Isolation of *Aspergillus fumigatus* with Y121H or Y121F single *cyp51A* mutations after isavuconazole or voriconazole treatment.

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INTRODUCTION

Currently, voriconazole and more recently isavuconazole are widely used as first line drugs for the treatment of Aspergillosis. However, the increasing detection of azole resistant *Aspergillus fumigatus* isolates is threatening the azole class effectiveness in the aspergillosis management[1]. Two different routes of azole resistance development in *A. fumigatus* have been described: a medical route in which azole resistance is generated during long periods of azole treatment in clinical settings and another route of resistance derived from environmental origin due to extended use of DMIs in agriculture. In the first setting, the described azole resistance mechanisms are mainly due to point mutations in *cyp51A* (G54, G138, P216L, M220 and G448). In the environmental route, *A. fumigatus showing* azole resistant mechanisms with combined Cyp51A modifications (TR_{34}/L98H or TR_{46}/Y12F/A289T) are generally isolated[2].

METHODS

A total of 116 *A. fumigatus* strains isolated from 57 patients were studied. All patients had at least two isolates and were or not under azole treatment. The ITS regions and the β-tubulin sequence analysis were indicative of an *A. fumigatus* sensu stricto isolates. Antifungal susceptibility testing was performed using a four wells screening methodology to detect azole resistant *A. fumigatus*[4]. The positive hit strains, were *cyp51A*, *cyp51B, hmg1 and hmg2* PCR amplified and sequenced. All the strains with *cyp51A* mutations and the related strains from the same patient were genotyped following the typing method TRESPERG[3] and antifungal susceptibility was confirmed by microdilution assay (EUCAST).

RESULTS

During voriconazole or isavuconazole treatment, azole resistant *A. fumigatus* were selected in two patients: Patient A and B. Patient A had four strains sequentially isolated: two azole susceptible (WT for *cyp51A*) and two azole resistant with different Cyp51A azole resistance mechanisms: TR_{46}/Y121F/A289T (strain 3-A) and point mutation Y121H (strain 4-A). All four isolates were genotypically different (Table 1). Patient B had two isogenics strains (t04Am1.1c07e07), but one ot them was azole susceptible and the other azole resistant with the Y121F mutation in Cyp51A (Table 2).

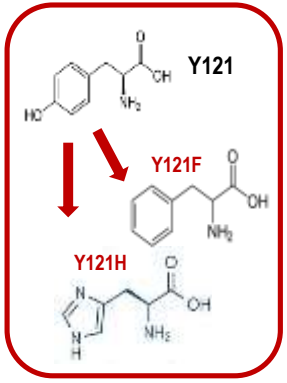
Table 1_ PATIENT A

STRAIN	DATE	*cyp51A*	*cyp51B*	CSP	Mp2	CFEM	erg4B	TRESPERG type	ITC	VCZ	POS	ISV	Hmg1	Hmg2
1-A	12/04/2020	WT	WT	t01	m3.4	c20	e11	t01m3.4c20e11	0.5	0.5	0.125	1	WT	WT
2-A	23/04/2020	WT	WT	t04A	m1.1	c07	e07	t04Am1.1c07e07	0.5	0.5	0.125	1	WT	WT
3-A	28/04/2020	TR_{46}/Y121F/A289T	WT	t01	m1.8	c05A	e16	t01m1.8c05Ae16	0.5	>8	0,5	8	WT	WT
4-A	14/05/2020	Y121H	WT	t05	m11.1	c08A	e09	t05m11.1c08Ae09	0.25	2	0.25	4	WT	WT

Table 2_ PATIENT B

STRAIN	DATE	*cyp51A*	*cyp51B*	CSP	Mp2	CFEM	erg4B	TRESPERG type	ITC	VCZ	POS	ISV	Hmg1	Hmg2
1-B	2016	WT	WT	t01	t04A	m1.1	c07	t04Am1.1c07e07	1	1	0.5	2	WT	WT
2-B	2017	Y121F	WT	t04A	t04A	m1.1	c07	t04Am1.1c07e07	2	2	0.25	4	WT	WT

Table 1 and **Table 2** show the TRESPERG genotype results, CMIs values obtained and *cyp51A, cyp51B, hmg1* and *hmg2* sequencing results of all strains isolated in both patients.



In *Saccharomyces cerevisiae* the Erg11 high-resolution X-ray crystal structure analysis demonstrated that the Y140F/H mutation disrupted the binding of short-tailed triazoles but not long-tailed ones. The resistance conferred by the Y140F/H mutations via the loss of hydroxyl group results in the disruption of a water-mediated hydrogen bond network between the tertiary hydroxyl groups of VCZ and the enzyme leading to a decrease in binding affinity[6]. Similarly, the structural analysis of the *A. fumigatus* Cyp51A showed that the Y121 residue stabilizes the interaction between voriconazole and the heme group supporting the assumption of this residue as very important site altering antifungal susceptibility when mutated[5].

The analysis of isolates obtained from Patient A strongly indicated that multiple resistant genotypes of *A. fumigatus* strains with several different resistance mechanisms might have been inhaled by the patient and subsequently selected in the lung due to the azole treatment. In Patient B, the genotyping results seems to indicate that the phenotype switch occurred in an isogenic background, supporting in-host selection due to the azole treatment

Due to the Y121F/H point mutations, observed in resistant strains, the stabilizing H-bond to the heme co-factor cannot be formed any more since the phenylalanine and histidine are both missing the hydroxyl group (**Figure**).

CONCLUSIONS

1.- Patient A genotyping results suggest that at the moment of the pulmonary aspergilosis diagnosis the patient was infected with a "mix" of *A. fumigatus* strains that can be selected or generated due to isavuconazole treatment.

2.-. Patient B genotyping results indicate that the phenotype switch occurred in an isogenic background, supporting in-host selection while the patient is under clinical azole treatments.

3.- The isolation of these *cyp51A* points mutations is very unusual. A) Y121H *cyp51A* point mutation has not been described before. B) Y121F *cyp51A* point mutation is the second time to be isolated[6].

4.- Cyp51A Mutation Y121F could be the "missing link" between the Tr_{46}/Y121F/A289T *A. fumigatus* strains and the wild-type *A. fumigatus* strains.

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