

How humans regulate *Aspergillus* colonization in fungal allergy

Dr Sara Gago

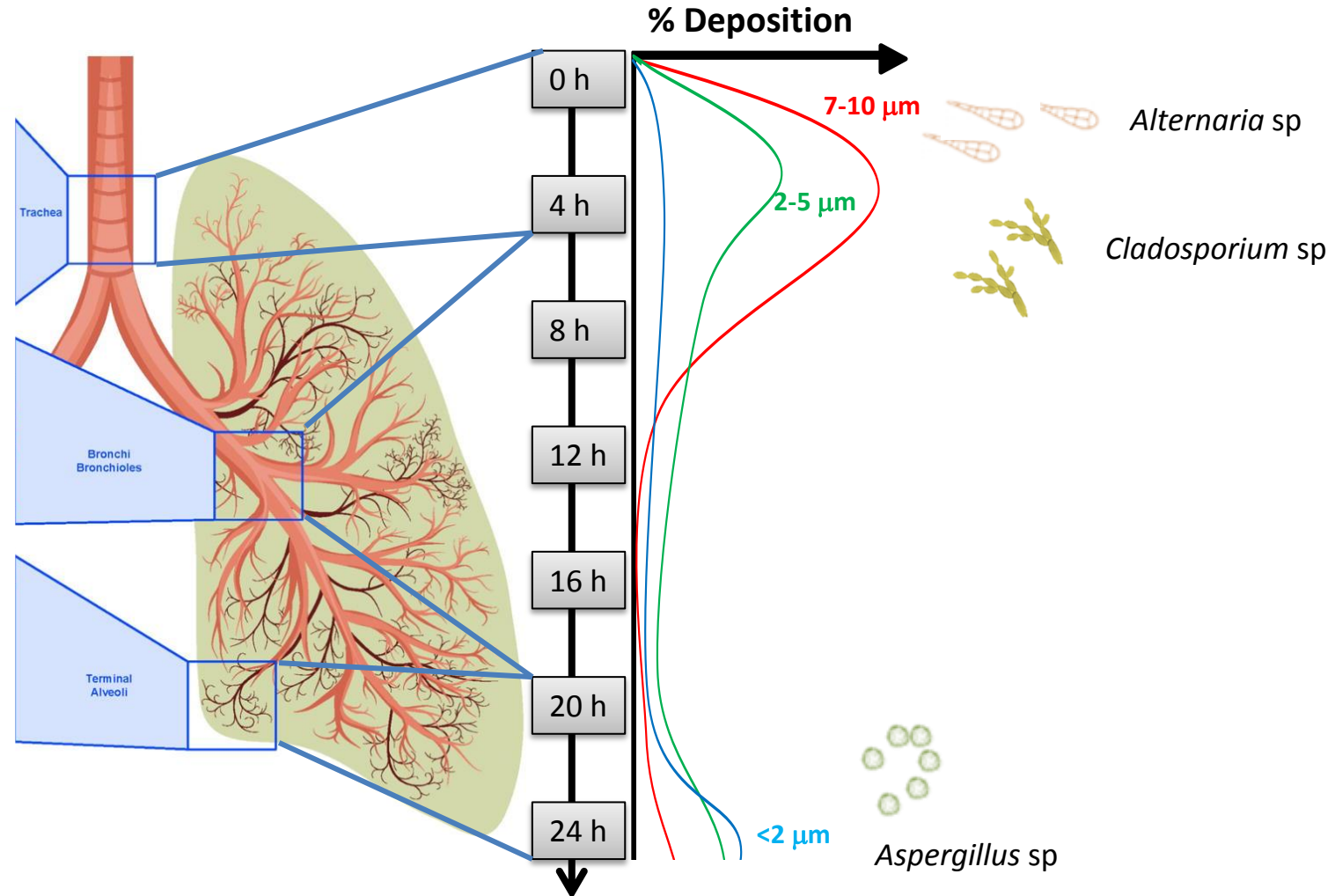
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8th AAA, Lisbon, 2018

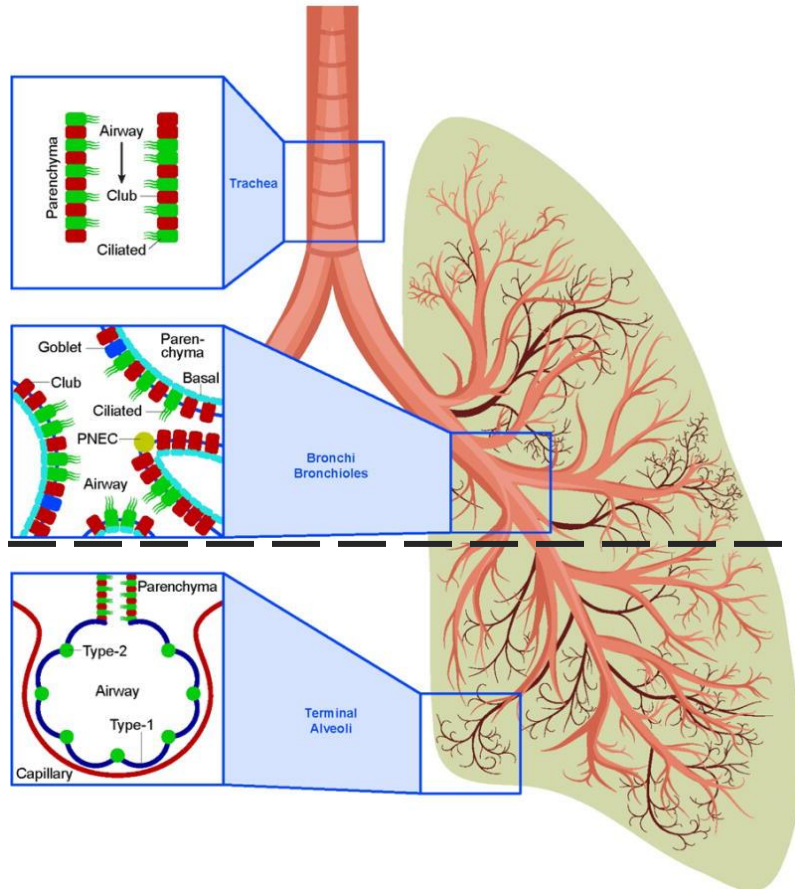


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DEPOSITION OF SPORES IN THE LUNG DEPENDS ON SPORE SIZE, BRANCHING AND TUBULE DIAMETER

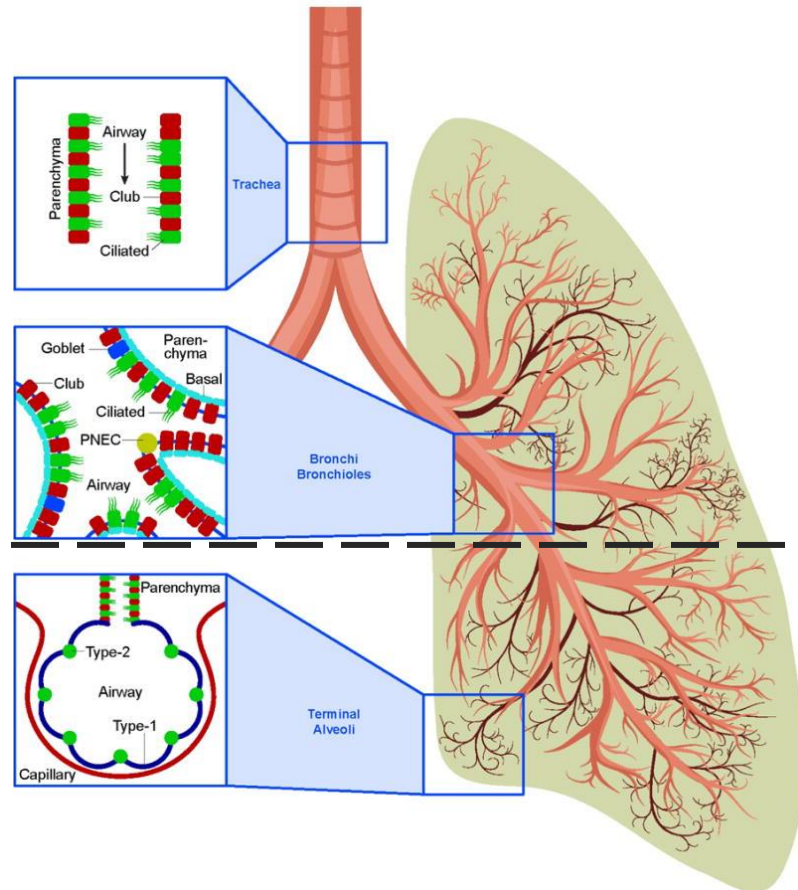


MECHANISMS FOR FUNGAL CLEARANCE



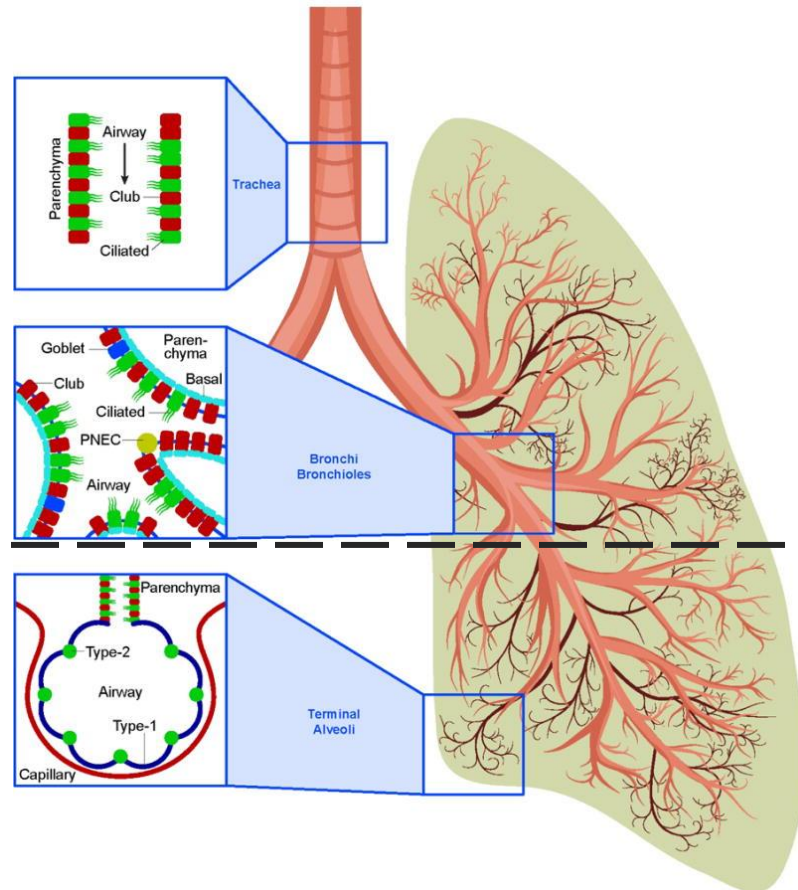
- ✓ *Mucous and cilia lining the airways capture and reverse the trajectory of inhaled pathogens.*
- ✓ *Different epithelial cell subsets will contribute to the secretion of antimicrobial peptides, mucous and surfactant to the lumen*

MECHANISMS FOR FUNGAL CLEARANCE



- ✓ *Alveolar macrophages and neutrophils will contribute to fungus killing.*
- ✓ *Increasing importance of the alveolar epithelial cells in fungal clearance.*

MECHANISMS FOR FUNGAL CLEARANCE ARE SIZE-DEPENDENT



***COLONIZATION OF THE
RESPIRATORY AIRWAYS
AND DISEASE***

BIOLOGICAL AND CLINICAL CONCEPT OF FUNGAL COLONIZATION

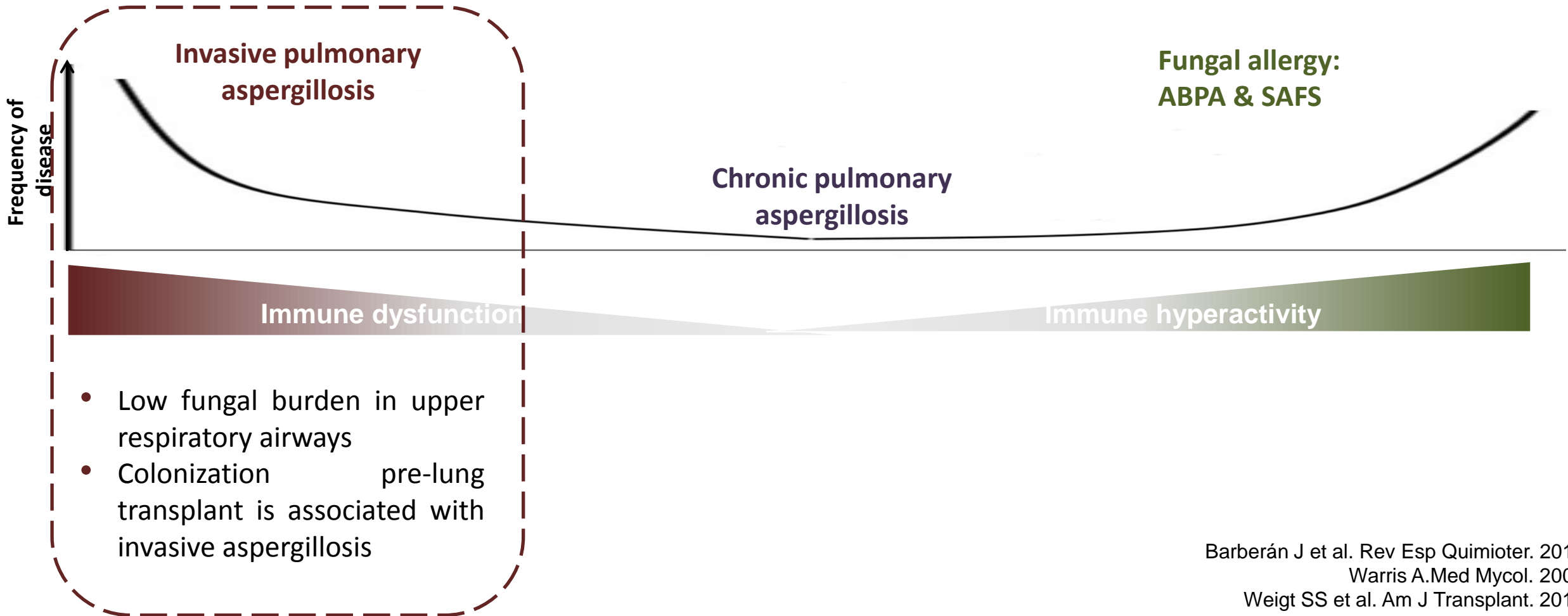
Biological

Fungus are established in the lung but it does not involve damage

Clinical

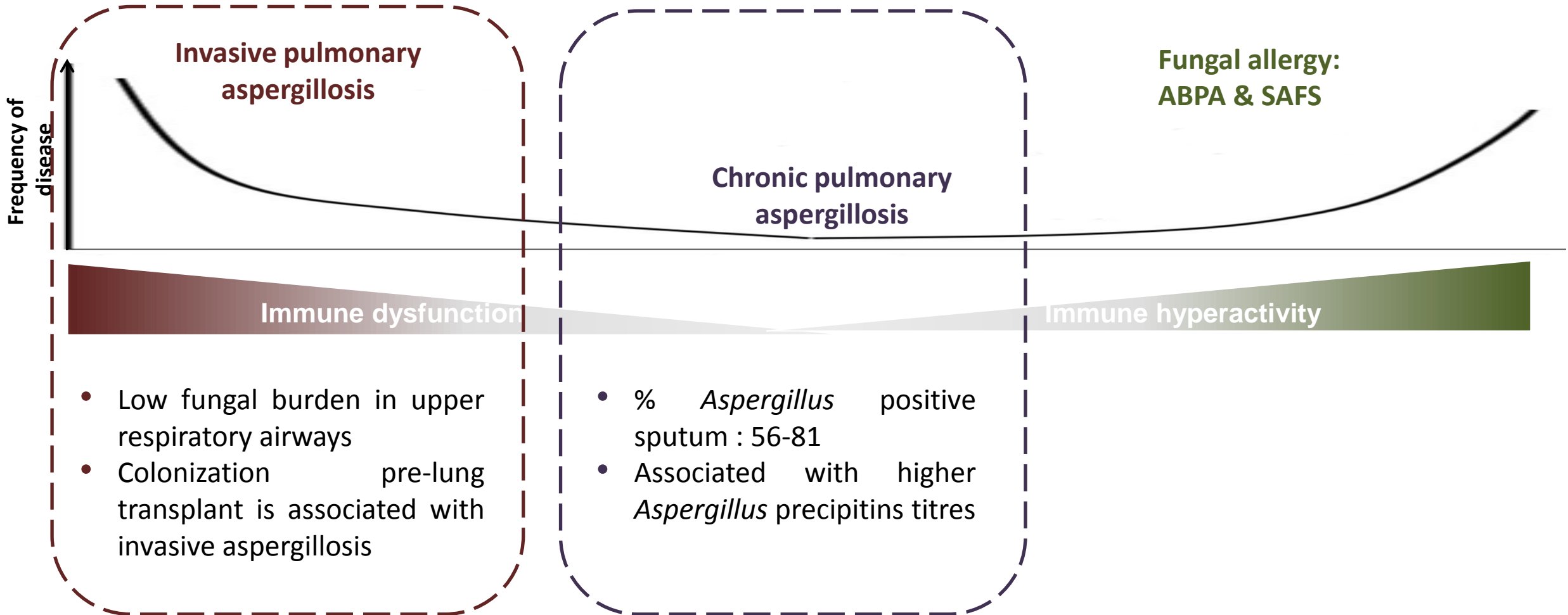
Presence of fungal species in respiratory samples (sputum or BAL)

ASPERGILLUS COLONIZATION AND ASPERGILLOSIS

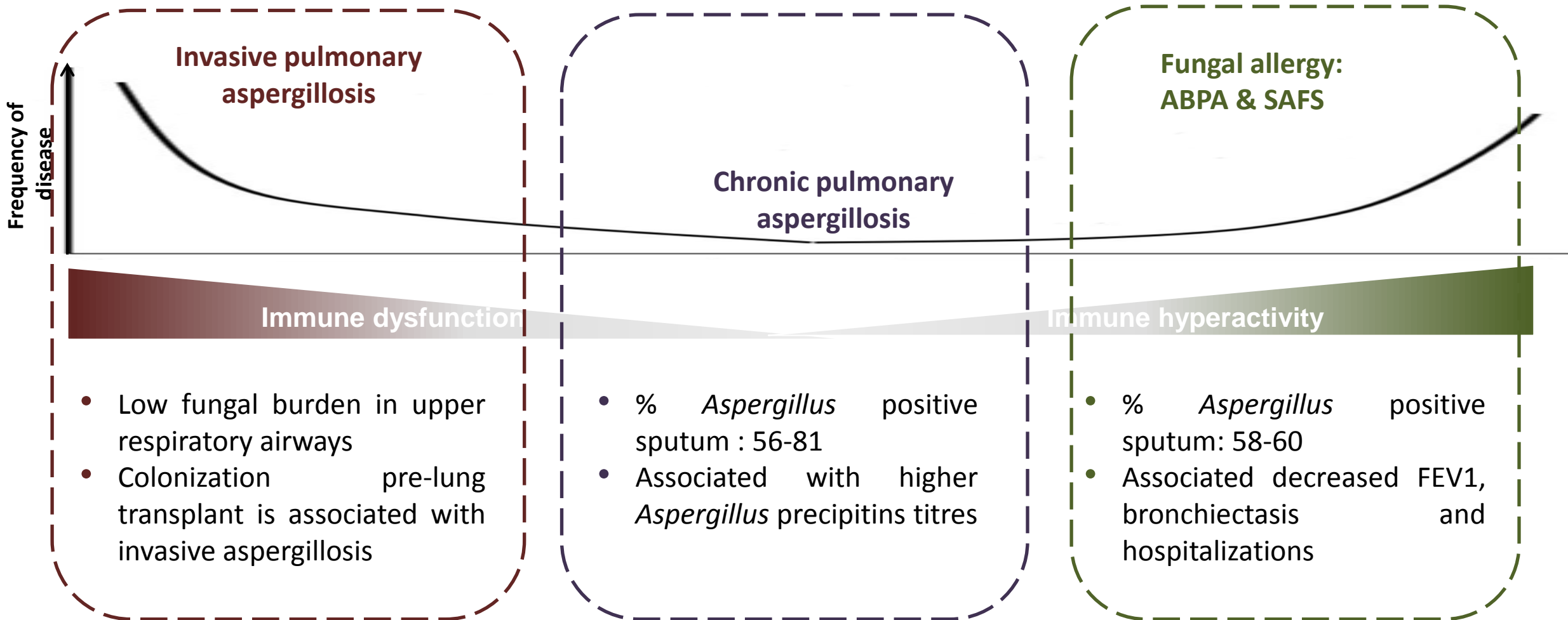


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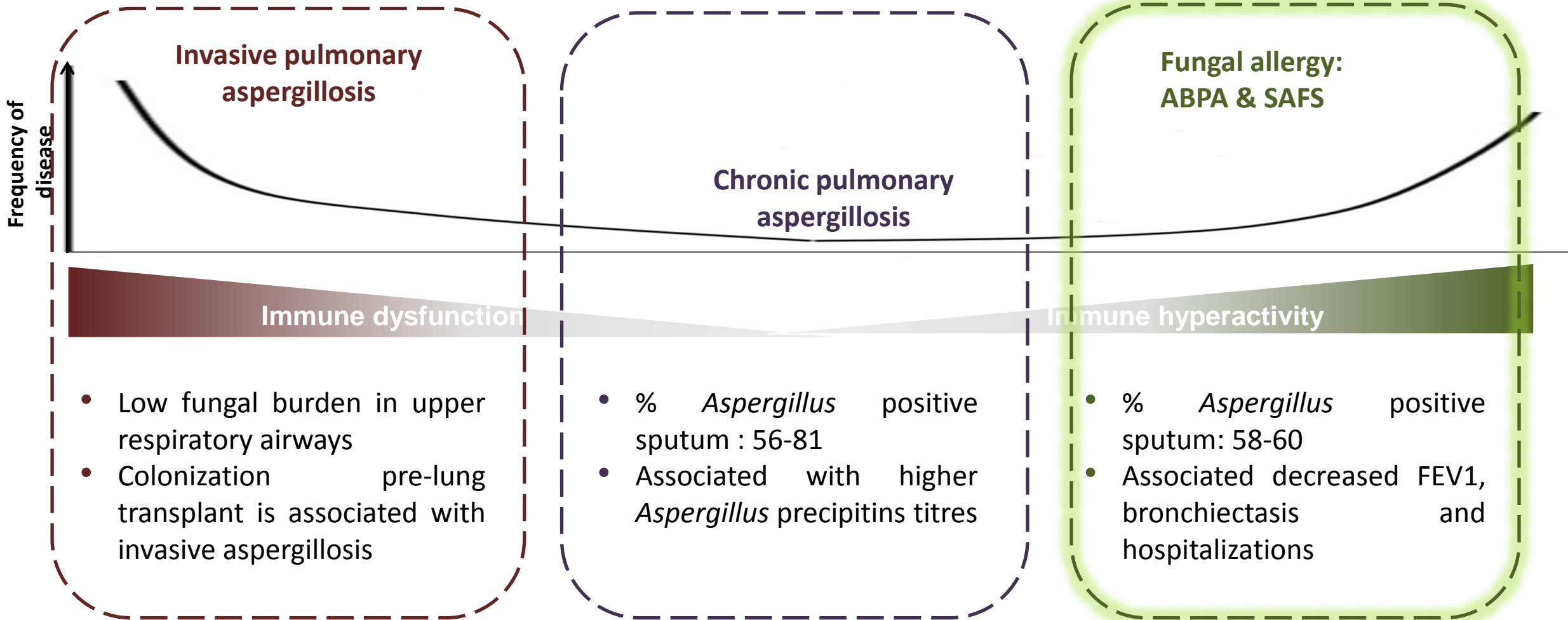
ASPERGILLUS COLONIZATION AND ASPERGILLOSIS



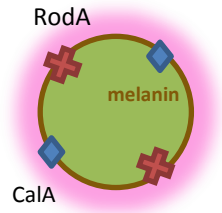
ASPERGILLUS COLONIZATION AND ASPERGILLOSIS



ASPERGILLUS COLONIZATION AND ASPERGILLOSIS

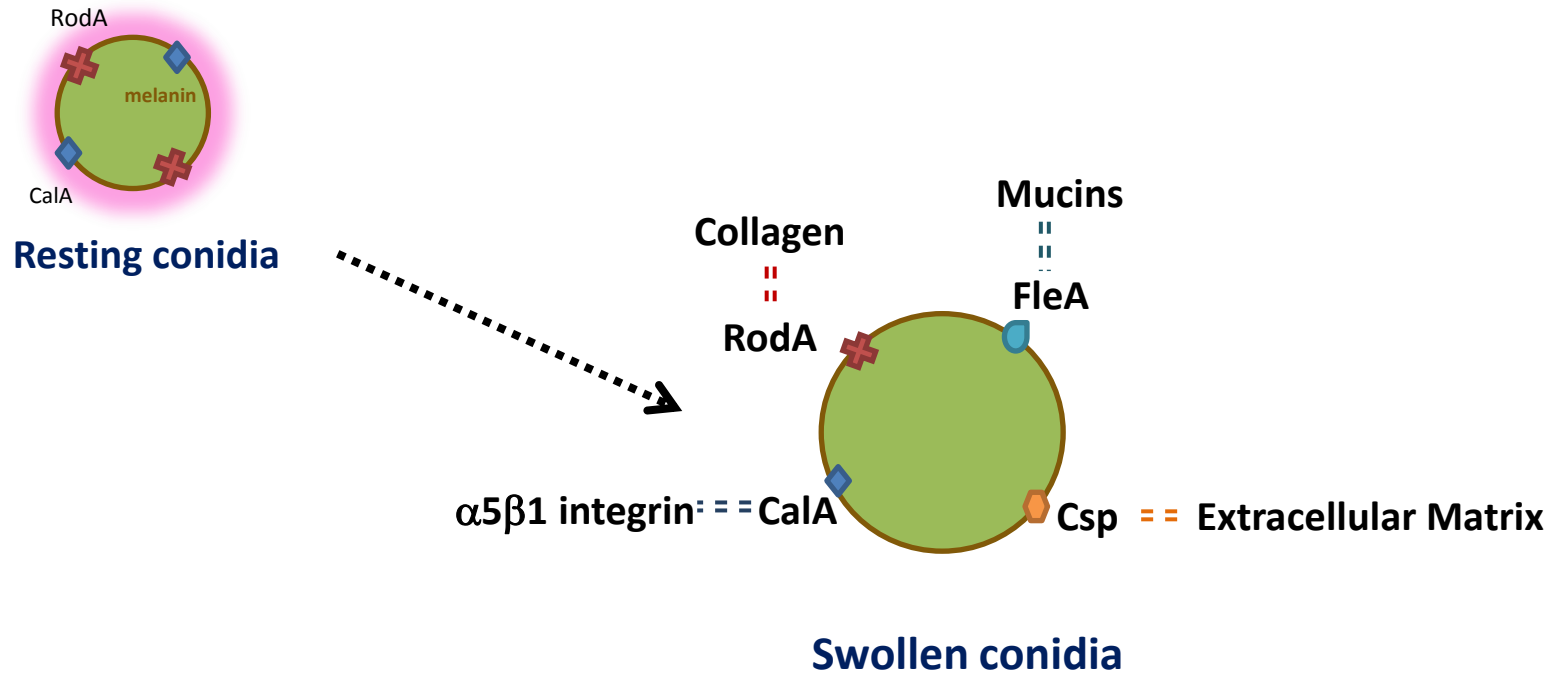


FUNGAL FACTORS CONTROLLING AIRWAY COLONIZATION: ADHESION

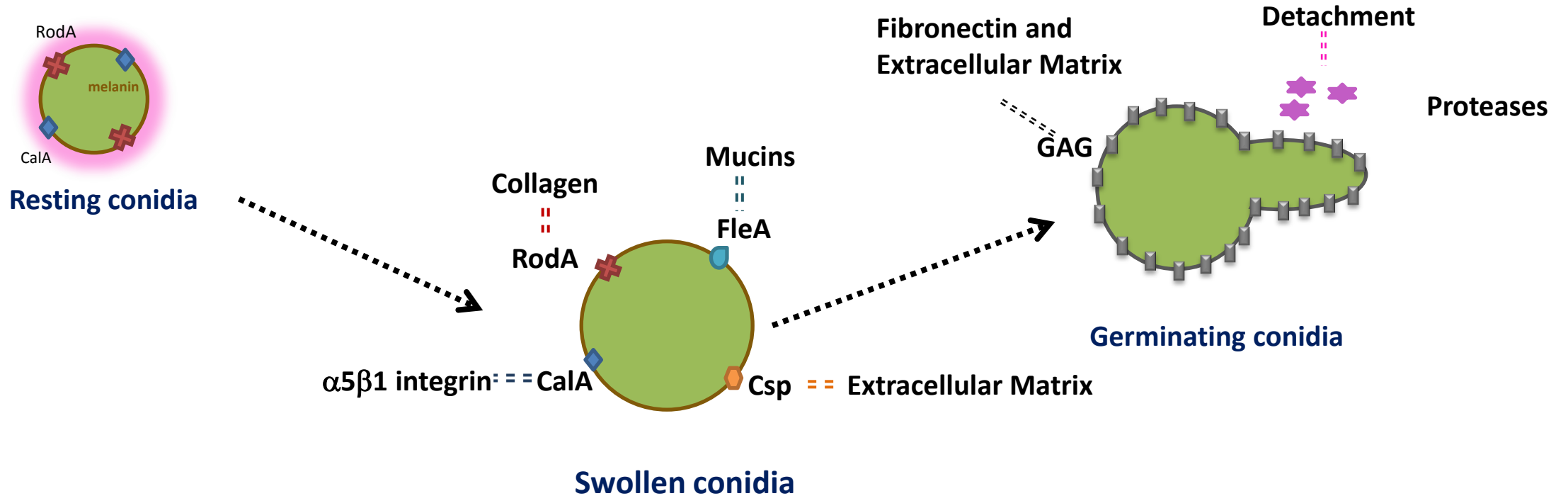


Resting conidia

FUNGAL FACTORS CONTROLLING AIRWAY COLONIZATION: ADHESION



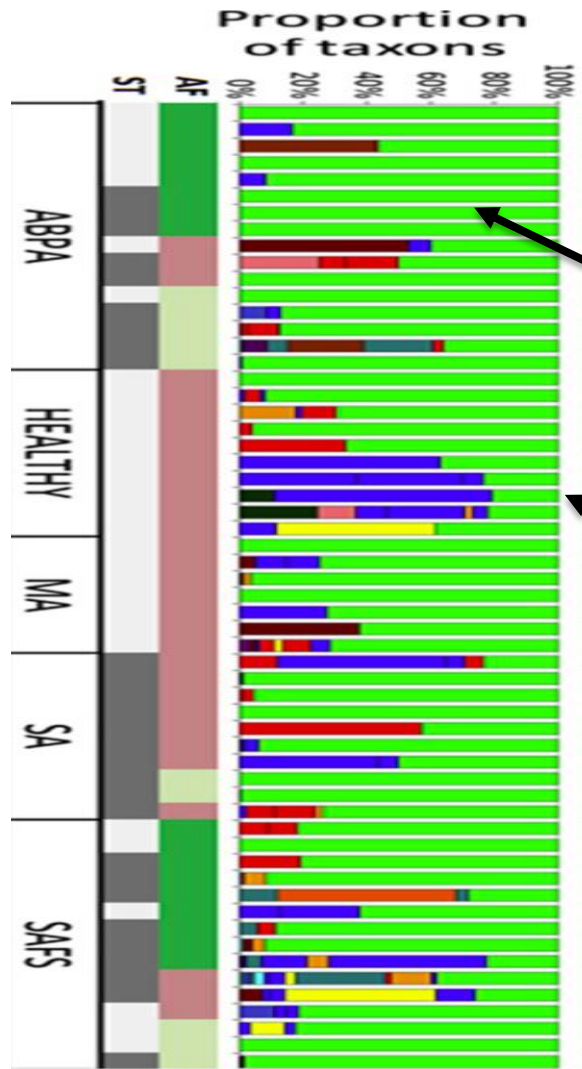
FUNGAL FACTORS CONTROLLING AIRWAY COLONIZATION: ADHESION



FUNGAL COLONIZATION HAS ALSO BEEN LINKED WITH...

- Defective epithelial clearance function in cystic fibrosis (CF) and asthmatic patients;
 - CF: mutations in the CTFR lead to dehydrated, suboptimal cleared mucus
 - asthma: compromised epithelial barrier
- Antibiotics misuse;
- Smoking status;
- Alteration of the microbiome composition (Dysbiosis);
- Corticosteroid treatment.

FUNGAL COLONIZATION IN DIFFERENT POPULATIONS OF PATIENTS



- Fluctuations in BAL fungal loads among patients with fungal disease:

— ABPA

- Fungal diversity is low
- *Aspergillus* spp complex are the main taxons (green).

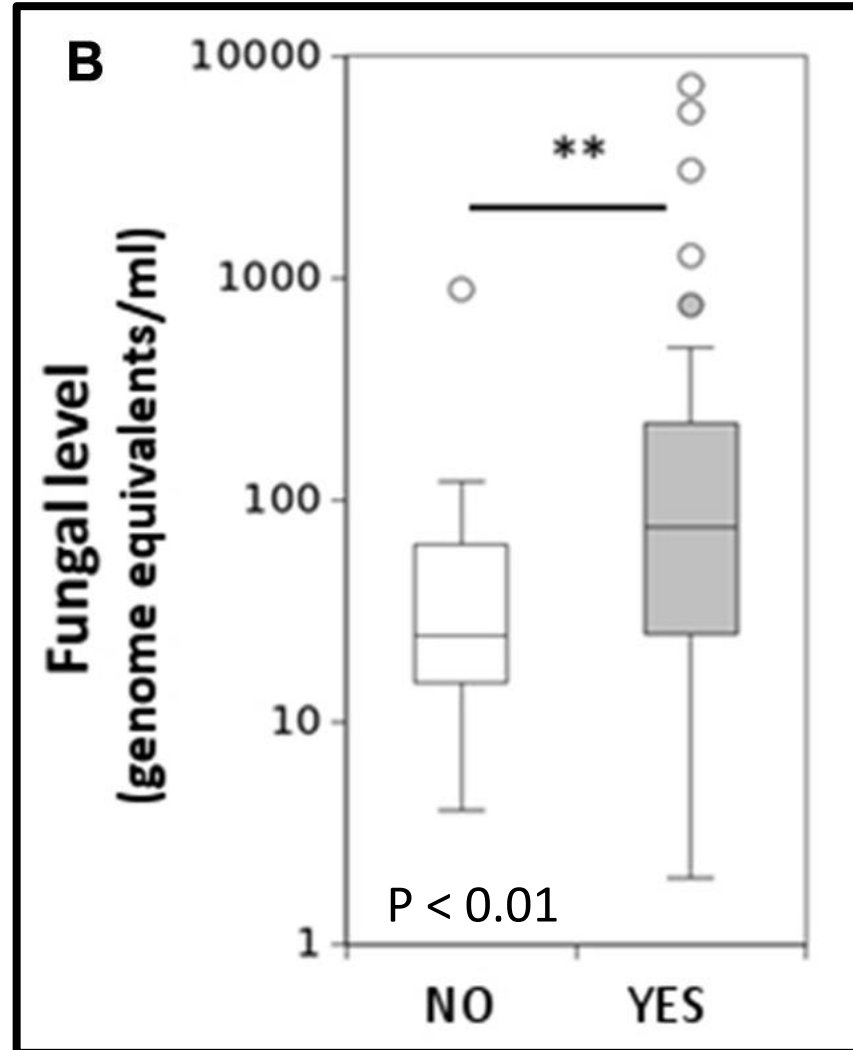
— Healthies

- Basidiomycota are highly represented (blue).

— Asthma and severe asthma

- Fungal diversity is higher.

CORTICOSTEROIDS & FUNGAL COLONIZATION



HOWEVER...

- Fungal exposure is universal and unescapable but not all asthmatics develop fungal allergy



- Development of ABPA and its pathological features might be genetically conditioned

SO FAR... POLYMORPHISMS IN IL-10 PROMOTOR HAVE BEEN ASSOCIATED WITH ASPERGILLUS COLONIZATION IN PATIENTS WITH ABPA AND CYSTIC FIBROSIS

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

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

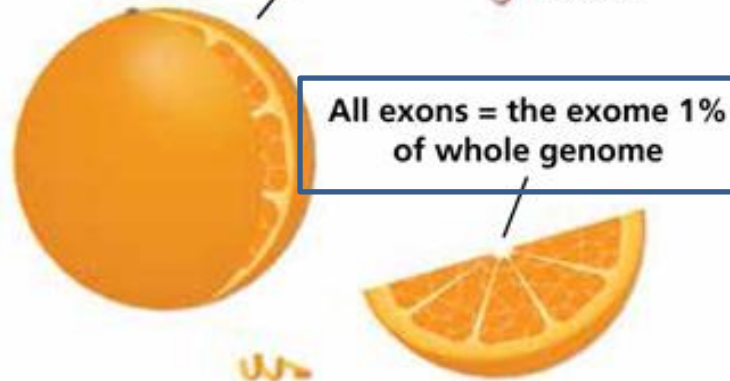
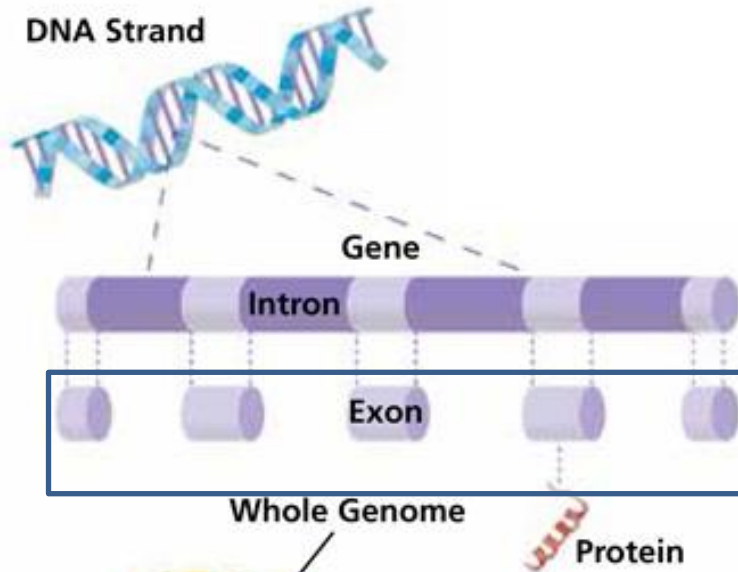
SO FAR... POLYMORPHISMS IN THE COLLAGEN REGION OF SP-A2 HAVE BEEN ASSOCIATED WITH EOSINOPHILIA AND INCREASED IGE IN ABPA

- 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 *SPA2* 91st and 94th codon polymorphisms

	Patients with ABPA with genotype: 91st codon (GCT/NCT), 94th codon (AGM), 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

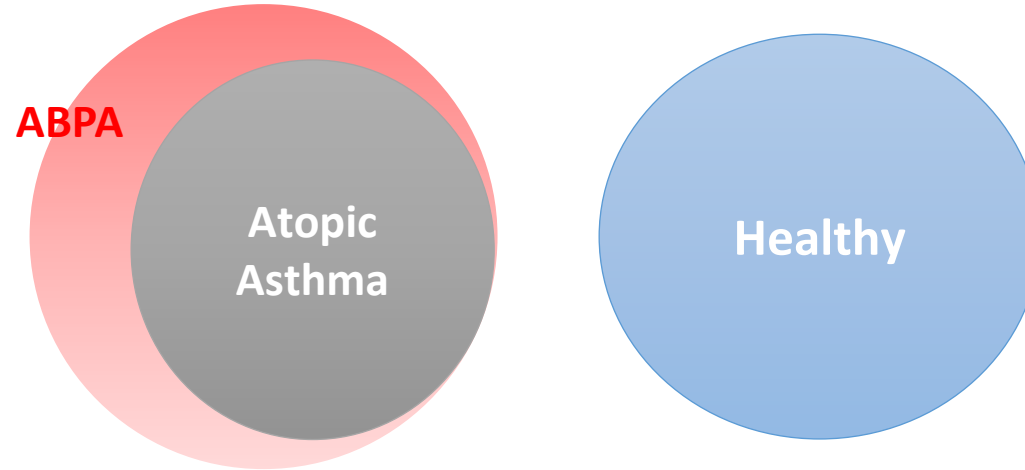
WHOLE EXOME SEQUENCING TO IDENTIFY GENETIC RISK FACTORS ASSOCIATED WITH ABPA



CONTAINS 90% GENETIC VARIABILITY

ALLOW FOR THE IDENTIFICATION OF GENETIC VARIANTS THAT ALTER PROTEIN SEQUENCES AT A LOWER COST THAN WGS

WHOLE-EXOME SEQUENCING IN PATIENTS WITH ABPA, COMPARED TO BOTH ATOPIC ASTHMATICS AND HEALTHY.



Differences in genetics factors underlying ABPA and atopic asthma might be the key to understand why not all asthmatics develop ABPA.

The role of these genetic variants in predisposition to *Aspergillus* colonization and the development of ABPA is unknown.

KNOWN TF-ABPA FUNCTIONS

- WHY TF?
 - ✓ It was significantly associated with ABPA
 - ✓ It was a high impact variant as introduces a premature stop codon in TF.
 - ✓ Good candidate for phenotype analyses.
- It has been associated with alterations in the secretion of growth hormones and increased cytokine response in fibromyalgia.

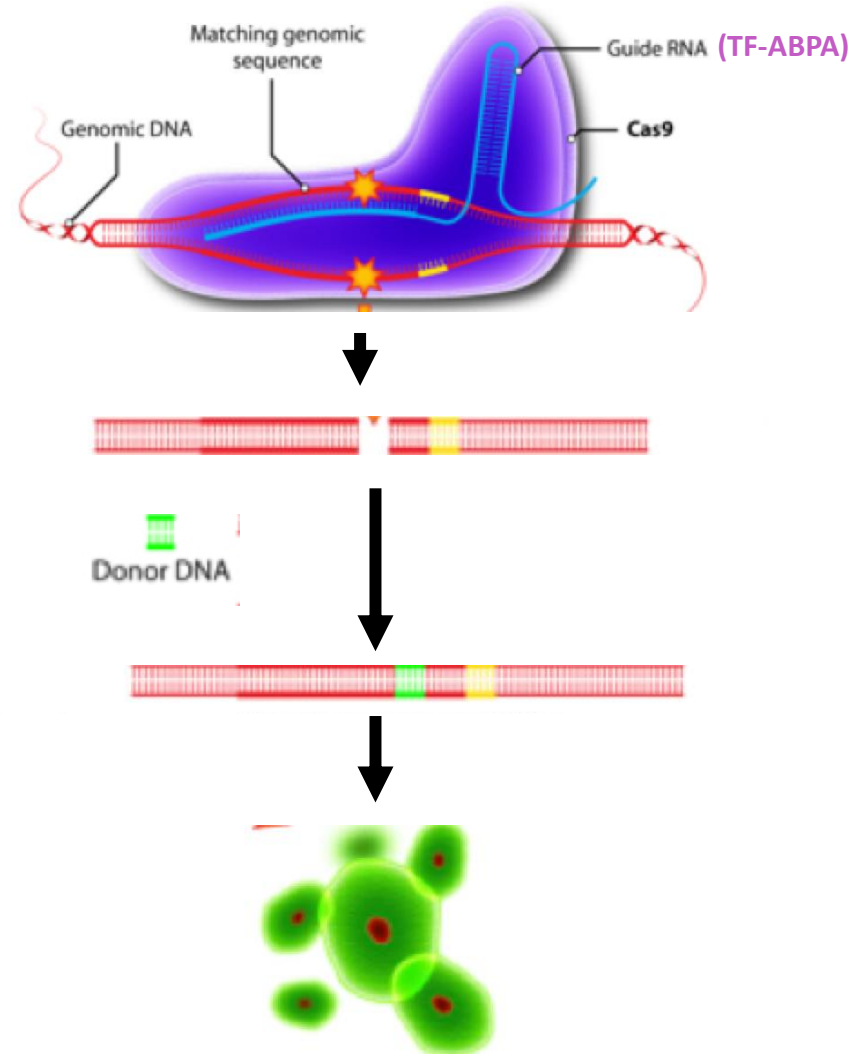
GENERATION OF 16HBE CELLS CARRYING THE ABPA-ASSOCIATED GENOTYPE IN TF-ABPA

Transfect the cells with a all-in-one CRISPR/Cas9 plasmid targeting a region 3bp upstream the genomic region containing the TF-ABPA*

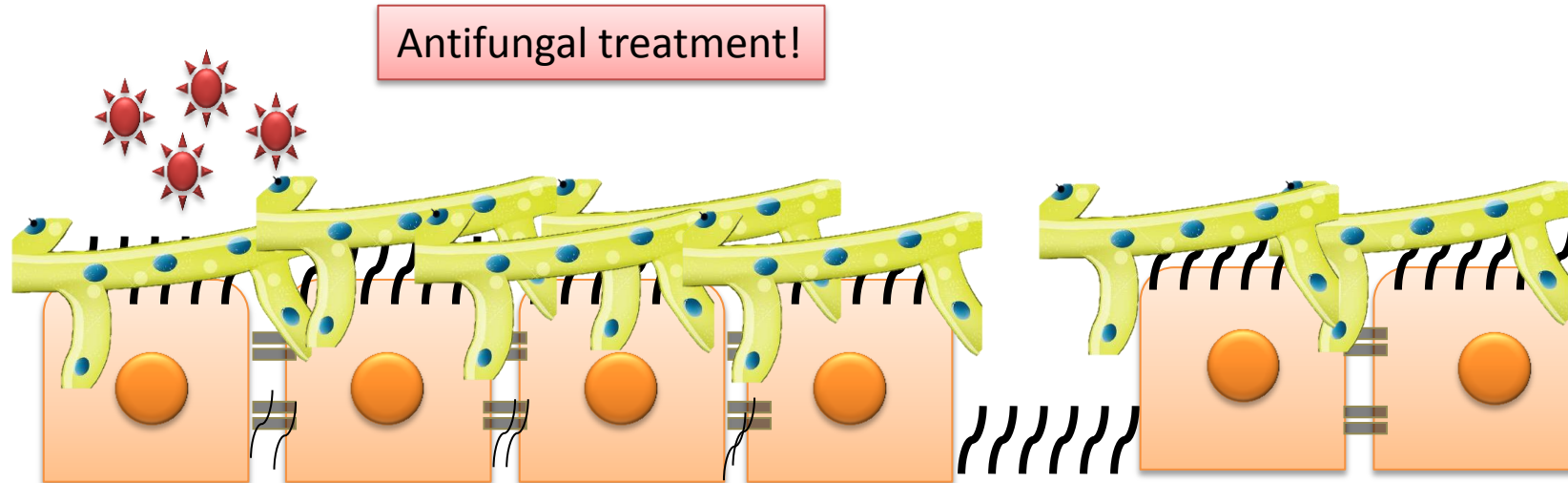
Cas9 specifically cuts in the TF-ABPA targeted sequence

A repair template allows to introduce the specific mutation by homologous recombination

Cell selection, clonal expansion, propagation and confirmation



TF-ABPA IMPAIRS EPITHELIAL INTEGRITY AND FACILITATES ASPERGILLUS FUMIGATUS ADHESION AND GROWTH



Extracellular components: fibrinogen, collagens, etc



Tight Junction Proteins

Aspergillus adhesion,
germination and growth

CONCLUSIONS...

- Fungal colonization of the respiratory airways by *Aspergillus* spp is frequent in ABPA;
- We have shown how TF-ABPA* leads to an abnormal epithelium structure facilitating *Aspergillus fumigatus* adhesion, germination and growth;
- Therefore, TF-ABPA-genotyping of patients with ABPA can be used as a fungal colonization risk-marker to guide interventions to minimize disease;
- However, other factors may play a role.

FUTURE WORK...

- Increase the cohort size to study associations between TF-ABPA and fungal colonization in ABPA;
- Study the role of TF-ABPA in controlling fungal colonization in other fungal disease e.g. SAFS, *Aspergillus* bronchitis or *Aspergillus* rhinosinusitis;
- Investigate other variants controlling fungal colonization derived from exome sequencing.

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 - Dr Jenni Hankinson
- **Patients from the NAC**

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THANKS!

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