of NSCLC with propionate regulates survivin, an inhibitor of the apoptosis protein family and suppresses p21 expression and the cell cycle (77). To date, the full regulatory mechanisms of the gut microbiome in systemic inflammation and lung diseases are not yet completely known; thus, it is necessary to take a multidisciplinary approach to elucidate these mechanisms.

COPD and asthma. COPD is a fatal lung disorder and an irreversible obstruction in the respiratory tract potentially accompanied with progressive decline in the function of lungs. The pathologies include chronic bronchitis, airway remodeling and emphysema. One of the major causes of COPD is smoking. There is a visible difference between the gut microbiota of healthy smokers and non-smokers (78). The intestinal levels of Firmicutes are decreased and the count of Bacteroidetes increases in smokers, whereas when an individual quits smoking, the effects are vice versa. The production of SCFAs is highly variable between smokers, non-smokers and in those who have quit smoking, and this has direct response to the immune response exerted in the lungs. The core microbiome of the lungs is mainly composed of *Pseudomonas, Streptococcus, Prevotella, Fusobacterium, Haemophilus, Veillonella* and *Porphyromonas*; these also play a protective role by producing SCFAs. For example, butyrate secreted by the gut microbiota can reduce the lung damage caused by cigarette smoke-induced-COPD, by inhibiting the mevalonate pathway in the gut, lungs and liver. However, the inflammation/emphysema pathological condition can be reduced by supplementing with a high fiber diet and probiotic intake of *Lactobacillus rhamnosus* and *Bifidobacterium* (79,80). The lung microbiota of patients with COPD exhibits an increase in *Gammaproteobacteria*, whereas healthy lungs exhibit Bacteroidetes (81).

The lung microbiome is frequently assessed through samples, such as serum, sputum, bronchoalveolar lavage fluid (BALF) and lung tissue, whereas for the gut microbiome, fecal samples are used. In a number of respiratory disease conditions, the spatial heterogeneity in the composition of the lung microbiome is highly variable; however, within an individual, the microbial spatial variation is less among healthy populations and either the segment of lingula or BALF from the right middle lobe are used for microbial assessment (82). Of note, in COPD the microbial diversity varies with the sampling from upper and lower bronchial regions of the lung (83). Exacerbations of COPD, are often caused by respiratory viruses or bacteria; these exacerbations are associated with markedly increased morbidity and mortality rates (84). Studies on the dynamics of the microbiome in different exacerbation phenotypes have indicated that diverse patterns of microbiota existence are associated with the types of exacerbation events (types 1, 2 and 3) (85,86). Therefore, for the understanding of disease etiology, associated changes in the microbial community and cytokine profiles are key features to be explored towards the development of more effective therapeutic strategies. The microbial niches and their associated mediators of inflammation for *Haemophilus* are IL-1β and tumor necrosis factor-α in sputum, as well as neutrophilic inflammation and *Moraxella,* which are related to Th1 pathways such as interferon signaling (87). The evaluation of the gut microbiome does not indicate the severity of COPD; however, the varied prevalence of microbiota at different stages of COPD has been observed (88). Diet plays a major role in maintaining microbial diversity; mortality rates due to COPD have been found to increase with western diets and may be reduced among populations consuming diets rich in antioxidants and fiber (88).

Medications for COPD include antibiotics, steroids and the use of bronchodilators, which exert effects on microbial composition. Recent day challenges, such as the emergence of multidrug resistance for several antibiotics and metal-based drugs with the capability of modulating the microbiome in

COPD have been previously discussed (71). A comprehensive elucidation of the existing evidence, challenges and the future concerns regarding the possible use of the lung microbiome either as a potential biomarker or therapeutic target for COPD has been previously provided (89). The microbial distribution of the lung and gut in COPD has also bene previously detailed (83).

COPD and asthma are the two most frequently diagnosed chronic respiratory diseases and it has been suggested that the oral, lung and gut microbiota are associated with these conditions. Asthma is a multifactorial and heterogeneous disease characterized by the presence of airflow limitation associated with chronic bronchitis or emphysema. Microbiota composition in the respiratory tract mainly consists of Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, etc. and it is suggested that the development of allergic diseases such as asthma is dependent on these, as they influence inflammation via the gut-lung axis (90). As regards gut microbial dysbiosis, the lower abundance of Firmicutes increases the risk of asthma and as with COPD lung microbial composition, *Proteobacteria* such as *Haemophilus* and *Moraxella* are the predominant among the lung microbiome (91).

Delivering probiotics orally has its own efficiency in immune cell regulation in the respiratory system; the most common probiotics include lactic acid-producing bacterial species such as *Lactobacillus, Streptococcus, Bifidobacterium* and *Enterococcus*, as well as the non-pathogenic yeast, *Saccharomyces boulardii*. The systemic immune modulating capability of Lactobacilli has been found to affect the cytokine profile, T-cell proliferation, and the phagocytic activity of mononuclear cells and natural killer (NK) cells (92,93). Research has demonstrated the involvement of immune cells like Th17 (T-helper) and Treg (regulatory T-cells), which indicates the role of $CD4^+$ T-cells in the production of pro-inflammatory cytokines, as well as the transcription factor FoxP3 + CD4 cells in the immune homeostasis. The administration of *Lactobacillus reuteri, Bifidobacterium lactis* Bb12, *Lacticaseibacillus rhamnosus* strain GG (LGG) and *Lactobacillus casei* increase the levels of these immune cells (94,95). In asthmatic conditions, the reduction of airway hyperresponsiveness and the number of inflammatory cells in BALF and in lung tissue have been reported with the supplementation of *Lactobacillus reuteri*, LGG and *Bifidobacterium breve* (96). Furthermore, probiotic supplements with the heat-killed *Mycobacterium vaccae* confer protection against airway allergic inflammation (97). Intaking *Enterococcus faecalis* FK-23 attenuates TH17 cell development as an asthmatic response, whereas NK cell impairment in lungs due to cigarette smoking indicates an increase in NK cell activity with the supplementation of *Lactobacillus casei* (98). The markers of systemic inflammation and oxidative stress, such as plasma C-reactive proteins and 8-isoprostane are significantly reduced by probiotic treatment. Moreover, multistrain probiotics stabilize the neuromuscular junction by improvising muscle strength and its functional performance by reducing the intestinal permeability (a randomized controlled clinical trial no. GMC-CREC-00263) (99,100). The therapeutic application of the gut bacteriome for the treatment of COPD and asthma has been previously discussed (101).

The bacterium *Parabacteroides goldsteinii* ameliorates COPD by reducing intestinal inflammation and enhancing cellular mitochondrial activities, restoring the abnormal amino acids metabolism through ribosomal activities. The derived lipopolysaccharides of *P. glodsteinii* are efficient to interact with Toll-like receptor (TLR)4 signaling pathways, thereby acting as an anti-inflammatory agent (102). Similarly, the derived saponins of the plant American ginseng have been reported to ameliorate COPD (103). Butyrate has pleiotropic functionality and has several beneficial effects on the maintenance of homeostasis of the gut and lung microbiota. Despite the short half-life of butyrate, rendering it a poor oral supplement for lung disorders, a pre-clinical evaluation of the administration of butyrate and metformin through inhalation was performed, to directly target the lung (103).

COVID-19. COVID-19 is the recent pandemic caused by a virus mainly affecting the respiratory tract. Recent research has indicated the association between gut microbiome alterations and COVID-19 severity, providing evidence that the translocation of bacteria into blood is associated with gut microbiome dysbiosis (104). This can lead to the occurrence of secondary infections. In this recent study, alterations in Paneth cells, goblet cells and markers of permeability barriers in association with alterations in the gut microbiome were observed. The gut-lung crosstalk is bidirectional, where the metabolites produced by the altered gut microbes transfuse through and exert affect the lungs, and lung inflammation affects the gut microbiota, and vice versa (104). Angiotensin converting enzyme (ACE2) is the enzyme that is located on the outer surface and functions as the gateway for viral entry and it regulates intestinal inflammation (105). The components of the bacterial envelope (lipopolysaccharides and peptidoglycans) facilitate the interaction of viral proteins, which enhances thermostability in Polio and other viruses (106). This indicates the importance of commensals in the increasing/decreasing of viral infections.

The gut microbiota of patients with COVID-19 are abundant with opportunistic pathogens, such as *Acinetobacter baumannii* and *Candida* spp. Furthermore, the functional loss of ACE2 results in gut microbiome imbalance and a leaky gut. These changes affect the host immune responses to SARS-CoV-2 infection (107). The pro- and pre-biotic treatment for other viral influenza confirms their potency to combat COVID-19. The flagellin of commensal bacteria are capable of activating pattern-recognition receptors (PRRs) of the pathogen and stimulate the release of IL-18 and IL-22, which are cytokine derivatives of the intestinal epithelium and significantly contribute to host defense mechanisms against inflammation and intestinal infection (108). Treatment with retinoic acid mediates the production of interferons through the bacterium *Lactobacillus* sp. and also increases the antiviral activity of vitamin A (109,110). Gut commensal *Clostridium orbiscindens* promotes host immune protection against influenza infection by enhancing the type I interferon signaling (111). The antiviral role of TLRs are scientifically proven mechanisms of host immune responses and the gut commensal microbiomes are expected to activate the TLRs and the release of antiviral protein cathelicidin from mast cells (112). Through TLR ligand stimulation, the death

Table II. Active clinical trials utilizing pre- and probiotics for lung cancer, COPD, asthma and COVID-19 (https://clinicaltrials.gov/).

Clinical Trial Gov. identifier	Subject or title
NCT04699721	Clinical study of neoadjuvant chemotherapy and immunotherapy combined with probiotics in patients with potential/resectable NSCLC
NCT05094167	*Lactobacillus* Bifidobacterium V9(Kex02) improving the efficacy of carilizumab combined with platinum in non-small cell lung cancer
NCT03642548	Probiotics combined with chemotherapy for patients with advanced-stage NSCLC
NCT03068663	Microbiota and the lung cancer
NCT04871412	The thoracic peri-operative integrative surgical care evaluation trial- Stage II (POISE)
NCT05037825	The gut microbiome and immune checkpoint inhibitor therapy in solid tumors (PARADIGM)
NCT05303493	Camu-Camu prebiotic and immune checkpoint inhibition in patients with non-small cell lung cancer and melanoma
NCT05492448	Probiotic on type 2 diabetes and chronic obstruction pulmonary disease
NCT05126654	Partially hydrolyzed Guar Gum (PHGG) for amelioration of chronic obstructive pulmonary disease (COPD)
NCT05523180	A study to evaluate the effect of probiotic supplement on quality of life
NCT04366089	Oxygen-ozone as adjuvant treatment in early control of COVID-19 progression and modulation of the gut microbial flora (PROBIOZOVID)
NCT04368351	Bacteriotherapy in the treatment of COVID-19 (BACT-ovid)
NCT00298337	Use of probiotic bacteria in prevention of allergic disease in children 1999-2008
NCT01419587	Ketogenic diet with chemoradiation for lung cancer (KETOLUNG)

rate due to viral infections is significantly reduced with the restoring responses of interferon gamma and $CD4^+$ T-cell responses (113). The bacterial species *Lactobacillus paracasei* and *Lactobacillus plantarum* activate the release of the cytokine IL-10 and suppress the inflammatory responses in the lungs (114,115).

Apart from bacterial balances within the gut, the intra-kingdom crosstalk between fungi, virus and bacteria has a marked influence in maintaining the gut homeostasis (116). A significant attenuation of COVID-19 infection and a reduced risk of respiratory failure was observed when patients were provided with standard medication and additional supplements with bacterial strains (*Bifidobacterium longum, Bifidobacterium animalis subsp. Lactis and Lactobacillus rhamnosus,* in addition vitamin D, zinc and selenium); this clinical trial was registered with the number NCT04666116 (117).

Dietary fibers, such as fructans and galactooligosaccharides increase the prevalence of *Bifidobacteria* and *Lactobacilli* (118). The intestinal commensals, *Bifidobacteria* and *Lactobacilli*, which are essential for inhibiting the colonization of pathogenic organisms in the gut epithelial cells. Adhesin production by commensals and probiotic supplements regenerates the intestinal mucosa, thereby suppressing viral adhesion and replication, and reducing the risk of respiratory tract infections. Peptides produced by *Lactobacillus* and *Paenibacillus* block the binding of SARS-CoV-2 with ACE2 (119). The lesions in the lung affects the gas exchange capability among COVID-19-infected patients, where the probiotic formulation SLAB51 combats the breathing difficulty by reducing nitric oxide synthesis within the gut (120). Polyphenols of beverages and other foods favor the growth of beneficial gut bacteria and provide intestinal barrier function (120). The active clinical trials for lung cancer, COPD, asthma and COVID-19 are presented in Table II.

Bacterial pneumonia and its impact on the gut microbiota. Bacterial pneumonia remains a leading cause of respiratory illness and mortality worldwide, with treatment relying heavily on antimicrobial agents (121). According to recent research, the human gut microbiota plays a critical role in protecting the body from infections due to antimicrobial resistance. The body is protected by a balanced microbiota through defense mechanisms, such as nutritional competition, niche exclusion, colonization resistance by secreting antibacterial substances, restoring the mucosal barrier, suppressing inflammation and immune modulation etc., rendering it a vital ally in the battle against antimicrobial resistance. However, this protection is compromised by gut dysbiosis, which makes it easier for resistant infections to persist, colonize and spread (122). While antibiotics are essential for managing the infection, they can significantly disrupt the gut microbiota, leading to dysbiosis and potential long-term health consequences (123). Antibiotic resistance genes are derived from the gut microbiome, which has developed into a major part of the resistome. Antibiotics alter the natural microbiota of the host by favoring resistant microorganisms that may cause opportunistic illnesses (124). The dysbiosis in the upper respiratory tract microbiome has been shown to be a major cause of bacterial pneumonia in the elderly and in young adults (125). Gut dysbiosis may be linked to subtherapeutic antibiotic treatment, the consumption of low-dosage antibiotics from food and horizontal gene transfer, leading to gene acquisition or loss, bacterial biofilms, quorum sensing and other environment factors (125).

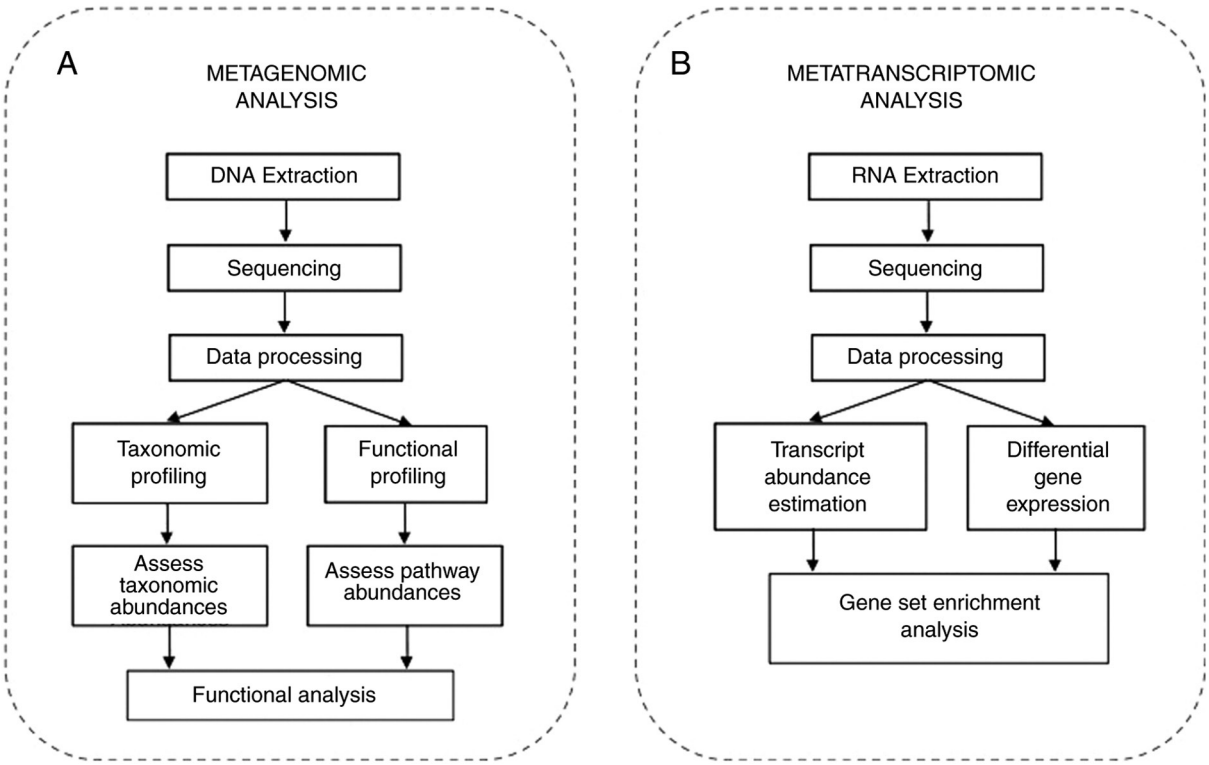

Figure 2. (A) Metagenomic and (B) metatranscriptomic analysis workflow.

Commonly prescribed antibiotics, including β-lactams (such as penicillins and cephalosporins), macrolides (such as azithromycin and clarithromycin), fluoroquinolones (such as levofloxacin and ciprofloxacin), tetracyclines and aminoglycosides, have been shown to reduce microbial diversity and deplete beneficial gut bacteria, such as *Bifidobacterium* and *Lactobacillus*. This disruption weakens immune function, promotes the growth of opportunistic pathogens, and increases the risk of developing antibiotic-resistant infections, such as *Clostridium difficile*. Over time, these alterations can contribute to metabolic disorders and chronic health issues (126). Restoring the gut balance through probiotics, prebiotics, fecal microbiota transplantation and dietary interventions may help mitigate these effects and support overall gut and immune health.

7. OMICS techniques: Metagenomics, metatranscriptomics, metaproteomics and metabolomics

The human intestine is colonized by >1,000 microbial species. The gut microbial community plays a critical role in the protection of the host against pathogenic microbes, modulating immunity and regulating metabolic processes (127,128). Since the beginning of microscopy, several human gut microbial communities have been identified. Recent technological advances in genomics and microbial genetics have led to the identification of human microbiota associated with various diseases.

Molecular biological technology plays a vital role in the intestinal microbiome analysis; in particular, metagenomic sequencing using the next-generation sequencing (NGS) techniques has led to notable developments in the study of the human gut microbiome. Metagenomics is widely used in the study of human gut microbiome diversity and to gain the knowledge of its insight with relation to various diseases, as well as its association with health and disease (129,130). This aids in the identification of taxonomic identification, and in the detection and characterization of microbial species (131). Metagenomic analysis involves the extraction of genomic DNA and sequencing. The next step involves annotation after assembly and the mapping of sequences to the reference database (Fig. 2A). Further, computational annotation is performed using bioinformatics tools, such BLAST, COG and KEGG, and statistical analysis can be performed using R and Bioconductor (132,133).

The application of metagenomics involves the identification of novel functional genes, microbial pathways, antibiotic resistance genes, functional dysbiosis of the intestinal microbiome, and the determination of interactions and co-evolution between microbiota and host (134,135). Although the complete bacterial repertoire of the human microbiome is unidentified, the study by Almeida *et al* (136) provided information of a vast diversity of uncultured gut bacteria, and supports the expansion of the gut microbiome repertoire of the human gut microbiota. Metagenomics also enables the profiling of whole gene repertoire and plays a vital role in the pan-genomic analyses and also enables the identification of whole genome content from a sample and determination of closely related taxa (137).

Metatranscriptomics involves the sequencing of the complete transcriptome of the microbial community and determines the genes that are expressed by the microbiome and helps determine the functional profile of the community under different conditions (138). In brief, metagenomics helps to identify the composition of a microbial community under

Table III. A brief comparison of the different omics techniques.

Omics techniques	What is analyzed	Advantages	Limitations
Metagenomics	DNA (microbial genomes)	Identifies microbial composition and potential functional genes.	Does not indicate active gene expression; unable to assess actual function.
Metatranscriptomics	RNA (microbial transcripts)	Captures gene expression and functional activity under specific conditions.	RNA is unstable; requires careful sample handling; highly dynamic and contextual.
Metaproteomics	Proteins (microbial proteome)	Provides direct evidence of expressed proteins and metabolic function.	Technically challenging; limited by protein extraction and database completeness.
Metabolomics	Metabolites (small molecule end-products)	Reflects functional output of microbiome and host-microbe interactions.	Difficult to link metabolites to specific microbes; complex data interpretation.

different conditions, while metatranscriptomics helps identify the genes that are collectively expressed under different conditions (139). A metatranscriptome experiment involves the isolation of total RNA from a sample and its sequencing followed by annotation, assembly and bioinformatics analysis (140), as illustrated in Fig. 2B.

Other novel omics approaches, such as metatranscriptomics (141), metaproteomics (142) and metabolomics (143) also complement the study of the human gut microbiome and support the identification of the microbiota community. Metaproteomics provides the knowledge of the entire protein complement of the microbiota communities, provides insight into the genes expressed and helps in the identification of the key metabolic activities (144). Metabolomics provides details of the metabolite composition of the microbiota community, and provides an understanding of the functional dynamics of the microbiome community and the host interactions (145). A brief comparison of the different omics techniques, including advantages and disadvantages to understand the choices and limitations of each approach is provided in Table III.

8. Machine learning for the development of microbiome therapeutics

Machine learning is an artificial intelligence (AI) technique that focuses on developing the intelligence to imitate humans for analyzing a large volume of data. The technique is widely applied to answer various biological and biomedical queries including the microbiome field. A simple boolean search using the query 'gut microbiome' and 'machine learning' retrieved 513 PubMed articles (on May 31, 2025). The first article was published in 2013 and the study focused on predicting the genes in metagenomic fraction by using support vector machines (SVM), one of the standard machine learning approaches for classification (146). The authors of that study presented a novel gene prediction method called MetaGUN and included three different stages: i) classifying the fragments into different phylogenetic groups with k-mer based sequence binding approach; ii) identifying protein coding sequences from each phylogenetic group using SVM that uses entropy density profiles, translation initiation site, and open reading frame length as input patterns; and iii) adjusting the translation initiation site (146).

Human health is greatly affected by the gut microbiota, which affects immune responses, metabolism and disease prevention. Numerous diseases, including cancer, are associated with microbial imbalance, or dysbiosis, a key observation that can be utilized for the early detection of disease. AI methods are well suited for large-scale biomedical big data mining, including microbiome data mining. Foundation models and transfer learning (ML and DL models) have been shown to have immense success with microbiome-based classification and prediction (147). Random Forest (RF), an ensemble learning technique, has been used for feature selection and classification in microbiome datasets to identify key microbial taxa associated with diseases such as type 2 diabetes and inflammatory bowel disease (148). SVMs and other machine learning techniques have markeldy enhanced the predictive power of cancer diagnosis and prognosis, especially in research involving gut microbiota. When trying to understand the complex interaction between the gut microbiota and cancer prevention, the SHapley Additive exPlanations (SHAP) algorithm is a crucial part of the eXplainable Artificial Intelligence (XAI) architecture (146,149). Machine learning models such as Ridge regression, Elastic Net, LASSO, Random Forest, Ridge regression and LASSO were constructed using the SIAMCAT v_2.0 and v_2.10 toolbox and have been used to accurately classify patients with Parkinson's diasease, with an average AUC of 71.9%. However, it was found that the trained models were study-specific and do not generalize well to datasets from other neurodegenerative diseases research (150). The increasing application of these varied algorithms highlights their potential for improving integrative multi-omics analysis, precision medicine and microbiome-based diagnostics.

Recent research has focused on disease prediction (151-153), the association between the gut microbiome and disease (e.g. obesity, constipation, type 2 diabetes, cardiovascular diseases) (154-157), the epigenetic regulation of brain disorders (158), and the role of the gut microbiome in promoting precision medicine for cancer (159). The interaction

between the gut microbiome and the intestinal cells is well known. Recently, the interaction between the gut microbiome and nervous system, particularly the enteric nervous system and central nervous system has gained increasing attention among researchers and neurologists (158). Studies have shown the involvement of the gun microbiome in anxiety (160), autism (161), depression (162), and a number of neurodegenerative diseases (163-167). The influence of the gut microbiome on the host metabolism by activating epigenetic regulators plays a crucial role in understanding the pathogenesis of numerous neurological disorders, including brain disorders (143). The integration of multi-omics data, including genomics, metabolomics, connectomics and gut microbiome multi-omics can provide a path for moving forward towards the prevention of disease and for the development of personalized therapeutics, as opposed to merely the prediction of diseases. Both supervised and unsupervised machine learning approaches can be used for such studies (158). Thus, machine learning approaches find a wide range of applications in gut microbiome research.

9. Conclusion and future perspectives

Respiratory diseases are markedly affected both by genetic and external factors. Where the genetic factors are difficult to control, the immunological response to the environmental factors can be mediated. In this regard, the human microbiota serves as a source towards this approach. The health of the gut microbiota is sustained with fibrous food intake, probiotics, etc. This cascades the immunological response in chronic respiratory diseases. The omics approach provides details of the metabolite composition and provides an understanding of the functional dynamics of the microbiome community and host interactions. The association between diet and the gut microbiome is analyzed using machine learning approaches, and much of the hidden information in literature can be explored using text mining approaches. This survey highlights the diagnostic aspects of personalized medicine based on the gut-lung axis microbiome of an individual by integrating various areas of research, such as molecular biology, immunology, omics, machine learning and text mining.

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Authors' contributions

All the authors (SM, ORI, AP, BB and KR) were involved in the conceptualization of the study. All the authors (SM, ORI, AP, BB and KR) were also involved in the formal analysis (collecting the relevant data and articles from the literature), in the writing and preparation of the original draft of the manuscript, and in the writing, review and editing of the manuscript. SM, ORI and AP were involved in visualization (preparation of the figures). KR supervised the study. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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