Stepwise Development of a Homozygous S80P Substitution in Fks1p, Conferring Echinocandin Resistance in Candida tropicalis

Rasmus Hare Jensen,a Helle Krogh Johansen,b Maiken Cavling Arendrupa

Mycology Unit, Statens Serum Institut, Copenhagen, Denmarka; Department of Clinical Microbiology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmarkb

Three Candida tropicalis isolates were obtained from a patient with acute lymphoblastic leukemia. The first isolate was susceptible to all drug classes, while isolates 2 and 3, obtained after 8 and 8.5 weeks of caspofungin treatment, respectively, were resistant to the three echinocandins. Multilocus sequence genotyping suggested a clonal relation among all isolates. FKS1 sequencing revealed a stepwise development of a heterozygous and finally a homozygous mutation, leading to S80S/P and S80P amino acid substitutions.

It is well recognized that long-term antifungal treatment entails a risk for in vivo selection of resistant fungi. Accordingly, an increasing number of reports demonstrate acquired echinocandin and azole resistance associated with both hetero- and homozygous mutations in the FKS and ERG11 genes, which encode antifungal target proteins in Candida (1–6). This is of clinical importance, as resistant Candida isolates are associated with breakthrough candidiasis, treatment failures, and increased mortality (7). Candida tropicalis is identified as one of the five most common pathogenic Candida species, with a geographically determined proportion ranging from 3 to 66% of candidemia cases (8–10). Unfortunately, acquired fluconazole resistance is increasing, with ranges from approximately 7% in Denmark (9) to 9% in a global study (11) and 40% in Japan (12). Based on such findings, echinocandins are increasingly being utilized in the management of candidiasis caused by C. tropicalis (10, 13–15).

In this study, we analyzed three sequential C. tropicalis isolates (isolates 1, 2, and 3) obtained over a 4-month period from a patient with acute lymphoblastic leukemia who had been referred for allogeneic bone marrow transplantation. The patient was initially blood culture positive on 19 December 2010 for C. tropicalis (isolate 1) while receiving voriconazole prophylaxis. Caspofungin treatment was initiated (70/50 mg/day [70 mg on day 1 as a loading dose, followed by 50 mg daily thereafter]) (Fig. 1) and continued for a total of 8.5 weeks, interrupted by a 3-week fluconazole step-down treatment (Fig. 1). During the initial caspofungin treatment, nine serum samples tested positive for Candida mannan antigen, peaking at 479 pg/ml but stabilizing around 250 pg/ml on 20 January 2011 (Fig. 1). C. tropicalis was again detected in the blood on 5 March 2011 (isolate 2, after approximately 8 weeks of caspofungin treatment), and treatment was switched to amphotericin B (3 mg/kg/day) on 9 March. The patient was blood culture negative from 16 March, but the final C. tropicalis isolate (isolate 3, after approximately 8.5 weeks of caspofungin treatment) was obtained on 18 March from an oral swab, and treatment was changed to posaconazole (800 mg/day) on 31 March 2011. A Hickman catheter was kept in place, but sterilization was attempted with acid and fluconazole lock. Susceptibility testing was done according to EUCAST EDef 7.2 (azoles, anidulafungin, and micafungin) (16) and by Etest (amphotericin B and caspofungin).

FIG 1 Systemic antifungal treatment of the leukemic patient illustrated in boxes with drugs administered as daily doses (dd.). Nine serum samples were positive for Candida mannan antigen during the first caspofungin treatment period, and subsequently, several positive blood cultures were obtained. Three isolates (isolates 1, 2, and 3) were chosen and sequenced for resistance mechanisms and genotyping.
Table 1: Origins, resistance mechanisms, genotypes, and susceptibility data for the three study and two control C. tropicalis isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Specimen origin</th>
<th>Collection date (day, mo, yr)</th>
<th>FKS1 resistance mechanism</th>
<th>Allelic profile according to PubMLST (ICL1-MDR1-SAPT2-SAPT4-XYR-ZWF)</th>
<th>MIC (µg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EURCAST (Edif 7.1)</td>
<td>Etest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ANI MICA POS VOR ITR FLU AMB CAS</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>BC</td>
<td>19.12.10</td>
<td>Wild type</td>
<td>16-20-4-10-25-5</td>
<td>≤0.03</td>
</tr>
<tr>
<td>2</td>
<td>BC-CVC</td>
<td>05.03.11</td>
<td>5805/P</td>
<td>16-20-4-10-25-5</td>
<td>≤0.03</td>
</tr>
<tr>
<td>3</td>
<td>Cavum oris</td>
<td>18.03.11</td>
<td>S80P</td>
<td>16-20-4-10-25-5</td>
<td>≤0.03</td>
</tr>
<tr>
<td>REF-1‡</td>
<td>BC‡</td>
<td>08.07.10</td>
<td>Wild type</td>
<td>1-7-4-6-2-4</td>
<td>≤0.03</td>
</tr>
<tr>
<td>REF-2‡</td>
<td>BC‡</td>
<td>23.01.11</td>
<td>Wild type (99.7%)</td>
<td>1-3-1-7-2</td>
<td>≤0.03</td>
</tr>
</tbody>
</table>

* ANI, anidulafungin; MICA, micafungin; POS, posaconazole; VOR, voriconazole; ITR, itraconazole; FLU, fluconazole; AMB, amphotericin B; CAS, caspofungin.

† Unspecified blood culture.
‡ Blood culture obtained via the intravenous Hickman catheter.

A homozogous S80P mutation has not been described previously. This mutation may be slightly more resistant to echinocandins (at least 1 dilution step, as suggested by the increase in anidulafungin and micafungin MICs). Other homozogous mutations in C. tropicalis fks1 have been associated with elevated echinocandin MICs and amino acid substitutions, including L79W (4), F76S (6), and F76L (34). Moreover, heterozygous S80P/R mutants that display echinocandin resistance have been found (34, 35), but interestingly, the homozogous S80P mutation has not been described previously. This is in contrast to the findings for C. albicans, where a homozogous alteration at the corresponding codon (S645) has been detected in several resistant isolates (17, 36–38). Several factors may contribute to this difference. First, fitness cost when the second allele is mutated may vary, as supported by previous observations associating homozogous fks1 mutations in C. albicans with both fitness and virulence costs (39). Second, the resistance conferred by the heterozygous mutation may be sufficient to allow escape in S805/P C. tropicalis during caspofungin treatment, whereas the homozogous variant may be required for high-level echinocandin resistance in C. albicans (37, 40).

Our and related studies contribute to the overall understanding of resistance development in vivo as a consequence of antifungal treatment, including understanding the duration of treatment and which compounds allow selection of resistant mutants. Finally, this study may assist in determining treatment guidelines for the management of C. tropicalis infections, as the development of echinocandin resistance should be acknowledged as a rising concern in the treatment of patients with long-term echinocandin exposure.

Acknowledgments
We thank Birgit Brandt for her invaluable technical assistance in the laboratory.
R.H.J. has received a research grant from Gilead and travel grants from Astellas and MSD.
M.C.A. has received research grants from Astellas, Gilead, MSD, and Pfizer, been an advisor or consultant for Gilead, MSD, and Pfizer, and received a speaker’s honorarium for talks from Astellas, Gilead, MSD, and Pfizer.
H.K.J. has nothing to declare.

References


