# The transcriptomic landscape of diffuse radiological bronchiectasis

**Wei-jie Guan[1,2,3], Pei-cun Hu[3] and Miguel Angel Martinez-Garcia[4,5,6]**

[1]State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, China. [2]Department of Thoracic Surgery, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China. [3]Department of Respiratory and Critical Care Medicine, Foshan Second People's Hospital, Affiliated Foshan Hospital of Southern Medical University, Foshan, China. [4]Pneumology Department, University and Politechnic La Fe Hospital, Valencia, Spain. [5]Centro de Investigación Biomédica En Red de Enfermedades Respiratorias – CIBERES – Instituto de Salud Carlos III, Madrid, Spain. [6]Senior author.

Corresponding author: Wei-jie Guan (battery203@163.com)

Shareable abstract (@ERSpublications)
**Transcriptomic profiling helps to unveil the pathophysiology of bronchiectasis**
https://bit.ly/3C1B6JA

**Cite this article as:** Guan W-J, Hu P-C, Martinez-Garcia MA. The transcriptomic landscape of diffuse radiological bronchiectasis. *Eur Respir J* 2023; 61: 2201733 [DOI: 10.1183/13993003.01733-2022].



Received: 4 Sept 2022
Accepted: 16 Sept 2022

Bronchiectasis is a heterogeneous chronic structural lung disease in which four canonical elements of a vicious circle, *i.e.* recurrent airway infections, impaired mucociliary clearance, chronic airway inflammation and irreversible airway dilatation, have been implicated [1, 2]. Unravelling the underlying causes of bronchiectasis, which can only be ascertained in approximately 50% of patients despite exhaustive diagnostic efforts [3], is clinically relevant for optimising therapeutic interventions by targeting the core pathophysiology. There are a number of primary underlying causes, for instance, primary ciliary dyskinesia, which affects the motile cilia, cystic fibrosis, and congenital malformation. Several studies have indicated a role of genetic mutations in bronchiectasis [4–6], evidenced by the possible link with the clinical phenotypes and disease severity. These studies also suffered from a limited capacity of thoroughly identifying the genes broadly representative of pathophysiology.

Our perceptions of the pathophysiology of bronchiectasis are about to be revolutionised. In this issue of the *European Respiratory Journal*, Xu *et al.* [7] comprehensively profiled the transcriptome of study participants from the Detection of Early Lung Cancer Among Military Personnel (DECAMP) cohort. Participants who had matched RNA sequencing (RNA-seq) data and chest computed tomography scans, including patients with radiologically diagnosed bronchiectasis (none was diagnosed as having physician-diagnosed bronchiectasis upon sample collection), were enrolled. RNA-seq was conducted based on the bronchial brushing or biopsy tissues and tracheal epithelial cells, yielding important insights into the pathophysiology of bronchiectasis.

Analysis of the differentially expressed genes (DEGs) suggested that diffuse (three lobes or more) radiological bronchiectasis was associated with a decreased expression of genes reflecting cell adhesion (*e.g.* protocadherin) and an increased expression of inflammatory genes (*e.g.* the interferon family). Notably, patients with diffuse radiological bronchiectasis yielded an upregulated expression of Wnt signalling (modulating cell development and differentiation), ciliogenesis (cilia assembly and organisation) and interferon-γ signalling pathways. Apart from these distinct transcriptomic signatures, gene clustering analysis has identified the role of elevated threonine-type endopeptidase activity, which might be instrumental in interpretation of the molecular mechanisms of bronchiectasis development. Many of these transcriptomic changes were linked to the deuterosomal cells and multiciliated cells whose numbers were expanded compared with basal cells. Furthermore, hierarchical clustering analysis has unveiled three genomic clusters (normal *versus* intermediate *versus* bronchiectatic), in which the bronchiectatic cluster was associated with a greater respiratory symptom burden, more lung lobes being affected and,

interestingly, higher SERPINA1 mutation (mostly the Glu342Lys mutant) rates. Hence, the transcriptomic changes are intimately associated with the clinical phenotype of bronchiectasis.

These findings can be interpreted by comparison to published studies related to other chronic airway inflammatory diseases. Specifically, some studies have profiled the transcriptomic landscape of airway epithelium in patients with cystic fibrosis, whose pulmonary manifestations frequently involved bronchiectasis. Most earlier studies profiled the transcriptomes of leukocytes, lymphoblasts, mononuclear cells, neutrophils and bronchial epithelium with microarray assays [8]. The transcriptomic pathways were mainly related to inflammatory responses (*e.g.* signalling of interleukins, PI3K/Akt and GPCR) and cell growth or differentiation (*e.g.* signalling of Wnt/β-catenin and mTOR) [8]. A transcriptome meta-analysis which was mostly based on microarray data has revealed the genes associated with proteolysis, cell proliferation, apical junction formation, lung development and epithelial–mesenchymal transformation [9]. By performing RNA-seq, one study has detected the enrichment of inflammatory and apoptosis genes coupled with the depletion of immune cell-related genes in peripheral blood from patients with cystic fibrosis, regardless of treatment with lumacaftor/ivacaftor [10]. Another study has applied RNA-seq to determine the association between the peripheral blood transcriptome landscape and the severity of cystic fibrosis, revealing the over-representation of type-1 interferon responses and anti-viral responses in patients with mild cystic fibrosis [11]. However, the evidence pertaining to the RNA-seq data of bronchial epithelium in cystic fibrosis remains scarce.

To further illustrate the clinical relevance of the RNA-seq transcriptome, the findings in patients with diffuse radiological bronchiectasis can be appraised alongside the published findings pertaining to chronic rhinosinusitis with nasal polyps (CRSwNP) [12]. Both diseases are characterised by exaggerated inflammatory responses and airway remodelling. Strikingly, RNA-seq of the airway epithelium has revealed the transcriptomes associated with defective ciliogenesis, heightened interferon responses and altered cell adhesion as DEGs or signalling pathways shared between bronchiectasis and CRSwNP [7, 12]. There was found to be a differential upregulated PCD gene expression (42 out of 310 candidate genes) in radiological bronchiectasis [7], which indicated a greater turnover of motile cilia repair (in which upregulated ciliogenesis genes might have been implicated) due to excessive ciliary impairment arising from chronic inflammatory insults and/or pathogen infections. Furthermore, most ciliogenesis genes were related to the tubulin assembly and orientation that had not been previously detected, although the specific DEG expression profile differed considerably between bronchiectasis and CRSwNP [7, 12]. The heightened interferon responses suggested a common, and perhaps neglected, role of chronic pathogen (*e.g.* viral, mycobacterial) infections or heightened cytokine responses in driving the progression of chronic airway inflammatory diseases, including bronchiectasis [7, 12]. Therefore, the disruption of the airway barrier and exaggerated inflammatory responses might be viewed as universal mechanisms of the underlying pathophysiology.

Despite these similarities, the transcriptomic landscape of bronchiectasis differed somewhat from that of CRSwNP in terms of the heightened Wnt signalling pathways in patients with diffuse bronchiectasis [7] and extracellular matrix accumulation in patients with CRSwNP (markedly downregulated in bronchiectasis) [12]. Additionally, the heightened oxidative phosphorylation signals and decreased k-ras signalling in bronchiectasis should be regarded as the novel findings. These might imply cellular activities reflecting oxidative stress responses and airway destruction. By contrast, the transcriptome of CRSwNP was characterised by heightened angiogenesis, O-glycan deposition and ossification activity [12], which were not detected in bronchiectasis, suggesting the differential consequences of airway structural changes associated with heightened inflammatory responses. However, the molecular underpinnings of these different outcomes between bronchiectasis and CRSwNP merit further investigation.

The transcriptomic profiling of diffuse radiological bronchiectasis has just begun to open up the Pandora's box of the pathophysiology of bronchiectasis (figure 1). Before embarking on future investigations, there remain several caveats that we should bear in mind. First, patients were not selected on the basis of clinically evident bronchiectasis and most study participants were males in the diffuse radiological bronchiectasis group. The findings might have been biased because most study participants were male smokers (the veterans), which contradicted with the female non-smoker predominance in the bronchiectasis patient populations as reported previously. Because respiratory symptoms were not mandatory as inclusion criteria, the diffuse bronchiectasis could only be diagnosed *via* radiological assessment. The study recruited current and former smokers, while bronchiectasis is diagnosed for the most part in non-smokers, thus dampening the generalisability of the principal findings. Second, comparison of the DEGs did not reveal substantial differences between patients with at least one lobe of bronchiectasis and those without. The DEGs reported herein could only be applied to patients with diffuse bronchiectasis only. Third, the over-representation of

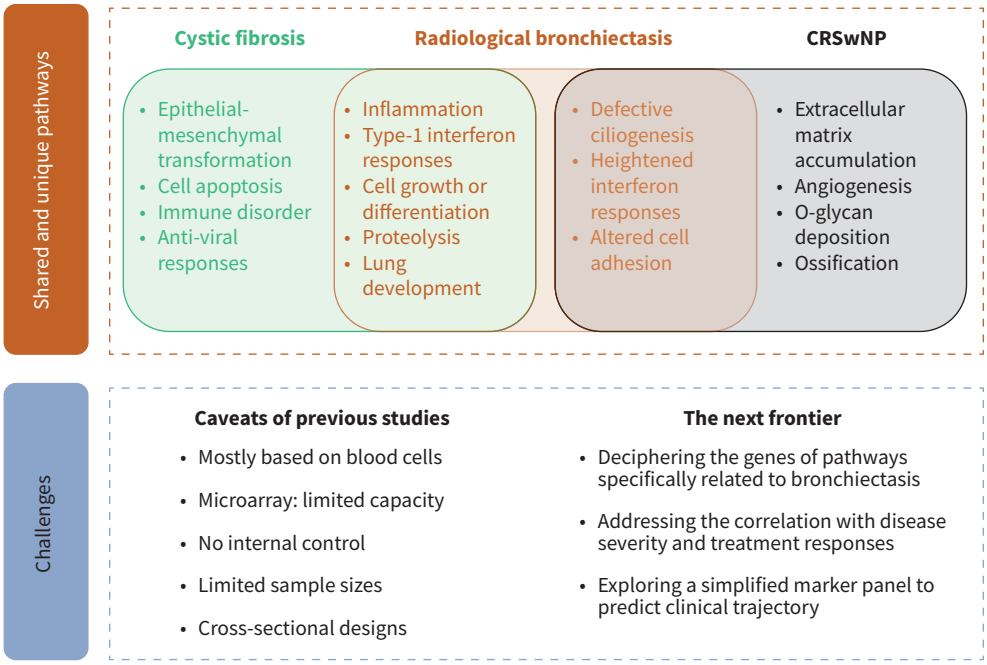


**FIGURE 1** Summary of the current evidence regarding the transcriptome of bronchiectasis and other chronic airway inflammatory diseases. The upper panel highlights the shared and the unique transcriptomic pathways of radiological bronchiectasis, cystic fibrosis and chronic rhinosinusitis with nasal polyps (CRSwNP). The shared pathways are shown in the middle column. The lower panel demonstrates (from left to right) the core caveats of previous studies which attempted to profile the transcriptome of bronchiectasis and other chronic airway inflammatory diseases such as cystic fibrosis, and the next frontiers and research goals.

patients with the "normal" transcriptomic cluster (accounting for 119 cases) and the limited number of study participants with the "bronchiectatic" transcriptomic cluster constrains the generalisability of the findings. A considerable proportion of study participants did not have matched chest computed tomographic records, and most patients did not have diffuse bronchiectasis, thus reducing the sample size and presumably the statistical power of the target population (patients with diffuse bronchiectasis). Fourth, dysregulated immune responses did not appear to play a crucial role in patients with diffuse radiological bronchiectasis, as previously documented in patients with bronchiectasis [13] and CRSwNP [12]. Whether this is a mere chance discovery or a consequence of biased sampling is unclear. Finally, the right main bronchus was included solely for transcriptomic profiling, precluding the comparison of the samples derived from the most significant bronchiectatic lobe and the least significant bronchiectatic, or even normal, lobe within an identical individual participant. Hence, the findings should be interpreted with caution.

In summary, RNA-seq has provided an important avenue for identifying the possible molecular mechanisms underlying the development and progression of bronchiectasis. The current study has demonstrated several key transcriptomic markers related to airway barrier disruption, heightened inflammation, and oxidative stress and dysregulated enzymatic responses, which might have collectively contributed to the development of bronchiectasis. Future studies which seek to unlock the association between transcriptomic changes and the inflammatory endotype and clinical phenotype of bronchiectasis are needed. Efforts which seek to determine the transcriptomic signals heralding the clinical progression or remission of bronchiectasis are also welcome.

Author contributions: W-J. Guan, P-C. Hu and M.A. Martinez-Garcia drafted and critically reviewed the manuscript. All authors have approved for the final submission.

Conflict of interest: All authors declare no potential conflict of interest related to this paper

Support statement: National Natural Science Foundation – Outstanding Youth Fund (number 82222001), National Natural Science Foundation (number 81870003), Guangdong Science and Technology Foundation (number

2019B030316028) and Zhongnanshan Medical Foundation of Guangdong Province (number ZNSA-2020013) (to W-J. Guan). M.A. Martinez-Garcia has no source of funding related to this paper. Funding information for this article has been deposited with the Crossref Funder Registry.

## References

1 Xu JF, Gao YH, Song YL, *et al.* Research advances and clinical management of bronchiectasis: Chinese perspective. *ERJ Open Res* 2022; 8: 00017–2022.
2 Flume PA, Chalmers JD, Olivier KN. Advances in bronchiectasis: endotyping, genetics, microbiome, and disease heterogeneity. *Lancet* 2018; 392: 880–890.
3 Araújo D, Shteinberg M, Aliberti S, *et al.* Standardised classification of the aetiology of bronchiectasis using an objective algorithm. *Eur Respir J* 2017; 50: 1701289.
4 Guan WJ, Li JC, Liu F, *et al.* Next-generation sequencing for identifying genetic mutations in adults with bronchiectasis. *J Thorac Dis* 2018; 10: 2618–2630.
5 Notarangelo LD, Plebani A, Mazzolari E, *et al.* Genetic causes of bronchiectasis: primary immune deficiencies and the lung. *Respiration* 2007; 74: 264–275.
6 Chau JFT, Lee M, Chui MMC, *et al.* Functional evaluation and genetic landscape of children and young adults referred for assessment of bronchiectasis. *Front Genet* 2022; 13: 933381.
7 Xu K, Diaz AA, Duan F, *et al.* Bronchial gene expression alterations associated with radiographic bronchiectasis. *Eur Respir J* 2023; 61: 2200120.
8 Ideozu JE, Zhang X, McColley S, *et al.* Transcriptome profiling and molecular therapeutic advances in cystic fibrosis: recent insights. *Genes (Basel)* 2019; 10: 180.
9 Clarke LA, Botelho HM, Sousa L, *et al.* Transcriptome meta-analysis reveals common differential and global gene expression profiles in cystic fibrosis and other respiratory disorders and identifies CFTR regulators. *Genomics* 2015; 106: 268–277.
10 Kopp BT, Fitch J, Jaramillo L, *et al.* Whole-blood transcriptomic responses to lumacaftor/ivacaftor therapy in cystic fibrosis. *J Cyst Fibros* 2020; 19: 245–254.
11 Kormann MSD, Dewerth A, Eichner F, *et al.* Transcriptomic profile of cystic fibrosis patients identifies type I interferon response and ribosomal stalk proteins as potential modifiers of disease severity. *PLoS One* 2017; 12: e0183526.
12 Peng Y, Zi XX, Tian TF, *et al.* Whole-transcriptome sequencing reveals heightened inflammation and defective host defence responses in chronic rhinosinusitis with nasal polyps. *Eur Respir J* 2019; 54: 1900732.
13 Bienvenu T, Sermet-Gaudelus I, Burgel PR, *et al.* Cystic fibrosis transmembrane conductance regulator channel dysfunction in non-cystic fibrosis bronchiectasis. *Am J Respir Crit Care Med* 2010; 181: 1078–1084.