AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

MECHANISMS OF RESISTANCE

Comparative Genomics of Serial *Candida glabrata* Isolates and the Rapid Acquisition of Echinocandin Resistance during Therapy

Amelia E. Barber,[a] Michael Weber,[a,b,c] Kerstin Kaerger,[a] Jörg Linde,[b,c] Hanna Gölz,[d] Daniel Duerschmied,[e,f] Antonie Markert,[f] Reinhard Guthke,[b] Grit Walther,[a] Oliver Kurzai[a,g]

[a]National Reference Center for Invasive Mycoses, Leibniz Institute for Natural Product Research and Infection Biology–Hans Knoell Institute, Jena, Germany
[b]Systems Biology and Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology–Hans Knoell Institute, Jena, Germany
[c]Research Group PiDOMICs, Leibniz Institute for Natural Product Research and Infection Biology–Hans Knoell Institute, Jena, Germany
[d]Institute of Medical Microbiology and Hygiene, Faculty of Medicine, Medical Center-University of Freiburg, Freiburg, Germany
[e]Department of Cardiology and Angiology I, Heart Center, University of Freiburg, Freiburg, Germany
[f]Department of Medicine III: Medical Intensive Care, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[g]Institute for Hygiene and Microbiology, Julius Maximilian University of Wuerzburg, Wuerzburg, Germany

ABSTRACT The opportunistic pathogen *Candida glabrata* shows a concerning increase in drug resistance. Here, we present the analysis of two serial bloodstream isolates, obtained 12 days apart. Both isolates show pan-azole resistance and echinocandin resistance was acquired during the sampling interval. Genome sequencing identified nine nonsynonymous SNVs between the strains, including a S663P substitution in FKS2 and previously undescribed SNVs in *MDE1* and *FPR1*, offering insight into how *C. glabrata* acquires drug resistance and adapts to a human host.

KEYWORDS *Candida*, *Candida glabrata*, candidiasis, comparative genomics, fungus, resistance

Candida glabrata is a commensal of the human microflora but is also a significant cause of both superficial and invasive fungal infections. *C. glabrata* is the second most common causative agent of invasive candidiasis, accounting for 27% of *Candida* bloodstream infections in the United States (1). Despite its name, *C. glabrata* is more closely related to the nonpathogenic model organism *Saccharomyces cerevisiae* than other *Candida* family members. *C. glabrata* is also unique among other *Candida* species for its haploid genome that forces asexual reproduction. Perhaps as a result, *C. glabrata* shows high levels of genomic plasticity and chromosomal rearrangements as a means to generate genetic diversity are common (2–4).

C. glabrata possesses MIC_{90} values five to seven dilution steps higher than *C. albicans* for azole class antifungals, independent of acquired resistance determinants (5). Beyond that, it can also develop bona fide resistance against azoles, which is often mediated by the upregulation of efflux pumps such as *CDR1* and *SNQ2* and a corresponding gain-of-function mutation in *PDR1* (4). In addition to its reduced azole susceptibility, the echinocandin resistance rate for *C. glabrata* in the United States more than doubled in the period of 2001 to 2010, going from 4.9 to 12.3% (6). Echinocandin resistance in *C. glabrata* is frequently the result of mutations in the "hot spot" region of *FKS2*, one of the catalytic subunits of the β-1,3-glucan synthase target complex (7). Importantly, *FKS* mutations correlate not only with higher *in vitro* MICs but also with treatment failure and increased mortality *in vivo* (6). Here, we present the analysis of two serial *C. glabrata* bloodstream isolates obtained at 12-day intervals. Both strains show pan-azole resistance and echinocandin resistance evolved during the sampling

Citation Barber AE, Weber M, Kaerger K, Linde J, Gölz H, Duerschmied D, Markert A, Guthke R, Walther G, Kurzai O. 2019. Comparative genomics of serial *Candida glabrata* isolates and the rapid acquisition of echinocandin resistance during therapy. Antimicrob Agents Chemother 63:e01628-18. https://doi.org/10.1128/AAC.01628-18.

Address correspondence to Oliver Kurzai, okurzai@hygiene.uni-wuerzburg.de.

Received 10 August 2018
Returned for modification 23 September 2018
Accepted 18 November 2018
Accepted manuscript posted online 26 November 2018
Published 29 January 2019

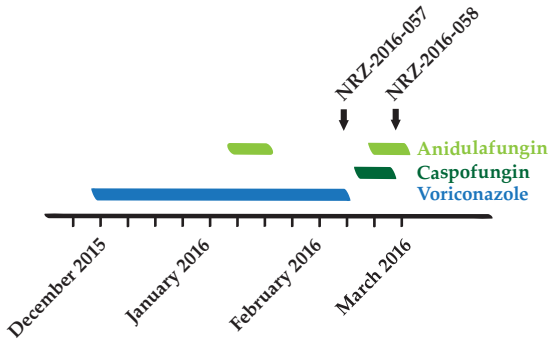

FIG 1 Schematic overview of the patient case report, including antifungal history and isolation from the bloodstream of NRZ-2016-057 and NRZ-2016-058.

interval, presumably as a result of the FKS2 S663P mutation identified through whole-genome sequencing in the second isolate. Our results highlight the ability of *C. glabrata* to rapidly acquire antifungal resistance, underscoring the increasing problem of resistant infections, and provide a global view of the genetic determinants necessary for *C. glabrata* to survive and persist in the bloodstream.

Patient case report and the development of echinocandin resistance in serial *C. glabrata* isolates. *Candida glabrata* strains NRZ-2016-057 and NRZ-2016-058 are serial isolates from the bloodstream of an AML patient obtained at a 12-day interval. The patient was treated with voriconazole prophylactically from 8 December 2015 to 13 February 2016. During this period, she also received a short course of anidulafungin from 15 to 21 January. On 13 February 2016 the patient was switched from azole to echinocandin therapy and received 70 mg caspofungin on the initial day of treatment and then 50 mg on subsequent days from 14 to 20 February. On 20 February, antifungal therapy was switched to 100 mg of anidulafungin, which was maintained until patient death on 28 February 2016. To help manage the source of infection, the central venous catheter was removed on 14 February 2016 and all other catheters replaced. NRZ-2016-057 was isolated from a blood culture on 13 February 2016 while the patient was on prophylactic voriconazole therapy, and NRZ-2016-058 was obtained on 25 February 2016 from a follow-up blood culture after the patient had been switched to echinocandin therapy (Fig. 1). The relatedness of the isolates was confirmed by multilocus sequence typing (MLST) as described previously (8) and using the allele definitions and MLST profiles described by PubMLST (https://pubmlst.org/cglabrata/) (Table 1). NRZ-2016-057 and NRZ-2016-058 were deposited in and are publicly available via the Jena Microbial Resource Collection under numbers JMRC:NRZ:0308 and JMRC:NRZ:0309, respectively.

The antifungal susceptibility of NRZ-2016-057 and NRZ-2016-058 was determined according to the EUCAST broth dilution method. Briefly, strains were precultured on yeast extract-peptone-dextrose (YPD) plates at 35°C, and then the susceptibility to amphotericin B (European Pharmacopoeia, Strasbourg, France), isavuconazole (Basilea Pharmaceutica International, Ltd., Basel, Switzerland), itraconazole (Janssen-Cilag GmbH, Neuss, Germany), posaconazole (MSD, Rahway, NJ), voriconazole (Pfizer, Inc., Peapack, NJ), fluconazole (Pfizer), anidulafungin (Pfizer), and caspofungin (MSD) was

TABLE 1 Antifungal susceptibility of *C. glabrata* strains NRZ-2016-057 and NRZ-2016-058

Strain	Strain type	MIC (mg/liter)[a]								Time elapsed (days)
		AMB	POS	VRC	ITC	FLC	ISA	AFG	CAS	
NRZ-2016-057	3	0.25	>8	>8	>8	>64	>8	0.03	0.5	
NRZ-2016-058	3	0.5	>8	>8	>8	>64	>8	4	>8	12

[a]AMB, amphotericin B; POS, posaconazole; VRC, voriconazole; ITC, itraconazole; FLC, fluconazole; ISA, isavuconazole; AFG, anidulafungin; CAS, caspofungin.

TABLE 2 Whole-genome comparison of CBS 138 and NRZ-2016-057

Parameter	Synonymous SNVs	Nonsynonymous SNVs	Indels	Indels in CDS
Total no.	26,235	11,471	4,168	171
Percentage vs CBS 138	0.21	0.09	0.03	0.003

assessed. MICs were determined using a Nepheloskan Ascent type 750 (Labsystems, Helsinki, Finland) after 24 h of incubation at 35°C and are defined as 50% reductions in growth compared to drug-free wells. Two reference strains, *Aspergillus fumigatus* ATCC 204305 and *Candida parapsilosis* ATCC 22019 were included as quality control in each set of tests. NRZ-2016-057 showed elevated MICs to all azoles examined but was still susceptible to amphotericin B and echinocandin-class drugs (Table 1). NRZ-2016-058 acquired resistance to anidulafungin and caspofungin during the sampling interval, in addition to its previously obtained azole resistance, but it remained susceptible to amphotericin B.

Whole-genome sequencing of NRZ-2016-057 and NRZ-2016-058. To determine the genetic changes that accompanied the development of echinocandin resistance, as well as any potential adaptations to the host environment, whole-genome sequencing was performed on NRZ-2016-057 and NRZ-2016-058 using 2x125bp Illumina paired-end sequencing by GATC Biotech (Constance, Germany). Raw genome sequence files (fastq) were uploaded to the NCBI Sequence Read Archive (SRA) and are publicly available under BioProject PRJNA483064.

Raw Illumina reads were processed by Trimmomatic (v0.32) (9) using specific parameters (leading:10 trailing:10 slidingwindow:4:15 minlen:50), ensuring the removal of adapter sequences and reads of low quality. After trimming, the read quality was checked with FastQC (v0.11.5) (10). Reads were aligned to the *Candida glabrata* reference genome CBS 138 (www.candidagenome.org) applying Novoalign (V3.07.01) in paired-end mode (Novocraft). From 4.4 and 5.5 million paired reads, we obtained 50- and 110-fold coverages for NRZ-2016-057 and NRZ-2016-058, respectively, covering 99% of the genome. Genetic variants, including single nucleotide variants (SNVs) and insertions and deletions (indels), were searched by SAMtools (v1.2; Samtools mpileup –uf) in conjunction with BCFtools using the multiallelic calling model (bcftools call –mv) (11). A minimum of five mapped reads for all reported variants was required. Processing and filtering of vcf files, allowing for the detection of amino acid substitutions in protein sequences, was carried out with the R package VariantAnnotation from Bioconductor (12). Compared to CBS 138, we identified 26,235 synonymous SNVs and 11,471 non-synonymous SNVs in NRZ-2016-057 (Table 2). This strain also showed 4,168 indels, 171 of which were in coding regions. We detected a 10.8-kb deletion on chromosome A of NRZ-2016-057, containing the uncharacterized open reading frame (ORF) CAGL0A02255g, as well as deletions of similar size on chromosomes H, J, and K, which did not contain any annotated ORFs (Fig. 2). These results indicate that NRZ-2016-057 displays a relatively high degree of genetic diversity compared to CBS 138, an observation in line with previous studies showing high genetic diversity between *C. glabrata* strains (2, 13). A complete list of genetic changes detected in NRZ-2016-057 compared to CBS 138 can be found in Data Set S1 in the supplemental material.

C. glabrata contains a large number of adhesins and the ability of the organism to adhere to host cells and other structures is a key virulence determinant for the organism (reviewed in reference 14) and, in general, the number of adhesins correlates with pathogenicity within the *Nakaseomyces* clade (15). Interestingly, we observed large spikes in read depth that predominantly corresponded to the genomic location of annotated adhesin genes in CBS 138, suggesting increases in the copy number of adhesin genes (Fig. 2). To confirm spikes in read coverage were not PCR duplicates, duplicates were marked and removed using Picard (v1.134; remove_duplicates=true assume_sorted=true).

Genomic comparison between NRZ-2016-057 and NRZ-2016-058. Genomic analysis of NRZ-2016-058 compared to NRZ-2016-057 with reference to CBS 138 identified

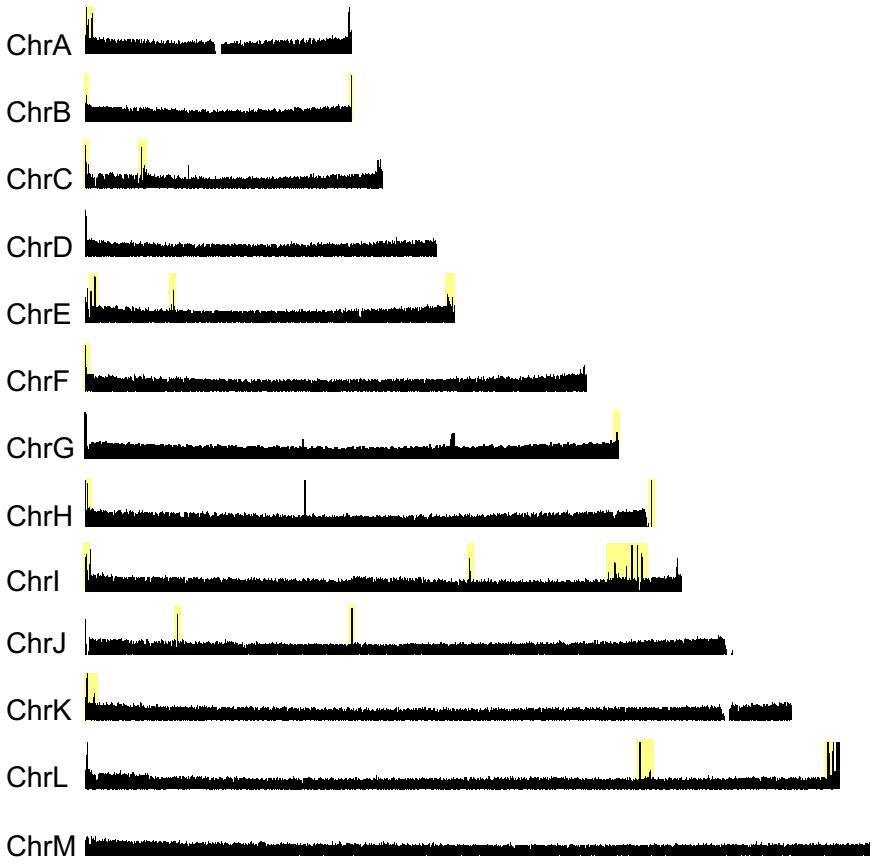

FIG 2 Read depth of NRZ-2016-057 across each chromosome when aligned to the CBS 138 genome. Highlighted regions indicate where spikes in read depth colocalize with the genomic location of putative or confirmed adhesin genes, suggesting increases in copy number.

two synonymous and nine nonsynonymous SNVs between the putative parent and offspring strain (Table 3). No indels were observed in NRZ-2016-058 that were not also present in the parent strain. In agreement with the development of phenotypic echinocandin resistance, we identified a T1987C (S663P) substitution in the *FKS2* coding sequence of NRZ-2016-058, a mutation previously shown to be sufficient to induce echinocandin resistance in *C. glabrata* (16). However, we also detected a nonsynonymous SNV in *MDE1*, a gene that is downregulated in response to caspofungin in *S. cerevisiae* and results in enhanced resistance to caspofungin upon its deletion in this organism, suggesting that there could potentially be additional mechanisms contributing to the echinocandin resistance observed in NRZ-2016-058 (17, 18). Another previously undescribed nonsynonymous SNV between the two isolates is in *FPR1*, a TOR signaling member whose expression is upregulated in biofilm versus planktonic growth (19). The *S. cerevisiae* ortholog of *FPR1* binds to rapamycin and FK506, both of which show antifungal activity. We also identified nonsynonymous SNVs in genes associated with metabolism and nutrient acquisition and the core stress response, including CAGL0M09999g and *HXT4/6/7*. The exact function of the genomic changes observed between NRZ-2016-057 and NRZ-2016-058 remains undefined; however, they presumably and collectively play a role in adapting the fungus to the host environment and the unique environmental stresses and antifungal therapy encountered there. It is also possible that these genetic changes conferred early or small increases in echinocandin resistance before the *FKS2* S633P mutation was acquired.

Interestingly, aside from the *FKS2* mutation, none of the changes observed between NRZ-2016-057 and NRZ-2016-058 overlapped those seen in another study following the

TABLE 3 Unique nonsynonymous SNVs observed in NRZ-2016-057 and NRZ-2016-058 compared to CBS 138[a]

Locus	Gene	Nucleotide position[b]	Nucleotide change	Amino acid change	GO biological process annotation(s)	GO molecular function annotation(s)
CBS 138 vs NRZ-2016-057						
CAGL0G02541g		ChrG: 234003	G→A	S→N	ND	ND
CAGL0L13002g		ChrL:1391142	T→C	F→L	Mismatch repair, mitochondrial DNA repair	ATP-binding, DNA-dependent ATPase activity, dinucleotide insertion or deletion binding, guanine/thymine misrepair binding
CBS 138 vs NRZ-2016-058						
CAGL0A02233g	*HXT4/6/7*	ChrA:236338	C→A	A →S	Transmembrane transport	Glucose transmembrane transporter activity
CAGL0G02519g		ChrG:229079	G→A	Q→stop	Protein kinase C-activating G-protein coupled receptor signaling pathway	Diacylglycerol kinase activity
CAGL0I04774g		ChrI:428298	G→A	A→V	Cellular calcium ion homeostasis	Enzyme regulator activity
CAGL0K04037g	*FKS2*	ChrK:375361	T→C	S→P	Cellular response to drug, 1,3-β-D-glucan biosynthesis pathway, ascospore wall assembly, pathogenesis	1,3-β-D-glucan synthase activity
CAGL0K09724g	*FPR1*	ChrK:952002	C→T	Q→stop	TOR signaling, cellular response to drug, chromatin reorganization, conjugation with cellular fusion, protein folding, regulation of homoserine biosynthetic process.	Macrolide binding, peptidyl-prolyl *cis-trans* isomerase activity
CAGL0M07876g	*MDE1*	ChrM:785919	T→C	I→V	L-Methionine salvage from methylthioadenosine	Metal ion binding, methylthioribulose 1-phosphate dehydratase activity
CAGL0M09999g		ChrM:992392	T→C	L→P	Negative regulation of Ras protein signal transduction, negative regulation of cAMP biosynthetic process, positive regulation of GTPase activity, regulation of adenylate cyclase activity	GTPase activator activity

[a]Lists of nonsynonymous SNVs observed in NRZ-2016-057 and NRZ-2016-058 compared to CBS 138 strain were compiled, and unique variants for each strain were identified by overlap analysis. ND, none determined.
[b]Nucleotide position from the CBS 138 genome.

genomics changes that accompanied the development of echinocandin resistance in a different set of *C. glabrata* isolates (16), highlighting the diverse mechanisms by which this organism can adapt to the selective pressures applied by the host and antifungal therapy. Moreover, despite the fact that the S633P mutation in FKS2 comes at a fitness cost to the organism in the absence of echinocandins (16, 20), we did not observe any significant growth differences between CBS 138, NRZ-2016-057, and NRZ-2016-058 in RPMI or YPD medium (data not shown), suggesting that one or more of the unique SNVs identified in NRZ-2016-058 could serve as a compensatory change for the reduced fitness resulting from the FKS2 mutation, potentially by upregulating *FKS* gene expression to compensate for the reduced catalytic potential of the mutated 1,3-β-D-glucan synthase complex, as has been shown for other *C. glabrata* isolates containing *FKS* hot spot mutations (20).

Finally, though NRZ-2016-057 and NRZ-2016-058 were resistant to all azoles tested, they do not possess any of the previously described mutations associated with azole resistance in *C. glabrata*, which are most commonly medicated via gain of function mutations in *PDR1* (21). However, we did identify a novel R761M polymorphism in this gene not previously described among the sequences present in the GenBank nr collection, in addition to V91I, L98S, and D243N polymorphisms which have been previously described in both azole-susceptible and azole-resistant isolates (22). Finally, neither strain showed any genetic changes in the mismatch repair complex gene *MSH2* that has also been shown to drive multidrug resistance in *C. glabrata* (23). The not-yet-deciphered mechanism by which azole resistance is mediated in NRZ-2016-057 and NRZ-2016-058 is an intriguing question warranting further investigation.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01628-18.

SUPPLEMENTAL FILE 1, XLS file, 7.1 MB.

ACKNOWLEDGMENTS

We thank Basilea Pharmaceutica International, Ltd. (Basel, Switzerland); Janssen-Cilag GmbH (Neuss, Germany); MSD (Rahway, NJ); and Pfizer, Inc. (Peapack, NJ), for providing the National Reference Center for Invasive Mycoses (NRZMyk) with antifungal agents at no cost.

The work of the NRZMyk is supported by the Robert Koch Institute from funds provided by the German Ministry of Health (grant 1369-240). In addition, this project was supported by The Federal Ministry for Education and Science (Bundesministerium für Bildung und Forschung) within the framework of InfectControl 2020 (Project FINAR, grant 03ZZ0809A), the German Research Foundation (Deutsche Forschungsgemeinschaft) CRC/Transregio 124: FungiNet, the Free State of Thuringia, and the European Regional Development Fund.

REFERENCES

1. Cleveland AA, Harrison LH, Farley MM, Hollick R, Stein B, Chiller TM, Lockhart SR, Park BJ. 2015. Declining incidence of candidemia and the shifting epidemiology of *Candida* resistance in two US metropolitan areas, 2008–2013: results from population-based surveillance. PLoS One 10:e0120452. https://doi.org/10.1371/journal.pone.0120452.
2. Carrete L, Ksiezopolska E, Pegueroles C, Gomez-Molero E, Saus E, Iraola-Guzman S, Loska D, Bader O, Fairhead C, Gabaldon T. 2018. Patterns of genomic variation in the opportunistic pathogen *Candida glabrata* suggest the existence of mating and a secondary association with humans. Curr Biol 28:15–27. https://doi.org/10.1016/j.cub.2017.11.027.
3. Bader O, Schwarz A, Kraneveld EA, Tangwattanachuleeporn M, Tangwattanchuleeporn M, Schmidt P, Jacobsen MD, Gross U, De Groot PWJ, Weig M. 2012. Gross karyotypic and phenotypic alterations among different progenies of the *Candida glabrata* CBS138/ATCC 2001 reference strain. PLoS One 7:e52218. https://doi.org/10.1371/journal.pone.0052218.
4. Healey KR, Jimenez Ortigosa C, Shor E, Perlin DS. 2016. Genetic drivers of multidrug resistance in *Candida glabrata*. Front Microbiol 7:1995. https://doi.org/10.3389/fmicb.2016.01995.
5. Castanheira M, Messer SA, Rhomberg PR, Dietrich RR, Jones RN, Pfaller MA. 2014. Isavuconazole and nine comparator antifungal susceptibility profiles for common and uncommon *Candida* species collected in 2012: application of new CLSI clinical breakpoints and epidemiological cutoff values. Mycopathologia 178:1–9. https://doi.org/10.1007/s11046-014-9772-2.
6. Alexander BD, Johnson MD, Pfeiffer CD, Jimenez-Ortigosa C, Catania J, Booker R, Castanheira M, Messer SA, Perlin DS, Pfaller MA. 2013. Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis 56:1724–1732. https://doi.org/10.1093/cid/cit136.
7. Pham CD, Iqbal N, Bolden CB, Kuykendall RJ, Harrison LH, Farley MM, Schaffner W, Beldavs ZG, Chiller TM, Park BJ, Cleveland AA, Lockhart SR.

2014. Role of FKS Mutations in *Candida glabrata*: MIC values, echinocandin resistance, and multidrug resistance. Antimicrob Agents Chemother 58:4690–4696. https://doi.org/10.1128/AAC.03255-14.
8. Dodgson AR, Pujol C, Denning DW, Soll DR, Fox AJ. 2003. Multilocus sequence typing of *Candida glabrata* reveals geographically enriched clades. J Clin Microbiol 41:5709–5717. https://doi.org/10.1128/JCM.41.12.5709-5717.2003.
9. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
10. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Babraham Bioinformatics, Cambridge, United Kingdom. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
11. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352.
12. Obenchain V, Lawrence M, Carey V, Gogarten S, Shannon P, Morgan M. 2014. VariantAnnotation: a Bioconductor package for exploration and annotation of genetic variants. Bioinformatics 30:2076–2078. https://doi.org/10.1093/bioinformatics/btu168.
13. Vale-Silva L, Beaudoing E, Tran VDT, Sanglard D. 2017. Comparative genomics of two sequential *Candida glabrata* clinical isolates. G3 (Bethesda) 7:2413–2426. https://doi.org/10.1534/g3.117.042887.
14. Timmermans B, De Las Penas A, Castano I, Van Dijck P. 2018. Adhesins in *Candida glabrata*. J Fungi (Basel) 4.
15. Gabaldon T, Martin T, Marcet-Houben M, Durrens P, Bolotin-Fukuhara M, Lespinet O, Arnaise S, Boisnard S, Aguileta G, Atanasova R, Bouchier C, Couloux A, Creno S, Almeida Cruz J, Devillers H, Enache-Angoulvant A, Guitard J, Jaouen L, Ma L, Marck C, Neuveglise C, Pelletier E, Pinard A, Poulain J, Recoquillay J, Westhof E, Wincker P, Dujon B, Hennequin C, Fairhead C. 2013. Comparative genomics of emerging pathogens in the *Candida glabrata* clade. BMC Genomics 14:623. https://doi.org/10.1186/1471-2164-14-623.
16. Singh-Babak SD, Babak T, Diezmann S, Hill JA, Xie JL, Chen YL, Poutanen SM, Rennie RP, Heitman J, Cowen LE. 2012. Global analysis of the evolution and mechanism of echinocandin resistance in *Candida glabrata*. PLoS Pathog 8:e1002718. https://doi.org/10.1371/journal.ppat.1002718.
17. Reinoso-Martin C, Schuller C, Schuetzer-Muehlbauer M, Kuchler K. 2003. The yeast protein kinase C cell integrity pathway mediates tolerance to the antifungal drug caspofungin through activation of Slt2p mitogen-activated protein kinase signaling. Eukaryot Cell 2:1200–1210. https://doi.org/10.1128/EC.2.6.1200-1210.2003.
18. Lesage G, Sdicu AM, Menard P, Shapiro J, Hussein S, Bussey H. 2004. Analysis of beta-1,3-glucan assembly in *Saccharomyces cerevisiae* using a synthetic interaction network and altered sensitivity to caspofungin. Genetics 167:35–49. https://doi.org/10.1534/genetics.167.1.35.
19. Seneviratne CJ, Wang Y, Jin L, Abiko Y, Samaranayake LP. 2010. Proteomics of drug resistance in *Candida glabrata* biofilms. Proteomics 10:1444–1454. https://doi.org/10.1002/pmic.200900611.
20. Garcia-Effron G, Lee S, Park S, Cleary JD, Perlin DS. 2009. Effect of *Candida glabrata* FKS1 and FKS2 mutations on echinocandin sensitivity and kinetics of 1,3-β-D-glucan synthase: implication for the existing susceptibility breakpoint. Antimicrob Agents Chemother 53:3690–3699. https://doi.org/10.1128/AAC.00443-09.
21. Vale-Silva LA, Sanglard D. 2015. Tipping the balance both ways: drug resistance and virulence in *Candida glabrata*. FEMS Yeast Res 15:fov025. https://doi.org/10.1093/femsyr/fov025.
22. Ferrari S, Ischer F, Calabrese D, Posteraro B, Sanguinetti M, Fadda G, Rohde B, Bauser C, Bader O, Sanglard D. 2009. Gain of function mutations in CgPDR1 of *Candida glabrata* not only mediate antifungal resistance but also enhance virulence. PLoS Pathog 5:e1000268. https://doi.org/10.1371/journal.ppat.1000268.
23. Healey KR, Zhao Y, Perez WB, Lockhart SR, Sobel JD, Farmakiotis D, Kontoyiannis DP, Sanglard D, Taj-Aldeen SJ, Alexander BD, Jimenez-Ortigosa C, Shor E, Perlin DS. 2016. Prevalent mutator genotype identified in fungal pathogen *Candida glabrata* promotes multi-drug resistance. Nat Commun 7:11128. https://doi.org/10.1038/ncomms11128.