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LETTER TO THE EDITOR

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 μg of amphotericin B, 8 μg of itraconazole, and 1 μg 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, ≤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 cutoff 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 (STR*Af* 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, and 4C) (2), and the STR*Af* type 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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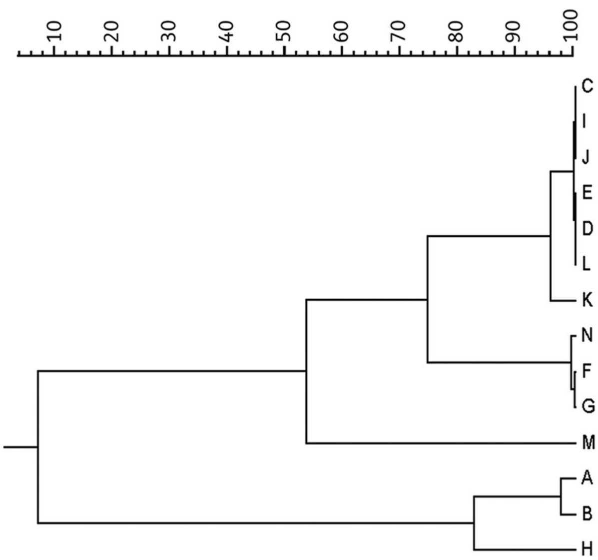

	2A	2B	2C	3A	3B	3C	4A	4B	4C		
C	26	21	9	33	11	22	8	14	10	The Netherlands	Clinical
I	26	21	9	33	11	22	8	14	10	The Netherlands	Clinical
J	26	21	9	33	11	22	8	14	10	Tanzania	Environmental
E	26	21	9	34	11	21	8	14	10	India	Environmental
D	26	21	9	33	11	21	8	14	10	India	Environmental
L	26	21	9	33	11	21	8	14	10	India	Environmental
K	26	26	9	33	11	22	8	14	10	The Netherlands	Clinical
N	26	21	12	25	9	19	14	9	9	China	Clinical
F	26	21	12	26	9	20	13.3	9	9	The Netherlands	Clinical
G	26	21	12	27	9	20	13.3	9	9	The Netherlands	Environmental
M	26	21	16	32	9	10	8	14	10	Denmark	Clinical
A	10	20	8	42	9	10	12	10	20	Germany	Clinical
B	10	20	12	42	8	11	12	9	20	Germany	Clinical
H	9	20	13	43	9	10	12	9	9	The Netherlands	Environmental

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.

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