Emergence of TR46/Y121F/T289A in an *Aspergillus fumigatus* Isolate from a Chinese Patient

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Azole resistance in *Aspergillus fumigatus* is increasingly reported and evolving into a global health problem (1). A substitution of histidine for leucine 98 in the *cyp51A* gene, together with a 34-bp sequence in tandem in the gene promoter (TR34/L98H), is currently the predominant resistance mechanism in azole-resistant *A. fumigatus* of environmental origin (2, 3). A new environmental *cyp51A*-mediated resistance mechanism consisting of a 46-bp tandem repeat and Y121F and T289A substitutions (TR46/Y121F/T289A), which might lead to voriconazole therapy failure, was identified first in The Netherlands and then reported in Belgium, Denmark, Germany, India, and Tanzania (4–7). We describe here the first isolation of a TR46/Y121F/T289A mutant strain from a patient in Beijing. This study was approved by the institutional ethics committees of the Academy of Military Medical Sciences of the Chinese People's Liberation Army (PLA), Beijing, China.

A 52-year-old male patient was diagnosed with hyperthyroidism in 2008. The patient complained of edema of the lower limbs and dyspnea and was admitted into the intensive care unit of a university hospital in Beijing in September 2013 for serious drug-induced liver injury in combination with jaundice and cardiac insufficiency. After admission, the patient received liver protection and jaundice removal therapy with magnesium isoglycyrrhizinate, coenzyme complex, ursodeoxycholic acid, and some traditional Chinese herbs, as well as five plasma exchanges. He was suspected of having invasive pulmonary aspergillosis in the middle of November 2013. Tests for both 1,3- β -D-glucan and galactomannan in serum were positive on 13 November 2013. The result of 1,3- β -D-glucan testing (Beijing Gold Mountain River Tech Development Company, Beijing, China) was 366.1 ng/liter (normal, <60 ng/liter), and the index of galactomannan testing (Platelia *Aspergillus*; Bio-Rad, France) was 3.52 (normal, <0.5). A chest X-ray indicated the presence of pneumonia. Voriconazole at 100 mg twice daily because of low body weight (<40 kg) was started on 15 November 2013. The sputum sample taken on 16 November 2013 was culture positive for *A. fumigatus* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany) and the MALDI Biotyper 3.0 database. Fungal spores and hyphae were observed in a sputum sample under a microscope. The Rosco Neo-Sensitabs tablet assay was performed according to the commercial antifungal disk diffusion method (8). Nine-millimeter tablets (containing 10 μ g of amphotericin B, 8 μ g of itraconazole, and 1 μ g of voriconazole) provided by Rosco Laboratory (Rosco Neo-Sensitabs; Key Scientific, TX) were applied to the inoculated agar. The resistance breakpoints used were <10 mm for amphotericin B, <14 mm for voriconazole, $\leq$ 9 mm for itraconazole. The results suggested that the isolate was sensitive to amphotericin B but resistant to voriconazole and itraconazole.

Caspofungin was then administered instead of voriconazole starting on 21 November 2013 (70 mg once every 2 days). On 18, 20, and 23 November 2013, three sputum samples were taken, respectively, and all were culture positive for *A. fumigatus* with the same resistance phenotype as the first *A. fumigatus* isolate. It is a pity that the later three isolates had not been stored. The patient's death on 30 November 2013 was probably due to acute liver failure and infectious shock.

The first isolate was sent to the Chinese PLA Institute for Disease Control and Prevention for molecular identification and genetic analysis. The strain was identified as *Aspergillus fumigatus sensu stricto* by sequencing of the gene for β -tubulin and a segment of the rRNA genes with internal transcribed spacers (9, 10). The isolate was retested for drug susceptibility by broth microdilution in accordance with the CLSI M38-A2 protocol. The MICs and category of the isolate according to suggested epidemiological cut-off values (11, 12) were as follows: voriconazole, >16 mg/liter (non-wild type); itraconazole, 0.5 mg/liter (wild type); posaconazole, 2 mg/liter (non-wild type); amphotericin B, 0.5 mg/liter (wild type). Sequencing of the *cyp51A* gene (13) showed that the non-wild-type MIC was associated with the TR46, Y121F, and T289A mutations. Microsatellite genotyping of the TR46/Y121F/T289A mutant *A. fumigatus* strain was performed with a panel of nine short tandem repeats (STRAf2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, and 4C) (2), and the STRAftype was shown to be 26-21-12-25-9-19-14-9-9. Genetic analysis based on microsatellite genotyping data from the literature showed that this strain is related to two strains from clinical and environmental samples obtained in The Netherlands (Fig. 1).

This study is the first identify the emergence of a new resistance mechanism, TR46/Y121F/T289A, in *A. fumigatus* from a Chinese patient. As the patient lacked any host factors for the diagnosis of invasive aspergillosis, this case can only be diagnosed as suspected pulmonary aspergillosis. However, it raises concern for the wide spread of TR46/Y121F/T289A mutant *A. fumigatus* in China, as it has been reported to be associated with invasive infection and therapy failure (14). This study also highlights the importance of

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FIG 1 Genotypic relationships of representative TR46/Y121F/T289A mutant *A. fumigatus* isolates from China, The Netherlands, Denmark, Germany, India, and Tanzania. The dendrogram is based on a categorical analysis of nine microsatellite markers in combination with clustering based on the unweighted-pair group method using average linkages. The microsatellite types of *A. fumigatus* from countries other than China were extracted from previous published results (5, 6, 15). The scale bar shows percentages of identity.

tracking and preventing the transmission of azole-resistant *A. fumigatus* of clinical significance in different countries and regions.

Nucleotide sequence accession numbers. The sequences of the β -tubulin gene and a segment of the rRNA genes with internal transcribed spacers have been submitted to the GenBank database and assigned accession no. [KT001539](#) and [KT162917](#).

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L. Han, Y. Chen, L. Huang, and F. Qu conceived and designed the experiments. Y. Chen, H. Wang, Z. Lu, Q. Zhang, J. Zhao, S. Tian, X. Han, F. Chen, C. Zhang, and X. Jia performed the experiments. Y. Chen, P. Li, and L. Han analyzed the data. H. Wang, T. Jia, and F. Qu contributed reagents, materials, and analysis tools. Y. Chen and L. Han wrote the paper.

All of the authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

REFERENCES

- Vermeulen E, Lagrou K, Verweij PE. 2013. Azole resistance in *Aspergillus fumigatus*: a growing public health concern. *Curr Opin Infect Dis* 26:493–500. <http://dx.doi.org/10.1097/QCO.0000000000000005>.
- Snelders E, van der Lee HA, Kuijpers J, Rijs AJ, Varga J, Samson RA, Mellado E, Donders AR, Melchers WJ, Verweij PE. 2008. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 5:e219. <http://dx.doi.org/10.1371/journal.pmed.0050219>.
- Lockhart SR, Frade JP, Etienne KA, Pfaller MA, Diekema DJ, Balajee SA. 2011. Azole resistance in *Aspergillus fumigatus* isolates from the ARTEMIS global surveillance study is primarily due to the TR/L98H mutation in the *cyp51A* gene. *Antimicrob Agents Chemother* 55:4465–4468. <http://dx.doi.org/10.1128/AAC.00185-11>.
- van der Linden JW, Camps SM, Kampinga GA, Arends JP, Debets-Ossenopp YJ, Haas PJ, Rijnders BJ, Kuijper EJ, van Tiel FH, Varga J, Karawajczyk A, Zoll J, Melchers WJ, Verweij PE. 2013. Aspergillosis due to voriconazole highly resistant *Aspergillus fumigatus* and recovery of genetically related resistant isolates from domiciles. *Clin Infect Dis* 57:513–520. <http://dx.doi.org/10.1093/cid/cit320>.
- Chowdhary A, Sharma C, van den Boom M, Yntema JB, Hagen F, Verweij PE, Meis JF. 2014. Multi-azole-resistant *Aspergillus fumigatus* in the environment in Tanzania. *J Antimicrob Chemother* 69:2979–2983. <http://dx.doi.org/10.1093/jac/dku259>.
- Steinmann J, Hamprecht A, Vehreschild MJ, Cornely OA, Buchheidt D, Spiess B, Koldehoff M, Buer J, Meis JF, Rath PM. 2015. Emergence of azole-resistant invasive aspergillosis in HSCT recipients in Germany. *J Antimicrob Chemother* 70:1522–1526. <http://dx.doi.org/10.1093/jac/dku566>.
- Chowdhary A, Kathuria S, Xu J, Meis JF. 2013. Emergence of azole-resistant *Aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. *PLoS Pathog* 9:e1003633. <http://dx.doi.org/10.1371/journal.ppat.1003633>.
- Espinel-Ingroff A. 2006. Comparison of three commercial assays and a modified disk diffusion assay with two broth microdilution reference assays for testing zygomycetes, *Aspergillus* spp., *Candida* spp., and *Cryptococcus neoformans* with posaconazole and amphotericin B. *J Clin Microbiol* 44:3616–3622. <http://dx.doi.org/10.1128/JCM.01187-06>.
- Balajee SA, Gribskov JL, Hanley E, Nickle D, Marr KA. 2005. *Aspergillus lentulus* sp. nov., a new sibling species of *A. fumigatus*. *Eukaryot Cell* 4:625–632. <http://dx.doi.org/10.1128/EC.4.3.625-632.2005>.
- Chen J, Li H, Li R, Bu D, Wan Z. 2005. Mutations in the *cyp51A* gene and susceptibility to itraconazole in *Aspergillus fumigatus* serially isolated from a patient with lung aspergilloma. *J Antimicrob Chemother* 55:31–37.
- Espinel-Ingroff A, Cuenca-Estrella M, Fothergill A, Fuller J, Ghannoum M, Johnson E, Pelaez T, Pfaller MA, Turnidge J. 2011. Wild-type MIC distributions and epidemiological cutoff values for amphotericin B and *Aspergillus* spp. for the CLSI broth microdilution method (M38-A2 document). *Antimicrob Agents Chemother* 55:5150–5154. <http://dx.doi.org/10.1128/AAC.00686-11>.
- Espinel-Ingroff A, Diekema DJ, Fothergill A, Johnson E, Pelaez T, Pfaller MA, Rinaldi MG, Canton E, Turnidge J. 2010. Wild-type MIC distributions and epidemiological cutoff values for the triazoles and six *Aspergillus* spp. for the CLSI broth microdilution method (M38-A2 document). *J Clin Microbiol* 48:3251–3257. <http://dx.doi.org/10.1128/JCM.00536-10>.
- Camps SM, van der Linden JW, Li Y, Kuijper EJ, van Dissel JT, Verweij PE, Melchers WJ. 2012. Rapid induction of multiple resistance mechanisms in *Aspergillus fumigatus* during azole therapy: a case study and re-

- view of the literature. Antimicrob Agents Chemother 56:10–16. <http://dx.doi.org/10.1128/AAC.05088-11>.
14. Vermeulen E, Maertens J, Schoemans H, Lagrou K. 2012. Azole-resistant *Aspergillus fumigatus* due to TR46/Y121F/T289A mutation emerging in Belgium, July 2012. Euro Surveill 17:20326. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20326>.
15. Astvad KM, Jensen RH, Hassan TM, Mathiasen EG, Thomsen GM, Pedersen UG, Christensen M, Hilberg O, Arendrup MC. 2014. First detection of TR46/Y121F/T289A and of TR34/L98H in azole naive patients in Denmark despite negative findings in the environment. Antimicrob Agents Chemother 58:5096–5101. <http://dx.doi.org/10.1128/AAC.02855-14>.