




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

Pilar Escribano,^{a,b} Patricia Muñoz,^{a,b,c,d} Pedro Montilla,^{a,b} Belén Padilla,^{a,b} Emilio Bouza,^{a,b,c,d}  Jesús Guinea^{a,b,c,d}

Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Universidad Complutense de Madrid, Madrid, Spain^a; Instituto de Investigación Sanitaria del Hospital Gregorio Marañón, Madrid, Spain^b; CIBER de Enfermedades Respiratorias (CIBERES CB06/06/0058), Madrid, Spain^c; Medicine Department, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain^d

KEYWORDS *Aspergillus fumigatus*, genotyping, disseminated lung infection

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.*

Accepted manuscript posted online 26 October 2016

Citation Escribano P, Muñoz P, Montilla P, Padilla B, Bouza E, Guinea J. 2017. Genotyping of *Aspergillus fumigatus* reveals compartmentalization of genotypes in disseminated disease after invasive pulmonary aspergillosis. *J Clin Microbiol* 55:331–333. <https://doi.org/10.1128/JCM.02037-16>.

Editor Daniel J. Diekema, University of Iowa College of Medicine

Copyright © 2016 American Society for Microbiology. All Rights Reserved.

Address correspondence to Pilar Escribano, pilar.escribano.martos@gmail.com, or Jesús Guinea, jguineaortega@yahoo.es.

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^a

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)	AF-1 (10)
				Brain biopsy (30 September 2006)	AF-1 (2)
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)	AF-2 (2) , AF-3 (2)
				Blood culture (18 January 2009)	AF-2 (3)
				BAS (19 January 2009) BAS (19 January 2009)	AF-2 (1) , AF-3 (1), AF-4 (1) AF-4 (2)
3	HIV (11 CD4 cells/mm ³) 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), AF-6 (1)
				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)	AF-6 (2)

^aGenotypes 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.

ACKNOWLEDGMENTS

This study was supported by grant numbers PI14/00740 and MS15/00115 from the Fondo de Investigación Sanitaria (FIS, Instituto de Salud Carlos III; Plan Nacional de I+D+I 2013-2016) and cofunded by the European Regional Development Fund (FEDER) "A way of making Europe." The funding bodies had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. P.E. (CPI15/00115) and J.G. (CPII15/00006) hold a Miguel Servet contract.

REFERENCES

- Cheon S, Yang MK, Kim CJ, Kim TS, Song KH, Woo SJ, Kim ES, Park KU, Kim HB. 2015. Disseminated aspergillosis in the immunocompetent host: a case report and literature review. *Mycopathologia* 180:217–222. <https://doi.org/10.1007/s11046-015-9903-4>.
- Liu YS, Wang NC, Ye RH, Kao WY. 2014. Disseminated infection in a patient with acute lymphoblastic leukemia: a case report and review of the literature. *Oncol Lett* 7:334–336.
- de Valk HA, Meis JF, de Pauw BE, Donnelly PJ, Klaassen CH. 2007. Comparison of two highly discriminatory molecular fingerprinting assays for analysis of multiple *Aspergillus fumigatus* isolates from patients with invasive aspergillosis. *J Clin Microbiol* 45:1415–1419. <https://doi.org/10.1128/JCM.02423-06>.
- Girardin H, Sarfati J, Traore F, Dupouy Camet J, Derouin F, Latge JP. 1994. Molecular epidemiology of nosocomial invasive aspergillosis. *J Clin Microbiol* 32:684–690.
- De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Munoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE. 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 46:1813–1821. <https://doi.org/10.1086/588660>.
- Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselsaers N, Dimopoulos G, Paiva JA, Misset B, Rello J, Vandewoude K, Vogelaers D. 2012. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med* 186:56–64. <https://doi.org/10.1164/rccm.201111-1978OC>.
- Escribano P, Pelaez T, Muñoz P, Bouza E, Guinea J. 2013. Is azole resistance in *Aspergillus fumigatus* a problem in Spain? *Antimicrob Agents Chemother* 57:2815–2820. <https://doi.org/10.1128/AAC.02487-12>.
- Guinea J, Garcia de Viedma D, Pelaez T, Escribano P, Muñoz P, Meis JF, Klaassen CH, Bouza E. 2011. Molecular epidemiology of *Aspergillus*

- fumigatus*: an in-depth genotypic analysis of isolates involved in an outbreak of invasive aspergillosis. *J Clin Microbiol* 49:3498–3503. <https://doi.org/10.1128/JCM.01159-11>.
9. Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
 10. Escribano P, Muñoz P, Montilla P, Padilla B, Bouza E, Guinea J. 2013. Abstr 23rd Eur Congr Clin Microbiol Infect Dis (ECCMID), Berlin, Germany, 27 to 30 April 2013, abstr O-467.
 11. Giannella M, Muñoz P, Guinea J, Escribano P, Rodriguez-Creixems M, Bouza E. 2013. Growth of *Aspergillus* in blood cultures: proof of invasive aspergillosis in patients with chronic obstructive pulmonary disease? *Mycoses* 56:488–490. <https://doi.org/10.1111/myc.12045>.