

[Background]

The increase of azole-resistant *Aspergillus fumigatus* is global healthcare concern. The most common causative mutations of azole resistance in the fungus are TR34/L98H and TR46/Y121F/T289A, particularly in European countries. In Japan, however, such point mutations in the ORF region of *cyp51A* as G54, M220 or G448 are also important causes of azole resistance of *A. fumigatus*.

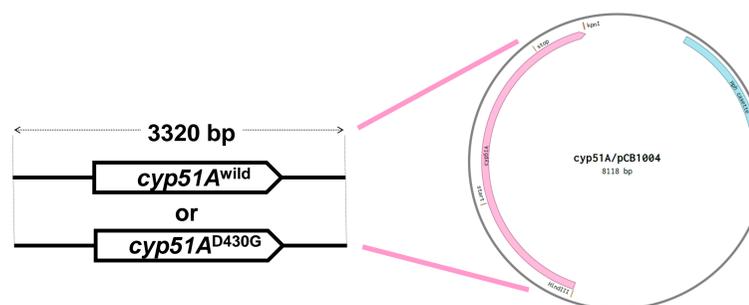
[Materials and Methods]

Clinical strains of *A. fumigatus* (IFM64503) were isolated from a patient with chronic pulmonary aspergillosis. He had a history of receiving voriconazole (VRCZ) for 4 months. Nine months after discontinuation of VRCZ, *A. fumigatus* (IFM 65189) was re-isolated from his sputum. Antifungal susceptibility test revealed that this strain was susceptible to VRCZ. Although administration of VRCZ was restarted, VRCZ-resistant *A. fumigatus* (IFM65685) was isolated again after 10 months.



Reference number	Duration of azole exposure	Mutation in <i>cyp51A</i> gene	MIC (mg/L)		Number of STR								
			ITCZ	VRCZ	2A	2B	2C	3A	3B	3C	4A	4B	4C
① IFM64503	Before receiving VRCZ	No mutation	1	1	18	12	20	28	10	20	8	9	10
② IFM65189	VRCZ 5M and discontinuation 9M	No mutation	0.5	0.5	24	22	23	25	26	32	10	11	10
③ IFM65685	VRCZ 10M	D430G	2	4	24	22	23	25	26	32	10	11	10

The mutated and wild *cyp51A* gene were transfected to $\Delta cyp51A$ strain using the shuttle vector pCB1004 by the PEG-mediated protoplast transformation method.



[Results]

Strain	Genotype	ITCZ	VRCZ	reference
<i>cyp51A</i> ^{wild}	$\Delta akuA$ -loxP $\Delta cyp51A::ptrA::cyp51A^{wild}::hph$	0.5	1	this study
<i>cyp51A</i> ^{D430G1}	$\Delta akuA$ -loxP $\Delta cyp51A::ptrA::cyp51A^{D430G}::hph$	8>	4	this study
<i>cyp51A</i> ^{D430G2}	$\Delta akuA$ -loxP $\Delta cyp51A::ptrA::cyp51A^{D430G}::hph$	8>	>8	this study
$\Delta cyp51A$	$\Delta akuA$ -loxP $\Delta cyp51A::ptrA$	0.25	0.25	1)
AfS35 (Background strain)	$\Delta akuA$ -loxP	0.5	0.5	

1) PLoS Pathog 2017;13:e1006096

Colony size of each strain (PDA, 35°C, 48h, data triplicate)



D430G strain (IFM65685)
28.7 mm



cyp51A^{D430G}
34.3 mm



$\Delta cyp51A$
35.0 mm

[Discussion and Conclusion]

The D430G mutation in CYP51A had not reported previously as the cause of azole-resistance, and we tried to check to see if this mutation is responsible for azole-resistance. In our study, MICs of ITCZ and VRCZ to the *cyp51A*^{D430G} transformant were higher than the ones to the D430G strain (IFM65685). This finding would be partially caused by an ectopic integration of transforming *cyp51A* gene. In fact, growth of the D430G strain was slower than the transformant. The D430G mutation in CYP51A is one of the responsible mutations for azole resistance of *A. fumigatus*.