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

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**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. Through the large genetic exchange, sexual and/or parasexual reproduction could be essential for this adaptation. The persistence 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.

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 occasionally colonized patients, a median of 41% of their samples were positive for AF (range: 33 to 50%).
  Finally, 7 environmental strains were also characterized.

- **Strain genotyping**
  Genotyping was performed by the mean of Cell Surface Protein (CSP) sequencing as previously described.

- **Mating type determination**
  Mating type (Mat1-1 or Mat1-2) was determined using fragment length analysis as described.

Figure 1: AF Strain genotypes according to the clinical context: Chronically infected, occasionally infected and environmental strains.

<table>
<thead>
<tr>
<th>Strain type</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronically infected (n=73)</td>
<td>51%</td>
</tr>
<tr>
<td>Occasionally infected (n=15)</td>
<td>25%</td>
</tr>
<tr>
<td>Environmental strains (n=7)</td>
<td>10%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain type</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF isolated from children</td>
<td>15%</td>
</tr>
<tr>
<td>Environmental strains</td>
<td>14%</td>
</tr>
</tbody>
</table>

**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.

**Poly morphic 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/or 2 CSP genotypes and 1 mating type.

<table>
<thead>
<tr>
<th>CSP genotype</th>
<th>Single</th>
<th>Double</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>55</td>
<td>4</td>
</tr>
<tr>
<td>Double</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

**Genotyping**

- MAT1-1: 60%
- MAT1-2: 40%
- T01: 40%
- T02: 40%
- T03: 20%

**Discussion**

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 airways 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.