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Aspergillus colonisation of the airways – it is partly in your genes

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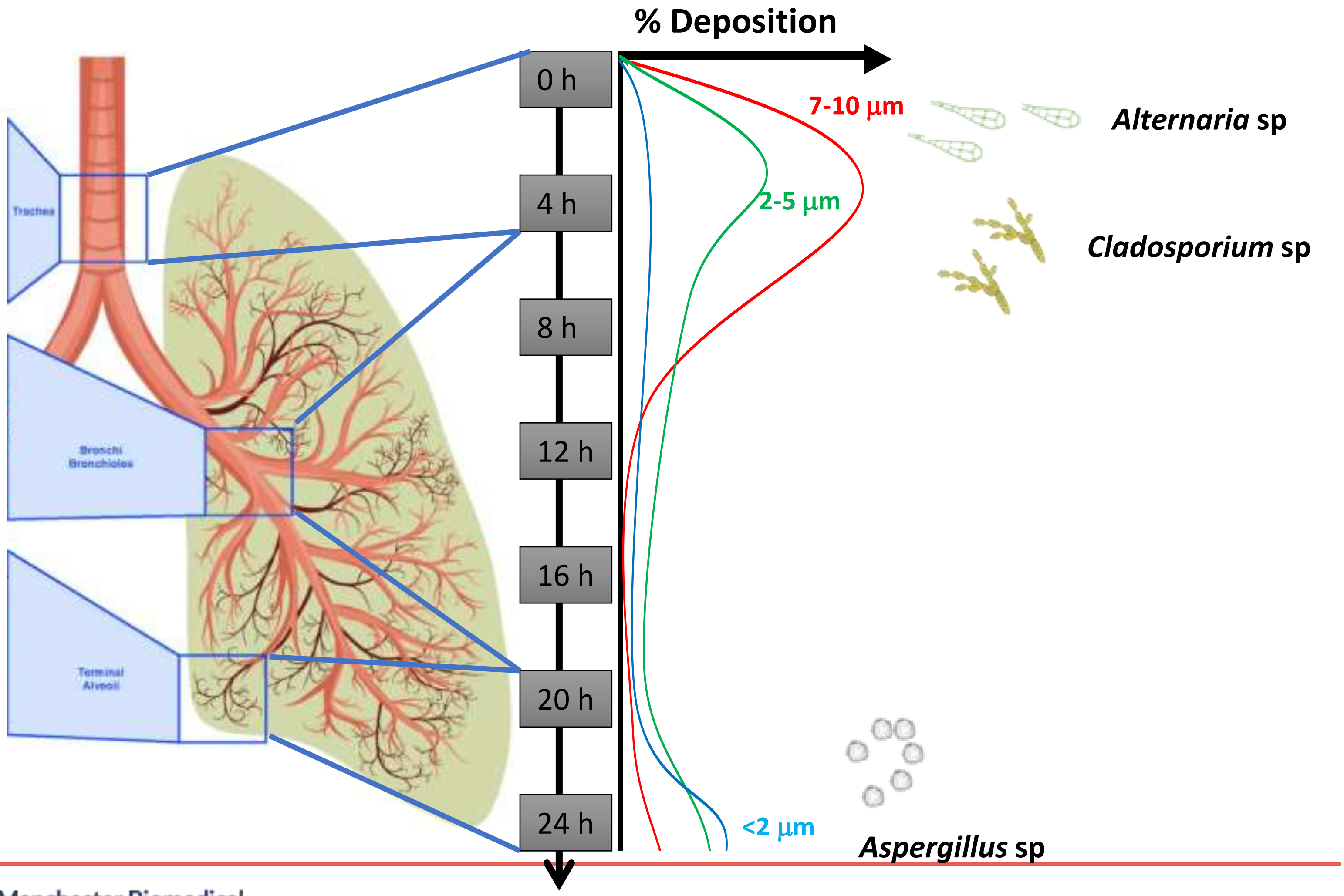
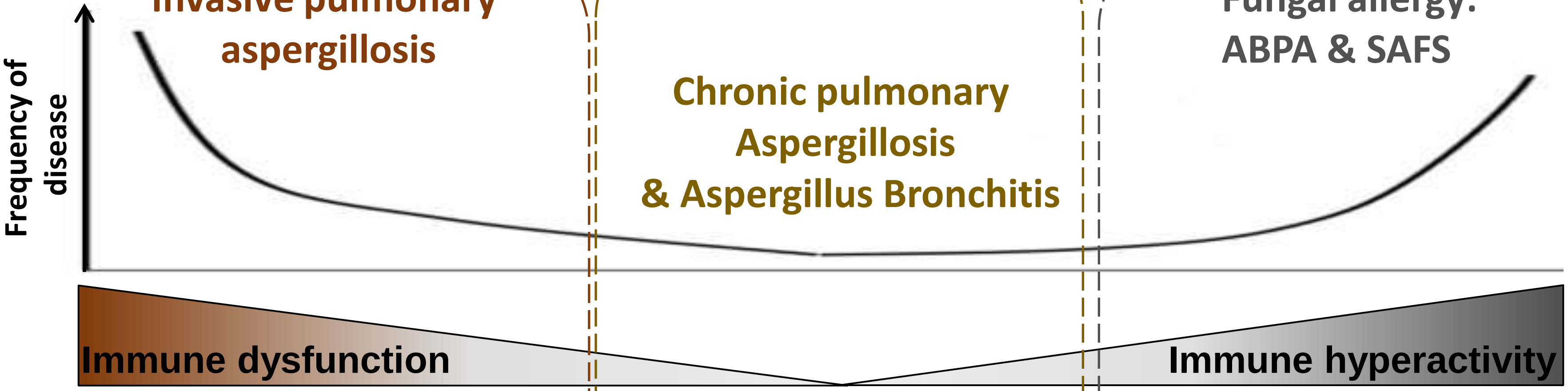
Deposition of fungal spores in the lungs depends on spore size, branching and tubule diameter



Wiesner D and Klein B, Curr Opin Microbiol. 2017
Gago S et al, Med Mycol. 2019

Aspergillus colonisation and Aspergillosis



Invasive pulmonary aspergillosis

- No frequent
- Risk for IPA in pre-lung transplant

Chronic pulmonary Aspergillosis & Aspergillus Bronchitis

- Frequent
- High Aspergillus - IgG

Fungal allergy: ABPA & SAFS

- Frequent
- Decreased lung function

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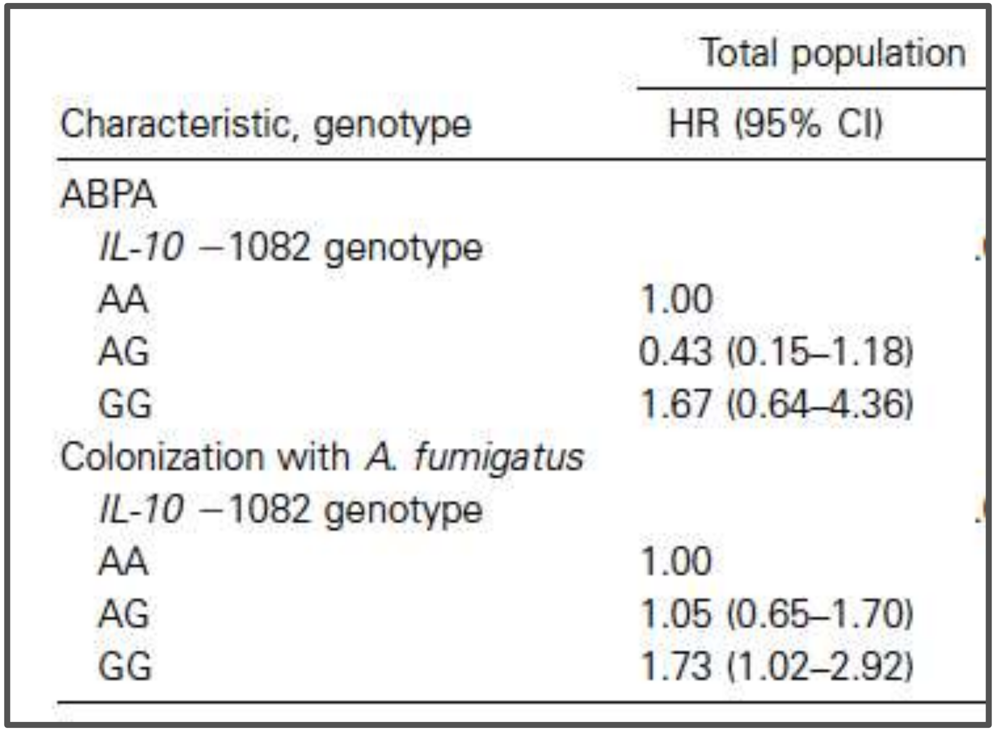
Denning DW, et al. Clin Infect Dis. 2011
Farrant J et al, J Asthma. 2016

Denning DW et al. Eur Respir J. 2006.
Menzies et al, Allergy. 2011

IL-10 & risk of ABPA and fungal colonisation in cystic fibrosis

- IL-10-1082GG genotype is associated with ABPA and *Aspergillus* colonization and high levels of IL-10 in serum



	Total population
Characteristic, genotype	HR (95% CI)
ABPA	
IL-10 −1082 genotype	
AA	1.00
AG	0.43 (0.15–1.18)
GG	1.67 (0.64–4.36)
Colonization with *A. fumigatus*	
IL-10 −1082 genotype	
AA	1.00
AG	1.05 (0.65–1.70)
GG	1.73 (1.02–2.92)

Brouart et al. The Journal of Infectious Diseases, 2005,

SP-A2 and ABPA

- Two non redundant polymorphisms in SP-A2 are associated with ABPA (A1660G and G1649C: P = .0079, OR = 10.4).
- Patients carrying both polymorphisms had high IgE levels and eosinophilia.

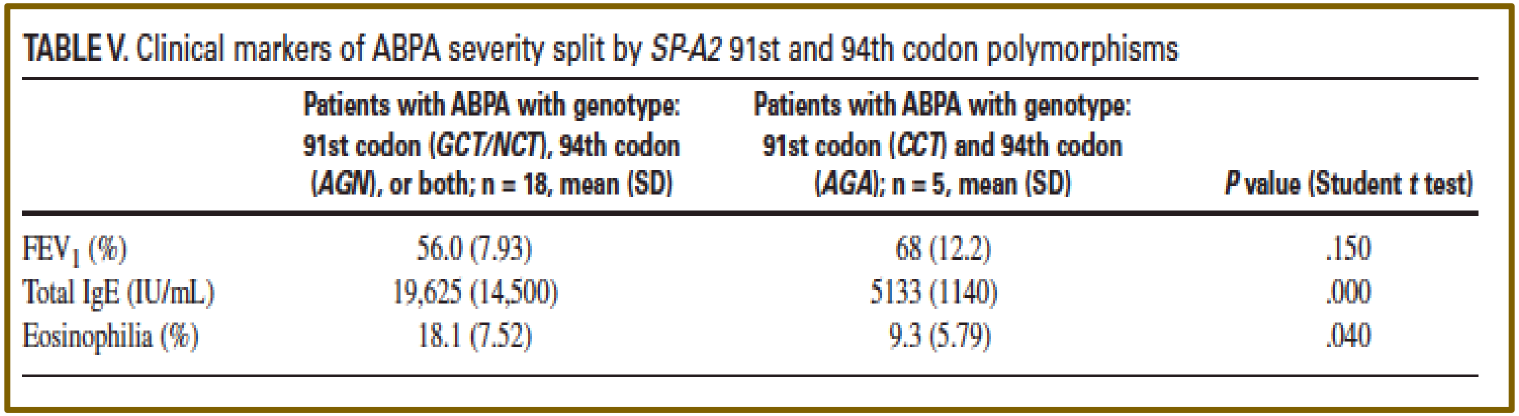


TABLE V. Clinical markers of ABPA severity split by *SP-A2* 91st and 94th codon polymorphisms

	Patients with ABPA with genotype: 91st codon (*GCT/NCT*), 94th codon (*AGN*), or both; n = 18, mean (SD)	Patients with ABPA with genotype: 91st codon (*CCT*) and 94th codon (*AGA*); n = 5, mean (SD)	*P* value (Student *t* test)
FEV_1 (%)	56.0 (7.93)	68 (12.2)	.150
Total IgE (IU/mL)	19,625 (14,500)	5133 (1140)	.000
Eosinophilia (%)	18.1 (7.52)	9.3 (5.79)	.040

Saxena, et al. Journal of Allergy and Clinical Immunolog
2003.

Whole exome sequencing to identify genetic risk factors associated with ABPA

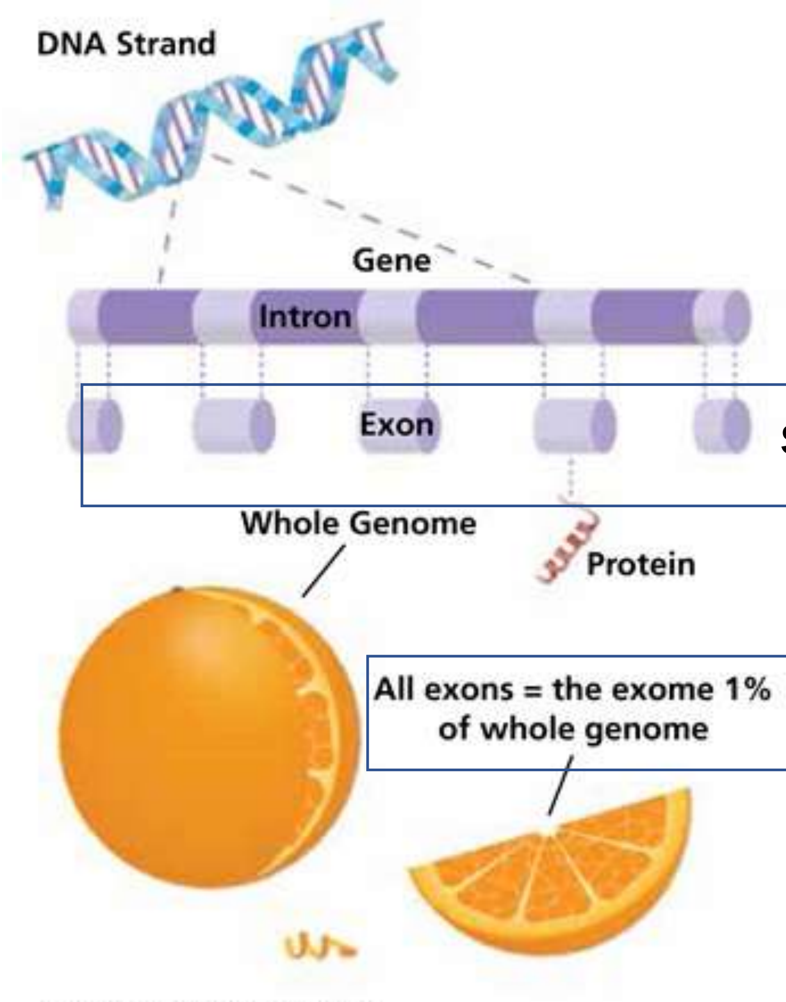


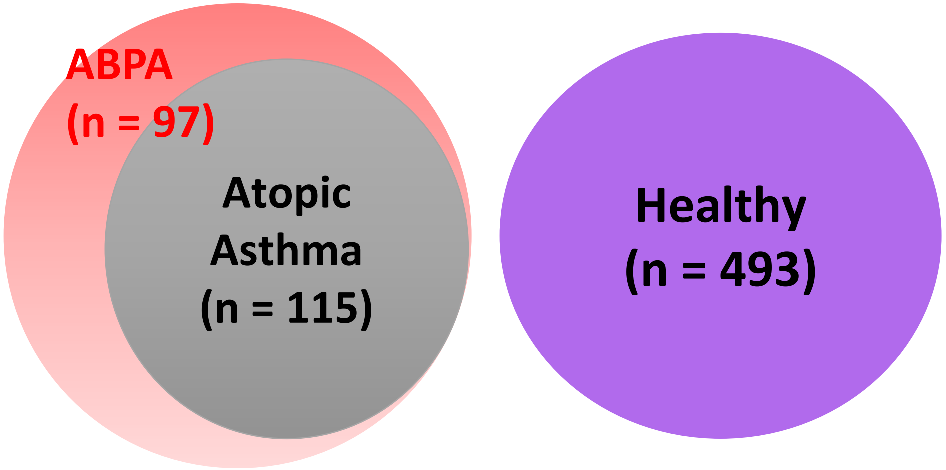
ILLUSTRATION BY MEAHGAN HARRIGAN

SEQUENCING OF ALL PROTEIN CODING GENES

CONTAINS 90% GENETIC VARIABILITY

IDENTIFICATION OF GENETIC VARIANTS THAT ALTER PROTEIN SEQUENCES

Whole-exome sequencing in patients with ABPA, compared to both atopic asthmatics and healthy.



- Common genetic signatures in ABPA and atopic asthma;
- Differences might be the key to understand why only 2 % of asthmatics develop ABPA.
- Role of genetic variability in the host antifungal response

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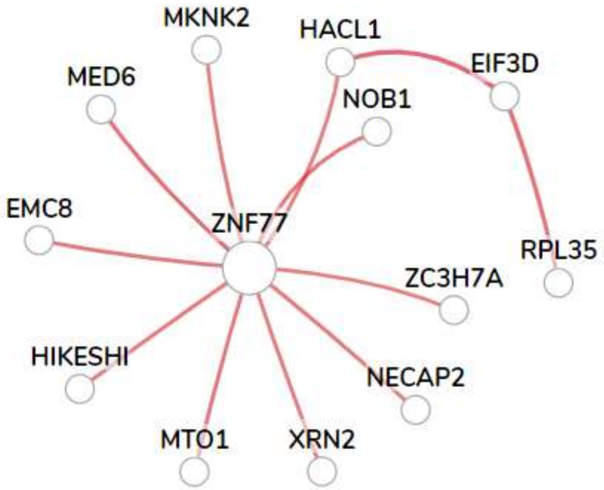
Overton, Gago et al, unpublished

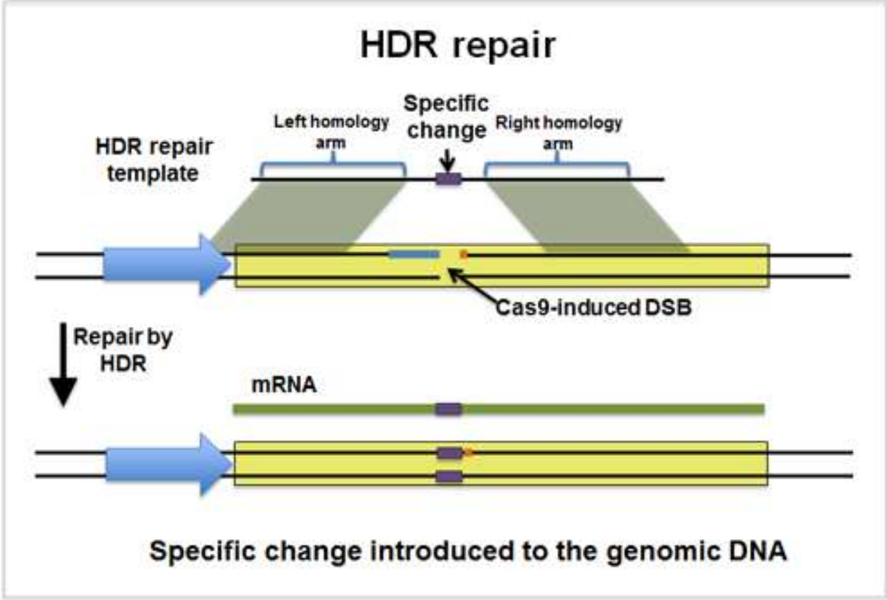
Is there a mechanistic link between ZNF77 and fungal allergy?

- ZNF77 belongs to the zinc finger transcription factors family
- It has been associated with alterations in cytokine release and endocytosis.
- **<u>WHY ZNF77?</u>**
  - It was significantly associated with ABPA
  - It was a high impact variant as introduces a premature stop codon in ZNF77.
  - Good candidate for phenotype analyses.



Overton, Gago et al, unpublished

Generation of 16HBE cells carrying the ABPA-associated genetic variant (rs35699176) in ZNF77



Gago Nat Comms, 2018

Gago S, 10.1007/978-1-0716-1182-1 2020

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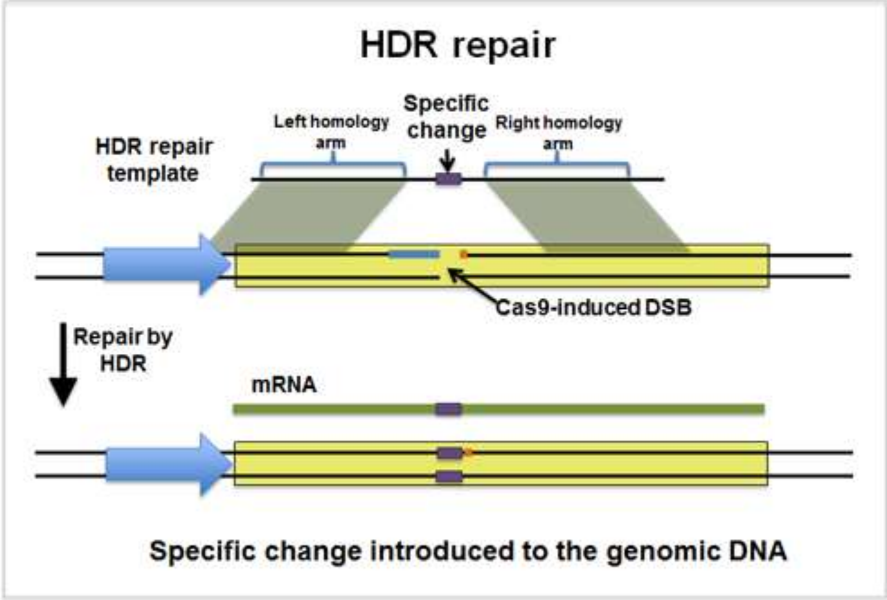
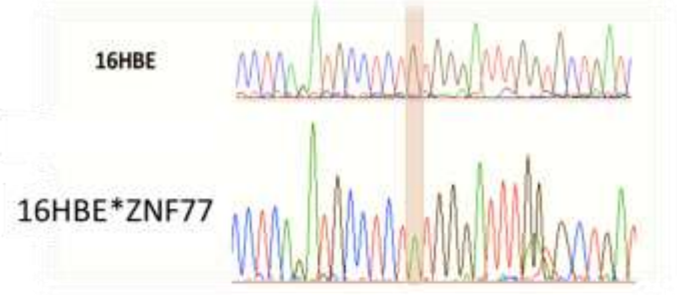
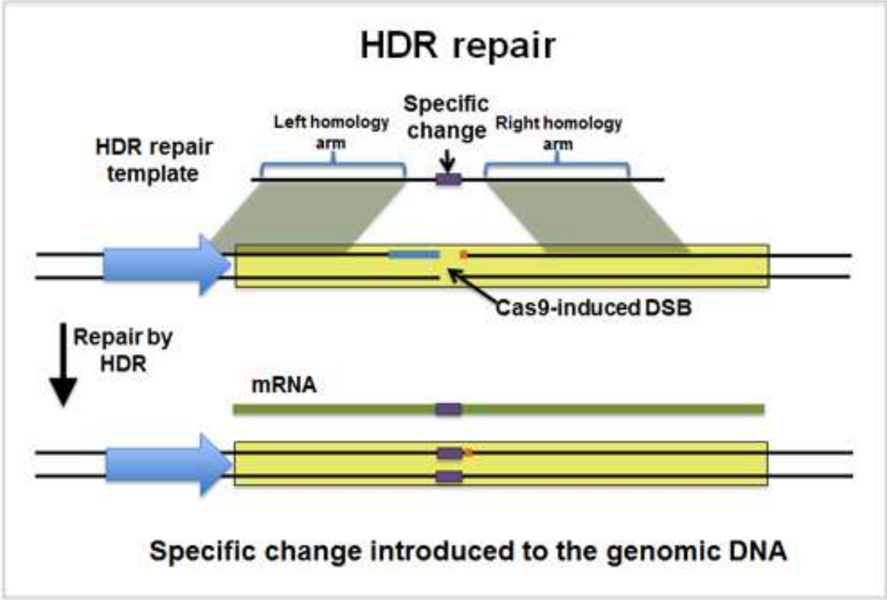


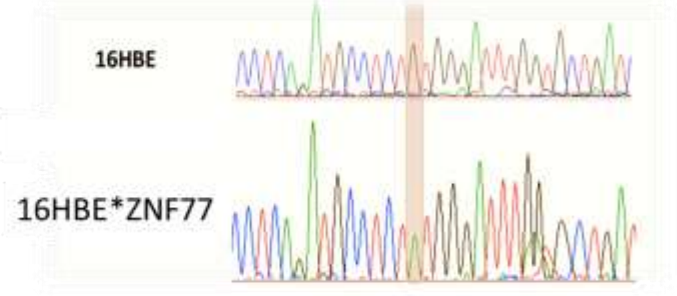


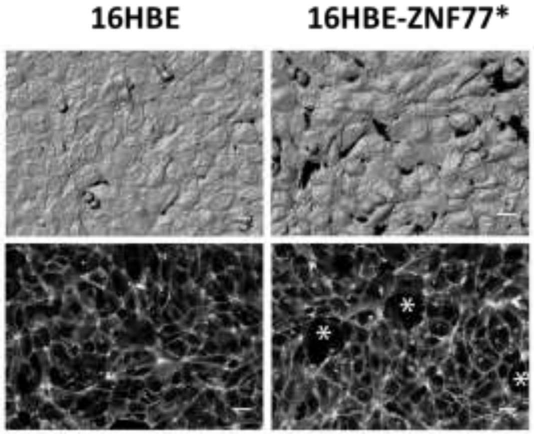
Gago Nat Comms, 2018

Gago S, 10.1007/978-1-0716-1182-1 2020

ZNF77 regulates epithelial integrity



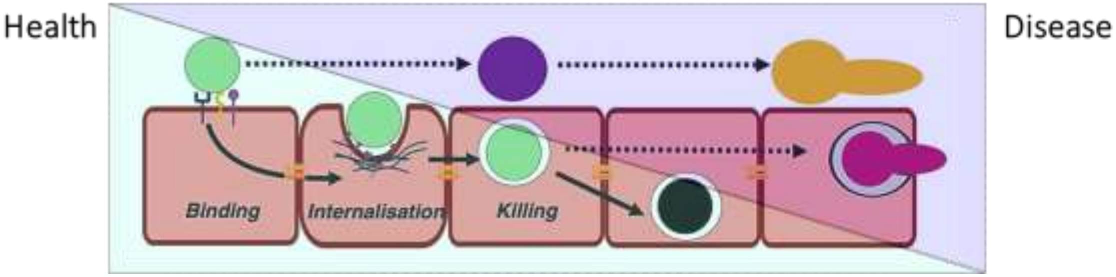




Gago Nat Comms, 2018

Gago S, 10.1007/978-1-0716-1182-1 2020

Impact of rs35699176 in the *Aspergillus* – epithelia interaction



Gago Nat Comms, 2018

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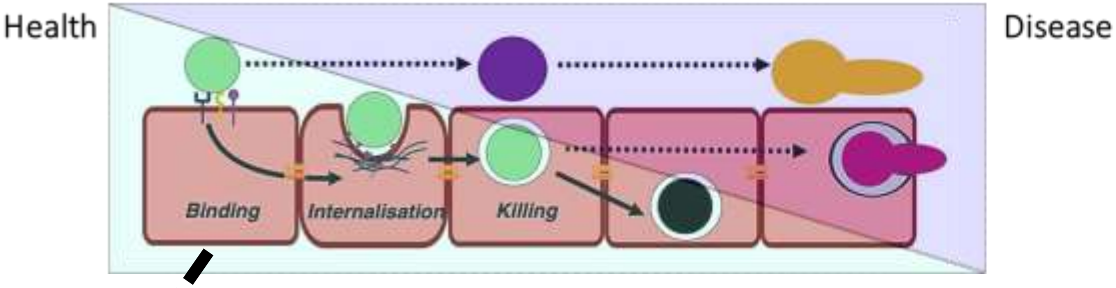
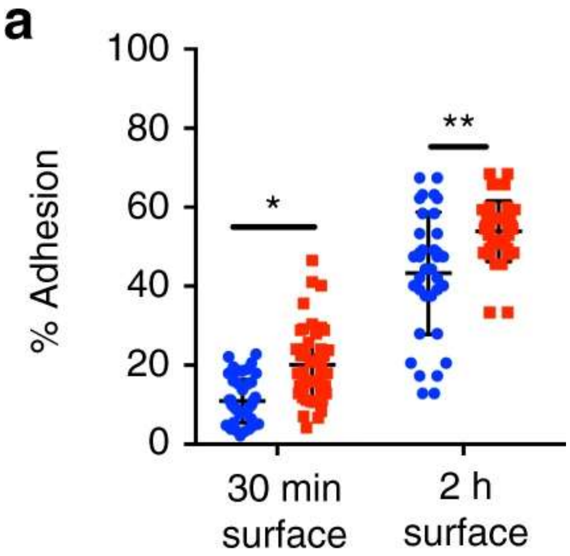


Cells with the mutation in ZNF77 (red) facilitate conidia adhesion compared to cells with the normal genotype (blue)



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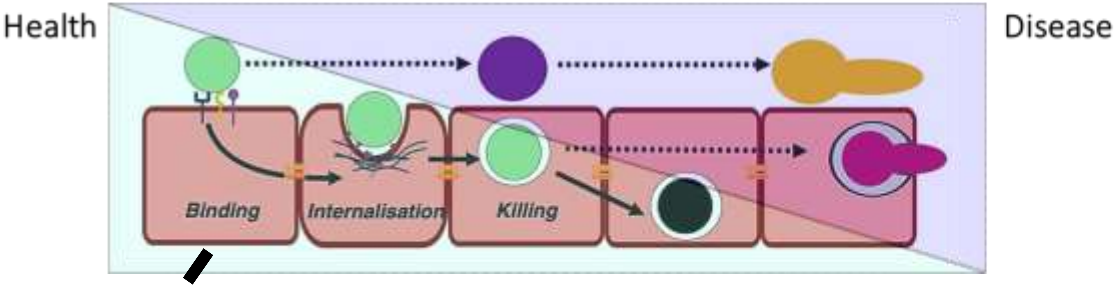

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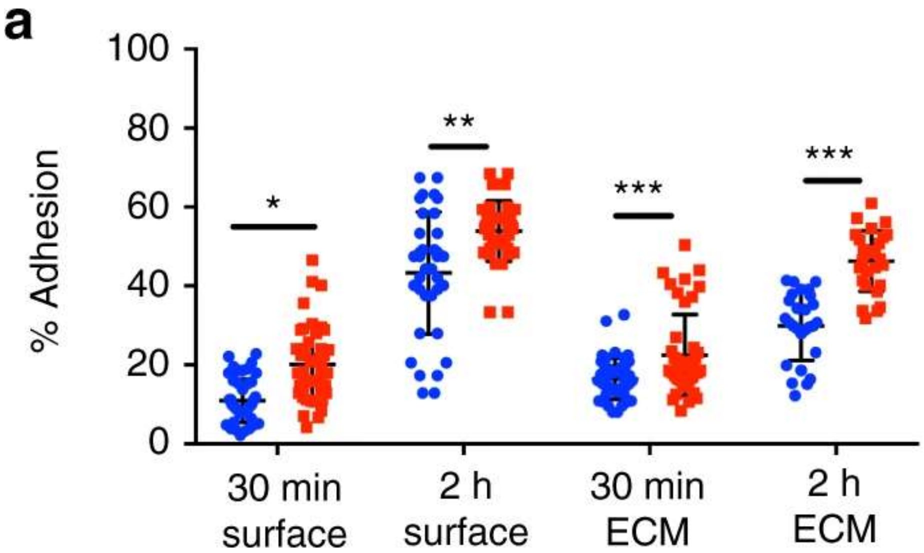

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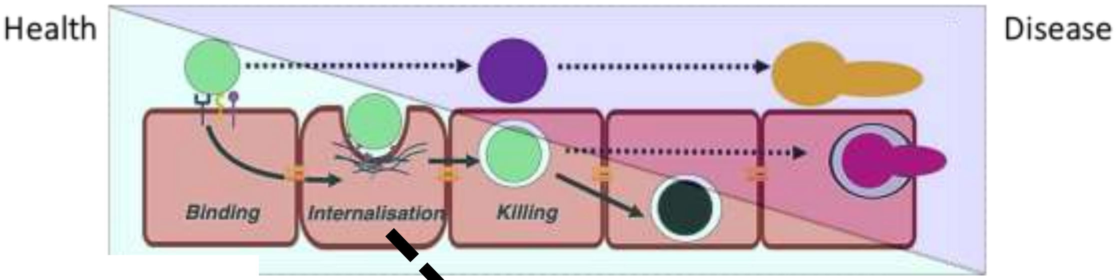
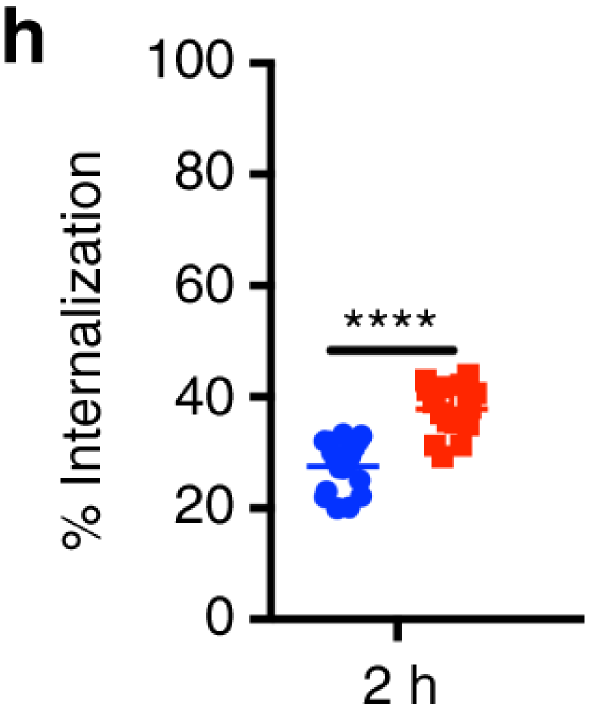
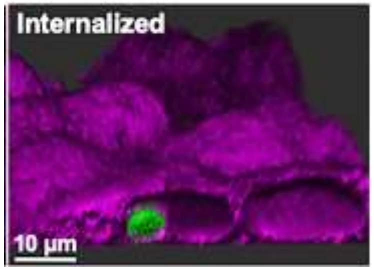


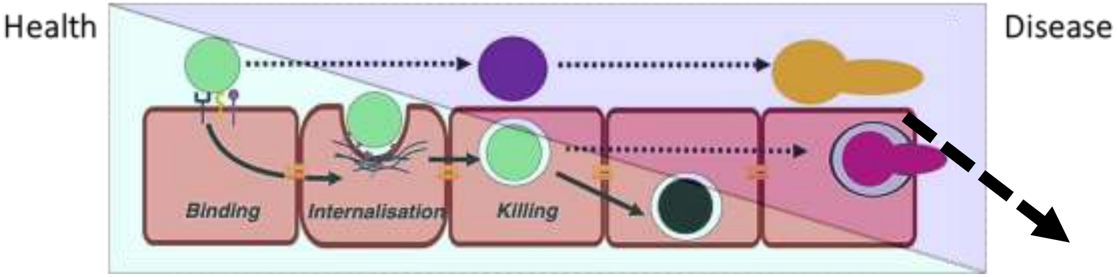


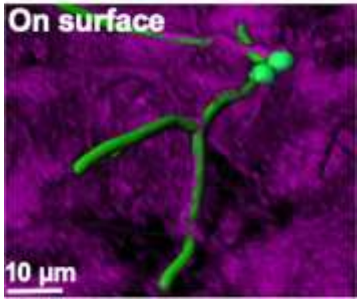


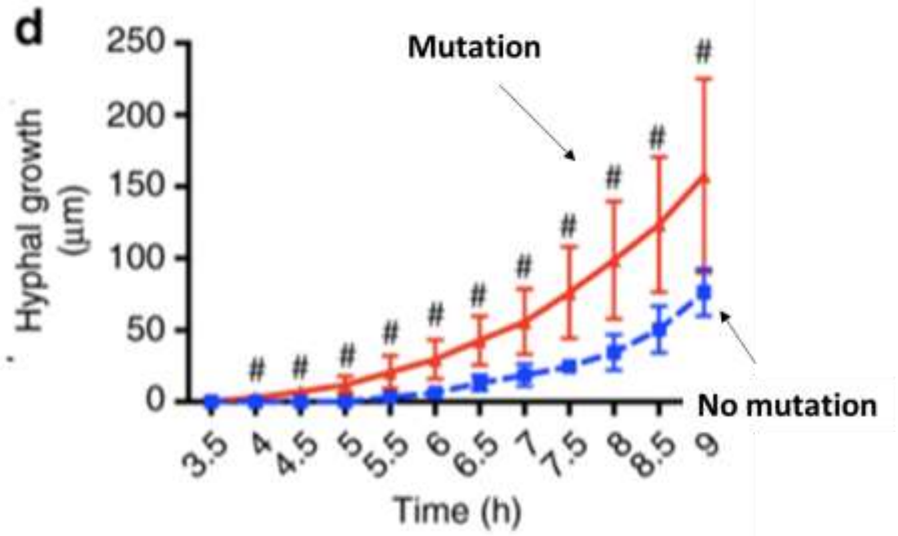
A. fumigatus **spores are more frequently internalised by cells carrying the mutation in ZNF77 (red) compared to cells with the normal genotype (blue) early after exposure**

Gago Nat Comms, 2018

Impact of rs35699176 in the *Aspergillus* – epithelia interaction







A. fumigatus **growth on cells with the mutation in ZNF77 (red) is higher than in cells with the normal genotype (blue)**

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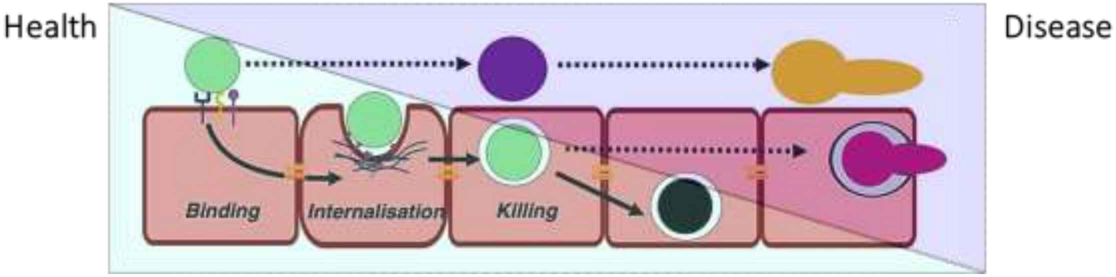
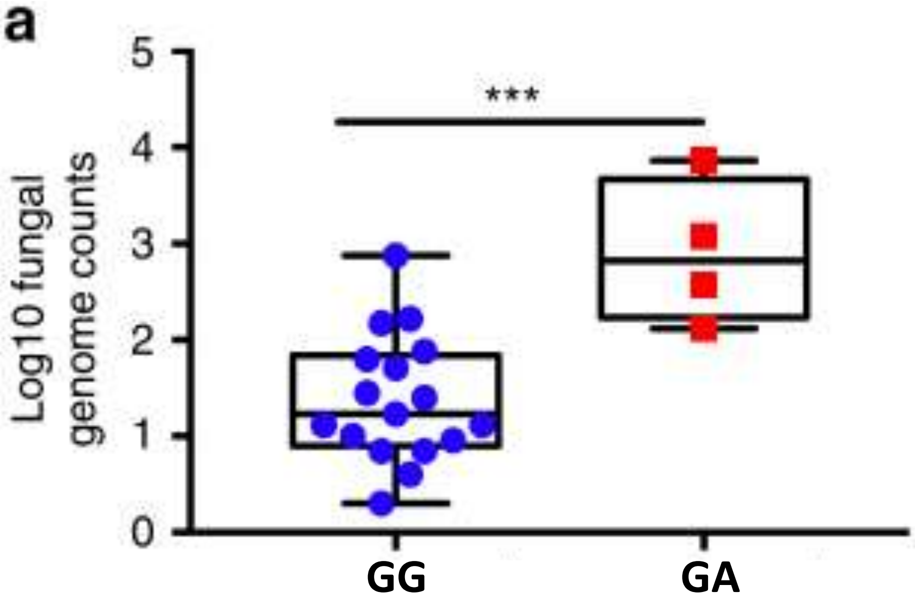
Impact of rs35699176 in the *Aspergillus* – epithelia interaction



Patients with the mutation (red) have higher fungal burden in the airways than patients without the mutation (blue)



Gago Nat Comms, 2018

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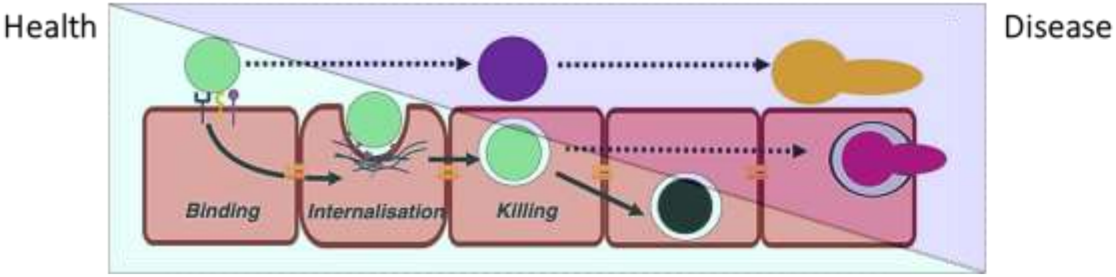

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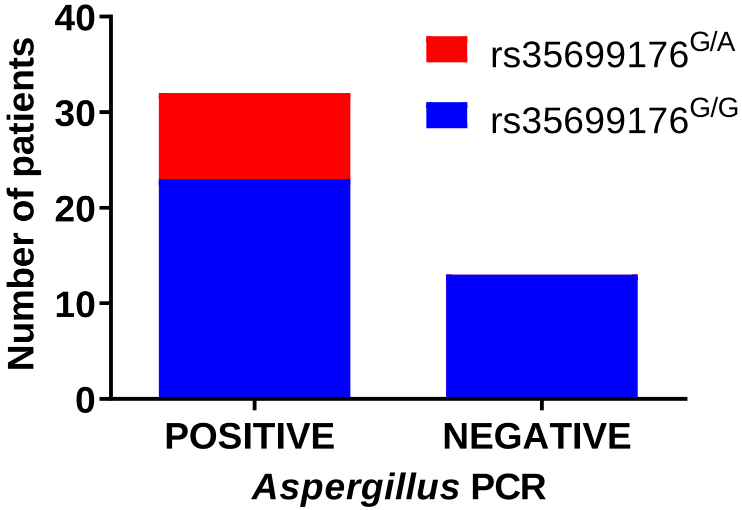

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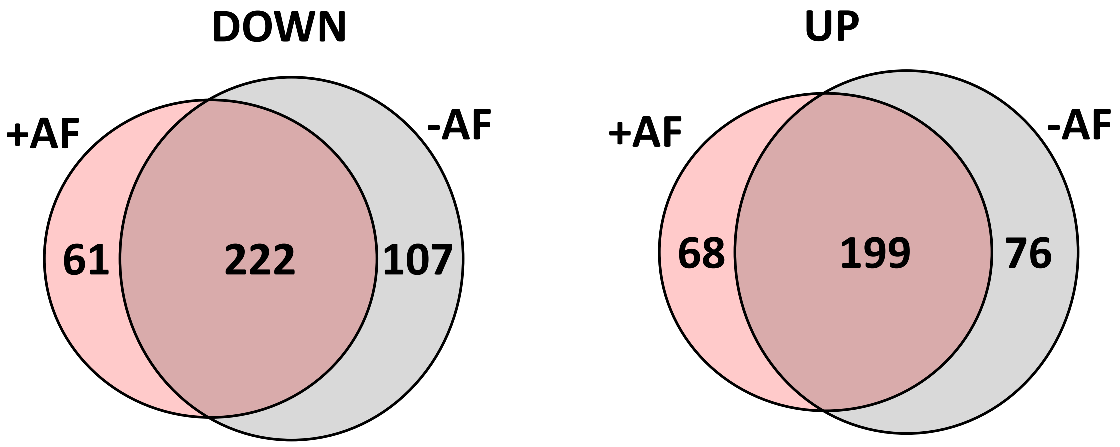
How does ZNF77 regulate fungal colonisation?



ZNF77* vs 16HBE

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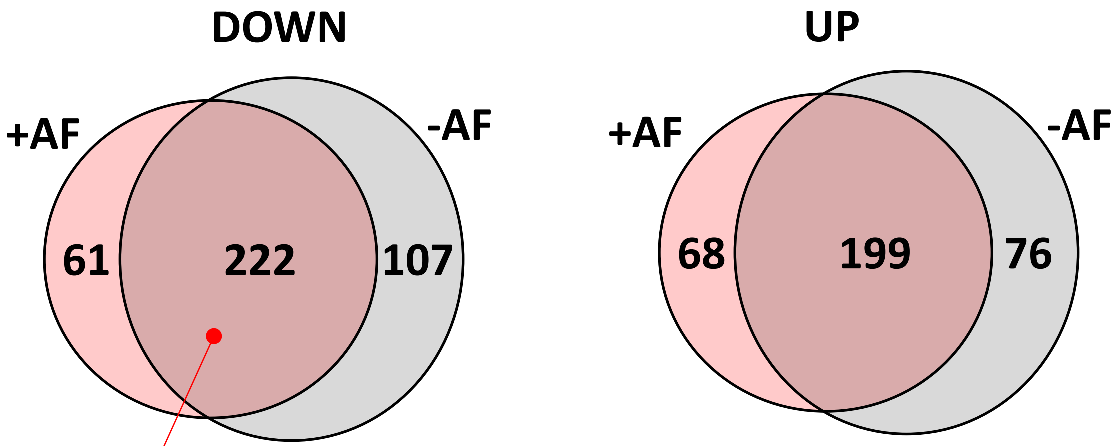
How does ZNF77 regulate fungal colonisation?

ZNF77* vs 16HBE



61/222
GO:0009653/0030334
“Migration/morphogenesis”
Padj=0.0005

Impaired epithelial structure

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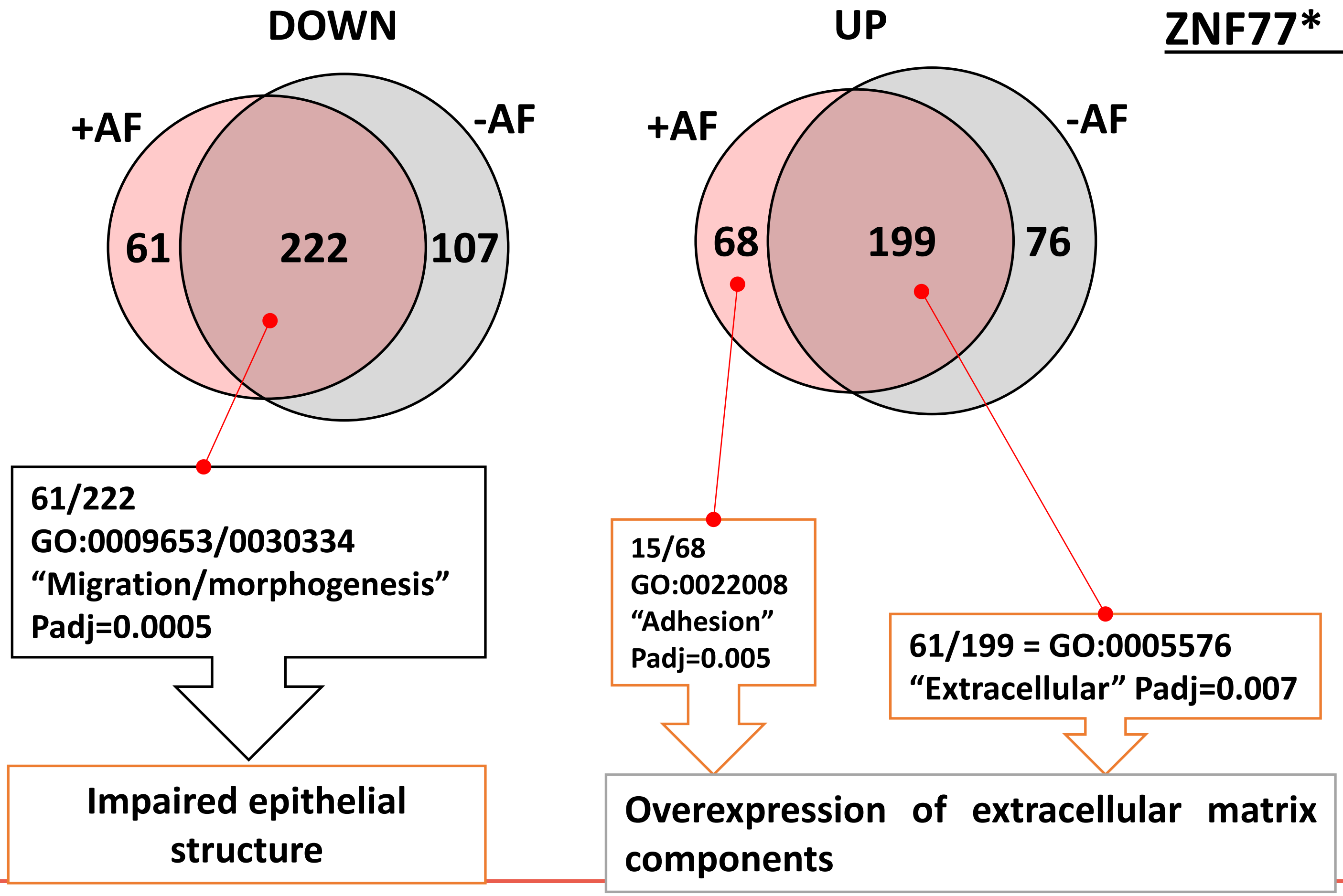
How does ZNF77 regulate fungal colonisation?

ZNF77* vs 16HBE



61/222
GO:0009653/0030334
“Migration/morphogenesis”
Padj=0.0005

15/68
GO:0022008
“Adhesion”
Padj=0.005

61/199 = GO:0005576
“Extracellular” Padj=0.007

Impaired epithelial structure

Overexpression of extracellular matrix components

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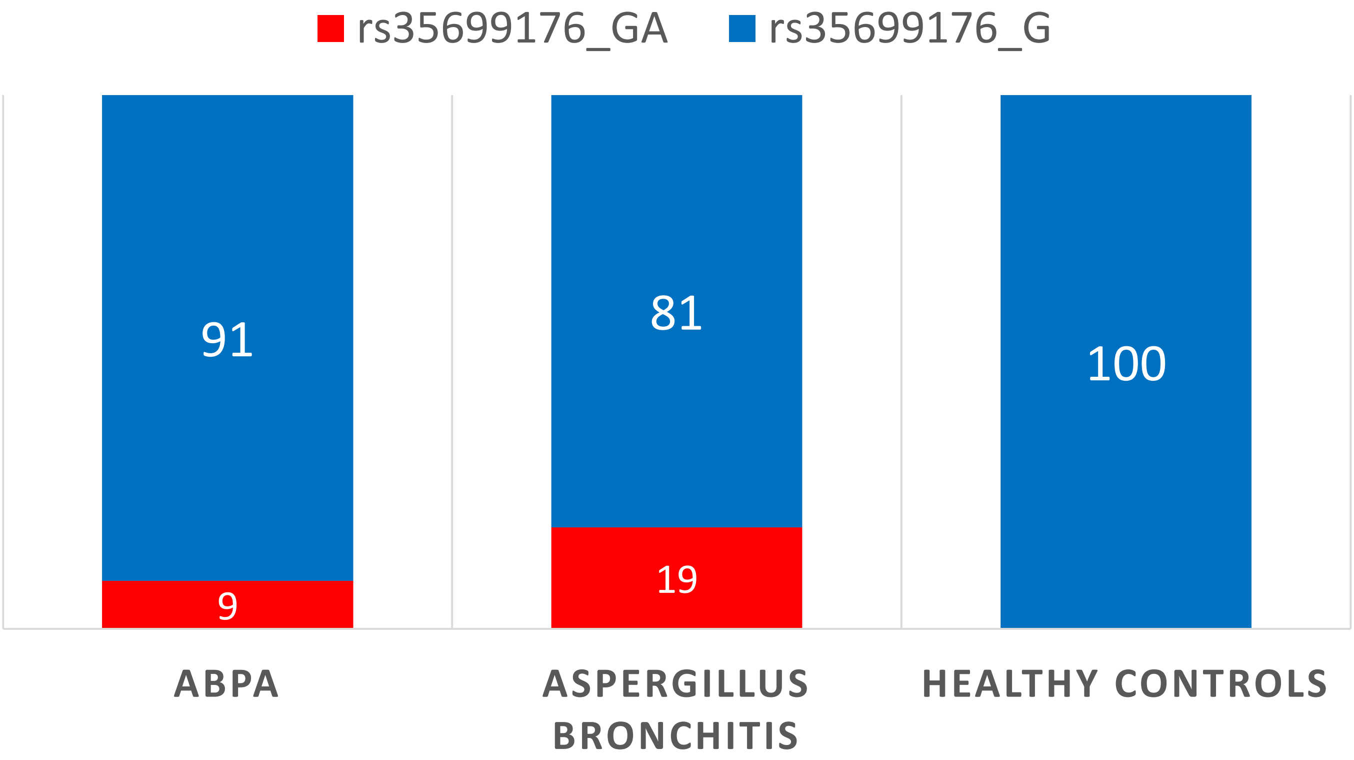
ZNF77 & *Aspergillus* bronchitis



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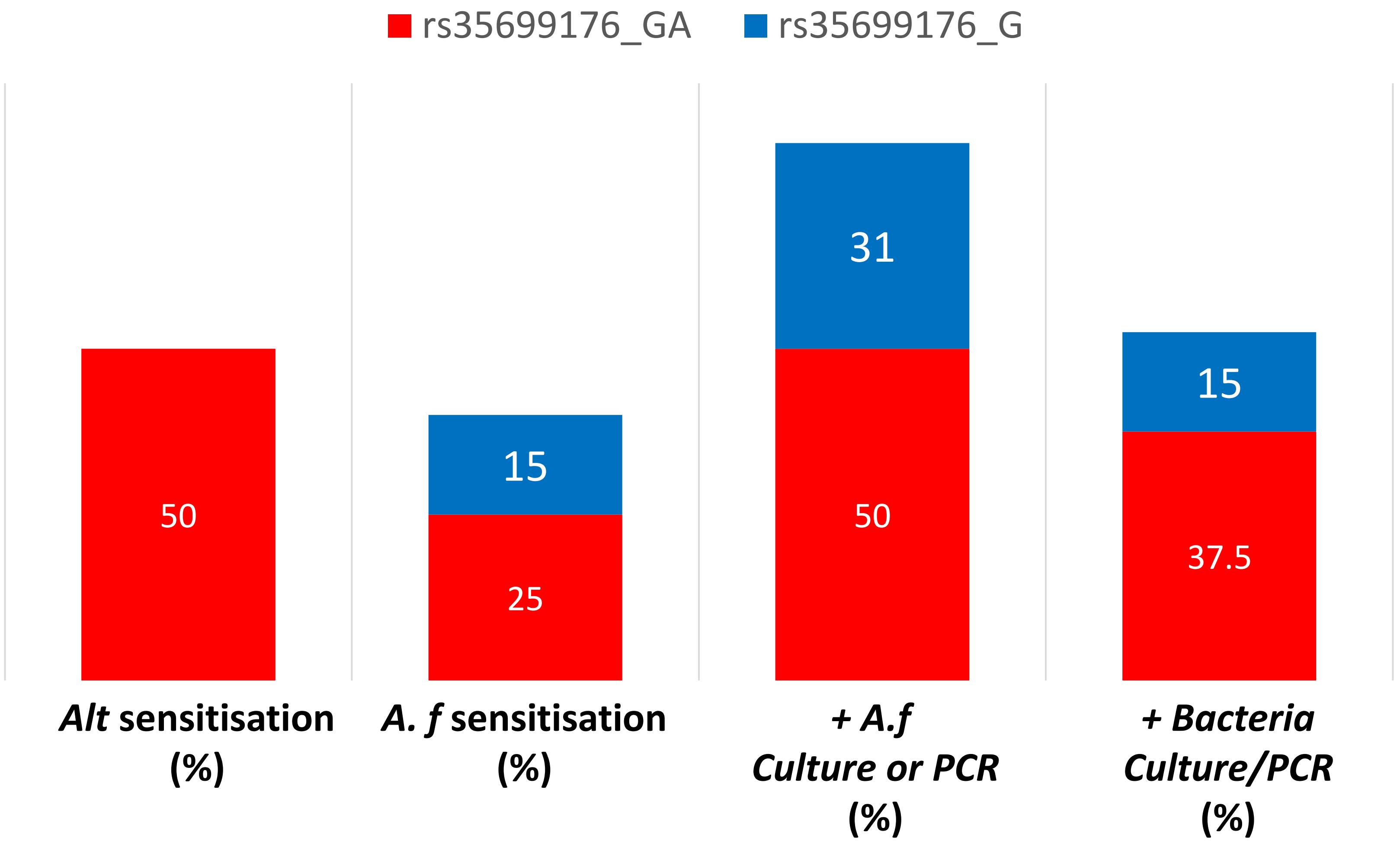

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Gago unpublished

Conclusions

- Fungal colonisation of the respiratory airways by *Aspergillus* spp is frequent in ABPA and *Aspergillus* bronchitis;
- Mutations in ZNF77 causes loss of epithelial integrity and make the airway epithelium more receptive to *A. fumigatus*;
- ZNF77 is a key controller of *A. fumigatus* colonization.

Future Directions

- Define genetic susceptibility factors for other aspergillosis clinical forms;
- Identify the role of genetic-comorbidities in the antifungal response;
- Explore the role of genetic susceptibility factors in co-infection.

Acknowledgements

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- **Flow Cytometry and Genomics Facility UoM**

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FEMS

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National Aspergillosis Centre

Mycology Reference Centre Manchester

Fungal Infection Trust

NC3Rs

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