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MECHANISMS OF RESISTANCE

First Clinical Isolation of an Azole-Resistant *Aspergillus fumigatus* Isolate Harboring a TR46 Y121F T289A Mutation in South America

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ABSTRACT One of the most recently described *Aspergillus fumigatus CYP51A*-mediated azole resistance mechanisms is TR46 Y121F T289A. Clinical *A. fumigatus* strains harboring these substitutions have been reported worldwide, with the exception of South America. We describe the first clinical *A. fumigatus* strain with this resistance mechanism isolated from an Argentinian patient. The strain was isolated in 2009 (1 year after the first-described mutant in United States), demonstrating that these alleles were scattered worldwide earlier than previously thought.

KEYWORDS *Aspergillus fumigatus*, resistance, CYP51A, TR46, South America, TR46 Y121F T289A mutation, azole

Invasive aspergillosis (IA) is an important cause of mortality in immunocompromised patients, especially those undergoing chemotherapy and those who have received a bone marrow transplant (1, 2). *Aspergillus fumigatus* is the main causative agent of the mycoses, and triazoles are the drugs of choice for its treatment (3). However, clinical azole resistance may arise in patients exposed to these drugs and in azole-naive individuals (4–6). *A. fumigatus* azole resistance is mainly linked to *CYP51A* mutations that may or may not be associated with alterations in its promoter (tandem repeats [TR]) (7, 8). One of the most recently described *CYP51A*-mediated resistance mechanisms is a double substitution combined with a 46-bp tandem duplication in the *CYP51A* promoter (named TR46 Y121F T289A). These mutations lead to high levels of voriconazole (VRC) resistance associated with increased MIC values for the other azoles (9). Clinical *A. fumigatus* strains harboring these substitutions were reported mainly in Europe but also in Asia, Africa, and North America (6, 10–14). Recently, these mutants were found in environmental samples in Colombia (13, 15). In this work, we describe the first clinical *A. fumigatus* strain harboring the TR46 Y121F T289A mutation found in South America.

The aforementioned strain was isolated from a 25-year-old patient diagnosed in November 2007 with acute lymphoblastic leukemia. He developed a lung lesion in the course of febrile neutropenia in November 2008. Following 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) definitions, the patient was diagnosed with possible aspergillosis (no culture was obtained and no other mycology tests were performed) (16, 17), and empirical VRC treatment was initiated (two 6-mg/kg intravenous doses followed by 200

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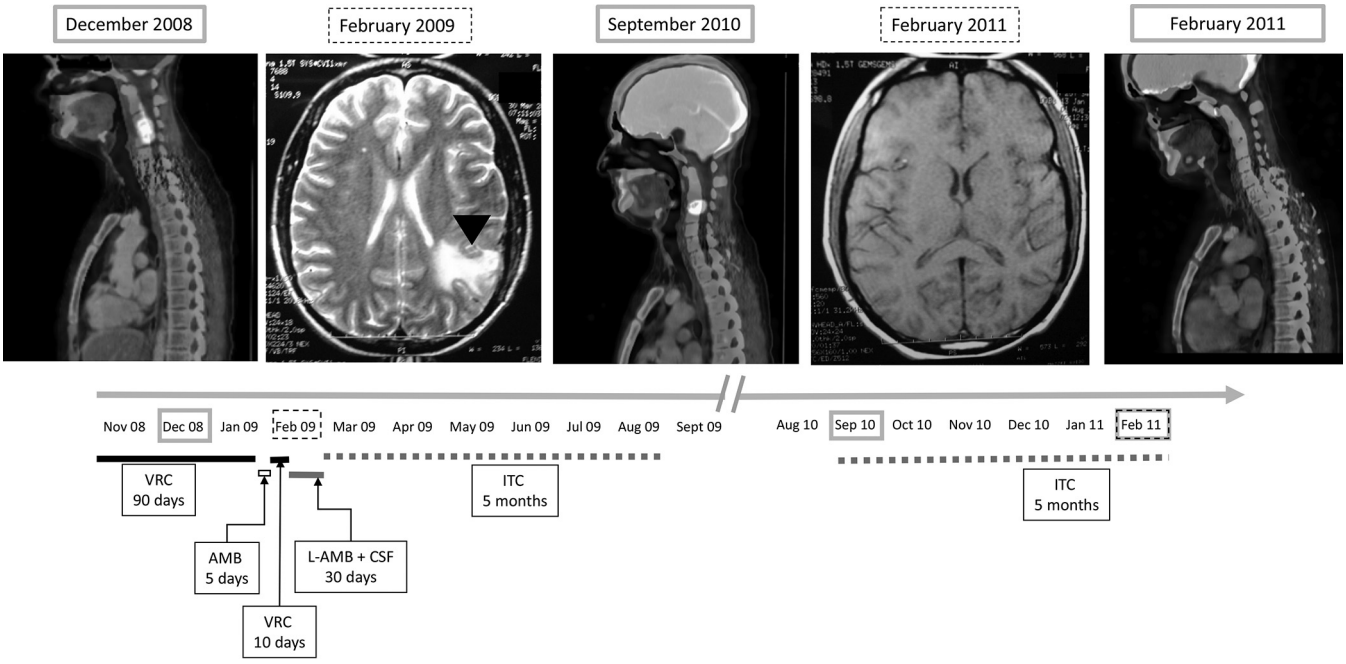

FIG 1 Gray boxes, positron emission tomography scans showing the patient's neck lesion as of December 2008, September 2010, and February 2011 (cured). Dotted boxes, brain magnetic resonance images as of February 2009 and February 2011 (no lesions). Arrowhead, brain lesion in February 2009 MRI. Lines represent antifungal treatment: black, initial 90-day (November 2008 to January 2009) voriconazole (VRC) treatment; white, 5-day amphotericin B (AMB) treatment; short black line, 10-day VRC treatment; gray line indicates 30-day liposomal AMB and caspofungin (CSF) combined treatment and dotted gray lines indicate two 5-month itraconazole (ITC) treatments between the end of February 2009 and August 2009 and between September 2010 and February 2011.

mg/day for 90 days). In December 2008, he started to have cervical pain, and a positron emission tomography computed tomography (PET-CT) scan showed augmented metabolic activity in his cervical area (Fig. 1). In February 2009, he developed aphasia, and a left parieto-occipital lesion was observed on magnetic resonance imaging (MRI) (Fig. 1). A brain biopsy was performed, and septated hyaline hyphae were observed. Liposomal amphotericin B (AMB) treatment was started. On the 5th day, the initial antifungal treatment was shifted to VRC because an *A. fumigatus* isolate was identified in the cultured specimens. Antifungal susceptibility testing was performed by agar diffusion (VRC) and microdilution (VRC, itraconazole [ITC], posaconazole [POS], AMB, caspofungin [CAS], and anidulafungin [AFG]) following CLSI documents M51A and M38, respectively (18, 19). No inhibition halo was obtained when VRC susceptibility was assessed by agar diffusion. VRC resistance was confirmed by microdilution (VRC MIC, $>$16 μg/ml). The POS MIC was also elevated (0.50 μg/ml,). On the other hand, the strain showed low AMB and ITC MICs and CAS and AFG minimum effective concentrations (1, 1, 0.03, and 0.06 μg/ml, respectively). Based on the susceptibility testing results, treatment was shifted to liposomal AMB combined with 50 mg/day CAS. Treatment was changed again after a month to ITC (400 mg/day), which was discontinued after 5 months because of liver toxicity. Twelve months after this last azole treatment (September 2010), the neck pain reappeared. On the PET-CT scan, a significant metabolic increase was observed (similar to the one observed in December 2008) (Fig. 1). The ITC treatment was repeated for 5 months, after which the symptoms disappeared and metabolic activity, according to PET-CT scan, returned to basal values (Fig. 1). During the described antifungal treatment, the patient's underlying disease was treated and considered clinically cured. In April 2018, 9 years after treatment of the neck and brain aspergillosis and almost 8 years after completing his last antifungal treatment, the patient was asymptomatic and had not relapsed. The patient provided written informed consent for inclusion in this study.

The azole-resistant strain was identified as *A. fumigatus sensu stricto* by β-tubulin and calmodulin gene sequencing and by matrix-assisted laser desorption ionization–

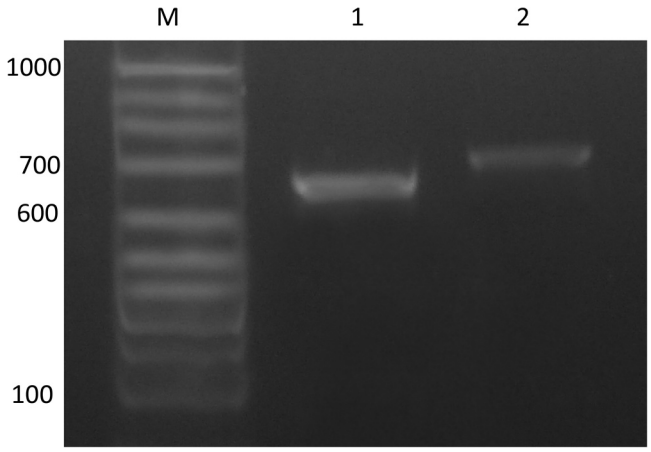

FIG 2 Electrophoresis of the A7-A5R PCR fragment of the *CYP51A* gene of the *A. fumigatus* strain resolved with 2% agarose gel. Lane M, 100-bp marker; lane 1, *A. fumigatus* LMDM-31 (wild type, azole susceptible); lane 2, TR46 Y121F T289A (DMIC-093515, VRC resistant).

time of flight mass spectrometry (20, 21). It is deposited and conserved at the Culture Collection of the Departamento Micologia (Instituto Nacional de Enfermedades Infecciosas [INEI] "Dr. C. G. Malbrán," Buenos Aires, Argentina) under number DMIC-093515. A PCR designed to amplify a fragment of the *CYP51A* promoter (A7, 5′-TCATATGTTGC TCAGCGG-3′; A5R, 5′-TCTCTGCACGCAAAGAAGAAC-3′) was used to assess the presence of *CYP51A* promoter alterations. This PCR was performed in an Applied Biosystems thermocycler (Tecnolab-AB, Argentina) using the Pegasus DNA polymerase (PBL, Argentina) following the manufacturer's protocol. The PCR bands were resolved in 2% agarose gel. In the studied clinical strain, the amplified promoter was bigger than its wild-type counterpart (Fig. 2), demonstrating that an alteration in this region would be responsible for the resistance phenotype (9, 22).

The *CYP51A* open reading frame (ORF) and promoter (5′-UTR, 1,000 bp upstream of the start codon) were sequenced by Sanger methodology as described previously using ABI genetic analyzer 3500 (Applied Biosystems, CA) (22, 23). Compared with the wild-type *A. fumigatus CYP51A* sequence (GenBank accession no. AF338659.1), a tandem duplication of a 46-bp sequence (5′-GTCTAGAATCACGCGGTCCGGATGTGTGCTGA GCCGAATGAAAGTT-3′) was found between 282 and 327 nt downstream of the start codon. In addition, sequence analysis of the *CYP51A* ORF showed two substitutions (A433T and A936G), resulting in the amino acid changes at residues 121 and 289 (Y121F and T289A) (Fig. 2).

In this work, we report the first case in Argentina of an azole-resistant *A. fumigatus* strain harboring the *CYP51A* TR46 Y121F T289A mutation. Although these *A. fumigatus* mutants are considered to have an environmental origin (15, 24–26), the source of our strain is not clear because it was isolated 1 year after finishing 90 days of VRC treatment.

Interestingly, the studied strain was isolated in February 2009. Thus, it was isolated in the same year as the first European isolate harboring these mutations and only 1 year after the first reported strain harboring this *CYP51A* allele (14, 26), demonstrating that these mutations were scattered worldwide earlier than previously thought. In addition, the data presented here suggest that ITC treatment would be a plausible option for infections caused by this particular *A. fumigatus* mutant. However, ITC reaches low levels of concentration in cerebrospinal fluid, and it is not a treatment option for central nervous system infections (27). This azole is also not recommended as first-line treatment for any type of IA due to *A. fumigatus* (27, 28). The observed microbiological cure seems more likely to be due to recuperation of the patient's immune status than to the specific antifungal treatment. The finding of this mutant allele in South America is important clinically and epidemiologically, considering that antifungal susceptibility testing is not routinely performed and VRC is used as empirical therapy for pulmonary

aspergillosis (3). Moreover, our finding highlights the need to acknowledge the extent of azole resistance in *A. fumigatus* worldwide.

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