# The mutation in insulin-induced protein (Insig) gene is a novel factor that contributes additively to azole resistance in collaboration with the Cyp51A mutation.

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## Purpose

The most studied azole-resistant mechanism of *Aspergillus fumigatus* is decreased affinity of the drug for Cyp51A, the drug target molecule, due to its amino acid substitutions. Typically, each azole resistance caused by the designated amino acid substitution of Cyp51A has a specific pattern depending on the substitution site. However, different azole susceptibility patterns have been reported even among the strains possessing the same mutation in Cyp51A. In this way, the overall picture of molecular mechanisms inducing azole resistance remains unclear. This study reports a novel factor that has been shown to contribute additively to azole resistance in collaboration with the Cyp51A mutation.

**Table 1 Strains with different resistance patterns isolated from the patient with interstitial pneumonia**

| | | MEC | MIC | | | Azole exposure | geno type | | STRs | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IFM | Isolate Date | MCFG | AMPH | ITCZ | VRCZ | | cyp51A | hmg1 | 2A | 2B | 2C | 3A | 3B | 3C | 4A | 4B | 4C |
| 63559 | 2014.6. | <0.015 | 2 | 2 | >8 | VRCZ 7 M | G448S | no mutation | 19 | 22 | 22 | 29 | 13 | 15 | 10 | 9 | 5 |
| 63560 | 2015.6. | <0.015 | 2 | 8 | >8 | VRCZ 19 M | G448S | no mutation | 19 | 22 | 22 | 29 | 13 | 15 | 10 | 9 | 5 |

## Method

Comparative genomic analysis was performed between IFM63559 and IFM63560. To investigate the association between the novel mutation and azole resistance, the mutant allele was replaced with the wild-type allele by the CRISPR-Cas9 system. Antifungal susceptibility tests were performed according to the CLSI-M38.

## Conclusion

This study confirmed the novel genetic changes related to azole resistance. The unfinished Insig contributes additively to azole resistance in collaboration with the Cyp51A mutation but does not alone itself. Our results indicate that focusing on the phenotypes of multiple genes is essential to clarify the overall picture of the azole resistance mechanism of *A. fumigatus*.

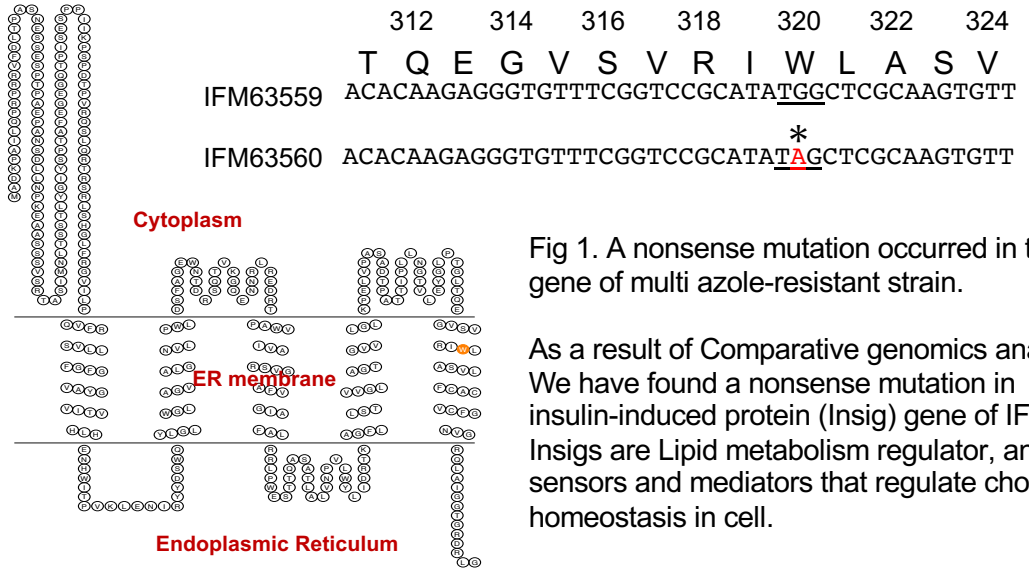


Fig 1. A nonsense mutation occurred in the *insig* gene of multi azole-resistant strain.

As a result of Comparative genomics analysis, We have found a nonsense mutation in the insulin-induced protein (Insig) gene of IFM63560. Insigs are Lipid metabolism regulator, and acts sensors and mediators that regulate cholesterol homeostasis in cell.

**Table 2 Verification of insig$^{W320*}$ using Clinical strain and Laboratory strain**

| | | MEC | MIC | | |
|---|---|---|---|---|---|
| Strain | genotype | MCFG | AMPH | ITCZ | VRCZ |
| complementary insig-*320W | cyp51A$^{G448S}$ Δinsig$^{W320*}$ ::insig$^{wild}$ ::hph | 0.015> | 1 | 1 | >8 |
| control insig-W320* | cyp51A$^{G448S}$ Δinsig$^{W320*}$ ::insig$^{W320*}$ ::hph | 0.015> | 1 | >8 | >8 |
| Background strain IFM63560 | cyp51A$^{G448S}$ insig$^{W320*}$ | 0.015> | 2 | 8 | >8 |
| AfS35 control insig-wild | cyp51A$^{wild}$ Δinsig$^{wild}$ ::insig$^{wild}$ ::hph | 0.015> | 0.5 | 0.5 | 0.5 |
| AfS35 insig-W320* | cyp51A$^{wild}$ Δinsig$^{wild}$ ::insig$^{W320*}$ ::hph | 0.015> | 0.5 | 1 | 1 |
| Background strain AfS35 | cyp51A$^{wild}$ insig$^{wild}$ | 1 | 2 | 0.5 | 0.5 |