Antifungal resistant isolates of *Candida glabrata* from the United States are enriched for specific sequence types with distinct *MSH2* alleles

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Rates of acquired resistance to triazoles and echinocandins are substantially higher among strains of *Candida glabrata* compared to other *Candida* species, particularly in the U.S. We previously demonstrated that disruption of the DNA mismatch repair (MMR) gene *MSH2* increases *C. glabrata* antifungal resistance frequencies. Here, we feature the diversity of *MSH2* polymorphisms identified in strains from around the globe and highlight their associated sequence types and relevance in resistance potential. We have performed multilocus sequence typing (MLST) on 110 U.S. strains and have found that echinocandin (ECH)-, fluconazole (FLC)-, and multidrug (MDR)- resistant strains are enriched for sequence types (STs) 10, 16, 8, 3, and 6, while susceptible isolates are comprised of a more diverse ST array. ST10, ST16, and ST8 are associated with partial loss of function Msh2 alleles that carry amino acid substitutions P208S/N890I, E231G/L269F, and V239L/A942T, respectively. Interestingly, ST10 and ST16 (and therefore their associated Msh2 alleles) are almost exclusively found within U.S. isolates. Other Msh2 alleles, primarily identified in international isolates, imparted partial (e.g. S346T, M651T) or no (e.g. E456D, E459K, R847C) Msh2 LOF. Greater genetic diversity in terms of *MSH2* sequences and STs (including many novel STs) were identified within international isolates.

Figure 1. Sequence type (ST) distribution of U.S.-based isolates.

- Resistant isolates from the U.S. are comprised of fewer STs than the susceptible isolate population (Fig. 1).
- STs 8, 10, and 16 contain partial LOF Msh2 alleles (yellow highlight).
- STs 10 and 16 are nearly exclusive to U.S. isolates.
- Non-U.S. isolates demonstrate greater ST (incl. new STs) and Msh2 profile diversity (Table 1).

Table 1. List of identified *MSH2* genotypes and associated geographical locations, phenotype, and sequence type(s)

<i>MSH2</i> genotype	Location(s)	LOF phenotype*	Sequence Type(s)
WT (as ATCC 2001)	1, 2, 3, 4, 5, 7, 8	-	3, 6, 15, 17, 19, 59, 76, new
E7K	6, 8	-	3
P27L/I156L	8	unknown	new
P208S/N890I	1, 2, 3, 6, 8	+++	10
P208S/N890I/Y949C	6	+++	.
L269F	1, 2, 3,	++	.
E231G/L269F	1, 2, 3, 4	++	16
E231G/L269S	3	unknown	16
V239L	1, 2, 3, 4, 5, 7, 8	++	46, 7, new
V239L/A942T	1, 2, 3, 4, 5, 6, 8	++	8, 2
V239L/S757T	2	unknown	.
V239L/D946E	7	unknown	.
V34E/V239L/A942T	6	unknown	.
V239L/K814E/A942T	5	unknown	.
Q259H	4	unknown	new
S346T	6	+	.
Q386K	7	+++	.
E456D	1, 2, 3, 6, 8	-	new
E459K	7, 8	-	.
E478Q	2, 6	++	.
S591Y	6	+++	.
E608D	8	unknown	.
M651T	6	+	.
T772S	7	unknown	.
R847C	7	-	.
V856A	8	unknown	new

Center locations: 1: U.S. (TX); 2: U.S. (GA/MD/TN); 3: U.S. (NC); 4: U.S. (MI); 5: Europe 1; 6: Europe 2; 7: South Asia; 8: Middle East

*Phenotype definition
(see definitions at right and Fig. 2 top)

MSH2 genotype identified in at least 10 isolates

- = 1-2 fold increase
+ = 3-4 fold increase
++ = 5-8 fold increase
+++ = ≥ 9 fold increase

Figure 2. Cloning assays used to determine significance of *MSH2* polymorphisms. Top: *MSH2* alleles expressed under native promoter in the *msh2Δ* strain. Bottom: *MSH2* WT expressed under constitutive promoter in clinical isolates. Selections on media containing 1 µg/ml caspofungin (CSF).

Conclusions: Compared to the genetically diverse international isolate populations, *C. glabrata* from U.S. centers is enriched for several STs and, therefore, specific Msh2 alleles. It is possible that specific STs (e.g. 16 and 10) that have the ability to become more pathogenic or acquire drug resistance at greater rates have become more widespread within the U.S. Our data shed light on factors, such as *MSH2*, that may influence ST distribution. We aim to identify additional factors that contribute to pathogenesis and enhanced antifungal tolerance or resistance among these dominant STs.