Repertoire of natural polymorphisms in genes involved in *Candida albicans* resistance to antifungal: a powerful tool to highlight resistance mutations

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Background: Azoles and echinocandins are the 2 main classes of antifungal agents used to treat candidiasis. Resistance of *Candida albicans* isolates is a major cause of breakthrough infections. *C. albicans* is a diploid yeast which displays a high genetic polymorphism. Point mutations found in genes involved in the resistance of *C. albicans* to antifungals could either be natural polymorphisms or could confer phenotypic resistance.

Objective: Our objective was, through whole genome sequencing of susceptible and resistant clinical strains, to establish a comprehensive repertoire of the non-synonymous polymorphisms (natural polymorphisms and/or mutations of resistance) in genes involved in resistance to azoles and echinocandins

RESISTANCE MUTATIONS or NATURAL POLYMORPHISMS?

Whole genome sequencing and MICs determination by Etest®

Localization of amino acid substitutions in Tac1p

Cartography of the amino acid substitutions (natural polymorphisms and resistance mutations) of Tac1p observed in the 161 *C. albicans* clinical strains compared to the already described resistance mutations.

Polymorphism diversity evaluation

Accumulation curve of mutations as a function of the random selection of clinical isolates of *C. albicans*. Solid lines present the mean accumulation of mutations with increasing number of strains (black: random resampling of all susceptible strains (n=151); red: random samples of 141 susceptible strains with final introduction of the 10 resistant strains). The grey envelope corresponds to the 2.5%/97.5% and 5%/95% percentiles.

The introduction of the 10 resistant strains causes an increase in cumulative mutations at the end of the curve. This discrepancy suggests the appearance of new polymorphisms in the resistance population strains.

Characteristics of the Resistance Mutations

Protein	Amino acid substitution	Reference
Tac1p	H263Y	New In this study
Tac1p	A736T	Known Coste et al. Eukaryotic cell 2009
Tac1p	A790V	New In this study
Tac1p	N823K	New In this study
Tac1p	H839Y	New In this study
Tac1p	C858Y	New In this study
Tac1p	P971S	New In this study
Erg11p	D9V ^h	New In this study
Erg11p	P30S ^h	New In this study
Erg11p	Y64H	New In this study
Erg11p	T123I	Known YongQin Wu et al. FEMS Yeast Research 2017
Erg11p	Y132H	Known Chau et al., 2004; Favre et al., 1999; Kakeya et al., 2000; Marichal et al., 1999; Sanglard et al., 1998; Xu et al., 2008
Erg11p	R157K	Known Sanglard and Bille 2002
Erg11p	P236S	New In this study
Erg11p	S405F	Known Wang et al. BMC Mic. 2009
Erg11p	G448E	Known Loffler et al. 1997
Erg11p	G450E	Known Chau et al., 2004; Favre et al., 1999; Goldman et al., 2004; Loffler et al., 1997; Perea et al., 2001
Erg11p	R467I	Known Lamb et al., 2000; Lee et al., 2004; Sanglard et al., 1998; White et al., 1997
Upc2p	G648D ^h	Known Flowers et al. AAC 2015
Upc2p	D654N	New In this study
Upc2p	G657D	New In this study
Fks1p	R647G	Known M. Lackner et al. AAC 2014

List of the 22 selected resistance amino acid substitutions found in the 10 resistant strains.

Bold formatting indicates newly described mutations potentially involved in antifungal resistance. h = heterozygous (i.e., mutation on a single allele).

Mutagenesis experiment

Of the putative new mutations described, 5 have been introduced by mutagenesis in a wild-type allele (*TAC1* (n=3) and *UPC2* (n=2)). The successful introduction of 1 of the mutations in *TAC1* allele results in a 7.8 fold change increase of the fluconazole MIC.

Overexpression level of *ERG11* and *CDR2* by RT-qPCR:

Transcript level measurements of *ERG11* and *CDR2* overexpression by reverse transcription quantitative PCR (RT-qPCR) for seven fluconazole resistant clinical isolates of *C. albicans*. Expression was compared to the SC5314 reference strain for the *ERG11* analysis and to resistant strain harboring the known A736T mutation for the *CDR2* analysis. The housekeeping gene *ACT1* was used as an expression control. The experiments were performed on 2 biological replicates and error bars show the SD.

Conclusion

Whole genome sequencing of a large panel of antifungal susceptible *C. albicans* strains allowed us to determine a repertoire of the natural polymorphisms within the main genes involved in the resistance to azoles and echinocandins.

Using this repertoire, we identified 22 resistance amino acid substitutions among them 12 new putative mutations conferring resistance. Mutagenesis experiments are in progress to confirm these mutations. The introduction of one of them in *TAC1* allele results in the increase of the fluconazole MIC. Thus this repertoire can serve as a tool helping to rapidly unveil mutations potentially linked to antifungal resistance.