Pathophysiology and genomics of bronchiectasis

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The understanding of bronchiectasis pathophysiology is challenging given its clinical and biological complexity and heterogeneity. Here we review current knowledge and propose a scientific roadmap of how genomics can identify new bronchiectasis endotypes. https://bit.ly/44zvxif

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Abstract

Bronchiectasis is a complex and heterogeneous inflammatory chronic respiratory disease with an unknown cause in around 30–40% of patients. The presence of airway infection together with chronic inflammation, airway mucociliary dysfunction and lung damage are key components of the vicious vortex model that better describes its pathophysiology. Although bronchiectasis research has significantly increased over the past years and different endotypes have been identified, there are still major gaps in the understanding of the pathophysiology. Genomic approaches may help to identify new endotypes, as has been shown in other chronic airway diseases, such as COPD.

Different studies have started to work in this direction, and significant contributions to the understanding of the microbiome and proteome diversity have been made in bronchiectasis in recent years. However, the systematic application of omics approaches to identify new molecular insights into the pathophysiology of bronchiectasis (endotypes) is still limited compared with other respiratory diseases.

Given the complexity and diversity of these technologies, this review describes the key components of the pathophysiology of bronchiectasis and how genomics can be applied to increase our knowledge, including the study of new techniques such as proteomics, metabolomics and epigenomics. Furthermore, we propose that the novel concept of trained innate immunity, which is driven by microbiome exposures leading to epigenetic modifications, can complement our current understanding of the vicious vortex. Finally, we discuss the challenges, opportunities and implications of genomics application in clinical practice for better patient stratification into new therapies.

Introduction

Bronchiectasis is a chronic airway disease characterised by its clinical and biological complexity and heterogeneity [1]. Patients suffer from cough, expectoration and recurrent airway infections that worsen their prognosis. A dysregulated inflammatory response contributes to the lung damage, resulting in an irreversible bronchial dilation. Together with airway mucociliary dysfunction, these alterations leave the respiratory epithelium vulnerable to recurrent airway infections. All these components are interconnected, perpetuating the inflammatory environment, in a model called the "vicious vortex" [2]. Due to this complex interaction, pharmacological therapies that individually target a component of the cycle do not show clear clinical benefits in these patients [3, 4]. Novel strategies are urgently needed since the incidence and prevalence of bronchiectasis have been increasing worldwide in the past decade. Although research has significantly increased over time, the underlying mechanisms of bronchiectasis are not fully understood. Consequently, there are no currently available specific therapies for bronchiectasis patients.

As for the potential causes of bronchiectasis, there is an extensive list including chronic infections such as tuberculosis and non-tuberculous mycobacterial infection, immunodeficiencies, cystic fibrosis (CF), primary ciliary dyskinesia (PCD) congenital syndromes, rheumatological disorders and allergic bronchopulmonary aspergillosis, among others [5–7]. However, data from the largest prospective registry of bronchiectasis for 16 963 patients from 27 European countries and Israel globally identified that in 38% of patients with bronchiectasis the cause is unknown, referred to as idiopathic [8]. Genomics may help to better characterise the molecular landscape of bronchiectasis, as happened in other chronic airway diseases that frequently coexist with bronchiectasis, such as COPD and asthma. This will lead to the identification of new causes of bronchiectasis.

Given the relevance of omics and multiomics approaches, this review will briefly describe the key components of the pathophysiology of bronchiectasis and how genomics may increase our knowledge. It will also address the epigenetic modifications that may influence gene expression and its association with the novel concept of trained innate immunity. Finally, it will discuss the implications of genomics application in clinical practice for the development of therapeutic strategies towards precision medicine.

Pathophysiology of bronchiectasis

Cole's "vicious cycle" has been central to the understanding of bronchiectasis progression since 1986 [9]. This model considers the following components as major drivers of the disease: airway infections, inflammation, mucociliary dysfunction and structural lung damage. However, as described earlier, it was renamed as the vicious vortex model due to the high interconnection between them [9]. Therefore, each component depends on all of the others. Due to this complexity, it is reasonable to suspect that genetic background (genome) and environmental factors (exposome) may play a role in the development and progression of bronchiectasis, although this has not yet been deciphered (figure 1).

Airway infection

Infections are a major driver of disease progression in bronchiectasis [10]. Bronchial infections are common events, and are associated with increased inflammation and poor clinical outcomes [11–13]. Bacteria, mycobacteria, fungi and viruses have been reported as causal agents of these infections, both at stable and at exacerbation phases. Among bacteria, the identification of *Pseudomonas aeruginosa* in

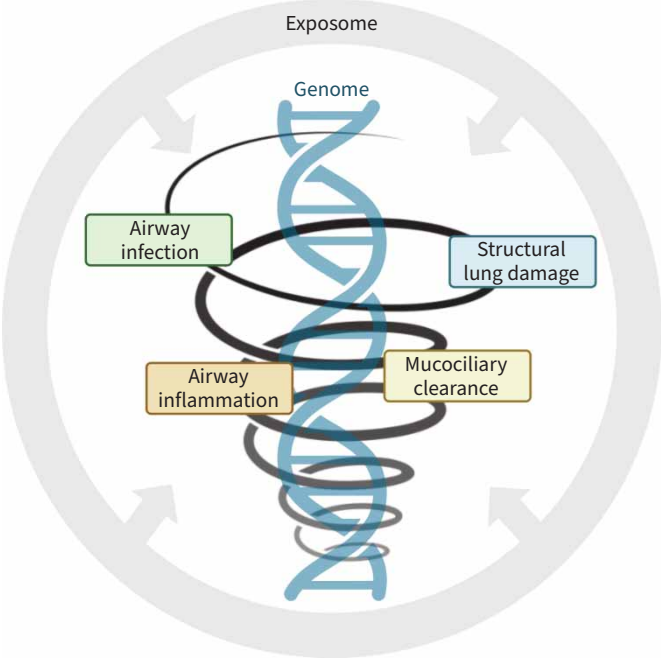

FIGURE 1 Model of the vicious vortex in bronchiectasis. Graphical representation of the vicious cycle for explaining the pathophysiology of bronchiectasis through the interconnection of the key components (airway infection, airway inflammation, mucociliary clearance and structural lung damage) and the potential influence of the genome and exposome on it. Figure partially created with BioRender.com.

sputum is the most frequent (15–50%) and is associated with worse prognosis in bronchiectasis, including lung function decline, high frequency of exacerbations and increased mortality [14–17]. This is due to the hypermutable nature of this Gram-negative bacteria that allows its adaptation to environmental, immune and antibiotic changes [17–20].

Over the past years, the emergence of advanced sequencing technologies has enabled the molecular identification of microorganisms and the profiling of bacterial communities in the lung [18]. Traditional culture methods are limited for exploring the microbial composition in greater detail. Multiple studies based on 16S rRNA techniques revealed that the bronchiectasis lung microbiome is highly complex, heterogeneous and less diverse, both at stable and at exacerbation episodes, and mainly dominated by Proteobacteria, which are associated with severe disease, higher frequency and severity of exacerbations, and higher risk of mortality [19–21]. As described earlier, airway infections in bronchiectasis are not limited to bacteria. Fungi are less explored but the first study profiling the lung mycobiome in bronchiectasis evidenced that they are also of clinical relevance in bronchiectasis [22]. Recently, the application of whole-genome shotgun metagenomics to the airway microbiome allowed for the first time the characterisation of the resistome in bronchiectasis to identify antibiotic resistance genes involved in the severity of bronchiectasis and in the underlying host microbiome [23].

Inflammation

Persistent airway inflammation is a hallmark of the pathophysiology of bronchiectasis. Abundant inflammatory cells, especially neutrophils, can be found in the airways of severe patients [24, 25]. Bronchial epithelium, under infectious or inflammatory stimuli, releases inflammatory mediators that attract and activate neutrophils. Once activated, neutrophils initiate their antimicrobial mechanisms such as the production of reactive oxygen species, phagocytosis, neutrophil extracellular trap (NET) release and degranulation [10]. Neutrophil serine proteases (NSPs) such as neutrophil elastase (NE), proteinase 3 (PR3), cathepsin G (CatG) and NSP4 are stored in neutrophil granules, released through degranulation, NET formation or cell death, and have key immunomodulatory and tissue remodelling properties [26, 27]. However, when a disruption in the endogenous antiproteases such as secretory leukocyte protease inhibitor (SLPI) and α_1-antitrypsin occurs, NSPs degrade lung tissue and perpetuate chronic inflammation [28].

To date, NSPs appear to be the most promising biomarkers evaluated in sputum for bronchiectasis. High NE activity in sputum is associated with severe bronchiectasis, risk and frequency of exacerbations, airway bacterial load, and treatment response [24, 29, 30]. PR3 concentrations are also increased during exacerbations and correlated with NE levels [31]. CatG activity induced ciliary dysfunction, destruction of airway epithelium and correlated with disease severity [32]. In contrast, low levels of one of the main NE antiproteases, SLPI, are associated with disease severity, airway infection and risk of exacerbation [33, 34]. Given the relevance of neutrophils in bronchiectasis, previous efforts to therapeutically block neutrophils were developed but account for high infection rates because of their essential antimicrobial role [35]. However, the pharmacological inhibition of dipeptidyl peptidase 1 (DPP-1), the enzyme responsible for NSP activation, showed positive results. Bronchiectasis patients treated with brensocatib (AZD7986/INS1007), which is an oral, selective and reversible DPP-1 inhibitor, decreased their sputum NE and improved their clinical outcomes in the phase 2 WILLOW study [36]. They also had a reduction in sputum PR3 and CatG activities, exerting a broad anti-inflammatory effect [26, 31]. These results from the WILLOW study highlight the potential for translating insights from basic research into novel therapies.

Although bronchiectasis is primarily considered a neutrophil-mediated disease, recent studies have also characterised eosinophilic inflammation in a subset of patients with bronchiectasis. Blood eosinophilia correlated with sputum counts and was associated with the lung microbiome and with a shorter time to next exacerbation [37]. Additionally, blood eosinophil counts were associated with clinical outcomes, lung function, nutritional status and inflammation [38] and with the benefits for using inhaled corticosteroids [39, 40]. Therefore, eosinophils may be a potential biomarker in bronchiectasis for helping in the identification of patients who require different management strategies [41]. Other inflammatory cells such as macrophages or cells from adaptive immunity can be found in the airways of patients with bronchiectasis. Their functions are key for the clearance of apoptotic neutrophils and for the proper immune response against respiratory infections [42].

The heterogeneity of bronchiectasis extends far beyond aetiology and it is known that the immune response of these patients is highly complex. Cluster tools can help to integrate biological data to identify patients with similar clinical and biological characteristics, called endotypes [43]. There is a wide variety of methods for classifying data, such as unsupervised *k*-means clusters, bottom-up agglomerative approaches and top-down divisive approaches [44]. Profiling patients according to airway antimicrobial

peptides allowed the identification of clusters of patients with distinct airway infections, severe disease and risk of exacerbation, together with distinct levels of inflammation and tissue damage [45]. In addition, a recent study integrating host inflammatory and lung microbiome data identified inflammatory molecular endotypes associated with distinct profiles of the microbiome and risk of exacerbations [46]. These studies highlight the need for the recognition of distinct molecular endotypes, which will guide us towards precision medicine.

Airway mucociliary dysfunction

Effective mucociliary clearance serves as a primary defence mechanism against infections. Mucus traps inhaled bacteria, viruses and particulate matter, and the coordinated, continuous beating of motile cilia plays a crucial role in clearing the overlying mucus from the airways [47]. Epithelial cells can also release pro-inflammatory mediators, antimicrobial peptides and mucins that contribute to mucus properties and to neutrophil migration to the site of infection. Impaired mucociliary clearance causes mucus accumulation in the airway, favouring the establishment of respiratory infections and perpetuating inflammation [10]. Therefore, cilia dysfunction and mucus hypersecretion, together with changes in viscoelastic mucus properties, leave the respiratory epithelium vulnerable to infection [48]. Ciliary dysfunction can be primary, like in bronchiectasis associated with PCD [49], or acquired. It has been reported that several interlinking environmental and physiological factors can also cause ineffective mucociliary clearance [50–52].

Airway mucosal immunity also involves immunoglobulins preventing bacterial adherence to the epithelium. Epithelial cells produce secretory IgA, the main airway immunoglobulin. Bronchiectasis patients can present primary or acquired immunoglobulin deficiencies, even in patients with normal total IgG levels, leading to a lack of specific antibody responses against certain pathogens [53, 54]. Further research is needed to restore normal epithelial defence by improving the imbalance of proteases and promoting the antimicrobial response of the airway epithelium [10].

Structural lung damage

The term bronchiectasis refers to both a clinical disease and a radiological appearance [55]. All the components described earlier contribute to the dilatation of the bronchi as a result of the destruction of the elastin layer, and to a lesser extent, the damage to the muscle and cartilage layers. The airways affected by bronchiectasis exhibit inflammation, tortuosity and frequent obstruction caused by an excess of mucus secretions [56, 57]. Studies in CF have shown that structural lung damage starts early in life with the presence of mucus plugging in the small airways, leading to obstruction and becoming fibrotic over time [58, 59]. This lung remodelling progresses towards alveoli and limits gas exchange. As in bronchiectasis, these alterations are irreversible [60]. Currently, there are no treatments to revert this structural lung damage in bronchiectasis, which has been demonstrated to affect both proximal and distal bronchioles and exhibit bronchiolar secretory cell features and mucus plugging but differs in mucin gene regulation and ectasia [61]. Some early-phase clinical trials are focused on regenerative medicine approaches [62].

A novel strategy is to focus research on the early stages of bronchiectasis instead of looking at the advanced stages of the disease. Before bronchiectasis is clearly established, a pre-bronchiectasis syndrome can occur including low symptomatology, persistent bacterial bronchitis (PBB) or genetic predisposition [63, 64]. Childhood PBB frequently evolves into bronchiectasis disease [65]. Future directions focused on a comprehensive understanding of the genetic background that influences lung tissue destruction will potentially improve the management of these patients.

From pathophysiology to multiomics

Despite the well-accepted vicious vortex model, major gaps remain in the understanding of the pathophysiology of bronchiectasis. High-throughput methodologies, analysing one or more layers of biological complexity, referred to as “omics”, and its integration as “multiomics”, can be used to investigate disease complexity and provide a holistic view [66]. As described earlier, multiple large-scale microbiome and proteomic studies have been performed in bronchiectasis and it has been proven that these methodologies can identify novel endotypes. However, other biological layers, such as genetics, epigenetics, transcriptomics and metabolomics, are still less explored (figure 2) [67], but have been investigated in other airway diseases, such as COPD [68].

Due to the fact that COPD and bronchiectasis coexist in 10–35% of bronchiectasis patients, we next review the state of the art of omics profiling in bronchiectasis and discuss what we can learn from the COPD literature [69–71].

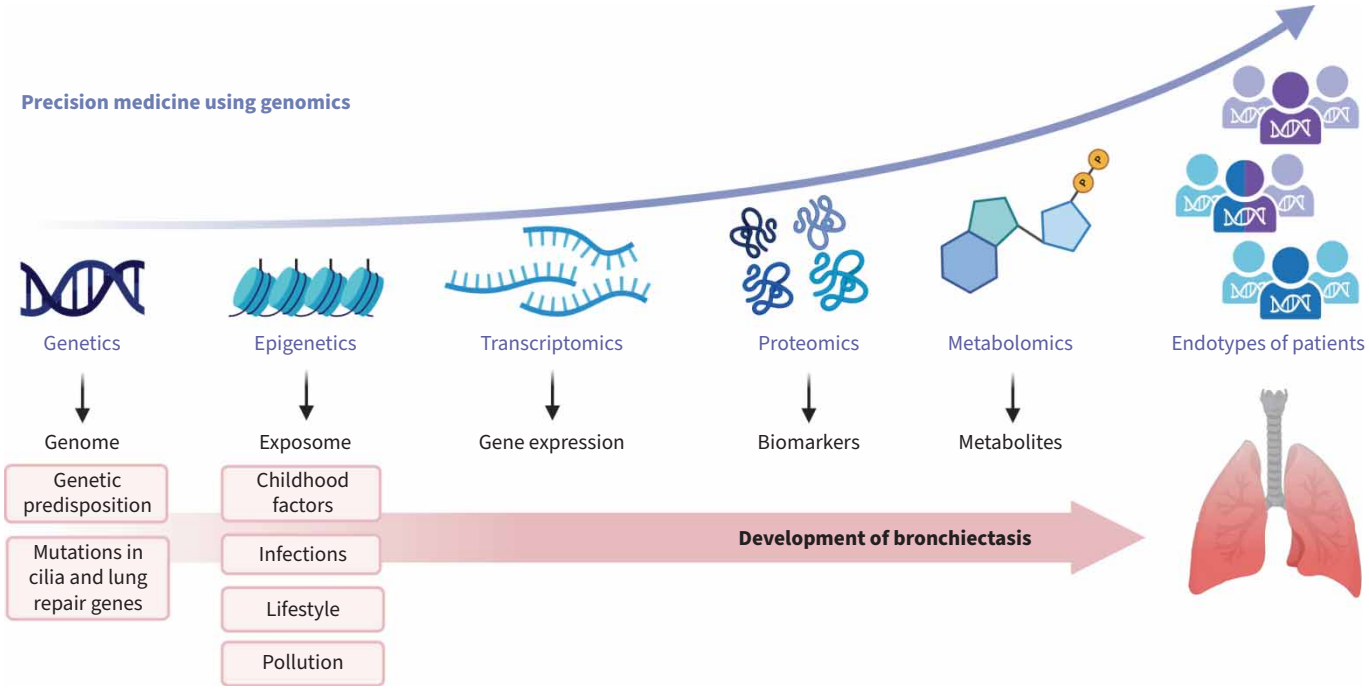

FIGURE 2 Conceptual overview of omics. Description of the relevance of genomics in the precision medicine of bronchiectasis by defining the contribution of genetics, epigenetics, transcriptomics, proteomics and metabolomics on the identification of endotypes of patients. Figure partially created with BioRender.com.

Genetics

Genomics is the study of all of a person's genes (the genome), including interactions of those genes with each other and with the person's environment; genetics, in turn, is the study of heredity. Currently, genetic analyses (genome-wide association studies (GWAS)) are performed by the identification of nucleotide variants called single nucleotide polymorphisms (SNPs) using SNP arrays or sequencing at the whole-genome scale, and the association of each individual SNP with clinical traits of interest is explored [44]. The largest COPD GWAS identified around 100 associated genetic loci, but the effect sizes of these loci were relatively small [72]. Interestingly, these loci were similar in smokers and non-smokers with COPD, suggesting that additional omics are needed to explain the interaction with the environment [73]. COPD loci have also been associated with fetal developmental processes [74], supporting the notion that the genetics of lung function are closely linked to lung growth and the achieved peak lung function [75].

Additionally, the categorisation of diseases based on genotype, as exemplified by CF, demonstrates how this can lead to successful therapeutic interventions. Genetic studies have enabled the identification of the CF transmembrane conductance regulator (*CFTR*) as a viable therapeutic target for restoring the defect. Recent progress in molecular therapy highlights this approach as the most promising one in CF [76, 77].

To date, GWAS have not been performed in bronchiectasis. To cope with this knowledge gap, the European Multicentre Bronchiectasis Audit and Research Collaboration (EMBARC), a European Respiratory Society Clinical Research Collaboration, is leading the GECCO (Genetics Encoding Complex Ciliopathies of Bronchiectasis) study to explore the whole exome in over 1000 patients with idiopathic bronchiectasis [78]. This study is looking for unrecognised PCD in these patients and for the identification of potential genetic contributors to the disease [79]. However, the prevalence-specific SNPs have been studied (table 1). For instance, mannose-binding lectin (*MBL*), a crucial component of innate immunity involved in bacterial and apoptotic cell clearance, shows a common genetic deficiency in the general population, correlating with disease severity in bronchiectasis, impacting quality of life, exacerbation frequency and hospital admissions [80]. Also, the secretion of α-1,2-fucosylated glycans, influenced by the secretor genotype (*FUT2*), becomes a risk factor affecting infection type and disease severity in bronchiectasis [81].

Finally, it is important to highlight the influence of ethnic variation and geographical differences on the genetic variants that may contribute to the underlying conditions in bronchiectasis [82].

TABLE 1 Key polymorphisms and genetic variants reported in bronchiectasis and other chronic airway diseases

Gene	Name	Airway disease	Implications	References
CFTR	CF transmembrane conductance regulator	Bronchiectasis without CF, CF, PCD	Defects in airway mucociliary clearance	[134, 135]
DNAH11	Dynein axonemal heavy chain 11	Bronchiectasis, PCD	Defects in airway mucociliary clearance	[136]
FUT2	Fucosyltransferase 2 (H blood group)	Bronchiectasis, COPD	Severe disease and infection susceptibility	[81]
MBL	Mannose-binding lectin	Bronchiectasis, CF, COPD	Severe disease and infection susceptibility	[80, 137, 138]
MMP1	Matrix metalloproteinase 1	Bronchiectasis, COPD	Severe disease	[139]
PI3KCD	Phosphoinositide 3-kinase δ	Bronchiectasis	Lymphocyte dysfunction, respiratory infections and extrapulmonary manifestations	[140, 141]

CF: cystic fibrosis; PCD: primary ciliary dyskinesia.

Transcriptomics

Transcriptomics quantifies the expression levels of cellular transcripts (*e.g.* mRNA), and thus is a surrogate of the cellular activity, and varies by age, sex, cell type, environmental factors and disease status. It is a widely used technology because mRNA arrays were one of the first omics introduced, currently replaced by RNA sequencing (RNA-seq) and single-cell RNA-seq methods that have more specificity. However, differences in mRNA expression do not necessarily translate to different protein levels [44].

In recent years, lung transcriptomics has helped to unravel differences in the molecular pathogenesis of COPD [83–85]. It has also helped to discern key features such as emphysema and bronchiolitis by the identification of unique signatures. For instance, it was demonstrated that B-cell-related genes were significantly enriched in emphysema, suggesting that these patients may benefit from different therapies based on their inflammatory pathways [86]. Additionally, the integrated Human Lung Cell Atlas, launched in 2023, is a key resource to show the diversity of cell types in healthy and diseased lungs [87]. Regarding the peripheral blood transcriptome, to date, there are no published works in bronchiectasis, whereas evidence is increasing in COPD [88–91]. Recently, a COPD study showing integrated blood transcriptome and proteome analyses revealed key blood-based biomarkers of emphysema to enhance its prediction [92].

However, transcriptomic data in bronchiectasis remain scarce. The DECAMP2 study (ClinicalTrials.gov: NCT02504697) conducted RNA-seq on bronchial brushings and biopsies from normal-appearing airway epithelium of participants with a high risk of lung cancer [93]. In this context, transcriptomic analysis revealed a gene expression signature associated with radiological bronchiectasis. This signature involved genes related to cell adhesion, Wnt signalling, ciliogenesis and interferon-γ pathways. However, this gene expression profile was also observed in patients with pulmonary symptoms (cough and phlegm production) but without significant radiological bronchiectasis, suggesting that it mainly reflects a bronchiectasis-associated process. Future studies looking to determine the association between blood/lung transcriptomic signals, inflammatory endotypes and clinical phenotypes of bronchiectasis are needed.

Proteomics

Proteomics refers to the comprehensive profiling of thousands of proteins present in a specific biological sample, including expression, structure, functions, interactions and modifications [94]. This can be done by untargeted approaches, such as high-performance liquid chromatography, or using panels of proteins determined by antibodies or “tags” (Olink/SomaScan platforms) [95]. To date, sputum proteomic studies in bronchiectasis have revealed an increased expression of neutrophil proteins in the airways of individuals with severe bronchiectasis [96]. In bronchiectasis research, proteome and microbiome data have been widely integrated to deeply characterise the disease. This approach allowed the recognition of dominant traits in patients with bronchiectasis and COPD overlap, leading to the identification of five distinct endotypes [97]. Proteomic studies also facilitated the discovery of a total of 80 proteins significantly associated with *P. aeruginosa* infection using sputum proteomics [98]. Among these proteins, pregnancy zone protein was highlighted by its association with airway infection, disease severity and NET release [98]. Examining sputum proteomics after antibiotic therapy also identified that those patients with *P. aeruginos*a had a lesser response to antibiotics in terms of reduced pro-inflammatory and increased anti-inflammatory proteins [30, 99]. This evidence shows that profound changes in the proteome are related to symptoms, which are different between patients with and without *P. aeruginosa*. In the near future, it is expected that this technology may facilitate the application of personalised medicine in clinical practice.

Metabolomics

Metabolomics determines the abundance profile of cellular and bacterial secreted metabolites and their relative ratios, helping to understand the cellular status of the host and microbiome. The metabolites profiled, among others, include carbohydrates, organic acids, amino acids, nucleic acids, vitamins and lipids. The prevalence of certain metabolites indicates alterations in synthesis, degradation and/or transport influenced by cellular metabolism, environmental factors and the microbiome [100]. Metabolomics has the potential to discover new pathways involved in the disease, both from the host and from the microbial composition. In COPD, metabolomics profiling has revealed panels of circulating biomarkers for the recognition of molecular subtypes of patients [101–103], and has also shown that the levels of bacterial metabolites associated with specific bacteria can mediate differences in the host airway transcriptome, defining new endotypes [104].

Few metabolome studies have been published in bronchiectasis. Recent studies with stable patients showed the association between host metabolites, *P. aeruginosa* infection and faecal microbial diversity [105, 106]. Future metabolomic studies on biological samples from different anatomical compartments are needed to elucidate the relationship with airway inflammation and for the identification of metabolomic pathways that may be predictive of severe bronchiectasis.

Epigenetics

The genome sequence of an individual (SNPs) does not change across their lifespan; epigenetics, in turn, refers to the mechanism that can modulate the expression of genes by environmental exposures (*i.e.* the exposome). The exposures that can drive epigenetic modifications can act across the lifespan, and include previous infections, childhood exposure factors, smoking, diet and pollution, among others [44]. Epigenetic regulation is crucial in driving cellular differentiation. The most comprehensively studied epigenetic modification is the addition of a methyl group to cytosines next to guanines in the DNA molecule (*i.e.* DNA methylation in CpG sites), but modifications of histone tails, non-coding RNAs such as microRNAs and long non-coding RNAs are also part of the epigenetic processes [107–109]. Epigenetic modifications have not yet been explored in bronchiectasis, but COPD studies have shown that mild-to-moderate COPD presents a smoking-related epigenetic signature that is absent in severe COPD [110], and recent studies have identified some shared epigenetics differences between blood and lung [111]. Different lung function trajectories across the lifespan differ in the methylation signals [112], and epigenetic markers identified in fetal lung tissue may increase the risk of developing COPD later in life, as co-methylation analysis revealed highly preserved features with enrichment in developmental and inflammatory pathways, including Hippo, Wnt, transforming growth factor-β and phosphoinositide 3-kinase/AKT pathways. Finally, smoking-related epigenetic alterations have been associated with hyperplasia of basal and goblet cells, squamous metaplasia, loss of club and ciliated cells, decrease in the periciliary layer, ciliary damage, junctional barrier loss and reduced polymeric immunoglobulin receptor expression which, in turn, leads to a deficiency in localised secretory IgA, bacterial colonisation and chronic airway inflammation [113–115].

Against the dogma: trained innate immunity in the vicious vortex framework

A key unsolved question in bronchiectasis is why some patients become chronically infected while others do not. Here we would like to propose a novel paradigm to address this issue, based on the addition of the trained innate immunity concept to the vicious vortex framework.

Trained innate immunity is a novel concept postulated a few years ago, where against a central dogma in classic immunology, not only adaptive but also innate immunity can have an immunological memory to repeated infections [116]. Following exposure to infectious agents (direct infection, pathogen-associated or damage-associated molecular patterns, referred to as “PAMPs” or “DAMPs”), trained immunity can mount a faster and greater response against a secondary stimulation [117]. Interestingly, both the magnitude and duration of the stimulus are critical for the strength of the immune response. The behaviour of the innate immune response is explained by different adaptive programmes: differentiation, priming, trained immunity and tolerance. Innate immune cells can undergo any of these functional adaptive programmes, so it is important to clearly characterise the immune response. However, the mechanisms underlying the activation of these programmes are the host epigenetic and metabolic remodelling, which will modify the ability of the host/cell to respond against future infections. That is, in the trained immunity paradigm, the first stimulus activates immune cells and returns to basal levels after its removal. During the training window, the induced epigenetic and metabolic alterations persist. After a homologous or heterologous stimulus, an increase in both gene transcription and cell function occurs at significantly elevated levels compared with those noted during the initial challenge. In contrast, in a tolerant state, immune cells do not activate gene transcription following the second challenge. Therefore, while trained innate immunity confers long-term

protection against lung infection, a tolerant state, which is its opposite programme, is unable to activate a proper inflammatory response against infections [117–119]. During trained immunity, an interesting interplay between metabolites and gene regulation occurs. In trained macrophages, an increase in succinate and fumarate levels has been associated with epigenetic changes [118].

In diseases such as bronchiectasis where patients suffer frequent airway infections, the concept of trained immunity may explain why some patients are unable to fight against these infections and eliminate the microorganisms from the airway.

Specifically, we propose that in the airways of some patients, the repeated bacterial exposure and the specific metabolites are unable to activate the innate immunity, not leading to the host epigenetic changes that are needed to enhance the immune system to repeated infectious exposures. Therefore, detrimental epigenetic modifications occur in these patients and lead to an immune system showing tolerant features, in order to allow the "survival" of the host (figure 3). Interestingly, the tolerant status can be reverted or modified with the proper stimulus. Among training stimuli, bacillus Calmette–Guérin (BCG) and β-1,3-D-glucan (β-glucan) have shown trained features in experimental studies [120]. BCG was initially proposed for enhancing non-specific protection against infections in both vaccinated children and *in vitro* studies, through a shift of the glucose metabolism towards glycolysis for the induction of the histone modifications, leading to epigenetic reprogramming [121–123]. Similarly, β-glucan, which is a cell wall component of *Candida albicans*, can revert the epigenetic state of lipopolysaccharide-induced tolerance by inducing modifications at the histone level and re-activations at the transcriptional level [124].

However, it is well known that the lung microbiome is not static and that the oral and intestinal microbiomes are closely related to the airways. In particular, the gut microbiome is increasingly recognised as an important modulator of immune memory through the effect of microbial products, ligands and metabolites, both locally and in other organs such as the lungs and mucosal sites [125–127]. In addition, it has recently been described that a dysregulated gut–lung axis in bronchiectasis is associated with disease severity and poor clinical outcomes [128]. Since there are some intestinal genetic conditions, such as CF and PCD, related to bronchiectasis [7], it may also be relevant to explore the potential role of the gut microbiota in the modulation of trained innate immunity in these patients.

This paradigm has never been explored in bronchiectasis and we propose that future research efforts should focus on the characterisation of the trained innate immunity programmes using both patient samples and experimental models, and eventually specific vaccination programmes. Understanding innate immune memory in bronchiectasis is an important step for deciphering the biological and clinical heterogeneity of chronic bronchial infection susceptibility, but also to be able to modify it, leading to a precision medicine approach.

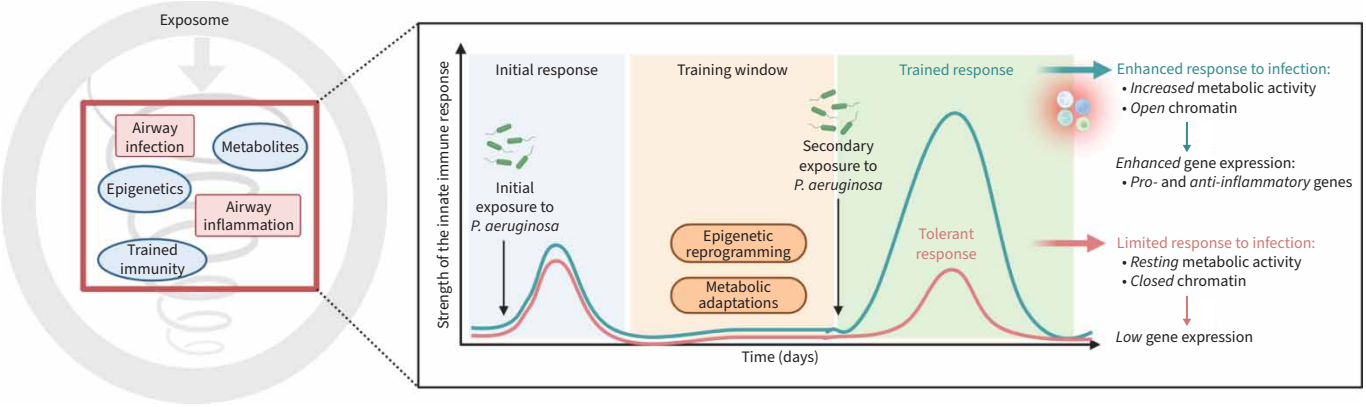

FIGURE 3 Hypothesis of trained innate immunity applied to the vicious vortex framework of bronchiectasis. This graphical representation shows the hypothesis about how the novel concept of trained innate immunity can contribute to the pathogenesis of bronchiectasis through epigenetic reprogramming and metabolic adaptations after exposures to microorganisms such as *Pseudomonas aeruginosa* (*P. aeruginosa*). Figure partially created with BioRender.com.

Early origins of bronchiectasis?

The latest 2024 version of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines acknowledges that early-life insults such as infections, genetic factors and exposures can influence the development of the aetiotypes of chronic lung disease [129], and COPD is defined as "a heterogeneous lung condition that results from gene (G)–environment (E) interactions occurring over the lifetime (T) damaging the lungs and/or altering their normal development/ageing processes" [73]. To refer to the interactions between the multiple environmental factors with the host, which are modulated by the host genetics and immune system, and might be mediated by epigenetics, and can lead to a wide range of lung function trajectories overtime in the general population, the term "GETomics" has been recently proposed.

GETomics involves integrating information from basic omics (*e.g.* genomics, epigenomics and proteomics), clinical omics (*e.g.* phenomics, physiomics and radiomics) and exposures (the exposome), across the life course [73]. In this setting, both age and biological memory become key factors for the understanding of disease pathophysiology [115, 130].

Finally, bronchiectasis and COPD are traditionally viewed as disease silos, but it is likely that the early-life risk factors are similar and overlapping; future endotyping efforts might need to consider why some patients develop one disease, the other or both, and how to integrate the GETomics concept to bronchiectasis, moving towards disease trajectories [131–133].

Moving towards the implementation of genomics in clinical practice

To date, there are no experimental models that can be used to study early bronchiectasis development or disease progression. Therefore, we can only deeply characterise patients to improve our understanding of the disease. We now discuss some considerations to take into account when multiomic research is applied.

International registries have contributed to great research progress in bronchiectasis. We need to ensure the accessibility to biobanks to improve our research. For this purpose, we need to keep working on the establishment of DNA biobanks linked to well-phenotyped patient cohorts. One of the key aims of EMBARC3 is to expand the BRIDGE (Bronchiectasis Research Involving Databases, Genomics and Endotyping) study (ClinicalTrials.gov: NCT03791086) incorporating a wide variety of samples and long-term follow-up [78]. These studies will allow us to deeply characterise patients and categorise them into endotypes to obtain meaningful outcomes.

Regarding the study design, first, it is important to decide which time-points are relevant for the understanding of bronchiectasis. While genetic data are constant across life, epigenetic marks are dynamic and influenced by the environment at a specific moment. Also, epigenetic marks can be tissue specific, and signals can come from many cell types, so the interpretation of the results should be done carefully.

Second, the study design should be clearly defined and include a large sample size to obtain robust and reproducible results. Since genetic studies have a high risk of false-positive associations, they should be replicated in validation and international cohorts.

Finally, instead of reporting single biomarkers associated with the disease, integrative data tools should be used to describe unique molecular signatures of bronchiectasis. This will significantly address bronchiectasis heterogeneity. Precision medicine based on multiomics approaches will allow the selection of the best therapeutic strategy for the appropriate patient.

Conclusions

Since bronchiectasis burden is increasing worldwide, clinicians and researchers need to keep working together to solve the knowledge gaps that remain in the understanding of the pathophysiology. Knowing the genetic background and the environmental factors that modify gene expression is crucial for the identification of new biomarkers based not only on genetics (including epigenetics and transcriptomics) but also on proteomics and metabolomics approaches. The new paradigm of trained innate immunity, which involves the microbiome, epigenetic modifications and metabolic changes, may help us to elucidate why some bronchiectasis patients are unable to fight against airway infections.

Given the progress made by genomics research in other chronic respiratory diseases, it is tempting to consider that in the near future we will significantly increase our understanding of bronchiectasis based on large genomics studies. This will help us to elucidate the molecular atlas of bronchiectasis, which is a crucial step towards precision medicine for allowing patients with bronchiectasis to benefit from new treatments.

Questions for future research

- What are the complex relationships between the genome and exposome in the pathophysiology of bronchiectasis?
- Is there a lack of trained innate immunity in patients with frequent infections and how can we can restore it?
- Will the concept of GETomics unravel the early origins of bronchiectasis?
- Will we be able to identify disease trajectories to predict clinical outcomes in our patients?

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