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Hmg1 mutation conferring multi-azole resistance in *Aspergillus fumigatus*

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Purpose

The recent increase in azole-resistant *A. fumigatus* is a global concern. The mutations in *cyp51A* gene have been mostly studied as the causes of azole-resistance in the fungus, but uncovering the unknown (non-*cyp51A*) mutations responsible for azole resistance should be essential for developing novel methods for prompt diagnosis and effective drug treatment. In our recent study, we reported results that mutation of *hmg1*, which encodes HMG-CoA reductase, the rate-limiting enzyme in ergosterol biosynthesis, would be mechanism conferring azole-drugs resistance.[1] The aim of this study is to delineate the relationship between *hmg1* mutation and multi azole-resistance.

Conclusion

In this study, we discovered novel genetic changes related to azole resistance. We proposed that some multi-azole drug resistance is imparted by a mutation of Hmg1. Future elucidation of the molecular mechanism of the hmg1 mutation will lead to a more complete understanding of the azole resistance mechanism in *A. fumigatus*.

Reference:
1 Emerg Infect Dis. 2018, 24(10), 1889-1897

Method

We used clinical azole-resistant A. fumigatus strains collected in Japan and investigated the sequences of hmg1 gene. To delineate the association between the hmg1 mutation and triazole resistance, the mutant hmg1 allele in two clinical multi-azole resistant strains (mutation; Hmg1S269F alone and, $Hmg1^{S305P}$ and $Cyp51A^{M220I}$ combination) were replaced with the wild-type Hmg1 allele by CRISPR-Cas9 system. Antifungal susceptibility testing was performed according to the CLSI-M38.

Results

Table1 Azole resistant clinical isolates with Hmg1 mutation

IFM	ITCZ	VRCZ	cyp51A	hmg1
62140	4	8	no mutation	F261_del
64258	4	8	no mutation	F390Y
63768	8	>8	no mutation	S269Y
65560	4	4	no mutation	L273F

Table2 Azole resistant clinical isolates with Cyp51A ^{M220} mutation

IFM	ITCZ	VRCZ	cyp51A	hmg1
62871	>8	>8	M220I	S305P
62105	>8	>8	M220K	G307D
64304	>8	1	M220V	no matation
65548	>8	2	M220I	no matation

Table3 Verification of hmg1 mutations using Laboratory strain

		MEC	MIC		
mutant	genotype	MCFG	AMPH	ITCZ	VRCZ
hmg-1-mut1-2	$\Delta hmg1^{wild}$ ::$hmg1^{wild}$ ::hph	0.015>	1	1	1
hmg1-S269F	$\Delta hmg1^{wild}$ ::$hmg1^{S269F}$ ::hph	0.015>	0.25	>8	8
hmg1-S305P	$\Delta hmg1^{wild}$ ::$hmg1^{S305P}$ ::hph	0.015>	0.5	2	4
Background strain Afs35		0.015>	1	0.5	0.5

Table4 Verification of $hmg1^{S269F}$ using Clinical strain

		MEC	MIC		
	genotype	MCFG	AMPH	ITCZ	VRCZ
$Hmg1^{S269F}$Complementary strain	$\Delta hmg1^{S269F}$ ::$hmg1^{wild}$ ::hph	0.015>	1	1	1
controle strain	$\Delta hmg1^{S269F}$ ::$hmg1^{S269F}$ ::hph	0.015>	0.5	>8	8
Background IFM63240	$hmg1^{S269F}$	0.015>	1	>8	>8

Table5 Verification of $hmg1^{S305P}$ using Clinical strain

		MEC	MIC		
	genotype	MCFG	AMPH	ITCZ	VRCZ
$Hmg1^{S305P}$Complementary strain	$cyp51A^{M220I}$, $\Delta hmg1^{S305P}$ ::$hmg1^{wild}$ ::hph	0.015>	2	>8	1
controle strain	$cyp51^{AM220I}$, $\Delta hmg1^{S305P}$ ::$hmg1^{S305P}$ ::hph	0.015>	1	>8	>8
Background IFM62871	$cyp51A^{M220I}$, $hmg1^{G305P}$	0.015>	1	>8	>8

Among azole-resistant strains with mutations in the 220th methionine of Cyp51A, the strains both with and without Hmg1 mutation were found. Multi azole-resistance was found only in the strains possessing mutations in Hmg1. Resistant strains with $Hmg1^{S269F}$ restored sensitivity to ITCZ and VRCZ by a genetic complementation test of Hmg1. On the other hand, resistant strain both with $Hmg1^{S305P}$ and $Cyp51A^{M220I}$ only restored sensitivity to VRCZ. Currently, genetic complementation test of other SNPs in hmg1 is underway.

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