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MECHANISMS OF RESISTANCE

Triazole-Resistant *Aspergillus fumigatus* in an Israeli Patient with Chronic Cavitary Pulmonary Aspergillosis Due to a Novel E306K Substitution in Hmg1

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ABSTRACT Triazole resistance in the pathogenic mold *Aspergillus fumigatus* has increased worldwide, posing a growing therapeutic challenge. Recently, mutations in the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase gene (*hmg1*) have been associated with triazole resistance. Here, we describe a novel E306K triazole resistance-conferring mutation in the HMG-CoA reductase gene from an Israeli patient with chronic cavitary pulmonary aspergillosis (CCPA).

KEYWORDS *Aspergillus fumigatus*, *Hmg1* resistance mutation, E306K substitution, triazole resistance, HMG1 mutations, antifungal resistance, azole resistance, mycology

The saprophytic fungus *Aspergillus fumigatus* is the most common cause of life-threatening mold infections in humans (1, 2). Triazoles, and voriconazole in particular, are the primary treatment for patients with invasive disease, including invasive pulmonary aspergillosis (IPA) and chronic cavitary pulmonary aspergillosis (CCPA). Triazoles inhibit the catalytic activity of fungal Cyp51/ERG11 14-alpha sterol demethylase, involved in ergosterol biosynthesis. Triazole resistance in *A. fumigatus* has markedly increased in the last decade, leading to a rise in the rates of treatment failure (3). The most common triazole resistance mechanism in *A. fumigatus* is alterations in the drug target *erg11A*/*cyp51A* gene or promoter (3, 4). Recently, triazole resistance mutations in the sterol-sensing domain (SSD) of the essential 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase gene (*hmg1*) have also been identified in *A. fumigatus* (5–12). Hmg1 catalyzes the first committed step in ergosterol biosynthesis by HMG-CoA reduction to mevalonic acid. The mechanism by which *hmg1* SSD mutations contribute to triazole resistance is still unknown (6).

Here, we describe a novel Hmg1 E306K substitution in a triazole-resistant isolate of *A. fumigatus* (strain TLV21) from an Israeli patient with CCPA.

Case description. A 32-year-old female student was admitted to hospital A for massive hemoptysis. She had a 7-year history of bronchiectasis, starting when she was 25 years old. Two years before admission, she had worsening cough and dyspnea and was diagnosed with *Mycobacterium kansasii* pulmonary disease. She was treated with isoniazid, rifampin, and ethambutol for a total of 18 months. Her condition improved, and she was able to resume her studies. Four months before admission to hospital A, she began experiencing cough and fever up to 38°C. Sputum culture prior to admission grew *A. fumigatus* and *Aspergillus flavus*. She refused medical treatment and was treated instead with acupuncture and yoga. A week before admission, her cough worsened with large quantities of blood. Her weight was only 36 kg, down from a baseline of 44 kg. On admission, her hemoglobin was 7.4 g/dl with a mean corpuscular volume

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TABLE 1 MICs of strains used in this study

Strain	Mutation	MIC[a] (mg/liter)				
		VRZ	ITZ	POS	CAS	AMB
TLV21	Hmg1 E306K	4	>16	2	0.25	0.125
Δ*KU80*	None	0.5	0.25	0.25	0.25	0.25
Δ*KU80*/Hmg1-E306K	Hmg1-E306K-*hph*	4	>16	2	0.25	0.125
Δ*KU80*/Hmg1-WT	Hmg1 WT-*hph*	0.5	0.25	0.25	0.25	0.25

[a]MICs were performed according to the CLSI M38-A2 guidelines. The CLSI epidemiological cutoff values (ECVs) used were 1 mg/liter for itraconazole, 1 mg/liter for voriconazole, and 0.25 mg/liter for posaconazole (13).

(MCV) of 82.9 fl. Chest computed tomography angiography showed extensive cavitary disease, with near total destruction of the left pulmonary parenchyma and large thick-walled cavitations in the right upper lobe. No source of bleeding was identified. Flexible bronchoscopy showed bronchorrhea with purulent secretions bilaterally. Treatment was started with piperacillin-tazobactam and voriconazole. She received packed red blood cell transfusion. Bronchoalveolar lavage fluid culture grew *A. fumigatus*. Mycobacterial culture was negative. Serum IgG, IgA, and IgM and alpha 1 antitrypsin levels were within normal ranges, HIV serology was negative, and genetic testing for immotile cilia syndrome and cystic fibrosis was negative. Whole-exome sequencing revealed a heterozygous STAT3 loss-of-function mutation, corresponding to autosomal-dominant hyper-IgE syndrome (Job's syndrome).

The patient was transferred to hospital B, where she underwent assessment for pulmonary lobectomy. Treatment with voriconazole was continued. She continued to have significant cough and sputum production. Sputum culture 5 months after starting voriconazole grew *A. fumigatus* (strain TLV21). Previous *Aspergillus* isolates were not available for analysis. Antifungal susceptibility testing using CLSI M38-A2 broth microdilution methodology showed the isolate to be resistant to itraconazole (MIC, 4 mg/liter), voriconazole (MIC, 2 mg/liter), and posaconazole (MIC, 0.5 mg/liter). Treatment was switched to posaconazole, and the patient underwent resection of the right upper lung lobe and was discharged to ambulatory follow-up. When she was last seen in the clinic 3 years later, the patient reported improved cough and sputum production, with bouts of respiratory exacerbation 1 or 2 times a year.

Antifungal susceptibility of TLV21 was subsequently retested three times using broth microdilution according to CLSI M38-A2 methodology and showed it to be resistant to itraconazole (MIC, >16 mg/liter), voriconazole (MIC, 4 mg/liter), and posaconazole (MIC, 2 mg/liter) but susceptible to the polyene antifungal amphotericin B (AMB) and the echinocandin antifungal caspofungin (CAS) (Table 1). To detect mutations associated with azole resistance, the promoter regions and coding sequences of *cyp51A* and *hmg1* were amplified and sequenced. TLV21 did not contain *cyp51A* mutations in the promoter region or coding sequence. Sequencing of the entire *hmg1* gene identified a novel E306K mutation (GAG to AAG) (GenBank accession number MZ436398) in the third transmembrane region of the SSD between two previously described mutations, S305P and P309L (6, 10). The S305P mutation was previously shown to confer triazole resistance upon introduction into a susceptible strain (6).

To assess the importance of the E306K mutation, we PCR-amplified TLV 21 *hmg1* containing the mutation and used it to replace the endogenous wild-type *Hmg1* gene in an azole-susceptible CEA10 background *KU80* null strain (Δ*KU80*) of *A. fumigatus* to generate Δ*KU80*/Hmg1 E306K (see details in the supplemental material). Antifungal susceptibility was tested by broth microdilution (Table 1) and Etest (Fig. 1). Broth microdilution results indicate that Δ*KU80*/Hmg1 E306K showed the same resistance profile as strain TLV21 and was resistant to VRZ, ITZ, and POS but not to CAS. AMB MIC decreased 2-fold in Δ*KU80*/Hmg1 E306K, as previously described for other *hmg1* mutations (6) (Table 1). The control background strains Δ*KU80* and Δ*KU80*/Hmg1-WT (Δ*KU80* transformed with a wild-type version of *hmg1*) showed an identical pattern of triazole susceptibility (Table 1). Etest susceptibility analysis generally supported these results but showed a slightly higher

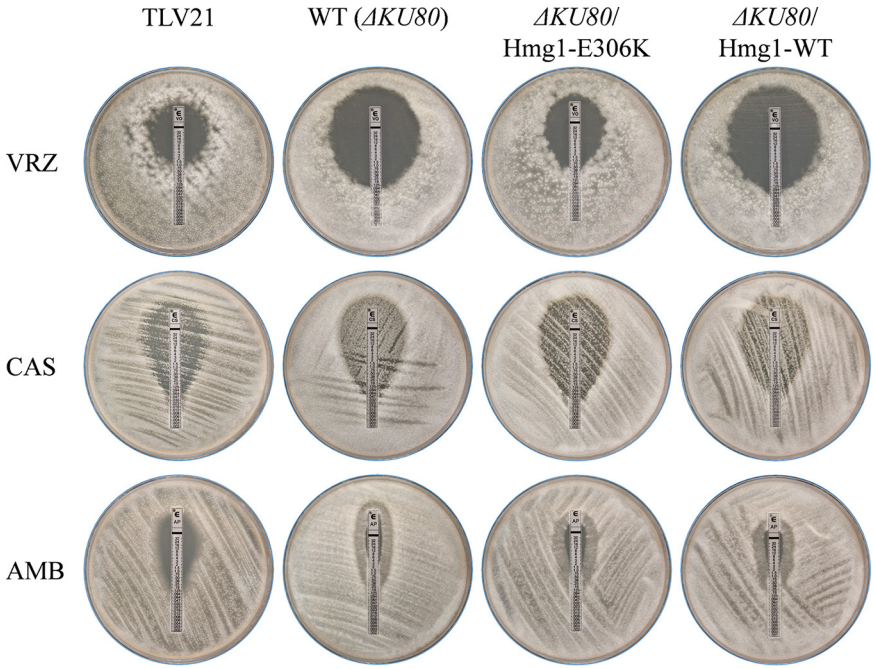

FIG 1 Drug susceptibility of *A. fumigatus* strains with and without *hmg1* mutations. Etest assay on agar plates with strips of voriconazole (VRZ), caspofungin (CAS), and amphotericin B (AMB). Each plate was streaked with a cotton swab dipped twice in 5×10^6/ml spore stock. Pictures were taken after 48 h.

voriconazole MIC for TLV21 (1.5 mg/liter) than Δ*KU80*/Hmg1 E306K (0.5 mg/liter) (Fig. 1). Together, the broth microdilution and Etest findings indicate that the Hmg1 E306K mutation is the main driver of resistance in the clinical isolate.

In conclusion, we describe the identification of a novel *A. fumigatus* Hmg1 E306K mutation responsible for triazole resistance and failure of voriconazole treatment in an Israeli patient with CCPA.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

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