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First Description of Azole-Resistant *Aspergillus fumigatus* Due to TR_{46}/Y121F/T289A Mutation in France

Rose-Anne Lavergne,[a,b] Florent Morio,[a,b] Loïc Favennec,[c] Stéphane Dominique,[d] Jacques F. Meis,[e,f] Gilles Gargala,[c] Paul E. Verweij,[f] Patrice Le Pape[a,b]

Département de Parasitologie et Mycologie Médicale, Université de Nantes, Nantes Atlantique Universités, EA1155-IICiMed, Faculté de Pharmacie, Nantes, France[a]; Laboratoire de Parasitologie-Mycologie, Institut de Biologie, CHU de Nantes, Nantes, France[b]; EA3800, Laboratoire de Parasitologie-Mycologie, Université de Rouen et CHU de Rouen, Rouen, France[c]; Department of Pneumology, Cystic Fibrosis Center, Rouen University Hospital, Rouen, France[d]; Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands[e]; Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands[f]

Azole resistance in *Aspergillus fumigatus* is an emerging public health concern. Recently, a novel fungicide-driven mutation in the *cyp51A* gene and its promoter, TR_{46}/Y121F/T289A, leading to high-level resistance to voriconazole has been identified in The Netherlands, Belgium, Germany, Denmark, Tanzania, and India in both clinical and environmental samples. Here we report the first description of *A. fumigatus* carrying this mutation in France, in a cystic fibrosis patient, underlining the need for extensive monitoring of *Aspergillus* resistance.

Azole-resistant *Aspergillus fumigatus* isolates have been increasingly reported in Europe since the later years of the first decade of the 2000s. This emerging public health concern occurs through two distinct routes of acquisition: *in vivo* selection of resistance as a consequence of long-term azole treatment and *de novo* acquisition of a resistant isolate directly from the environment, linked to the widespread use of azole fungicides in agriculture. Besides the TR_{34}/L98H mutation in the *cyp51A* gene first described in The Netherlands, a novel fungicide-driven mutation, TR_{46}/Y121F/T289A, has been recently identified. Until now, the TR_{46}/Y121F/T289A mutation has been reported in both environmental and clinical samples in four countries across Europe (1–7), in Asia (8), and, more recently, in Africa (9), suggesting a large geographical spread. Here we provide the first description of *A. fumigatus* carrying a TR_{46}/Y121F/T289A mutation in a cystic fibrosis patient in France.

A 23-year-old male cystic fibrosis patient with follow-up at the Pneumology Department at Rouen University Hospital, France, was seen in consultation in March 2014. This patient had high levels of total IgE and *Aspergillus*-specific IgE with positive *Aspergillus*-specific IgG antibodies, suggesting a diagnosis of allergic bronchopulmonary aspergillosis. He had a history of *A. fumigatus* airway colonization and exposure to mold-active azoles (itracona-

Received 17 January 2015 **Returned for modification** 17 February 2015
Accepted 14 April 2015
Accepted manuscript posted online 27 April 2015
Citation Lavergne R-A, Morio F, Favennec L, Dominique S, Meis JF, Gargala G, Verweij PE, Le Pape P. 2015. First description of azole-resistant *Aspergillus fumigatus* due to TR_{46}/Y121F/T289A mutation in France. Antimicrob Agents Chemother 59:4331–4335. doi:10.1128/AAC.00127-15.
Address correspondence to Patrice Le Pape, patrice.le-pape@univ-nantes.fr.
R.-A.L. and F.M. contributed equally to this article.

doi:10.1128/AAC.00127-15

TABLE 1 Overview of the characteristics of all *Aspergillus fumigatus* strains isolated from sputum samples of the patient

Strain no.	Reference in the dendrogram	Mo/yr of isolation	MIC (mg/liter) (EUCAST)[a]		*cyp51A* mutations
			ITC	VRC	
1	14-105-2468	March 2014	8	>8	TR_{46}/Y121F/T289A
2	14-148-2457	November 2013	0.5	0.5	Wild type
3	14-148-2460	February 2013	>8	>8	TR_{46}/Y121F/T289A
4	141428-459	January 2013	0.5	1	Wild type
5	14-148-2458	January 2013	0.25	0.25	Wild type
6	14-148-2456	December 2010	Not determined	Not determined	F46Y, G89G, M172V, N248T, D255E, L358L, E427K, C454C
7	14-148-2455	September 2010	Not determined	Not determined	F46Y, G89G, M172V, N248T, D255E, L358L, E427K, C454C
8	14-148-2454	July 2010	Not determined	Not determined	F46Y, G89G, M172V, N248T, D255E, L358L, E427K, C454C
9	None	July 2009	Not determined	Not determined	F46Y, G89G, M172V, N248T, D255E, L358L, E427K, C454C
10	14-148-2450	July 2009	0.25	1	Wild type
11	14-148-2448	March 2009	0.25	1	Wild type
12	14-148-2447	December 2007	0.25	1	Wild type
13	14-148-2445	May 2007	0.5	2	Wild type
14	None	February 2007	0.5	1	F46Y, G89G, M172V, N248T, D255E, L358L, E427K, C454C

[a] ITC, itraconazole; VRC, voriconazole.

TABLE 2 Literature review of all studies reporting TR_{46}/Y121F/T289A *A. fumigatus* isolates[a]

Reference	Date of isolation	Type of sample	Underlying condition(s)	Infection	Antifungal susceptibility MIC (mg/liter)[b]			Outcome	Country
					VRC	ITC	POS		
1	July 2012	BAL fluid	HSCT	Probable IA	>16	4	1	Death	Belgium
2	December 2009	Sputum	HSCT	Probable IA	>16	4	0.25	Persistent infection	The Netherlands
	January 2010	Ear	Chronic otitis externals/sinusitis	IA	>16	>16	2	Persistent infection	The Netherlands
	January 2010	Abdominal abscess	SOT	Proven IA	>16	2	0.5	Death	The Netherlands
	February 2010	Sputum	Cystic fibrosis	No IA	>16	4	0.5	Survival	The Netherlands
	February 2010	Sputum	Lung carcinoma	No IA	>16	>16	2	Survival	The Netherlands
	March 2010	Sputum	HSCT	Probable IA	>16	1	0.25	Death	The Netherlands
	March 2010	Sputum	Cystic fibrosis, SOT	Proven IA	>16	>16	0.5	Survival	The Netherlands
	May 2010	Biopsy specimen	Chronic otitis, surgery	Proven IA	>16	4	1	Survival	The Netherlands
	May 2010	Sputum	Lung fibrosis	None	>16	>16	1	Survival	The Netherlands
	June 2010	Sputum	Traumatism	None	>16	1	0.25	Death	The Netherlands
	July 2010	Brain biopsy specimen	Beta thalassemia, diabetes mellitus	Proven IA	>16	4	1	Death	The Netherlands
	September 2010	Sputum	Cystic fibrosis	ABPA	>16	2	0.5	Survival	The Netherlands
	Oct 2010	Sputum	COPD, SOT	None	>16	>16	2	Survival	The Netherlands
	November 2010	Sputum	COPD	No IA	>16	>16	2	Survival	The Netherlands
	January 2011	Sputum	HSCT	Probable IA	>16	>16	1	Death	The Netherlands
	December 2009 to January 2011	Air sampling			ND	ND	ND		The Netherlands
8	2012–2013	Soil sampling			>16	1 to 2	0.25 to 0.5		India
3	September 2012	Sputum	Cystic fibrosis	Colonization	>8*	>8*	2*	Survival	Germany
4	January 2014	Sputum	Bruton's agammaglobulinemia, SOT	Probable IA	>4*	0.25 to 0.5*	0.125 to 0.25*	Death	Denmark
9	Not reported	Soil sampling			16 to >16	1 to 2	0.25 to 0.5		Tanzania
5	November 2013	BAL fluid	HSCT	Probable IA	>8	>16	1	Death	Belgium
7	September 2012	BAL fluid	HSCT	Probable IA	16*	>16*	0.5*	Death	Germany
	July 2012	BAL fluid	HSCT	Proven IA	1*	>16*	0.5*	Death	Germany
Present report	February 2013	Sputum	Cystic fibrosis	Colonization	>8*	>8*	ND	Survival	France
	March 2014	Sputum	Cystic fibrosis	Colonization	>8*	8*	ND	Survival	France

[a] POS, posaconazole; BAL, bronchoalveolar lavage; HSCT, hematopoietic stem cell transplantation; IA, invasive aspergillosis; SOT, solid-organ transplantation; ABPA, allergic bronchopulmonary aspergillosis; COPD, chronic obstructive pulmonary disease; ND, not determined.

[b] Data represent results determined using CLSI breakpoints, except those indicated with an asterisk, which represent results determined using EUCAST breakpoints.

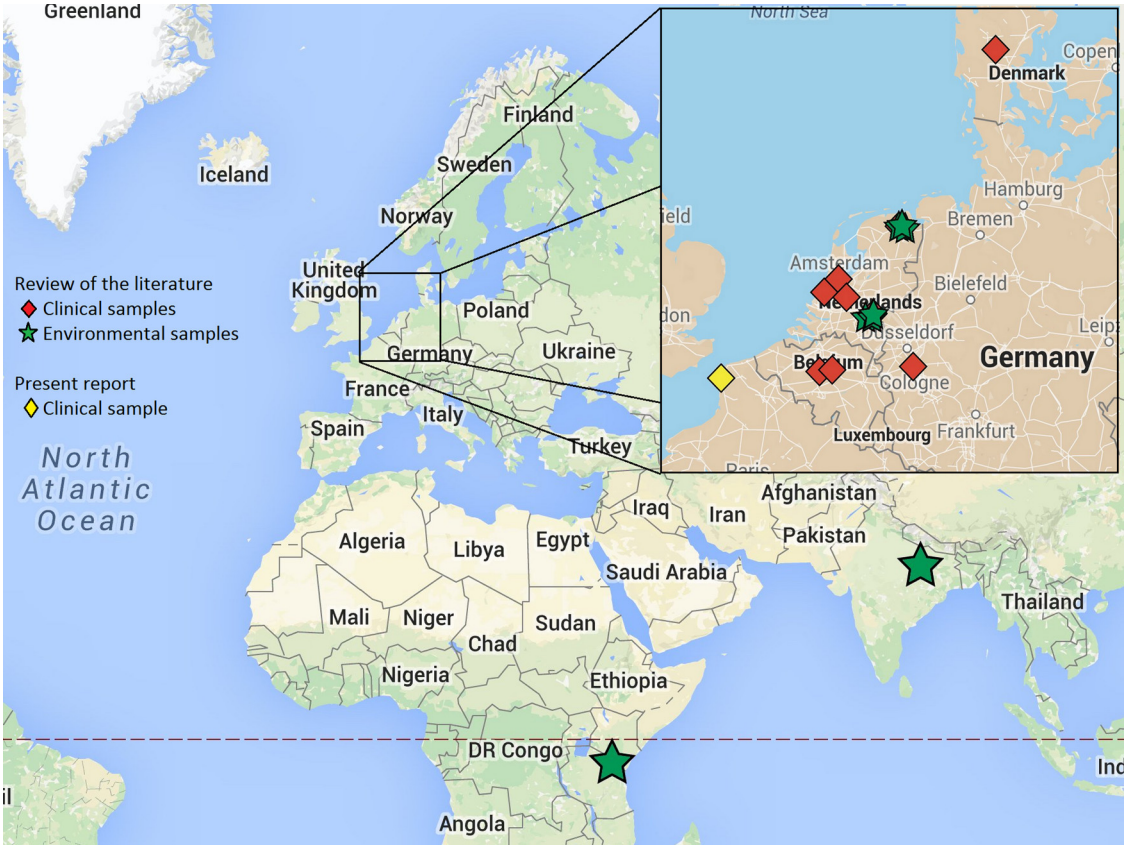
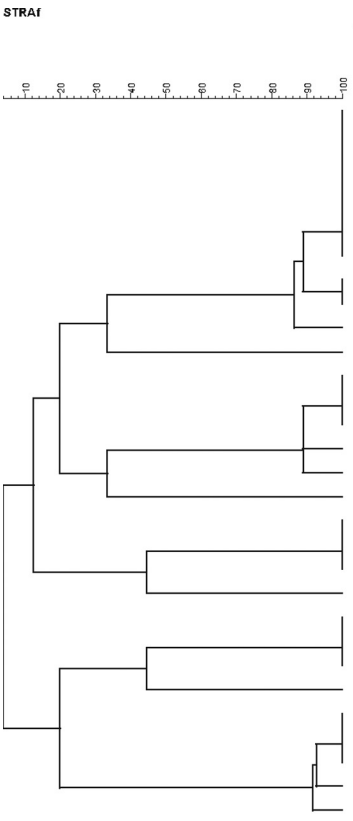


FIG 1 Geographical spread of the TR_{46}/Y121F/T289A resistance mechanism (for each strain, the exact location and origin [clinical or environmental] are indicated). Map data: Google GeoBasis-DE/BKG and Google, INEGI.

zole and voriconazole) since 2002. At the time of the consultation, he was treated with voriconazole. Mycological cultures of the sputum collected during the consultation grew *A. fumigatus* (strain 1). Species identification was obtained by both macroscopic and microscopic characteristics on Sabouraud's agar medium together with sequencing of the beta-tubulin gene (10). In accordance with a local research protocol aiming at the surveillance of azole resistance, this isolate was tested for antifungal susceptibility by the Etest method (bioMérieux, Marcy l'Etoile, France). Unexpectedly, this strain exhibited high-level resistance to voriconazole (MIC = >32 μg/ml) in comparison with itraconazole (MIC = 8 μg/ml) and posaconazole (MIC = 1 μg/ml). Antifungal susceptibility was confirmed by the EUCAST broth microdilution reference method (Table 1) (11, 12). Nucleotide sequencing of the *cyp51A* gene and its promoter, using previously described primers (13, 14) and in-house-designed primers (CYP51AF-F1 [5′-ATTTCCCTCATCACTGCAA], CYP51AF-R1 [5′-CATCATGTGCGCAATCTCTT], CYP51AF-F2 [5′-AGAAGCGAGATGCTGCTCAT], and CYP51AF-R2 [5′-CCTTTGAAGTCCTCGATGGT]), showed the TR_{46}/Y121F/T289A mutation. Antifungal therapy was therefore switched to posaconazole in April 2014 and then to caspofungin (50 mg per day) in July 2014 because of pulmonary exacerbation.

Given these findings, we retrospectively analyzed all *A. fumigatus* strains that had been isolated from this patient since 2007 ($n = 13$) for itraconazole and voriconazole susceptibility, *cyp51A* sequencing, and microsatellite genotyping. As shown in Table 1, our patient had already been colonized by a TR_{46}/Y121F/T289A isolate 1 year before, in February 2013 (strain 3). All remaining isolates collected before February 2013 were azole susceptible, either being wild type for the *cyp51A* gene or carrying mutations previously found in both azole-resistant and azole-susceptible isolates (15). As only a single colony was subjected to *in vitro* susceptibility testing, other azole-resistant isolates could have been missed. Microsatellite genotyping was performed using a panel of nine short tandem repeats as described previously (16). As illustrated in Table 1, both TR_{46}/Y121F/T289A isolates from our patient had the same genotype as a strain previously isolated in Germany (7) (Table 1). To gain further insights into the route of acquisition of this azole-resistant isolate in our patient, we conducted an environmental study by performing soil samplings next to the patient's home as described previously (17), as well as surface samplings (contact agar plates) in his office. Neither *A. fumigatus* carrying TR_{46}/Y121F/T289A nor *A. fumigatus* carrying TR_{34}/L98H was identified.

Aspergillus fumigatus isolates carrying the TR_{46}/Y121F/T289A mutation were first described in December 2009 in The Netherlands (2). Since then, such isolates have been evidenced in three other European countries, namely, Belgium (1, 5), Germany (3, 7), and Denmark (4), and recently in India (8) and Tanzania (9) (Table 2 and Fig. 1). Taken together, these findings suggest, as discussed previously for TR_{34}/L98H isolates, a large geographical spread of this resistance mechanism. Several lines of evidence indicate that, as in the case of TR_{34}/L98H, TR_{46}/Y121F/T289A has emerged through a fungicide-driven route (18), such isolates being found in both azole-naive patients (1, 2, 6, 7) and azole-exposed patients (2, 3,



2A	2B	2C	3A	3B	3C	4A	4B	4C			
26.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL1659	The Netherlands	TR46/Y121F/T289A
26.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL1859	The Netherlands	TR46/Y121F/T289A
26.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL1866	The Netherlands	TR46/Y121F/T289A
26.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL2244	The Netherlands	TR46/Y121F/T289A
26.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL2340	The Netherlands	TR46/Y121F/T289A
26.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL2604	The Netherlands	TR46/Y121F/T289A
26.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL2992	The Netherlands	TR46/Y121F/T289A
25.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL0682	The Netherlands	TR46/Y121F/T289A
25.0	21.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL0723	The Netherlands	TR46/Y121F/T289A
26.0	26.0	9.0	33.0	11.0	22.0	8.0	14.0	10.0	CF/NL0645	The Netherlands	TR46/Y121F/T289A
18.0	12.0	16.0	25.0	11.0	22.0	8.0	8.0	5.0	141428-459	France	Wildtype
26.0	21.0	12.0	26.0	9.0	20.0	13.3	9.0	9.0	CF/NL0032	The Netherlands	TR46/Y121F/T289A
26.0	21.0	12.0	26.0	9.0	20.0	13.3	9.0	9.0	CF/NL2753	The Netherlands	TR46/Y121F/T289A
26.0	21.0	12.0	26.0	9.0	20.0	13.3	9.0	9.0	3F	The Netherlands	TR46/Y121F/T289A
26.0	21.0	12.0	26.0	9.0	22.0	13.3	9.0	9.0	CF/NL0840	The Netherlands	TR46/Y121F/T289A
26.0	21.0	12.0	26.0	9.0	21.0	13.3	9.0	9.0	CF/NL2889	The Netherlands	TR46/Y121F/T289A
25.0	21.0	12.0	47.0	9.0	11.0	8.0	10.0	19.0	14-148-2457	France	Wildtype
23.0	24.0	14.0	34.0	11.0	20.0	13.0	9.0	5.0	14-148-2448	France	Wildtype
23.0	24.0	14.0	34.0	11.0	20.0	13.0	9.0	5.0	14-148-2450	France	Wildtype
23.0	24.0	14.0	34.0	11.0	20.0	13.0	9.0	5.0	14-148-2447-1	France	Wildtype
23.0	20.0	14.0	37.0	11.0	7.0	10.0	9.0	8.0	14-148-2458	France	Wildtype
10.0	20.0	8.0	42.0	9.0	10.0	12.0	10.0	20.0	2107	Germany	TR46/Y121F/T289A
10.0	20.0	8.0	42.0	9.0	10.0	12.0	10.0	20.0	14-148-2460	France	TR46/Y121F/T289A
10.0	20.0	8.0	42.0	9.0	10.0	12.0	10.0	20.0	14-105-2468	France	TR46/Y121F/T289A
9.0	20.0	13.0	43.0	9.0	10.0	12.0	9.0	9.0	19B	The Netherlands	TR46/Y121F/T289A
15.0	20.0	11.0	18.0	10.0	7.0	15.3	10.0		14-148-2447-2	France	Wildtype
15.0	20.0	11.0	18.0	10.0	7.0	15.3	10.0	16.1	14-148-2454	France	Wildtype
15.0	20.0	11.0	18.0	10.0	7.0	15.3	10.0	16.1	14-148-2455	France	Wildtype
15.0	20.0	11.0	18.0	10.0	7.0	15.3	10.0	8.0	14-148-2456	France	Wildtype
15.0	20.0	11.0	18.0	10.0	7.0	15.3	10.0	5.0	14-148-2445	France	Wildtype

FIG 2 STR*Af* dendrogram highlighting the genetic relatedness between the *Aspergillus fumigatus* isolate collected from our patient and previously reported TR_{46}/Y121F/T289A isolates.

5, 7) as well as in samples from the environment (2, 8, 9). Here we report the first description of *A. fumigatus* carrying the TR_{46}/Y121F/T289A mutation isolated from a French patient.

Interestingly, our patient organized trips to The Netherlands as a tour operator. For these working purposes, he traveled to Amsterdam in November 2012, 3 months before the first isolation of the TR_{46}/Y121F/T289A strain from his sputum (February 2013). Moreover, he regularly received advertising postal packages from Dutch flower producers which were opened in his office. Three hypotheses can explain the route of acquisition of this TR_{46}/Y121F/T289A strain in our patient. (i) The first hypothesis is that he inhaled spores carrying TR_{46}/Y121F/T289A during his trip to The Netherlands (2). (ii) The second hypothesis is that colonization occurred after he inhaled spores carrying TR_{46}/Y121F/T289A from his environment in France. Our environmental study conducted next to the patient's home, less than 100 km from Belgium (where TR_{46}/Y121F/T289A strains have been recently identified [1]), failed to detect TR_{46}/Y121F/T289A environmental isolates. Nevertheless, environmental isolates carrying this mutation have been recently identified by our team in the same region in France, supporting this hypothesis (unpublished data). (iii) The last hypothesis is that colonization occurred after he inhaled *A. fumigatus* spores carrying TR_{46}/Y121F/T289A that had escaped while he was opening the packages received from The Netherlands. Though the French strains are genetically indistinguishable from the German isolates and genetically different from the Dutch isolates (Fig. 2), the route of acquisition in our patient is unclear, as the spores probably followed an airborne migration pattern as hypothesized previously for TR_{34}/L98H (19, 20).

The present report provides evidence that *A. fumigatus* voriconazole-resistant isolates carrying the TR_{46}/Y121F/T289A mutation can be now isolated from clinical samples in France. As observed with TR_{34}/L98H, a geographical spread of this resistance mechanism is ongoing across Europe and possibly worldwide. These findings, together with the high-level voriconazole resistance of the TR_{46}/Y121F/T289A strains both *in vitro* and *in vivo* (1, 2, 4–6), underline the need for intensive investigations to determine the prevalence of the mutation in both clinical and environmental samples. In line with this, as recommended by a European Centre for Disease Prevention and Control (ECDC) technical report (18), antifungal susceptibility testing of triazoles should be performed on all clinical *A. fumigatus* isolates before starting antifungal therapy.

ACKNOWLEDGMENTS

We are grateful to all the technicians of the Parasitology and Mycology Laboratories at Nantes University Hospital and Rouen University Hospital for technical assistance.

P.L.P. received grants from Astellas and Pfizer and speaker's fees from Merck and Gilead. F.M. received speaker's fees from Gilead and MSD and travel grants from Gilead, MSD, Pfizer, and Astellas. J.F.M. has received grants from Astellas, Basilea, and Merck, has been a consultant to Astellas, Basilea, and Merck, and has received speaker's fees from Merck and Gilead.

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