Genetic polymorphism and mating-type of *Aspergillus fumigatus* strains isolated from cystic fibrosis patients

A. Rolland[1], J. Bigot[1], S. Velaissamy[1], F. Botterel[2, 3], C. Hennequin[1], J. Guitard[1]

[1]Sorbonne Université, Inserm, CRSA, AP-HP, Hôpital Saint-Antoine, F-75012 Paris, France; [2]Hôpital Universitaire Mondor, laboratoire de Parasitologie-Mycologie, Créteil, France; [3]EA Dynamyc UPEC, EnvA, USC Anses, Faculté de Médecine de Créteil, 8 rue du Général Sarrail, 94010 Créteil, France.

Introduction

Bronchial airways of cystic fibrosis patients may be chronically colonized by *Aspergillus fumigatus* (AF). Clinical manifestations are various ranging from asymptomatic long term colonization to *Aspergillus* bronchitis or allergic manifestation[1]. Through the large genetic exchange, sexual and/or parasexual reproduction could be essential for this adaptation. The persistance of some strains over months or even years suggests a particular adaptation of the fungus to this specific environment. To occur, these modes of reproduction require compatible strain(s) that must harbour at least similar or opposite mating-type, respectively [2].

This study aimed to analyse the mating type and genotype of AF strains isolated from CF patients to test the possible existence of sexual/parasexual cycle in the bronchial airways of those patients.

Materials and Methods

Patients and strains **(Table 1)**

Six children chronically (≥ 4 isolations /year) colonized with AF and having a positive anti-AF IgG serology were selected. Sixty-nine clinical isolates (7 to 17/child; mean 11± 5) collected during a follow-up from 18 to 36 months were available for testing.

Also, 2 patients with occasional AF colonization (≤2 isolations/year), and an alternate of *Aspergillus* species during the follow up were also collected, leading to the collection of 5 AF isolates.

Concerning chronically colonized patients, a median of 100% of their samples were positive to AF (range: 63 to 100%) whereas in occasionnally colonized patients, a median of 41% of their samples were positive for AF(range: 33 to 50%).

Finally, 7 environmental strains were also characterized.

A. fumigatus sensu stricto identification was confirmed using apporpriate MALDI-TOF protocol

Strain genotyping

Genotyping was performed by the mean of Cell Surface Protein (CSP) sequencing as previously described[4].

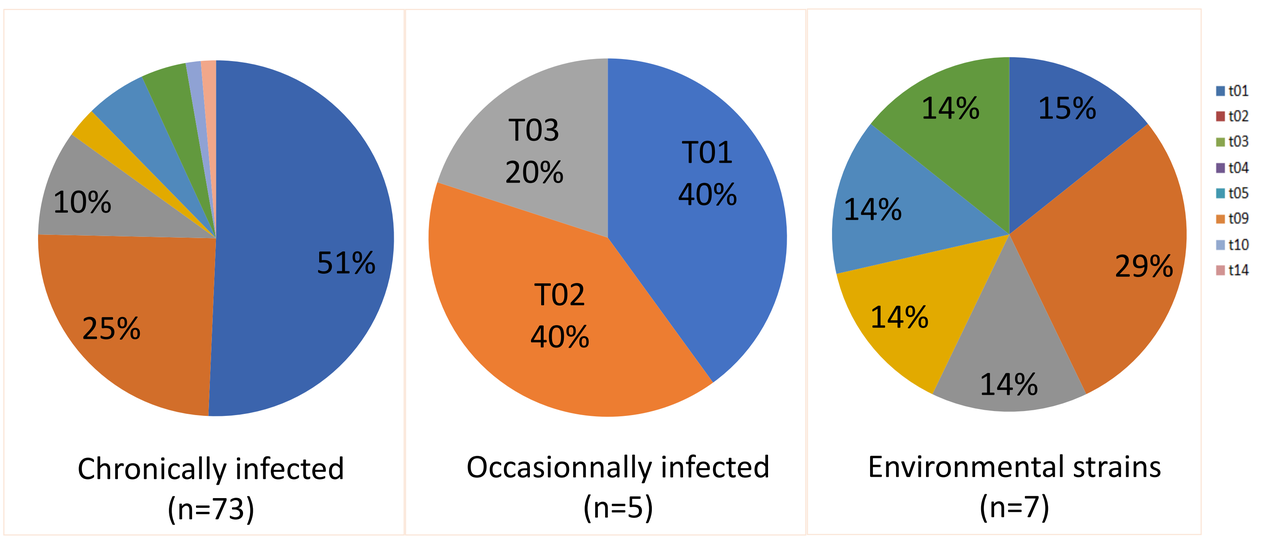
Mating type determination

Mating type (Mat1-1 or Mat1-2) was determined using fragment length analysis as described[5].

	Sex	Age (y)	Follow up (mo)	Clinical signs	Anti-AF serology	Number of AF studied strains	Number of AF positive samples during follow-up	% of AF positive samples during follow up	ATF
1 C	F	16	18		IgG+++	6	8	100%	No
2 C	M	15	33	Bronchitis	IgG+++	8	13	100%	No
3 C	M	12	33		IgG+, IgE+	17	27	100%	No
4 C	F	6	36	ABPA	IgG++ IgE++	10	15	63%	ITR
5 C	F	8	33		IgG+	17	25	100%	No
6 C	F	11	31		IgG++ IgE++	8	16	100%	No
1 O	F	15	18		IgG+	2	3	33%	No
2 O	F	9	19		N	3	5	50%	No

Table 1: Demographic and clinical data from our patients.
Y: year, mo: month, F: Female, M: male, ABPA: Allergic BronchoPulmonary Aspergillosis, ITR: itraconazole, N: negative , C: chronically infected, O : occasionally infected

Figure 1: AF CSP genotypes according to the clinical context: Chronically infected, occasionally infected patients, environment.



	n	Mat1-1	Mat1-2
Chronically colonizing isolates	110	20.9%	79.1%
Ocassionnally colonizing strains	6	50%	50%
Environmental strains	7	43%	57%

Table 2: Determination of mating type in AF strains isolated from chronically, occasionally infected patients and environment

	ATF	Predominant CSP genotype during FU	Alternative CSP during FU	Predominant mating type during FU
1 C		T01 100%	0	Mat1-2 100%
2 C		T02 62%	T05 25% ; T01 12,5%	Mat1-2 70%
3 C		T01 88%	T04 11% ; T05 11%	Mat1-2 94%
4 C	ITR	T01 50%	T09 20% ; T03 20% ; T14 10%	Mat 1-2 65%
5 C		T02 41%	T01 35% ; T09 11% ; T03 11% ; T05 6%	Mat1-2 78%
6 C		T02 50%	T01 25% ; T03 25%	Mat1-2 54%

Table 3: CSP genotyping and mating type determination of AF isolates during patients follow up, in chronically infected patients
ATF: antifungal, FU: Follow-up

Results

CSP genotyping (Figure 1)

In chronically infected patients, during longitudinal follow-up, patients are infected with a dominant CSP type.

T01 and T02 genotypes were the most frequent in our patient population, either in chronically colonized or occasionally colonized. In environmental strains, very diverse genotypes were found.

Mating type determination (Table 2)

AF isolated from chronically infected patients were mostly from *mat 1-2* genotype, whereas in occasionally infected patient only 50% of strains were *mat1-1* or *mat1-2*, as well as in the environmental strains.

Polymorphic bronchial samples in chronically infected patients (Table 4)

Patients with chronic colonisation appear to have a dominant AF strain (one CSP type and similar mating type) in the bronchial airways over the time.

15% of the bronchial samples retrieved either 2 different mating type and 1 CSP genotype, or 2 CSP genotypes and 1 mating type.

CSP genotype \ Mat genotype	Single	Double
Single	55	4
Double	6	1

Table 4: Repartition of CSP genotypes and mating types in bronchial samples from chronically infected patients

Conclusion

Colonization with strains of similar CSP genotype was mainly detected in the chronically infected patients.

CSP type 01 and 02, and Mat1-2 sexual type were predominant.

The presence, either during the follow-up or at the same time, of strains with same CSP and different mating-type or same mating type and different CSP, supports the hypothesis that recombination can occur in the bronchial airway of these patients.

Strains with either different mat type or CSP type alone or in combination should be tested to investigate in different models (biofilm, bronchial epithelial cells) a possible increase in fitness possibly due to sexual or parasexual reproduction.

References:
1 Delfino et al Clin Med Insights Circ Respir Pulm Med, 2019, 2 Verweij et al,Lancet Infect Dis, 2016, 3 Ranque et al, Mycoses, 2014, 4 Klaassen et al, J Microbiol Methods 2009, 5 Paoletti et al, Curr Biol 2005