Recreation of in-host acquired single nucleotide polymorphisms by CRISPR-Cas9 reveals an uncharacterised gene playing a role in *Aspergillus fumigatus* azole resistance via a non-cyp51A mediated resistance mechanism.

Ballard E, Weber J, Melchers WJG, Tammireddy S, Whitfield PD, Brakhage AA, Brown AJP, Verweij PE, Warris A (2019) Recreation of in-host acquired single nucleotide polymorphisms by CRISPR-Cas9 reveals an uncharacterised gene playing a role in *Aspergillus fumigatus* azole resistance via a non-cyp51A mediated resistance mechanism. *Fungal Genet Biol* 130, 98-106.

Abstract

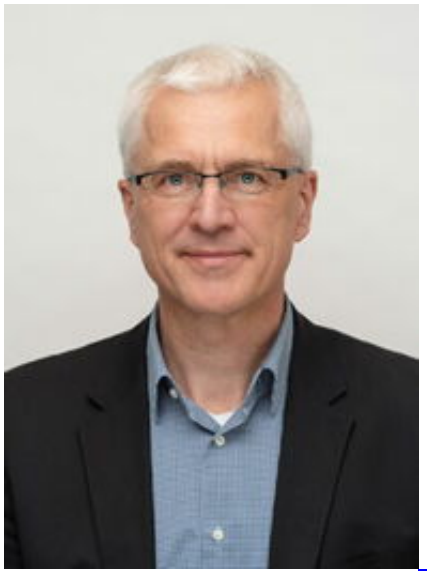
The human host comprises a range of specific niche environments. In order to successfully persist, pathogens such as *Aspergillus fumigatus* must adapt to these environments. One key example of in-host adaptation is the development of resistance to azole antifungals. Azole resistance in *A. fumigatus* is increasingly reported worldwide and the most commonly reported mechanisms are cyp51A mediated. Using a unique series of *A. fumigatus* isolates, obtained from a patient suffering

from persistent and recurrent invasive aspergillosis over 2 years, this study aimed to gain insight into the genetic basis of in-host adaptation. Single nucleotide polymorphisms (SNPs) unique to a single isolate in this series, which had developed multi-azole resistance in-host, were identified. Two nonsense SNPs were recreated using CRISPR-Cas9; these were 213* in svf1 and 167* in uncharacterised gene AFUA_7G01960. Phenotypic analyses including antifungal susceptibility testing, mycelial growth rate assessment, lipidomics analysis and statin susceptibility testing were performed to associate genotypes to phenotypes. This revealed a role for svf1 in *A. fumigatus* oxidative stress sensitivity. In contrast, recapitulation of 167* in AFUA_7G01960 resulted in increased itraconazole resistance. Comprehensive lipidomics analysis revealed decreased ergosterol levels in strains containing this SNP, providing insight to the observed itraconazole resistance. Decreases in ergosterol levels were reflected in increased resistance to lovastatin and nystatin. Importantly, this study has identified a SNP in an uncharacterised gene playing a role in azole resistance via a non-cyp51A mediated resistance mechanism. This mechanism is of clinical importance, as this SNP was identified in a clinical isolate, which acquired azole resistance in-host.

Involved units

Molecular and Applied Microbiology Axel Brakhage Read more

Leibniz-HKI-Authors



Axel A. Brakhage

Details

Jakob Weber

Details

Identifier

doi: 10.1016/j.fgb.2019.05.005

PMID: 31128273