A NOVEL COMBINATION OF Cyp51A MUTATIONS IN *Aspergillus fumigatus* INDUCES A SIGNIFICANT REDUCTION IN THE In-Vitro SUSCEPTIBILITY TO VORICONAZOLE AND POSACONAZOLE

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Introduction

Since the beginning of the first decade of this century, a steady increase in worldwide reports of azole resistance in *Aspergillus fumigatus* has been a matter of serious clinical concern. Some resistant strains were isolated during antifungal therapy, while others may arise in the environment through exposure to triazole drugs used in agriculture. The resistance mechanism most frequently identified in environmental and clinical *A. fumigatus* strains is the combination of a 34-base pair (bp) repetition in tandem (TR_{34}) located 322 pb downstream the start codon of the *cyp51A* gene along with a substitution of leucine 98 to histidine (L98H)[1,3]. The aim of the present study was to uncover the molecular mechanism responsible of the azole cross-resistance profiles of three *A. fumigatus* isolates obtained from a patient with cystic fibrosis (CF).

Methodology

Three *A. fumigatus* isolates were recovered from different sputum samples from an Argentinean CF patient in October 2015, November 2016 and June 2017 (identified as A, B and C, respectively). The patient had received treatment with itraconazole (ITC, 400 mg/day) during 2015-2016 and with voriconazole (VCZ, 200 mg/day) during 2017. MICs of ITC, VCZ and posaconazole (PSC) were determined according to CLSI M38-3.ed document. Species were identified by sequencing of the *β-tubulin* and *calmodulin* genes. In addition, the ORF and 5´UTR regions of the *cyp51A* genes of all the strains were sequenced. *CYP51A* gene replacement experiments were performed by electroporation using a KU80[2] defective strain. Two PCR fragments were transformed, one including the Tr_{34}/L98H mutations and the other including a newly described mutation (see results).

Results

Susceptibility testing showed that isolates A, B and C had MIC values above the reported epidemiological cut-off values (ECVs) to ITC (MIC > 2 µg/ml) and PSC (MIC > 1 µg/ml), while only isolate B showed MIC above the ECV for VCZ (MIC > 1 µg/ml) and a higher MIC value for PSC (MIC > 2 µg/ml) (table 1). Sequence analysis of the *CYP51A* promoter region showed the presence of the Tr_{34} duplication in the isolates A, B and C (figure 1) accompanied by the mutations L98H and S297T in the *cyp51A* coding region (figure 2). Isolate B had an additional point mutation at the residue 65 (R65K) which has been never reported in the literature (figure 2). To test whether this new mutation was responsible or not for the increased PSC and VCZ MIC values, gene replacement experiments were carried out using a WT strain of *A. fumigatus* (KU80 deletant). Transformants that incorporated the Tr_{34}-R65K-L98H mimicked the MIC increase to VCZ and PSC observed in the clinical strain B.

Isolate	MIC (µg/µl)			Cyp51A Genotype			
	VCZ	PSC	ITC	5`UTR	R65	L98	S297
KU80	0.12	0.25	0.12	WT	R	L	S
A	1.00	2.00	≥8.00	Tr34	R	H	T
B	2.00	4.00	≥8.00	Tr34	K	H	T
C	1.00	2.00	≥8.00	Tr34	R	H	T
KU80-A (Tr_{34}/L98H)	1.00	2.00	≥8.00	Tr34	R	H	S
KU80-B (Tr_{34}/L98H/R65K)	2.00	4.00	≥8.00	Tr34	K	H	S

Table 1 – MIC values and genetic profile of isolates. WT = wild type.

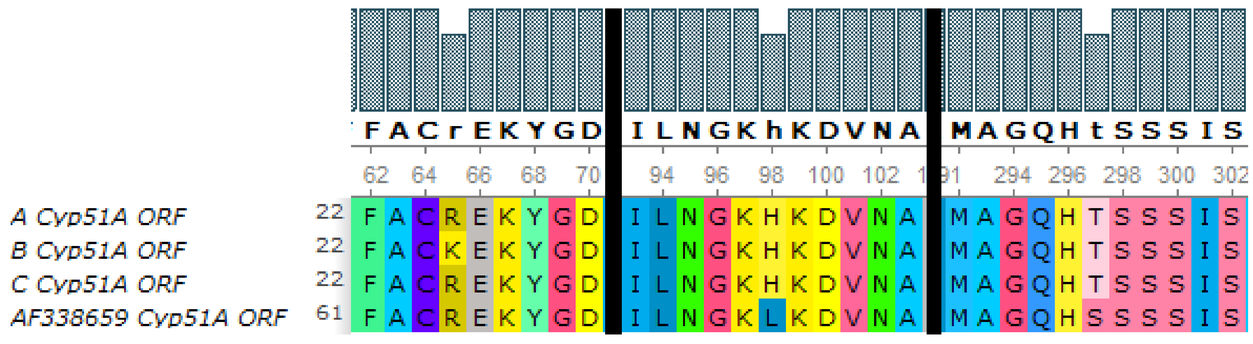

Figure 2 – A portion of the multiple alignment of the translated sequences of Cyp51A. Two amino acid changes can be observed at positions 98 and 297 in the sequence of A, B and C. An additional amino acid change is present in sequence B at position 65. All sequences were compared against reference sequence AF338659.

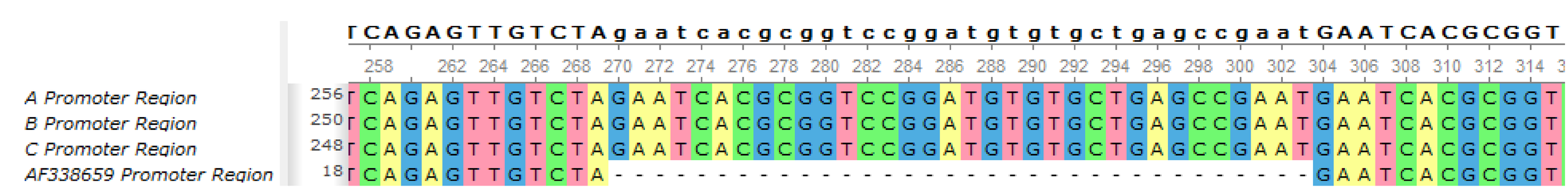

Figure 1 – Multiple alignment of a portion of the promoter region of Cyp51A. A 34 bp repetition can be observed in A, B and C. All sequences were compared against reference sequence AF338659.

Discussion and Conclusions

To the best of our knowledge, this is the first report of this novel combination of *CYP51A* mutations in *A. fumigatus.* The transformation experiments confirmed the association of these three modifications in the *cyp51A*'s promoter and coding region are responsible for the cross azole resistance phenotype. Further studies are warrant to determine whether this new mutants were selected by triazole clinical treatment or whether it has an environmental origin, possibly from agricultural use of fungicides.

References

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