




# Genotyping of *Aspergillus fumigatus* Reveals Compartmentalization of Genotypes in Disseminated Disease after Invasive Pulmonary Aspergillosis

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Invasive pulmonary aspergillosis (IPA) disseminated to distant organs (IPAd) is uncommon (1, 2) and has traditionally been considered a monomicrobial infection caused by a single *Aspergillus fumigatus* clone present both in the lower respiratory tract and at distant sites (3, 4). In order to explore the potential compartmentalization of genotypes at different anatomical sites, we studied available isolates collected from the lung and distant sites in 3 patients with proven IPAd (5, 6) (Table 1). *A. fumigatus* colonies ( $n = 38$ ) from 10 samples were subcultured for molecular identification (7), genotyping using short tandem repeats of *A. fumigatus* (STRAf genotyping) (8), and antifungal susceptibility testing (9).

(The findings of this study were partially presented at the 23rd European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Berlin, Germany, 27 to 30 April 2013 [abstr O-467] [10]).

Microsatellite typing of the isolates resulted in the detection of 10 genotypes. Lower respiratory tract samples tended to yield several genotypes, whereas nonrespiratory samples yielded a single genotype. Patient 1 was a 26-year-old woman who had received an allogeneic hematopoietic stem cell transplant for acute lymphocytic leukemia in 2004. Her disease relapsed in 2006, and she received chemotherapy and corticosteroids to control graft-versus-host disease. IPAd was proven in the autopsy, and a single genotype was found in the 2 samples. Data for patient 2 have been reported elsewhere (11). IPAd was diagnosed based on the isolation of *A. fumigatus* in lower respiratory tract samples and blood cultures. A total of 3 genotypes were found in the lung, but only 1 of them was present in the blood cultures; this genotype was found in several lower respiratory tract samples and was clonally related to another genotype found in 2 additional respiratory samples. Patient 3 was a homeless person who had received corticosteroids. He was diagnosed first with IPA (probable) and then with invasive aspergillosis of the brain 16 months later (proven). Of the 6 genotypes found in the lung during the first episode, 1 was detected in the central nervous system sample taken during the second episode.

All isolates were susceptible to itraconazole, voriconazole, and posaconazole. We did not find differences in antifungal susceptibility between different colonies from the same patient or between the genotypes. Genotyping enabled us to explore 3 issues with clinical impact. First, our data support the hypothesis of dissemination of disease from the lung. Second, the presence of identical genotypes in the blood and respiratory tract in patient 2 sustains the idea of the potential clinical significance of isolating *A.*

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**TABLE 1** Underlying conditions of and diagnostic criteria of aspergillosis for the 3 patients and genotypes found in samples from the lower respiratory tract and distant organs<sup>a</sup>

Patient	Underlying condition(s)	Radiological finding(s)	Mycological finding(s)	Sample (date of isolation)	Genotype(s) (no. of colonies)
1	Lymphocytic acute leukemia; allogeneic stem cell transplantation	Nodules in the lungs on CT scan; no lesions in the brain on CT scan	Positive serum galactomannan result; isolation of <i>A. fumigatus</i> from the brain sample and respiratory sample	Lung biopsy (30 September 2006)	<b>AF-1 (10)</b>
				Brain biopsy (30 September 2006)	<b>AF-1 (2)</b>
2	COPD	Opacity in the upper right lobe, with nodular lesions on the chest X-ray	Positive serum galactomannan result; isolation of <i>A. fumigatus</i> from the respiratory sample and blood culture	Sputum (15 January 2009)	<b>AF-2 (2)</b> , AF-3 (2)
				Blood culture (18 January 2009)	<b>AF-2 (3)</b>
				BAS (19 January 2009)	<b>AF-2 (1)</b> , AF-3 (1), AF-4 (1)
				BAS (19 January 2009)	AF-4 (2)
3	HIV (11 CD4 cells/mm <sup>3</sup> ) H1N1 infection	Bilateral cavitated infiltrates on CT scan; abscesses and ventriculitis on central nervous system CT scan	Positive BAL fluid galactomannan result; isolation of <i>A. fumigatus</i> from the respiratory sample and blood culture and CSF samples	Sputum (26 January 2011)	AF-5 (2), <b>AF-6 (1)</b>
				Sputum (28 January 2011)	AF-7 (2), AF-8 (1), AF-9 (1)
				Sputum (31 January 2011)	AF-5 (1), AF-8 (2), AF-10 (2)
				CSF (28 May 2012)	<b>AF-6 (2)</b>

<sup>a</sup>Genotypes in bold indicate matches between strains from the lower respiratory tract and distant organs. All 3 patients were receiving corticosteroids and had proven aspergillosis according to European Organization for Research and Treatment of Cancer (EORTC) criteria (patients 1 and 3) (5) or the criteria of Blot et al. (patient 2) (6). BAS, basophil; COPD, chronic obstructive pulmonary disease; CSF, cerebrospinal fluid; CT, computed tomography.

*fumigatus* from blood cultures in a patient with risk factors and symptoms compatible with invasive aspergillosis (5, 11). Finally, patient 3 had 2 episodes of IPA in 16 months; since the second episode was caused by 1 of the genotypes found in the lung during the first episode, the second episode was a relapse rather than a reinfection. We observed compartmentalization of *A. fumigatus* in patients with IPAd; the genotype found at distant sites was always present in the respiratory tract, and some genotypes were found exclusively in the lung. A refined and in-depth analysis of all visible *A. fumigatus* isolates grown on the plates was required to support these observations.

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