Defining new risk factors for *Aspergillus* bronchitis

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INTRODUCTION

- *Aspergillus* bronchitis is a chronic non-invasive infection of the lower respiratory airways that affects immunecompetent patients, although the global burden of disease is unknown (1).
- Patients with symptoms of chronic pulmonary disease, microbiological evidence of *Aspergillus* in the airways (culture or PCR) and *Aspergillus*-specific IgG antibodies that do not fulfil the diagnostic criteria for chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis or invasive aspergillosis may have *Aspergillus* bronchitis (2).
- Although *A. fumigatus* is the most common species isolated from lungs of individuals with *Aspergillus* bronchitis, other *Aspergillus* species have been described as causative agents. Bacterial pathogens are also commonly observed in *Aspergillus* bronchitis (2).
- **Our objective was to define host and pathogen factors contributing to *Aspergillus* bronchitis.**

HOST FACTORS

METHODS

- We have previously described a mutation (rs35699176) in the human transcription factor ZNF77 associated with lung colonisation of the respiratory airways of patients with allergic bronchopulmonary aspergillosis (3).
- The presence of the genetic variant in rs35699176 was analysed on 46 DNA samples from patients with *Aspergillus* bronchitis. A 663 bp amplicon of the genomic region containing the genetic variant rs35699176 was amplified by PCR and sequences were resolved by Sanger sequencing (3).
- Prevalence of the genetic variant was compared to that found in patients with allergic bronchopulmonary aspergillosis in our previous study or in the healthy population.

RESULTS

- **ZNF77$^{rs356991176+/-}$ (GA) was present in 19% of patients with *Aspergillus* bronchitis.** Allele frequency for the variant associated with fungal colonisation was higher in patients with *Aspergillus* bronchitis than in those with allergic bronchopulmonary aspergillosis (ABPA, 9%) or healthy controls (0%) **(Figure 1).** (ABPA vs *Aspergillus* bronchitis: P = 0.09; OR: 0.37 [95% IC = 0.1307 to 1.011] ; *Aspergillus* bronchitis vs healthy: P < 0.0001: OR: 0.02 [95% IC = 0.004 to 0.084].

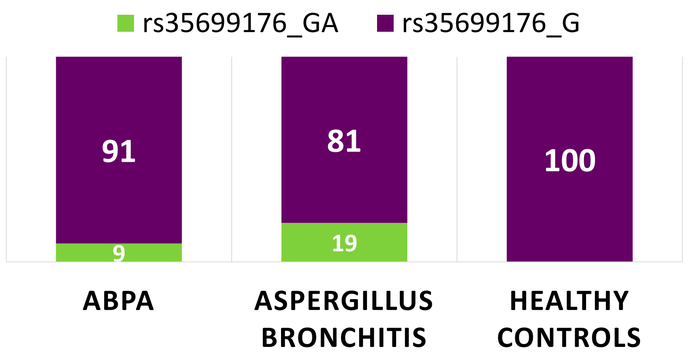

Figure 1: Allele frequency (%) of the variant GA in rs35699176 (ZNF77) in patients with *Aspergillus* bronchitis (n=46); Allergic bronchopulmonary aspergillosis (ABPA, n=95) and healthy controls (n=403).

CONCLUSIONS

- **The prevalence of the fungal-colonisation at risk allele rs35699176 in patients with *Aspergillus* bronchitis was higher than the reported for patients with fungal allergy.**
- **The mycobiome of patients diagnosed with *Aspergillus* bronchitis indicates high levels of *Cladosporium* and *Alternaria*, raising the possibility that this clinical entity is actually '*fungal bronchitis*', '*airway mycosis*' or '*fungal-associated airways disease*' rather than aspergillosis.**
- **Further research is needed to investigate the link between the presence of rs35699176 and the colonisation of the airways by non-*Aspergillus* species.**

REFERENCES

1. Chrdle et al. Ann N Y Acad Sci. 2012 Dec;1272:73-85. **2.** Brandt C et al. Mycopathologia. 2018 Feb;183(1):61-69. **3.** Gago S et al. Nat Commun. 2018 Sep 20;9(1):3835.**4.** Mac Aogain et al. Eur Respir J. 2018 Jul 27;52(1).

PATHOGEN FACTORS

METHODS

- Changes in the airway microbiome have been associated with the development and severity of lung diseases. However, little is known about the composition of the lung mycobiome in patients with *Aspergillus* bronchitis.
- The Internal transcribed spacer 1 (ITS1) region of DNA sputum samples from an independent cohort of patients with *Aspergillus* bronchitis was amplified by using ITS1 and ITS2deg primer pairs including Nextera XT adapters.
- PCR products were indexed and paired-end sequenced using Illumina MiSeq (2x250) .
- Raw sequencing reads were analysed using FastQC - Bbmerge - Cutadapt. An end-to-end alignment was performed against the ISHAM ITS database using Bowtie2 (v 2.3.4.3).

RESULTS

- **DNA quality is critical to get enough reads for mycobiome analyses. Long-term sample storage had a negative impact on Sequencing performance (Figure 2)**

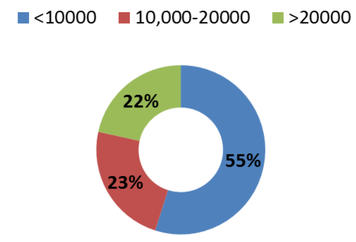

Figure 2: Distribution of samples according to mapped reads. Only 22% of the samples displayed an optimal number of reads for mycobiome analyses which correlated to samples in long term storage. Low number of reads correlated with poor assay reproducibility.

- **The pulmonary mycobiome in *Aspergillus* bronchitis is dominated by Ascomycota environmental genera (Figure 3).**

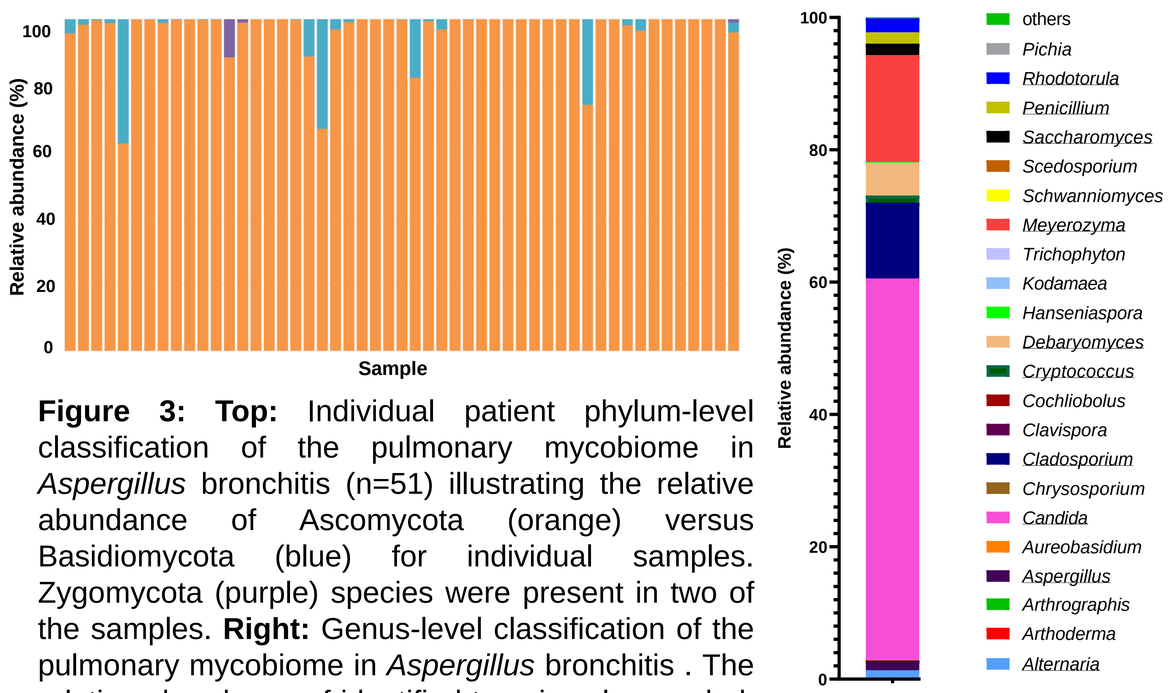

Figure 3: Top: Individual patient phylum-level classification of the pulmonary mycobiome in *Aspergillus* bronchitis (n=51) illustrating the relative abundance of Ascomycota (orange) versus Basidiomycota (blue) for individual samples. Zygomycota (purple) species were present in two of the samples. **Right:** Genus-level classification of the pulmonary mycobiome in *Aspergillus* bronchitis . The relative abundance of identified taxa is colour-coded. *Cladosporium* species represented the most abundant mould genera.

- **Percent prevalence of observed fungal genera in *Aspergillus* bronchitis samples compared to patients with bronchiectasis and non-disease controls available from published studies (Figure 4).**

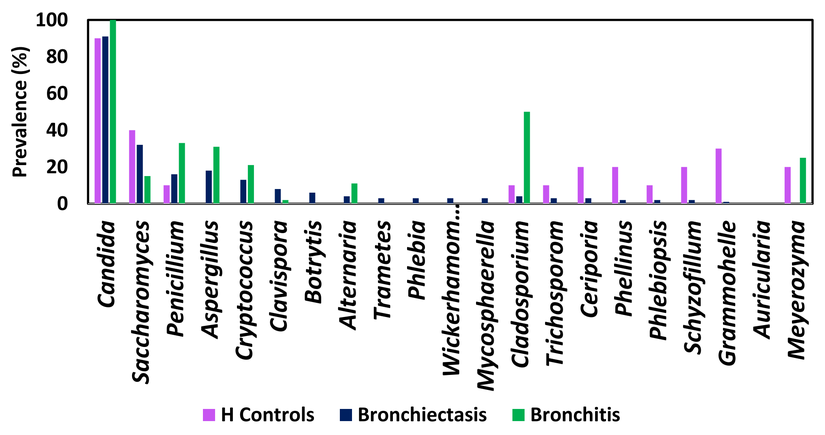

Figure 4: Percent prevalence of observed fungal genera/sample in *Aspergillus* bronchitis compared to patients with bronchiectasis and non-disease controls available from published studies (4). *Cladosporium, Aspergillus* and *Alternaria* were the most abundant mould genera compared to healthies and patients with bronchiectasis.

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