Genomewide Expression Profile Analysis of the Candida glabrata Pdr1 Regulon*†‡

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The ABC transporters Candida glabrata Cdr1 (CgCdr1), CgPdh1, and CgSnq2 are known to mediate azole resistance in the pathogenic fungus C. glabrata. Activating mutations in CgPDR1, a zinc cluster transcription factor, result in constitutive upregulation of these ABC transporter genes but to various degrees. We examined the genomewide gene expression profiles of two matched azole-susceptible and -resistant C. glabrata clinical isolate pairs. Of the differentially expressed genes identified in the gene expression profiles for these two matched pairs, there were 28 genes commonly upregulated with CgCDR1 in both isolate sets including YOR1, LCB5, RTA1, POG1, HF1, and several members of the FLO gene family of flocculation genes. We then sequenced CgCDR1 from each susceptible and resistant isolate and identified a set of commonly differentially expressed genes, including CgCDR1, YOR1, and YML1, as well as genes uniquely regulated by specific mutations. Our results demonstrate that while CgPdr1 activates a broad repertoire of genes, specific activating mutations result in the activation of discrete subsets of this repertoire.

Over the past 2 decades, there has been an increase in Candida infections caused by non-albicans species, with Candida glabrata being the second most common cause of mucosal and invasive fungal infections in humans (24). Development of high-level resistance to the azoles, the most widely used antifungal agents, has been reported in C. glabrata oral and bloodstream isolates from head and neck radiation patients, stem cell transplant patients, and human immunodeficiency virus (HIV) patients (2, 25, 27). Furthermore, the development of azole resistance has been implicated in the fluconazole treatment failure and death of a patient suffering from C. glabrata candidemia (18).

Studies to determine the mechanisms of high-level azole resistance in C. glabrata have demonstrated frequent constitutive overexpression of the ATP-binding cassette (ABC) transporters C. glabrata CDR1 (CgCDR1), CgPdh1, and CgSnq2, all of which contribute to this phenotype (21, 25, 27, 32, 36). These transporters are regulated by the zinc binuclear cluster transcription factor CgPdr1, a single-gene homolog of Saccharomyces cerevisiae Pdr1 (ScPdr1) and ScPdr3 transcription factors (35, 36). CgPDR1, CgCDR1, CgPdh1, and CgSnq2 all contain at least one pleiotropic drug response element sequence (PDRE) in their promoters, suggesting that CgPdr1 may regulate its own expression, as well as that of the transporters, through binding these regulatory elements (32, 35). Recently, it was shown that C. glabrata Pdr1 (and S. cerevisiae Pdr1) directly binds to fluconazole, resulting in activation of drug efflux pumps, a mechanism similar to regulation of multidrug resistance (MDR) by the pregnane X receptor (PXR), a nuclear receptor, in vertebrates (30). Furthermore, single point mutations in the putative functional domains of CgPDR1 result in increased transcription of CgCDR1, CgPdh1, and CgSnq2 as well as increased resistance to azoles (9, 33, 35). A recent study demonstrated a high variability in these mutations and showed that the CgPdr1 activating mutations have an effect on not onlyazole susceptibility but also virulence (9). Collectively, these studies indicate that CgPdr1 is uniquely important to the ability of C. glabrata to not only resist antifungal therapy but also cause disease.

With these aforementioned studies in mind, we endeavored to delineate genes of the CgPdr1 regulon by first identifying matched isolated pairs whose resistant isolates carry activating mutations in CgPDR1 that conferred resistance to fluconazole. We then identified genes that are differentially expressed in the presence of two unique mutations, one residing in the putative activation domain and one near the putative inhibitory domain. Our results demonstrate that while CgPdr1 activates a
broad repertoire of genes, specific activating mutations result in the activation of discrete subsets of this repertoire.

**Materials and Methods**

Antifungal agent. Fluconazole was obtained from MP Biomedicals (Solon, OH). Stock solutions of 10 mg/ml were made in dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO) and stored at –20°C.

Strains and growth media. The isolates and strains used in this study are listed in Table 1. Both of these matched fluconazole-susceptible and -resistant clinical isolate sets have been described previously (18, 25, 31). All isolates/strains used in these studies were grown in YPD (1% yeast extract, 2% peptone, and 1% dextrose) agar plates and incubated at 37°C, and the MIC was read at 24 and 48 h. The MIC was defined as the lowest concentration of drug that causes a significant decrease in turbidity (>50%) compared to growth of the control. Candida krusei ATCC 6258 served as the quality control isolate.

**Drug susceptibility tests.** Microdilution broth susceptibility testing was performed in duplicate according to the CLSI M27-A3 method (4) in RPMI 1640 growth medium (Sigma) buffered with morpholinopropanesulfonic acid (MOPS; Sigma). Microtiter trays containing serial dilutions of fluconazole were inoculated and incubated at 37°C, and the MIC was read at 24 and 48 h. The MIC was defined as the lowest concentration of drug that causes a significant decrease in turbidity (>50%) compared to growth of the control. *Candida krusei* ATCC 6258 served as the quality control isolate.

**Growth conditions for gene expression analysis.** A single colony of each strain was grown overnight in YPD medium. Cells were then grown to mid-log phase (optical density at 600 nm [OD_{600}] of 0.6 to 1.0) in 200 ml of YPD medium and were harvested by centrifugation at 3,500 × g at 4°C for 5 min. The medium was removed by aspiration, and the cell pellets were stored at –80°C until RNA preparation.

**RNA preparation, cRNA synthesis, and hybridization for microarrays.** Total RNA was isolated using the hot phenol method (28) and as previously reported (36). cRNA synthesis and hybridization for microarray were previously described (36). Two independent microarray experiments were performed for each comparison.

**Microarray data analysis.** The scaled gene expression values from GCOS software were processed and analyzed using GeneSpring, version 7.2, software (Agilent Technologies). Probe sets were removed from analysis if they were called absent by the Affymetrix criterion and displayed an absolute value below 20 in all experiments. The expression value of each gene was normalized to the median expression of all genes in each chip as well as to the median expression of that gene across all chips in the study. Pairwise comparison of gene expression was performed for each matched experiment. Genes were considered to be differentially expressed if their change in expression was at least 1.5-fold in both independent experiments.

**Quantitative real-time RT-PCR.** Three sets of independently obtained RNA samples were used for quantitative real-time reverse transcription-PCR (RT-PCR) analysis. Quantitative real-time RT-PCR was conducted as previously described (36). Statistical analysis was performed using Microsoft Office Excel 2007. Relative changes were compared using a Student’s t test. The statistical significance threshold was fixed at an α of 0.05.

**Sequence analysis of C. glabrata PDR1.** CgPDR1 coding sequences were amplified by PCR (Pfu DNA polymerase; Stratagene) of *C. glabrata* genomic DNA using the primers PDR1seq1 and PDR1seq2 (Table 2). Products were cloned into pCR-BluntII-TOPO using a Zero Blunt TOPO PCR Cloning Kit (Invitrogen) and transferred into *E. coli* TOP10 cells with selection on LB agar plates containing 50 μg/ml kanamycin. Plasmid DNA was purified (Qiagen; Chatsworth, CA) and sequenced on an ABI 3130XL Genetic Analyzer using the universal primers M13F and M13R and a set of 10 primers (Table 2) that span both strands of the CgPDR1 coding sequence. The sequencing was performed using three sets of cloned derivatives from each independent PCR for each strain/isolate sequenced.

**Microarray data accession number.** Data files for each sequenced chip were submitted to the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo). The accession number for the series is GSE24168.

**Results**

**Identification of PDR1 mutations in azole-resistant clinical isolates.** As overexpression of CgPDR1 and several efflux pump genes regulated by CgPdr1 (CgCDR1, CgPDR1, and

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**Table 1. C. glabrata isolates/strains used in this study**

| Strain or isolate | Matched isolate/parent | Relevant characteristic(s) or genotype* | Reference or source |
|-------------------|------------------------|----------------------------------------|---------------------|
| SM1               | SM1                    | Clinical isolate, fluconazole susceptible | 17                  |
| SM3               | SM1                    | Clinical isolate, fluconazole resistant  | 24                  |
| 6856              | 6856                   | Clinical isolate, fluconazole susceptible |                     |
| 6955              | SM1                    | Clinical isolate, fluconazole resistant  |                     |
| SM1Δpdr1          | SM1Δpdr1               | pdr1::FRT                              | This study          |
| SM1ΔPDR1SM1       | SM1Δpdr1               | pdr1A::PDR1SM1-caSAT1                  | This study          |
| SM1Δpdr1/PDR1SM3  | SM1Δpdr1               | pdr1A::PDR1SM3-caSAT1                  | This study          |
| SM1ΔPDR18656      | SM1Δpdr1               | pdr1A::PDR18656-caSAT1                 | This study          |
| SM1Δpdr1/PDR1G1225| SM1Δpdr1               | pdr1A::PDR1G1225-caSAT1                | This study          |

* caSAT1, C. albicans-adapted nourseothricin resistance marker.
CgSNQ2 have previously been shown to be a mechanism of high-level azole resistance (2, 27, 32, 35), we first examined expression of these genes by real-time RT-PCR in the two matched pairs of azole-resistant and -susceptible C. glabrata clinical isolates. As has been observed in other azole-resistant clinical isolates (9), we observed distinctly different expression patterns for CgCDR1, CgPDH1, and CgSNQ2 in both of the azole-resistant isolates we examined (Fig. 1). CgCDR1 was upregulated in both resistant isolates, but CgPDH1 and CgSNQ2 were upregulated only in isolate SM3.

Next, we sequenced the CgPDRI gene from each isolate. Both resistant isolates contained nucleotide changes in CgPDRI compared to their corresponding susceptible isolates. Isolate SM3 contained a T2837C mutation which is predicted to result in the amino acid substitution L946S, located in the putative transcriptional activation domain of CgPdr1, an assumption based on similarity with ScPdr1 and ScPdr3 (36). Isolate 6955 contained a G822T mutation which is predicted to result in the amino acid substitution K274N, located near the putative inhibitory domain (36) (Fig. 2).

TABLE 2. Primers used in this study

| Primer function and primer or gene name | Primer\(^a\) | Amplicon size (bp) |
|----------------------------------------|-------------|--------------------|
| Plasmid construction                    |             |                    |
| CgPDRI1A                                | F, 5'-AACATTTCCTCATAGGGCCCATCTTTATC-3', R, 5'-TTTGAAGCTTATCTATCT-3' | 1,080 |
| CgPDRI1B                                | F, 5'-TTTGAAGCTTATCTATCT-3', R, 5'-TTTGAAGCTTATCTATCT-3' | 1,000 |
| CgPDRI1C                                | F, 5'-AACATTTCCTCATAGGGCCCATCTTTATC-3', R, 5'-TTTGAAGCTTATCTATCT-3' | 4,295 |
| CgPDRI1D                                | F, 5'-TTTGAAGCTTATCTATCT-3', R, 5'-TTTGAAGCTTATCTATCT-3' | 52 |
| CgPDRI1E                                | F, 5'-TTTGAAGCTTATCTATCT-3', R, 5'-TTTGAAGCTTATCTATCT-3' | 52 |
| CgPDRI1F                                | F, 5'-TTTGAAGCTTATCTATCT-3', R, 5'-TTTGAAGCTTATCTATCT-3' | 52 |
| Quantitative real-time PCR expression analysis |              |                    |
| 18S rRNA                                | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| CgPDRI                                 | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 57 |
| CgCDRI                                 | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| CgPDH1                                 | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| CgSNQ2                                 | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| YOR1                                   | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| YML1                                   | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| YNL134c                                | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| RTA1                                   | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| RPN4                                   | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| ERG3                                   | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| ERG4                                   | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| ERG11                                  | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 55 |
| EPA1                                   | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 50 |
| Sequencing CgPDRI                      |              |                    |
| CgPDRIseq                              | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 3,393 |
| CgPDRIseqA1                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| CgPDRIseqA2                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| CgPDRIseqA3                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| CgPDRIseqA4                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| CgPDRIseqA5                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| CgPDRIseqB1                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| CgPDRIseqB2                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 51 |
| CgPDRIseqB3                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| CgPDRIseqB4                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |
| CgPDRIseqB5                            | F, 5'-GGTAAAGTCATTCTTTAGCT-3', R, 5'-GGTAAAGTCATTCTTTAGCT-3' | 52 |

\(^a\) F, forward; R, reverse. Restriction sites introduced into primers are underlined.
T2837C and G822T are activating mutations in CgPDR1, and each confers increased resistance to fluconazole. Single point mutations in CgPDR1 in C. glabrata have previously been shown to play a role in azole resistance (9, 35). Therefore, to test whether the L946S and K274N substitutions in CgPdr1 are responsible for the increase in the fluconazole MICs for isolates SM3 and 6955, respectively, we expressed the mutated CgPDR1 from SM3 and 6955 in the isolate SM1 background. For this purpose, we deleted CgPDR1 from isolate SM1 to generate strain SM1Δpdr1 (26). The mutated CgPDR1 alleles, PDR1T2837C and PDR1G822T, were then introduced individually into the CgPDR1 locus in strain SM1Δpdr1 to generate strains SM1Δpdr1/PDR1SM3 and SM1Δpdr1/PDR16955. We also created corresponding control strains by introducing CgPDR1 alleles from the susceptible isolates SM1 and 6856 to generate strains SM1Δpdr1/PDR1 to generate strains SM1Δpdr1/PDR1SM3 and SM1Δpdr1/PDR16955. Replacement of the different CgPDR1 mutations was verified by sequencing the cloned CgPDR1 ORF PCR product as detailed in the Materials and Methods section.

Introduction of the two CgPDR1 mutations into the susceptible background (SM1Δpdr1) resulted in fluconazole MICs equivalent to those of the strains from which each CgPDR1 was originally identified (Table 3). Specifically, introduction of PDR1T2837C resulted in a fluconazole MIC of 64 µg/ml, and introduction of PDR1G822T resulted in a fluconazole MIC of 16 µg/ml, whereas the fluconazole MICs for SM1Δpdr1/PDR1SM3 and SM1Δpdr1/PDR16955 were measured as 2 µg/ml and 1 µg/ml, respectively (Table 3). As introduction of the CgPDR1 mutated alleles in the SM1 isolate background resulted in increased fluconazole MICs compared to introduction of the alleles from isolates SM1 and 6856 in the same background, these CgPDR1 activating mutations are responsible for conferring decreased susceptibility to fluconazole in these C. glabrata isolates.

**Genes differentially expressed in matched sets of clinical isolates.** In order to determine which genes are differentially expressed in the presence of these activating mutations, we first compared the gene expression profiles of the resistant isolates to those of their respective susceptible matched isolates by microarray analysis. There were 539 upregulated genes (see Table S1A in the supplemental material) and 552 downregulated genes (see Table S2A) in isolates SM3 compared to isolate SM1 and 468 upregulated genes (see Table S3A) and 509 downregulated genes (see Table S4A) in isolate 6955 compared to isolate 6856. There were 28 genes commonly upregulated and 22 downregulated in both clinical sets (Tables 4 and 5).

**Identification of putative CgPDR1 target genes.** In order to identify putative CgPDR1 target genes whose expression is affected by these activating mutations, we compared gene expression profiles of SM1Δpdr1/PDR1SM3 and SM1Δpdr1/PDR16955 to those of SM1Δpdr1/PDR1SM1 and SM1Δpdr1/PDR16856, respectively. There were 133 genes upregulated in the strain expressing the SM3 allele, PDR1T2837C (see Table 1SB in the supplemental material), and of these genes 38 were also upregulated in isolate SM3 compared to isolate SM1 (Table 6). Thirty-two genes were upregulated in the reconstituted strain expressing the 6955 allele PDR1G822T (Table S3B), and of these genes only seven were also upregulated in isolate 6955 compared to isolate 6856 (Table 7). Likewise, 676 genes were downregulated in the strain expressing PDR1T2837C (Table S2B), and of these genes 51 were also downregulated in isolate SM3 compared to isolate SM1. Sixty-five genes were downregulated in the strain expressing PDR1G822T (Table S4B), and of these, nine were also downregulated in isolate 6955 compared to isolate 6856.}

**TABLE 3. Fluconazole MICs for isolates/strains used in this study.**

| Isolate or strain | MIC (µg/ml) |
|-------------------|-------------|
| SM1               | 2           |
| SM1Δpdr1          | 0.5         |
| SM3               | 64          |
| SM1Δpdr1/PDR1SM3  | 2           |
| SM1Δpdr1/PDR1SM5  | 64          |
| 6856              | 1           |
| 6955              | 16          |
| SM1Δpdr1/PDR16856 | 1           |
| SM1Δpdr1/PDR16955 | 16          |

**FIG. 1.** Quantitative real-time RT-PCR analysis of CgPDR1, CgCDR1, CgPDR1, and CgSNQ2 in isolate SM3 versus (vs) SM1 and in isolate 6955 versus 6856. Data are shown as means ± standard error. Relative changes were compared using a Student’s t test. Gene expression values marked with an asterisk are statistically significant (P < 0.05) in all three experiments.

**FIG. 2.** Location of gain-of-function mutations found in previous studies (9, 32, 33, 36) and in the present study (in bold). The putative DNA-binding domain (DBD) and middle homology region (MHR) were previously found by Pfam analysis (9). The putative transcriptional activation domain (AD) and inhibitory domain (ID) were previously inferred by similarity with Pdr1 and Pdr3 from S. cerevisiae (36). The region with diagonal lines corresponds to the xenobiotics binding domain (XBD) (30).
TABLE 4. Genes upregulated by at least 1.5-fold in both clinical isolate sets

| Function                        | S. cerevisiae homolog                   | C. glabrata designation | Description                   | Fold change in expression in: |
|---------------------------------|----------------------------------------|-------------------------|-------------------------------|-------------------------------|
|                                 |                                        |                         |                               | SM3 vs SM1 | 6955 vs 6856 |
| Transport                       | PDR5 (CgCDR1)                          | CAGL0J01760g            | Plasma membrane ABC transporter | 12.7      | 2.0          |
|                                 | YOR1b                                  | CAGL0G00242g            | Plasma membrane ABC transporter | 10.5      | 1.8          |
|                                 | HXT2                                   | CAGL0J00286g            | High-affinity glucose transporter of the major facilitator superfamily | 3.9       | 1.9          |
|                                 | IMH1                                   | CAGL0E03454g            | Protein involved in vesicular transport | 2.0       | 2.4          |
|                                 | ALR1                                   | CAGL0E01617g            | Plasma membrane Mg2⁺ transporter | 1.8       | 2.5          |
| Response to stress              | YIL077c                                | CAGL0J012947g           | Putative protein of unknown function | 17.7      | 2.5          |
|                                 | YIM1d                                  | CAGL0J09713g            | Protein of unknown function | 12.8      | 2.5          |
|                                 | RTA1d                                  | CAGL0K00715g            | Protein involved in 7-aminocolesterol resistance | 4.8       | 2.2          |
| Lipid metabolism process       | LCBSd                                  | CAGL0J05995g            | Minor sphingoid long-chain base kinase | 2.2       | 2.0          |
| Carbohydrate metabolism        | GLKI                                   | CAGL0F00605g            | Glucokinase                    | 4.2       | 3.0          |
|                                 | CDC25                                  | CAGL0E03470g            | Membrane bound guanine nucleotide exchange factor | 2.5       | 1.8          |
| Cell cycle                      | RSE1                                   | CAGL0L01507g            | Protein involved in pre-mRNA splicing | 1.9       | 1.7          |
|                                 | RMD54                                  | CAGL0M01958g            | DNA-dependent ATPase           | 1.7       | 1.6          |
| Cellular amino acid and         | FMO1                                   | CAGL0J07612g            | Polyamine oxidase              | 2.7       | 2.6          |
| derivative metabolism           |                                        |                         |                               |               |
| Cytoskeleton organization       | FLO10 (CgEPA1)                         | CAGL0J06644g            | Lectin-like protein, thought to be involved in flocculation | 3.9       | 1.9          |
|                                 | FLO5                                   | CAGL0E06600g            | Lectin-like cell wall protein (flocculin) involved in flocculation | 1.9       | 2.6          |
|                                 | APE2                                   | CAGL0E06226g            | Aminopeptidase yscII           | 1.0       | 1.5          |
| DNA metabolism                  | REV1                                   | CAGL0J05588g            | Deoxyectyldyl transferase involved in DNA repair | 2.3       | 2.2          |
| Protein catabolism              | ECM29                                  | CAGL0J03828g            | Major component of the proteasome | 2.5       | 2.4          |
|                                 | SAN1                                   | CAGL0J01441g            | Ubiquitin-protein ligase       | 1.7       | 2.0          |
|                                 | MNT3                                   | CAGL0C03916g            | Alpha-1,3-mannosyltransferase | 2.0       | 3.0          |
| Transcription                   | AZF1                                   | CAGL0L03916g            | Zinc-finger transcription factor | 2.0       | 2.2          |
| Vitamin metabolism              | ROG1                                   | CAGL0E04950g            | Protein with putative serine active lipase domain | 2.1       | 8.8          |
| Other                           | HFD1d                                  | CAGL0K03509g            | Putative fatty aldehyde dehydrogenase | 4.4       | 2.3          |
|                                 | FLO9                                   | CAGL0L03332g            | Lectin-like protein thought to be expressed and involved in flocculation | 3.9       | 15.9         |
|                                 | YCR061w                                | CAGL0K09218g            | Protein of unknown function | 2.5       | 1.6          |

a Parentheses indicate a previously named C. glabrata gene.
b Descriptions of S. cerevisiae homologs from the Saccharomyces Genome Database (http://www.yeastgenome.org).
c Fold change defined as the average ratio of gene expression levels in the isolates compared in two independent microarray experiments.
d Genes also upregulated in the laboratory-derived fluconazole resistant isolate F15 (35).
for this purpose as 1,000 bp immediately upstream from the start codon of the ORF) of each gene listed in Tables 6 and 7 for a match to the consensus S. cerevisiae PDRE (TCCRYGSR). Twenty-two of the 38 genes in Table 6 and 4 of the 7 genes in Table 7 contained at least one consensus sequence, suggesting that they may be direct targets of CgPdr1.

### DISCUSSION

*C. glabrata* Pdr1 is the single-gene homolog of two *S. cerevisiae* zinc cluster transcription factors ScPdr1 and ScPdr3, which regulate the expression of genes involved in multidrug resistance. ScPdr1 and ScPdr3 act by binding to PDREs in the promoters of target genes (14). Furthermore, it has been found that ScPdr1 is constitutively bound to its target DNA (8). In *S. cerevisiae*, activating mutations in Pdr1 and Pdr3 can result in decreased susceptibility to fluconazole by upregulation of multidrug efflux pumps (14). In *C. glabrata*, CgPdr1 has been shown to regulate to various degrees the expression of the ABC transporter genes *CgCDR1*, *CgPDH1*, and *CgSNQ2* and has been shown to be responsible for azole resistance in this organism (21, 26, 28, 33, 36). Moreover, a recent study investigated the incidence of *CgPDR1* mutations in a large collection of fluconazole-resistant clinical isolates and identified 57 distinct single amino acid substitutions. However, it was concluded that although *CgPDR1* regulates *CgCDR1*, *CgPDH1*, and *CgSNQ2*, these genes are not always coordinately expressed in fluconazole-resistant clinical isolates, suggesting that these genes are differentially regulated depending on the mutation (9). Of note, this study was the first to address the fitness cost of these mutations. Using engineered isogenic strains with unique *CgPDR1* mutations, this study also found that the amino acid substitutions in *CgPDR1* mutations from fluconazole-resistant isolates were identified that lead to constitutive upregulation of ABC transporter genes as well as decreased susceptibility to fluconazole (9, 32, 33, 36). Three of these mutations were found in the putative inhibitory domain, four were found in the xenobiotic binding domain, and five were found in the putative activation domain of CgPdr1. Two novel activating mutations resulting in amino acid substitutions in CgPdr1 were identified in the present study, one near the putative inhibitory domain (K274N) and one in the putative activation domain (L946S). These mutated *CgPDR1* alleles not only cause upregulation of *CgCDR1* when expressed in a susceptible wild-type isolate but also result in the same fluconazole MIC as the original isolate from which the mutated allele was identified. Interestingly, when examined collectively, activating mutations identified to...

### TABLE 5. Genes downregulated by at least 1.5-fold in both clinical isolate sets

| Function                          | *S. cerevisiae* homolog | *C. glabrata* designation | Description* | Fold change in expression in%^: |
|-----------------------------------|-------------------------|---------------------------|--------------|-------------------------------|
| Transport                         | BCP1                    | CAGL0A02673g              | Essential protein involved in nuclear export of Mss4p | 0.65 0.42 |
| Cell cycle                        | SPO12                   | CAGL0I01914g              | Nucleolar protein of unknown function | 0.41 0.36 |
| Protein catabolism                | NIN1                    | CAGL0A04807g              | Subunit of the 19S regulatory particle of the 26S proteasome lid | 0.46 0.44 |
| Translation                       | RPS13B                  | CAGL0H05511g              | Protein component of the small (40S) ribosomal subunit | 0.19 0.45 |
|                                  | PTH2                    | CAGL0A04323g              | Mitochondrially localized peptidyl-tRNA hydrolases | 0.48 0.46 |
|                                  | YMR831                  | CAGL0A04697g              | Mitochondrial ribosomal protein of the small subunit | 0.57 0.50 |
| Vitamin metabolism               | TAL1                    | CAGL0K04235g              | Transaldolase | 0.34 0.31 |
| Unknown                           | NA^a                    | CAGL0I00550g              | Putative protein of unknown function | 0.14 0.60 |
|                                  | YBR242w                 | CAGL0C01749g              | Putative protein of unknown function | 0.41 0.44 |
|                                  | NA                      | CAGL0I07777g              | Putative protein of unknown function | 0.42 0.50 |
|                                  | SNG1^c                  | CAGL0G09273g              | Putative protein of unknown function | 0.42 0.29 |
|                                  | YLR326w                 | CAGL0A01801g              | Putative protein of unknown function | 0.43 0.22 |
|                                  | NA                      | CAGL0K00341g              | Putative protein of unknown function | 0.45 0.34 |
|                                  | YOR186w                 | CAGL0G09603g              | Putative protein of unknown function | 0.54 0.46 |
|                                  | NA                      | CAGL0A02882g              | Putative protein of unknown function | 0.54 0.52 |
|                                  | NA                      | CAGL0A03688g              | Putative protein of unknown function | 0.58 0.51 |
|                                  | SUR7                    | CAGL0L01551g              | Putative integral membrane protein | 0.59 0.46 |
|                                  | YSC83                   | CAGL0A02134g              | Nonessential mitochondrial protein of unknown function | 0.59 0.51 |
|                                  | NIT3                    | CAGL0G01210g              | Nit protein | 0.65 0.59 |

* Descriptions of *S. cerevisiae* homologs from the Saccharomyces Genome Database (http://www.yeastgenome.org).

^b^ Fold change defined as the average ratio of gene expression in the isolates compared in two independent microarray experiments.

^c^ Genes also downregulated in the laboratory-derived fluconazole-resistant isolate F15 (35).

^d^ NA, not applicable.
date demonstrate various patterns of ABC transporter expression. For example, in one of the previous studies, one activating mutation led to the overexpression of CgSND2, while different amino acid substitutions in CgPdr1 result in the activation of different CgPDR1 target genes. Like Pdr1 in S. cerevisiae, CgPdr1 may bind constitutively to the promoters of a repertoire of genes and activate a subset of them, depending on the mechanism by which Pdr1 is activated (8).

**TABLE 6. Genes upregulated in SM1Δpdr1/PDR1<sup>SSM</sup> compared to SM1Δpdr1/PDR1<sup>SSM</sup> and SM3 compared to SM1**

| GO category | Systematic name | S. cerevisiae homolog | C. glabrata designation | Description<sup>c</sup> | PDRE position(s)<sup>d</sup> | Fold increase in expression<sup>f</sup> |
|-------------|-----------------|----------------------|-------------------------|--------------------------|---------------------------|-----------------------------------|
| Transport   | IPF1620         | QDR2                 | CAGL0G08624g            | MFS transporter          | 848                       | 3.5                               |
|             | IPF5884         | SNQ2 (CgSNQ2)        | CAGL0B04862g            | ABC transporter          | 218, 792                  | 3.7                               |
|             | IPF6352         | PDR5 (CgCDR1)        | CAGL0M01760g            | ABC transporter          | 134, 227, 387, 515, 669  | 10.2                              |
|             | IPF8922         | YOR1                 | CAGL0G001462g           | ABC transporter          | 648                       | 10.5                             |
|             | IPF2968         | POR1                 | CAGL0F00900g            | Mitochondrial porin     | 1.6                       | 2.6                               |
|             | IPF9171         | PDR15 (CgPDH1)       | CAGL0F02717g            | ABC transporter          | 521, 557                  | 10.5                             |
|             | IPF8982         | YOR1                 | CAGL0G013289g           | ABC transporter          | 450                       | 8.1                              |
| Transcription | IPF3325       | PDR1                | CAGL0A00451g            | Transcription factor     | 557, 707                  | 5.2                               |
|             | IPF4550         | UTH1                 | CAGL0L05434g            | Mitochondrial outer membrane | 294, 297  | 2.9                               |
|             | IPF5076         | RN4                  | CAGL0K01727g            | Transcription factor     | 378, 394, 552            | 3.1                               |
| Response to stress | IPF119       | DDR4                 | CAGL0H08844g            | DNA damage-responsive protein | 382, 382  | 5.1                               |
|             | IPF1380         | RHR2                 | CAGL0J05874g            | Di-Glycerol-3-phosphatase | 849                       | 2.0                               |
|             | IPF6087         | HSP12                | CAGL0J04202g            | Plasma membrane localized | 849                       | 4.1                               |
|             | IPF9036         | YMI1                 | CAGL0M09713g            | Protein of unknown function | 127, 179                  | 11.5                             |
|             | IPF9549         | FLO1                 | CAGL0E00209g            | Lectin-like protein       | 292, 427                  | 10.0                             |
|             | IPF4884         | ATG2                 | CAGL0D05918g            | Alcohol acetyltransferase | 30, 195, 560, 722         | 7.2                               |
| Lipid metabolic process | IPF5152       | RTA1                 | CAGL0K00175g            | Involved in 7-aminocolesterol resistance | 300, 379                 | 5.0                               |
|             | IPF6116         | YIL077c              | CAGL0M12947g            | Putative protein of unknown function | 472, 502                 | 23.5                             |
|             | IPF7689         | AGA1                 | CAGL0G10120g            | C-Subunit of a-agglutinin of α-cells | 472, 502                 | 4.7                               |
| Lipid metabolic process | IPF1834       | ERG6                 | CAGL0H04653g            | Δ(24)-Sterol C-methyltransferase | 515, 969                 | 1.6                               |
|             | IPF2503         | ERG11                | CAGL0E04334g            | Lanosterol 14-alpha-demethylase | 364, 364                 | 1.9                               |
|             | IPF2933         | PLB1                 | CAGL0J11770g            | Phospholipase B           | 754                       | 2.3                               |
|             | IPF3323         | ERG4                 | CAGL0A00429g            | C(24)-28 sterol reductase | 292, 427                  | 2.0                               |
|             | IPF5753         | ERG3                 | CAGL0J01795g            | C-5 sterol desaturase     | 30, 195, 560, 722         | 3.6                               |
|             | IPF7335         | ERG24                | CAGL0F02707g            | C-14 sterol reductase     | 226                       | 1.9                               |
|             | IPF8367         | LAC1                 | CAGL0M10219g            | Ceramide synthase component | 531                       | 2.3                               |
| Organelle organization | IPF4656       | HEM13                | CAGL0K12100g            | Coproporphyrinogen III oxidase | 291, 291                 | 2.9                               |
|             | IPF6034         | ADH6                 | CAGL0M14047g            | NADPH-dependent alcohol dehydrogenase | 300, 300                 | 3.0                               |
| Other       | IPF3204         | SED1                 | CAGL0I0164g             | Glycosylphosphatidylinositol wall glycoprotein | 1046                     | 2.6                               |
|             | IPF8044         | FRE8                 | CAGL0M07942g            | Similarity to iron/copper reductases | 291, 291                 | 2.0                               |
| Biological process unknown | IPF2180       | HDF1                 | CAGL0K03509g            | Putative fatty aldehyde dehydrogenase | 218                       | 4.4                               |
|             | IPF3173         | YNL134c              | CAGL0J09702g            | Putative protein of unknown function | 549                       | 13.0                              |
|             | IPF3854         | SGA1                 | CAGL0J02717g            | Uncharacterized           | 251                       | 2.6                               |
|             | IPF3721         | GA5                  | CAGL0F01287g            | 1,3-Beta-glucosyltransferase | 803, 793                 | 3.9                               |
|             | IPF3875         | NA                   | CAGL0J01122g            | Uncharacterized           | 450                       | 9.1                               |
|             | IPF8009         | YIL163c              | CAGL0M08426g            | Putative protein of unknown function | 450                       | 2.2                               |
|             | IPF8259         | PRY1                 | CAGL0G07667g            | Protein of unknown function | 222                       | 2.2                               |
|             | IPF8571         | MET2                 | CAGL0M06402g            | Methionine and cysteine synthase | 49.1                     | 1.7                               |

<sup>a</sup> Gene Ontology categories of *S. cerevisiae* homologs (Saccharomyces Genome Database [http://www.yeastgenome.org]).

<sup>b</sup> Parentheses indicate a previously named C. glabrata gene.

<sup>c</sup> Description of *S. cerevisiae* homologs (http://www.yeastgenome.org).

<sup>d</sup> Promoter regions (1,000 bp) were searched for matches to the *S. cerevisiae* PDRE consensus TCCRYGSR. Numbers indicate the distance (in bp) upstream of the ATG start codon of the PDRE.

<sup>e</sup> Fold change defined as the average ratio of gene expression levels in the isolates/strains compared in two independent microarray experiments.
fluconazole resistance. Further studies are needed to determine if these genes contribute to regulated in both matched sets of clinical isolates. Of note, may regulate their expression through binding these regulatory elements. Of note, CgCDR1, YOR1, YIM1, CgPDR1, and YNL134c were all upregulated in the presence of both of these two mutations (Table 8; Fig. 3A and B). In *C. glabrata*, YOR1 encodes an ABC transporter that is involved in oligomycin resistance (15), and YIM1 and YNL134c encode proteins of unknown function; however, both genes may play a role in the stress response to DNA-damaging drugs (3, 17). All five of these genes contain at least one PDRE in their promoters, suggesting that CgPdr1 may regulate their expression through binding these regulatory elements. Of note, CgCDR1, YOR1, and YIM1 were also upregulated in both matched sets of clinical isolates. Further studies are needed to determine if these genes contribute to fluconazole resistance.

As CgPdr1 is an ortholog of the ScPdr1 and ScPdr3 paralogs, it is important to compare the ScPdr1 and ScPdr3 gene targets to the genes found in the present study to be controlled by CgPdr1. Previously, analysis has been performed to determine the transcriptional targets of Pdr1 and Pdr3 in *S. cerevisiae* (19). Strains expressing an artificially activated Pdr1 (or Pdr3) chimera, consisting of the DNA-binding domain of either ScPdr1 or ScPdr3 fused to the transactivation domain of VP16, were compared to their wild-type parent strains for changes in their transcriptomes. Such comparisons revealed 7 genes that were induced by Pdr1 alone, 35 genes that were induced by Pdr3, and an additional 31 genes that were commonly induced by Pdr1 and Pdr3. Of these 73 *S. cerevisiae* genes induced by ScPdr1 and/or ScPdr3, 10 were orthologs of genes identified as being differentially expressed by CgPdr1 in the present study. These were the ScPdr1 targets ScRPN4 and ScRTA1, the ScPdr3 target gene ScSG1, and a number of genes controlled by both ScPdr1 and ScPdr3 (*ScHSP12, ScLAC1, ScPDR5, ScPDR9*).

### Table 8. Genes upregulated in SM1Δpdr1/PDR1^K955^ and SM1Δpdr1/PDR1^K556^ compared to SM1Δpdr1/PDR1^K555^ and 6955 compared to 6856

| GO category | Systematic name | S. cerevisiae homolog | C. glabrata designation | Description | PDRE position(s) | Fold increase in expression |
|-------------|-----------------|----------------------|-------------------------|-------------|------------------|----------------------------|
| Transport   | IPF6352         | PDR5 (CgCDR1)        | CAGL0M01760g            | ABC transporter          | 969, 515, 387, 227, 134 | 2.1 | 6.8 |
|             | IPF8922         | YOR1                 | CAGL0G00242g            | ABC transporter          | 648             | 1.8 | 2.4 |
|             | IPF9036         | YIM1                 | CAGL0M09713g            | Protein of unknown function | 179, 127 | 2.8 | 2.9 |
| Response to stress | IPF7412        | PRP39                | CAGL0L01441g            | U1 snRNP protein involved in splicing | 179, 127 | 2.8 | 2.9 |
| RNA metabolic process | IPF5973        | FLO10 (CgEPA1)      | CAGL0E06644g            | Lectin-like protein of unknown function | 571 | 1.9 | 3.3 |
|             | IPF6033         | FLO5                 | CAGL0M14069g            | Lectin-like cell wall protein (flocculin) | 179, 127 | 2.8 | 2.9 |
| Other       | IPF5979         | FLO5                 | CAGL0E06600g            | Lectin-like cell wall protein (flocculin) | 571 | 1.9 | 3.3 |

| GO category | Systematic name | S. cerevisiae homolog | C. glabrata designation | Description | PDRE position(s) | Fold increase in expression |
|-------------|-----------------|----------------------|-------------------------|-------------|------------------|----------------------------|
| Transport   | IPF6352         | PDR5 (CgCDR1)        | CAGL0M01760g            | ABC transporter          | 969, 515, 387, 227, 134 | 2.1 | 6.8 |
|             | IPF8922         | YOR1                 | CAGL0G00242g            | ABC transporter          | 648             | 1.8 | 2.4 |
|             | IPF9036         | YIM1                 | CAGL0M09713g            | Protein of unknown function | 179, 127 | 2.8 | 2.9 |
| Response to stress | IPF7412        | PRP39                | CAGL0L01441g            | U1 snRNP protein involved in splicing | 179, 127 | 2.8 | 2.9 |
| RNA metabolic process | IPF5973        | FLO10 (CgEPA1)      | CAGL0E06644g            | Lectin-like protein of unknown function | 571 | 1.9 | 3.3 |
|             | IPF6033         | FLO5                 | CAGL0M14069g            | Lectin-like cell wall protein (flocculin) | 179, 127 | 2.8 | 2.9 |
| Other       | IPF5979         | FLO5                 | CAGL0E06600g            | Lectin-like cell wall protein (flocculin) | 571 | 1.9 | 3.3 |

* Gene Ontology of *S. cerevisiae* homologs (*Saccharomyces* Genome Database [http://www.yeastgenome.org]).

* Parentheses indicate a previously named C. glabrata gene.

* Descriptions of *S. cerevisiae* homologs ([http://www.yeastgenome.org](http://www.yeastgenome.org)).

* Promoter regions (1,000 bp) were searched for matches to the *S. cerevisiae* PDRE consensus TCCRYGSR. Numbers indicate the distance (in bp) upstream of the ATG start codon of the PDRE.

* Fold change defined as the average ratio of gene expression levels in the compared isolates/strains in two independent microarray experiments.
ScPDR15, ScSNQ2, ScYOR1, and ScYC061W). It should be noted that while ScSNQ1 expression was induced in a ScPdr3-dependent manner, CgSNQ1 was actually found to be downregulated by an activated CgPdr1. Interestingly, of the small number of genes held in common between the CgPdr1 and ScPdr1/ScPdr3 transcriptional targets, only PDR5, PDR15, and SNQ2 are genes which encode proteins known to mediate fluconazole resistance.

Previously, we examined the gene expression profile of a set of fluconazole-susceptible and laboratory-derived fluconazole-resistant C. glabrata strains (35). In that analysis, we identified genes upregulated and downregulated in the laboratory-derived fluconazole-resistant strain F15, which carries a CgPdr1 allele with a P927L amino acid substitution. This mutation is located in the same putative activation domain as the L946S allele with a P927L amino acid substitution. This mutation is derived from a C. glabrata clinical resistant isolate. A previous study showed an increase in ERG11 mRNA in a fluconazole-resistant isolate compared to its susceptible parent that was due to duplication of the entire chromosome containing the ERG11 gene; however, loss of the duplicated chromosome was seen when the isolate was grown in fluconazole-free medium (19). Further study will be needed to determine if these ergosterol biosynthesis genes are directly or indirectly regulated by this hyperactive CgPDR1 allele and if this modest increase in ERG11 contributes to azole resistance in this isolate.

Other genes found to be unique to this activating mutation included the S. cerevisiae homolog of the transcription factor RPN4 which also contains two PDREs in its promoter. This was confirmed by quantitative real-time PCR (Fig. 3B). In S. cerevisiae, RPN4 is a transcription factor whose expression is dependent on ScPdr1 and ScPdr3 and regulates expression of proteosome genes. RPN4 is also required for normal cell tolerance to various cytotoxic compounds including cycloheximide, a known ABC transporter substrate (23). Together, these findings suggest that the proteosome may be influenced by CgPdr1 via RPN4 expression; however, its role in azole resistance has not been elucidated. Lastly, other genes found to be unique to this activating mutation included the homologs of the S. cerevisiae genes that encode proteins involved in stress response such as DDR48, HSP12, YIM1, FLO1, ATF2, and RTA1. As with YIM1, RTA1 is also upregulated in both matched pairs of clinical isolates. In S. cerevisiae, RTA1, which is also known to be regulated by ScPdr1 and ScPdr3, encodes a protein member of the fungal lipid-translocating exporter family and is involved in 7-aminocaproic resistance; however, the role of RTA1 in azole resistance has not been studied from Erg11p in this biosynthetic pathway encoded by the genes ERG6, ERG3, and ERG4. While quantitative real-time PCR confirmed that levels of expression for these genes were within the range observed by microarray analysis, these changes were modest and were not statistically significant (data not shown).

In C. albicans and S. cerevisiae, the overexpression of ERG11, which encodes lanosterol demethylase, the target of azole antifungals, can contribute to fluconazole resistance (7, 16). Overexpression of ERG11 in S. cerevisiae and C. albicans has been attributed to activating mutations in the transcription factor gene UPC2 (7, 11, 37). No studies have conclusively demonstrated constitutive overexpression of ERG11 in a C. glabrata clinical resistant isolate. A previous study showed an increase in ERG11 mRNA in a fluconazole-resistant isolate compared to its susceptible parent that was due to duplication of the entire chromosome containing the ERG11 gene; however, loss of the duplicated chromosome was seen when the isolate was grown in fluconazole-free medium (19). Further study will be needed to determine if these ergosterol biosynthesis genes are directly or indirectly regulated by this hyperactive CgPDR1 allele and if this modest increase in ERG11 contributes to azole resistance in this isolate.

Table 6 summarizes both genes found to be upregulated due to the L946S amino acid substitution and genes found to be upregulated in the resistant isolate SM3 compared to the susceptible isolate SM1. Interestingly, there were several ABC transporters coregulated with CgPDR1 by CgPdr1, including CgSNQ2, CgPDH1, YOR1, and YBT1. Also upregulated was the major facilitator superfamily (MFS) transporter, QDR2. All of these transporters contain at least one PDRE in their promoter region. The role of CgCdr1, CgPdh1, and CgSnq2 in azole resistance is well established in C. glabrata (12, 32, 35). The role of YBT1 and QDR2 in fluconazole resistance has yet to be studied in C. glabrata.

Surprisingly, several genes encoding proteins involved in the ergosterol biosynthetic pathway were also found to be CgPdr1 dependent unique to the CgPDR1 activating mutation in isolate SM3, including ERG11 and four enzymes downstream
in *S. cerevisiae* or *C. glabrata*. In *C. albicans*, overexpression of *RTA2*, which encodes an *S. cerevisiae* homolog of *RTA1*, in a *C. albicans* strain with deletions of *CDR1, CDR2*, and *MDR1* conferred increased resistance to azoles, suggesting a role for this protein family in azole resistance (13).

Recently, it was shown that *C. glabrata* strains expressing hyperactive *CgPDR1* alleles were more virulent in mice than the *C. glabrata* strains expressing wild-type alleles (9). Our study has also allowed for the identification of *CgPdr1* targets that may play a role in virulence. Of note, *PLB1*, the homolog of the *S. cerevisiae* gene that encodes phospholipase B, was upregulated in azole-resistant isolate SM3 and also found to be specific to the SM3 *CgPDR1* mutation when expressed in SM1. Moreover, *PLB1* contains a PDRE in its promoter. In *C. albicans*, phospholipase B activity is involved in the spread of the organism from the gastrointestinal tract as well as in hematogenous dissemination (6, 22). To date, no published studies have analyzed the role of *PLB1* in *C. glabrata*; however, one study did find an association between phospholipase B activity and persistent *C. glabrata* candidemia (3a).

Consistent with the idea that adhesion of *C. glabrata* to host epithelial tissue plays an important role in virulence, the genes *FLO1, FLO5*, and *FLO10* were found to be *CgPdr1*-dependent. The *C. glabrata* *FLO* genes are homologs of *S. cerevisiae* genes encoding proteins involved in cell adhesion (10). Only *FLO1* and *FLO10* contain PDREs in their promoters. In *S. cerevisiae*, *FLO1, FLO5*, and *FLO10* are all involved in cell-cell adhesion (floculation). Although *FLO10* in *S. cerevisiae* is involved in floculation, in *C. glabrata* this gene (named *CgEPA1*) is required for efficient in vitro adherence to human epithelial cells proving a role for *CgEPA1* in adherence to abiotic materials (5). *FLO5* has been reported as a member of the EPA family of adhesion proteins in *C. glabrata*; however, its role in adherence has not been directly studied. Interestingly, among genes found to be upregulated uniquely by the 6955 *CgPDR1* hyperactive allele was *CgEPA1*. This was verified by real-time RT-PCR (Fig. 3B). Adhesion proteins in *C. albicans* have been shown to confer distinct adhesion profiles toward human proteins and cells (29). This may explain the difference in adhesion gene expression levels in our study as these isolates were isolated from different human tissues. SM3 was isolated from the blood, and 6955 was isolated from the oral mucosa.

While the manuscript was under review, Tsai et al. reported the investigation by microarray analysis of seven paired azole-resistant and -susceptible *C. glabrata* oropharyngeal isolates from hematopoietic stem cell transplant recipients (34). All seven resistant isolates carried nonsynonymous mutations in *CgPDR1* occurring in one of three domains, five of which were verified to be activating mutations directly influencing azole resistance. While different isolate pairs with mutations in the same domain exhibited different transcriptional profiles, the authors identified 19 genes that were upregulated in the majority of resistant isolates. Interestingly, 11 of these genes were also among those found in our study to be upregulated by the SM3 *CgPDR1* hyperactive allele (*CgCDR1, CgPDH1, CgSNQ2, YOR1, YBT1, ATF2, HDF1, RTA1, ERG4, RPN4, and CgPDR1*), whereas only two were found to be upregulated by the 6955 *CgPDR1* hyperactive allele (*CgCDR1 and YOR1*). While the commonly upregulated genes found among these seven isolates and those regulated by the SM3 *CgPDR1* hyperactive allele suggest that these are, indeed, Pdr1 target genes, the differences observed in those regulated by the 6955 *CgPDR1* hyperactive allele suggest that this mutation results in a very different effect on Pdr1 function. Tsai et al. went on to show that five of the activating *CgPDR1* mutations when expressed in a common background strain differentially regulated the expression of *CgPDR1, CgCDR1, CgPDH1, CgSNQ2*, and *YOR1*. This is consistent with the variable expression levels of these genes that we observed in strains carrying the SM3 and 6955 *CgPDR1* hyperactive alleles as well as with similar variable expression levels among activating alleles originally observed by Ferrari et al. (9).

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