Phylogenetic and Transcripts Profiling of Glucose Sensing Related Genes in *Candida glabrata*

Tzu Shan Ng,[1] Mohd Nasir Mohd Desa,[2] Doblin Sandai,[3] Pei Pei Chong,[2] and Leslie Thian Lung Than[1,*]

[1]Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Penang, Malaysia

Corresponding author: Leslie Thian Lung Than, Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel: +60-389472373, Fax: +60-389413802, E-mail: leslie@upm.edu.my

Abstract

Background: The sensing mechanism of glucose in *Saccharomyces cerevisiae* is well studied. However, such information is scarcely found in other yeast species such as *Candida glabrata*.

Objectives: This study aimed to identify the glucose sensing pathway related genes of *C. glabrata* and to analyze the regulation pattern of these genes in response to different surrounding glucose concentrations through the quantitative real time polymerase chain reaction (qRT-PCR).

Materials and Methods: Phylogenetic analysis was carried out on predicted amino acid sequences of *C. glabrata* and *S. cerevisiae* to compare their degree of similarity. In addition, the growth of *C. glabrata* in response to different amounts of glucose (0%, 0.01%, 0.1%, 1% and 2%) was evaluated via the spot dilution assay on prepared agar medium. Besides, the *SNF3* and *RGT2*, which act as putative glucose sensors, and the *RGT1* and *MIG1*, which act as putative transcriptional regulators and selected downstream hexose transporters (*HXTs*), were analysed through qRT-PCR analysis for the gene expression level under different glucose concentrations.

Results: Comparative analysis of predicted amino acids in the phylogenetic tree showed high similarity between *C. glabrata* and S cerevisiae. Besides, *C. glabrata* demonstrated the capability to grow in glucose levels as low as 0.01% in the spot dilution assay. In qRT-PCR analysis, differential expressions were observed in selected genes when *C. glabrata* was subjected to different glucose concentrations.

Conclusions: The constructed phylogenetic tree suggests the close evolutionary relationship between *C. glabrata* and *S. cerevisiae*. The capability of *C. glabrata* to grow in extremely low glucose environments and the differential expression of selected glucose-sensing related genes suggested the possible role of these genes in modulating the growth of *C. glabrata* in response to different glucose concentrations. This study helps deepen our understanding of the glucose sensing mechanism in *C. glabrata* and serves to provide fundamental data that may assist in unveiling this mechanism as a potential drug target.

Keywords: Glucose Transport Proteins, Facilitative, Phylogeny, *Candida glabrata*

1. Background

The incidence of invasive yeast infection caused by non-*Candida albicans Candida* (NCAC) species including *Candida glabrata* has increased considerably (1) in the past two decades. The emergence of *C. glabrata* as an important threat has been reported in an epidemiological study conducted at a tertiary care hospital, whereby the prevalence of *C. glabrata* as a causative agent for healthcare-associated candidemia has increased drastically from 0% to 16% from 1983 to 2007 (2). The candidiasis disease ranges from superficial to life-threatening disseminated types such as deep-seated candidiasis (3, 4). The wide spectrum of disease found in various anatomical regions of the human body suggests that *C. glabrata* has flexibility in sensing nutrients such as glucose and thus can adapt to unique environmental niches of the host.

Glucose sensing and transportation are highly regulated processes in most organisms, as glucose is an important source of carbon and energy. Previous studies have shown that *Saccharomyces cerevisiae*, a model organism of eukaryotes, senses the uptake of glucose through the Sugar Receptor-Repressor pathway (SRR). Basically, SRR pathway uses two glucose sensors in the cell membrane, namely Snf3 and Rgt2 that govern and regulate the expression of genes encoding for hexose transporter (HXT) with the aids of transcription regulators: RGT1 and MIG1 (5-7). In a similar fashion, another important human pathogen, *C. albicans*, also employs the same glucose sensing pathways, yet instead of two glucose sensors in the SRR pathway, only one has been shown to be present in this species, namely the Hgt4 (8, 9). However, little is known about the function

of these orthologous genes found in *C. glabrata* in response to glucose. *Candida glabrata*, which was previously known as *Cryptococcus glabratus* and *Torulopsis glabrata* (10) has displayed a closer evolutionarily relationship with *S. cerevisiae* (11), in comparison to other *Candida* species. Even though these two yeasts have shared common ancestry yet there are differences between them as *C. glabrata* has an exclusively haploid life cycle while *S. cerevisiae* undergoes active sexual reproduction and has interchangeable haploid/diploid life cycle (12).

The availability of fully sequenced *C. glabrata* genome (13) has aided with the determination of the physiology of this organism through the phylogenomics approach (14). The availability of this data is advantageous for the investigation of the evolutionarily lineage between *C. glabrata* and *S. cerevisiae*. According to Yin et al. (15) and Rodaki et al. (16), *S. cerevisiae* and *C. albicans* have a high sensitivity response to glucose levels as low as 0.01%, suggesting the presence of a strictly regulated glucose sensing system in the corresponding yeasts. Owing to the importance of these processes, it would be interesting to decipher the key genes in this mechanism, which could serve as potential antifungal or antimicrobial targets (17).

2. Objectives

The present study aimed to identify glucose sensors, key regulators and hexose transporters of *C. glabrata* and also to predict their phylogenetic relationships with their counterparts found in *S. cerevisiae*. In addition, the growth of *C. glabrata* and expression pattern of selected glucose-sensing related genes in response to different concentrations of glucose was assessed.

3. Materials and Methods

3.1. Retrieval of Nucleotides and Protein Sequences

The nucleotide and protein sequences of *C. glabrata* (CAGL) and *S. cerevisiae* were retrieved from the *Candida* genome database (CDG) and yeast genome database (YGD), respectively. The accession number of the proteins of *C. glabrata* and *S. cerevisiae* are listed in Tables 1 and 2.

3.2. Phylogenetic Analysis

The multiple sequence alignment of selected amino acid sequences listed in Tables 1 and 2 was calculated and generated through Clustal Omega program (18-20). A phylogenetic tree was constructed using MEGA version 5 (21) with the maximum likelihood method based on the Poisson correction model (22). Bootstrap tests (100 replicates) were computed (23) for the confidence limits of the constructed phylogenetic tree.

3.3. Yeast Strain and Media Preparation

The standard laboratory reference wild type, *C. glabrata* strain ATCC2001, was used in this study. Two types of media, namely standard synthetic minimal glucose medium (SD), which contains Yeast Nitrogen Base (YNB) (Becton, Dickinson and Company, USA) plus glucose (Fisher Scientific, USA), and the standard Yeast extract Peptone Dextrose (YPD) (Becton, Dickinson and Company, USA) medium were used. Different glucose concentrations (0%, 0.01%, 0.1% 1%, 2% glucose) of SD was prepared as described by Sherman (24). The YPD medium that consists of 10 g of yeast extract, 20 g of peptone and 20 g of glucose per litre was used as routine growth media.

3.4. Yeast Culture Conditions and Spot Dilution Assay

The glucose response experiment was performed using a modified procedure described for *C. albicans* by Brown et al. (25) and Rodaki et al. (16). Briefly, *C. glabrata* cells were grown overnight in YPD medium at 37°C. The cells were reinoculated in 50 mL of fresh YPD (OD_{600} = 0.1) and regrown at 37°C to mid log phase (OD_{600} = 0.5). Yeast extract Peptone Dextrose was used in this study with a purpose to provide optimum growth for the cells and prevent possible stress responses triggered by inadequate carbon sources. Prior to reinoculation in the desired glucose concentrations, the cells were washed with Phosphate Buffered Saline (PBS), pH 7.4, three times to ensure complete removal of residues of YPD medium. For the spot dilution assay, mid log phase cells were adjusted to 10^6 and a ten-fold serial dilution was performed before growth on SD agar and incubated at 37°C for 24 hours.

3.5. Gene Expression Analysis and RNA Extraction

For the gene expression analysis, mid log phase cells were reinoculated in 15 mL of fresh SD with the mentioned glucose concentrations for two hours at 37°C. Collected cells were washed and RNA extraction was carried out as described by the Yeast Current Protocols in Molecular Biology (26). Verification of RNA integrity and quality was performed by visualization on 1% tris-acetate-EDTA gel and a NanoPhotometer (Implen, Germany). Furthermore, RNA was treated with Turbo DNase (Life Technologies, USA) and reverse transcribed using SuperScript® III First-Strand synthesis system (Life Technologies, USA). In order to generate full length transcripts (27), RNA was reverse transcribed with the mixture of Oligo (dT) 18 and random hexamer (Fermentas, Germany). A reaction mixture without reverse transcriptase (Non-Reverse Transcriptase; NRT) and a reaction mixture without RNA template (non-template control; NTC) were included as controls. Next, RNA isolation and subsequent cDNA synthesis were performed by three biological independent experiments for each glucose concentration tested.

3.6. Quantitative Polymerase Chain Reaction (qPCR)

Gene *ACT1* was used as the normalizing housekeeping gene (28) and all PCR primers listed in Table 3 were designed to amplify target genes based on the gene sequences sourced from the *Candida* genome database (29). The PCR efficiency using each set of primers of the respective genes was tested by two independent experiments by running a series of five-fold dilutions of *C. glabrata* DNA in MiniOpticon™ Real time PCR (Bio-Rad, USA). The acceptable amplification efficiency of the current study was between 90% and 110%. For the expression analysis of the genes, all samples were performed in triplicate. The total volume of each reaction was 20 μL where it contained the cDNA template, primers and 2X SensiFAST™ SYBR No-ROX master mix (Bioline, UK). Melting curve analysis was performed to ensure no non-specific PCR products were generated. For post-experimental expression analysis, normalized expression ratios were calculated based on the mathematical equation developed by Pfaffl (30) as shown below:

$$\textbf{(1)} \quad \text{Normalized expression ratio} = \frac{(E_{\text{target}})^{\Delta \text{Ct target (control − sample)}}}{(E_{\text{reference}})^{\Delta \text{Ct reference (control − sample)}}}$$

Where, E_{target} = the quantitative real-time PCR efficiency of target genes; $E_{\text{reference}}$ = the quantitative real-time PCR efficiency of the reference gene (*ACT1*); ΔCt = the crossing point difference of a particular gene derived from the control (0% or 2% glucose) and the sample (different glucose concentrations).

There were two glucose controls: no glucose control (0%) and high glucose control (2%), which were chosen as baselines when interpreting the results in accordance to the predicted glucose affinity of target genes for better representation of the transcript profiling. The calculated normalized expression ratios were transformed to logarithm based values (Log10) and averaged with standard error means (SEM). Statistical analysis was performed using relative expression software tool (REST©) version 2009 (31) in order to examine significant differences of sample expression ratios in relation to the control.

4. Results

4.1. Sequence Comparison and Phylogenetic Relationship Between C. glabrata and S. cerevisiae Glucose-Sensing Related Proteins

Two main groups of *C. glabrata* glucose-sensing related proteins were identified by bootstrap maximum-likelihood analysis with *S. cerevisiae*: first, transcriptional regulators and second, the hexose transporter -like proteins (Figure 1). The clade of *C. glabrata* transcriptional regulator consisted of CgMig1 and CgRgt1, which were grouped with ScMig1 and ScRgt1 (Figure 1). Besides, the clade of

hexose transporter like-protein group consisted of three sub clades: first, the CgHxt14, second, the *C. glabrata* glucose sensors, and third, the CgHxts (Figure 1). Sub clade *C. glabrata* glucose sensors contains two glucose sensor-like proteins (SNF3 (CAGL0J09020g) and Rgt2 (CAGL0I03872 g)) with a longer cytoplasmic tail. A copy of 25-amino acid motif was found in both CgSnf3 and CgRgt2 (Figure 2) as found in *S. cerevisiae* glucose sensors. On the other hand, there were ten hexose transporters found in *C. glabrata* within the subclade *HXTs* with approximately 530 - 590 amino acids in length. Generally, *C. glabrata* hexose transporters were found to be similar to each other and they could be categorized to three main groups: CgHxt2/10, CgHxt1/3/5 and CgHxt4/6/7 (Figure 1).

4.2. Growth Assay of Candida glabrata in Response to Different Glucose Concentrations

Candida glabrata was able to survive in glucose levels as low as 0.01% (Figure 3). This is far below the human blood glucose level (0.05% - 0.1%) (16) thus suggests that *C. glabrata* is likely to be equipped with a sensitive glucose sensing mechanism to sense and transport such low levels of glucose.

4.3. qRT-PCR analysis of Selected Glucose-Sensing Related Genes in Response to Different Glucose Concentrations

With the high glucose control experiments (2% glucose) as a baseline, the putative high affinity glucose sensor, *SNF3* (CAGL0J09020g), and the putative downstream transcriptional repressor, *RGT1* (CAGL0L01903g), were found to have similar expression patterns (Figure 4). Both genes were significantly up-regulated in no (0%), low (0.01%) and moderately high (1%) glucose conditions (Figure 4). On the other hand, the experiment with no glucose control (0% glucose) as baseline showed that the putative low affinity glucose sensor, *RGT2* (CAGL0I03872 g), was significantly up-regulated in the moderately high glucose condition (1%) (Figure 4). Meanwhile, the putative downstream transcriptional repressor, *MIG1* (CAGL0A01628g), was found to be responsive to the high surrounding glucose concentration (Figure 5). For the hexose transporters gene expression, only four candidates were selected for qRT-PCR analysis after screening through semi-quantitative reverse transcriptase-PCR. These hexose transporter genes were selected as they showed distinct gene expression levels (data not shown). In the high glucose control (2%), both expression of putative hexose transporter *HXT1* (CAGL0A01804g) and *HXT4* (CAGL0A01782g) were found to be higher in no glucose (0%) and high glucose conditions (2%) (Figure 6). Meanwhile, *HXT3* (CAGL0A0231g) and *HXT5* (CAGL0A01826g) were up-regulated significantly only under no (0%) glucose conditions (Figure 6).

Table 1. Details of Glucose Sensing-Related Genes of *Candida glabrata*

Target Gene Name	Systematic Name	Accession Number	No. of Amino Acids	Molecular Mass, kDa
SNF3	CAGL0J09020	CAG61052	856	95.2
RGT2	CAGL0I03872	CAG60354	762	84.0
RGT1	CAGL0L01903	CAG61822	1287	143.4
MIG1	CAGL0A01628	CAG57729	424	46.4
HXT1	CAGL0A01804	CAG57737	563	62.3
HXT3	CAGL0A02321	CAG57758	556	61.5
HXT4	CAGL0A01782	XP_444843	568	62.9
HXT5	CAGL0A01826	CAG57738	588	65.7
HXT4/6/7	CAGL0A02233	XP_444861	549	61.2
HXT6/7	CAGL0A02211	XP_444860	552	61.5
HXT6/7	CAGL0A00737	XP_444803	549	61.6
HXT2/10	CAGL0D02662	XP_445530	564	62.4
HXT2/10	CAGL0D02640	XP_445530	564	62.4
HXT2/10	CAGL0I00286	XP_447265	555	61.7
HXT14	CAGL0M04103	XP_449525	531	59.0

Table 2. Details of Glucose Sensing-Related Genes of *Saccharomyces cerevisiae*

Target Gene Name	Systematic Name	Accession Number	No. of Amino Acids	Molecular Mass, kDa
SNF3	YDL194W	CAA98771	884	96.7
RGT2	YDL138W	CAA65621	763	83.1
RGT1	YKL038W	CAA81873	1170	128.2
MIG1	YGL035C	CAA96736	504	55.6
HXT1	YHR094C	DAA06789	570	63.3
HXT2	YMR011W	DAA09909	541	59.8
HXT3	YDR345C	DAA12185	567	62.6
HXT4	YHR092C	DAA06788	576	63.9
HXT5	YHR096C	DAA06790	592	66.3
HXT6	YDR343C	DAA12184	570	62.7
HXT7	YDR342C	DAA12183	570	62.7
HXT8	YJL214W	DAA08597	569	63.5
HXT9	YJL219W	DAA08593	567	62.9
HXT10	YFL011W	DAA12428	546	60.7
HXT11	YOL156W	DAA10630	567	62.7
HXT13	YEL069C	DAA07586	564	62.7
HXT14	YNL318C	CAA96250	540	61.0
HXT15	YDL245C	DAA11622	567	62.9
HXT16	YJR158W	DAA08942	567	62.9
HXT17	YNR072W	DAA10613	564	62.8

Table 3. Target and Normalizing Genes Used in Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) With Their Respective Primer Sequences and Size of Amplicon

Target Gene Name	Accession Number	Direction	Sequence 5' - 3'	Amplicon Size
SNF3 (CAGL0J09020)	XM_448101	Forward	ACGACGCATCTCTGCTTTTT	182 bp
		Reverse	CGTGAATCCAACGTGATTTG	
RGT2 (CAGL0I03872)	XM_447417	Forward	CGTTTGTGGGACTTTTCGTT	203 bp
		Reverse	TGAAATCCATGGAGCAATGA	
RGT1 (CAGL0L01903)	XM_448852	Forward	CCAACTCAAAGGATGGAGGA	194 bp
		Reverse	TATCGTTGGCGTCATTTTGA	
MIG1 (CAGL0A01628)	XM_444836	Forward	CCGGGATGTGTCAAGAGATT	212 bp
		Reverse	CGTTTCGTCTTCCTCCTCAG	
HXT1 (CAGL0A01804)	XM_444844	Forward	AAACCAAGTCGGCAAGAATG	224 bp
		Reverse	ATTCAGTTCCGTCAGGATGC	
HXT3 (CAGL0A02321)	XM_444865	Forward	TGACCTTCGTTCCAGAATCC	165 bp
		Reverse	TACCAGCGGCATTAGCTTCT	
HXT4 (CAGL0A01782)	XM_444843	Forward	TCCTGGGGTGAATTGTTCTC	228 bp
		Reverse	GCCAAATCTACCGACCAAGA	
HXT5 (CAGL0A01826)	XM_444845	Forward	TATGTTTCGCATGGGCATTA	153 bp
		Reverse	CCAAAAGGACGATTGGAGAA	
ACT1	XM_448768	Forward	TTGCCACACGCTATTTTGAG	225 bp
		Reverse	ACCATCTGGCAATTCGTAGG	

Figure 1. Molecular Phylogenetic Analysis of *Candida glabrata* Glucose Sensing-Related Members by the Maximum Likelihood Method
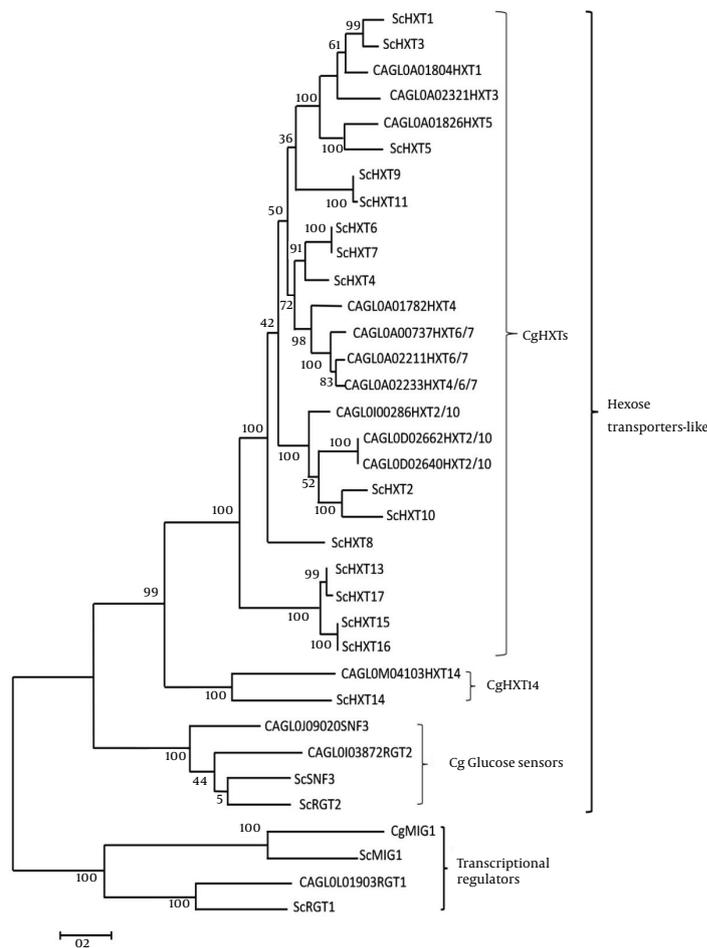
Initial trees for the heuristic search were obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using a Jones-Taylor-Thornton (JTT) model and then selecting the topology with superior log likelihood value. The analysis involved 35 glucose sensing-related amino acid sequences from *S. cerevisiae* and *C. glabrata*. The numbers on branches denote bootstrap values (100 replicates).

Sc 25 amino acids motif : Y V D L G N G L G L N T Y N R G P P S I S S D S S

CgSnf 3 : M M D F G N D M I L H I C N R G P P S I S T A A S

Sc 25 amino acids motif : Y V D L G N G L G L N T Y N R G P P S I S S D S S

CgRgt 2 : Y V E L G N G F G L N T Y R G G P P S I S   D S S

Figure 2. Alignment of the 25 Amino Acid Motifs in the C-Terminal Tail of *Sc* (*S. cerevisiae*) Glucose Sensors with CgSnf3 and CgRgt2
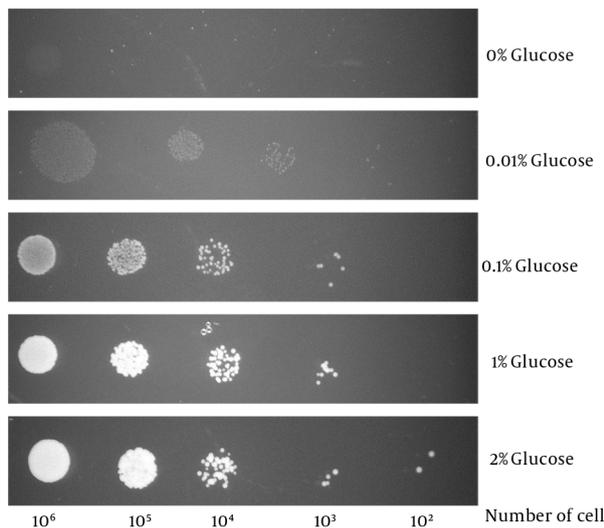
Figure 3. *Candida glabrata* Survival Under Extremely Low Glucose Levels (as low as 0.01%)

Figure 4. Comparison of Expression Ratios (Log10) for the *SNF3* and *RGT1* Genes During Exposure to Different Glucose Concentrations Relative to the High Glucose Control (2%)
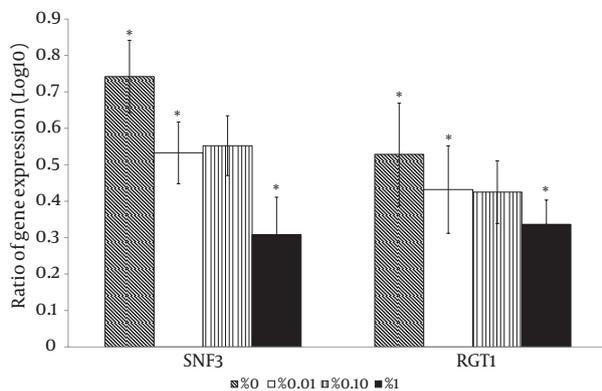
Data are presented as means ± standard error of the mean (SEM) with significant up-regulation as indicated by * (P-value < 0.05).

Figure 5. Comparison of Expression Ratios (Log10) for the *RGT2* and *MIG1* Genes During Exposure to Different Glucose Concentrations Relative to the No Glucose Control (0%)
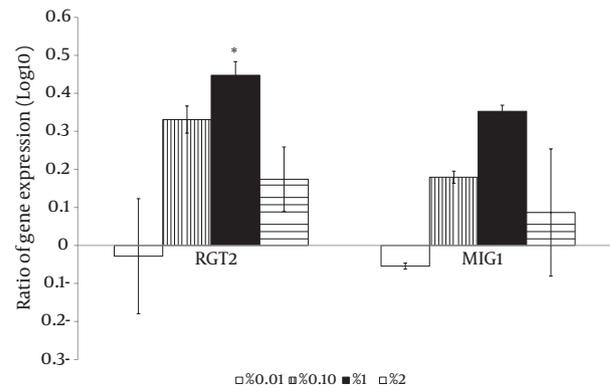
Data are presented as means ± standard error of the mean (SEM) with significant up-regulation as indicated by * (P-value < 0.05).

Figure 6. Comparison of Expression Ratios (Log10) for Putative Hexose Transporter Genes During Exposure to Different Glucose Concentrations Relative to the High Glucose Control (2%)
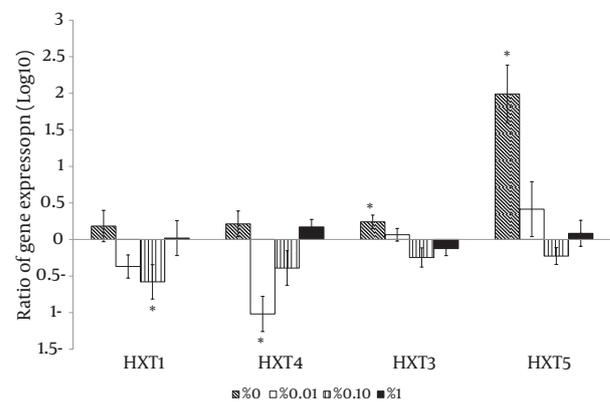
Data are presented as means ± standard error of the mean (SEM) with significant up- and down-regulation as indicated by * (P-value < 0.05).

5. Discussion

The ability of a microorganism to sense and transport glucose is crucial for its survival and pathogenicity in the host. Our study aimed to identify the key genes of the glucose sensing mechanism in *C. glabrata*, an emerging fungal pathogen, with the aid of the genomic profile available for *S. cerevisiae*. The results showed that *C. glabrata* SNF3 and Rgt2 protein were similar to their counterpart in *S. cerevisiae* (59% and 60%). Sc*SNF3* and Sc*RGT2* encode a protein with 12 predicted transmembrane-spanning domains and share only low similarity (30%) with other Hxt proteins (32). *Saccharomyces cerevisiae* with *SNF3*-deleted mutants was found to have lost the ability to grow fermentatively at low concentrations of glucose with impaired *HXT2* expression (33) and this has

led to the view that Snf3 is a high affinity glucose sensor in *S. cerevisiae*. On the other hand, deletion of Sc*RGT2* led to low expression of *HXT1*, which suggests the role of *RGT2* as a low affinity glucose sensor of *S. cerevisiae* (33). Both ScSnf3 and ScRgt2 were found with unusual long C-terminal segments (341 amino acids in Snf3 and 218 amino acids in Rgt2). This molecular structure is distinct from other members of the hexose transporter family as none of them possess C-terminal tail that is longer than 60 amino acids (32).

The role of the C-terminal tail to transmit intracellular signal for glucose sensing was further confirmed with the generated chimeric protein. The attachment of *SNF3* C-terminus to *HXT1* and *HXT2* has successfully complemented the hexose transporters expression defect found in Sc*SNF3*- and Sc*RGT2*-deleted mutants (34). The functional unit of this C-terminal tail was further discovered with a 25-amino acid sequence. Deletion of all C-terminal tail distal sequences and preservation of this critical 25-amino acid sequence has partially retained the functionality of ScSnf3 and ScRgt2. Interestingly, ScSnf3 was found to possess two copies of this 25-amino acid motif while ScRgt2 possessed only one (34).

In our study, distinct long C-terminal amino acid extensions were observed in both *SNF3* and *RGT2* of *C. glabrata* in comparison to other hexose transporter members. This observation suggests that *C. glabrata* may have retained this conserved region of glucose sensors, inherited from its common ancestor with *S. cerevisiae*. However, only a copy of a 25-amino acid motif was found in both CgSnf3 and CgRgt2 (Figure 2). In comparison to *C. albicans*, even though *HGT4* and *HGT12* were grouped in the same clade, yet only *HGT4* with long C-terminal tails was identified as a glucose sensor of *C. albicans* in a gene knock out study (17, 25). The missing C-terminal tail in *HGT12* is believed to support the observation that C-terminal tail is a signature characteristic of glucose sensors.

Besides, for the transcriptional regulator clade, CgRgt1 and CgMig1 are predicted to play the same role as ScRgt1 and ScMig1, where the latter serves as a glucose responsive transcription factor by regulating the expression of hexose transporters (7, 35). The qRT-PCR result is supportive of this prediction as both the *RGT1* and *MIG1* were found to be responsive to low and high surrounding glucose concentrations, respectively (Figures 4 and 5). The role of *RGT1* and *MIG1*, as the key links that control the expression of hexose transporters and glucose sensors in response to different glucose concentrations, was also determined in *C. albicans* (36). In addition, sub clade CgHxt14 (CAGL0M04103g) was found to be distinct from other hexose transporter-like proteins. This may be due to its native role as a glucose transporter for the mitochondria. Unlike other hexose transporters, which are located in the cell membrane, Sc*HXT14* was found to be part of a mitochondria protein as reported in a mitochondria proteomic study done for *S. cerevisiae* (37). Notably, no ortholog of *HXT14* was found in *C. albicans* (17). This may fur-

ther indicate the dissimilar evolutionarily relationship between *C. glabrata* and *C. albicans*.

Notably, the number of *C. glabrata* hexose transporters (11 *HXTs*) were found to be less than *S. cerevisiae* (17 *HXTs*) and *C. albicans* (20 *HGTs*) (17, 34). In addition, there were no orthologs of Sc*HXT11* or Sc*HXT9* found in *C. glabrata*, which were previously reported as drug resistance-related transporters in *S. cerevisiae* (38). This suggests that *C. glabrata* may not rely on this *HXT*-like transporter mechanism to counteract with the antifungal drug. In addition, Sc*HXT11* or Sc*HXT9* were also not found in *C. albicans* (17). Although the number of hexose transporters found in *C. glabrata* is different from *S. cerevisiae* and *C. albicans*, both hexose transporters and glucose sensors of *C. glabrata* were found to possess several sites of high amino acid conservation, such as GR and GRK/C/R, when compared to *C. albicans* and *S. cerevisiae* (17). Interestingly, both CAGL0D02662g and CAGL0D02640g were found to be indistinguishable in terms of nucleotide sequences and length yet different in their chromosomal location. These two copies of identical genes are unusual to be seen in *C. glabrata*, a haploid organism. Besides, both CAGL0D02233g and CAGL0D02211g were also found to be highly similar with 96% identity.

For the gene expression analysis, this study revealed that *C. glabrata*, like *S. cerevisiae* and *C. albicans* (15, 16) was sensitive to a wide range of glucose concentrations from 0% to 2%, in its living environment. Changes in expression of target genes were observed within two hours of the incubation time upon exposure to different concentrations of glucose. These findings showed that *C. glabrata* is able to detect and respond according to the available glucose in its surrounding, which in turn, may greatly aid the species in the process of the colonization and establishment of infections in human niches. A similar study done by Brown et al. (25) on *C. albicans* showed a high degree of concordance with our data, where *C. albicans* putative glucose sensor, *HGT4*, was expressed significantly when the glucose source was low. A similar observation was made for *C. glabrata* on the linkage between glucose sensors and transcriptional regulators. For the high glucose affinity lineage, the expression patterns of the *SNF3* and *RGT1* were in high degree of concordance and the same was observed for the low glucose affinity lineage, *RGT2* and *MIG1*. These observations reveal a possible collaboration among glucose sensors and transcriptional regulators in responding to the dynamic environment in which the amount of glucose is changing. Notably, the putative *HXT3* (CAGL0A02321g) and *HXT5* (CAGL0A01826g) genes were highly expressed in the presence of no glucose and this might suggest the potential role of these two transporters as high affinity hexose transporters. The expression of putative *HXT1* (CAGL0A01804g) and *HXT4* (CAGL0A01782g) was high under both no glucose (0%) and high glucose (2%) conditions. This highlights the possible role of *HXT1* (CAGL0A01804g) and *HXT4* (CAGL0A01782g) in glucose transportation at two extreme conditions (0% and 2%) with regulation from both low and high affinity glucose sensors.

In conclusion, *C. glabrata* was found to have the ability to withstand low glucose environments and this points to the possible role of glucose-sensing in aiding the survival of *C. glabrata*. The findings imply the interrelatedness of glucose-sensing-related members between *C. glabrata* and *S. cerevisiae*. In addition, the gene expression analyses of this study provided better insight into the glucose sensing key members of *C. glabrata*. The low glucose affinity glucose sensor and the downstream transcription factor, *RGT2* and *MIG1*, were sensitive to high glucose concentrations while the high glucose affinity sensor and the downstream transcription factor, *SNF3* and *RGT1*, were up-regulated at low glucose concentrations. This study also demonstrated the possible role of glucose sensing by *SNF3* and *RGT2* and the potential transcriptional regulators, *RGT1* and *MIG1*, in the glucose uptake mechanisms in *C. glabrata*. Our results for expression profiling of these genes serve as exploratory data for forthcoming studies in deciphering the complex mechanism of glucose sensing and transporting in *C. glabrata*. Future works, such as investigating protein expression and performing gene knockout experiment by involving more isolates of *C. glabrata* will further assist in explaining the operative role of these genes in the physiological process of *C. glabrata* and thus shed light on the understanding of the comprehensive picture of glucose sensing mechanisms in the species.

Acknowledgments

Footnotes

References

1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev.* 2007;**20**(1):133–63. doi: 10.1128/CMR.00029-06. [PubMed: 17223626]

2. Diekema D, Arbefeville S, Boyken L, Kroeger J, Pfaller M. The changing epidemiology of healthcare-associated candidemia over three decades. *Diagn Microbiol Infect Dis.* 2012;**73**(1):45–8. doi: 10.1016/j.diagmicrobio.2012.02.001. [PubMed: 22578938]

3. Molero G, Diez-Orejas R, Navarro-Garcia F, Monteoliva L, Pla J, Gil C, et al. Candida albicans: genetics, dimorphism and pathogenicity. *Int Microbiol.* 1998;**1**(2):95–106. [PubMed: 10943347]

4. Fidel PL, Vazquez JA, Sobel JD. Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. *Clin Microbiol Rev.* 1999;**12**(1):80–96. [PubMed: 9880475]

5. Rolland F, Winderickx J, Thevelein JM. Glucose-sensing and -signalling mechanisms in yeast. *FEMS Yeast Res.* 2002;**2**(2):183–201. [PubMed: 12702307]

6. Gancedo JM. The early steps of glucose signalling in yeast. *FEMS Microbiol Rev.* 2008;**32**(4):673–704. doi: 10.1111/j.1574-6976.2008.00117.x. [PubMed: 18559076]

7. Santangelo GM. Glucose signaling in Saccharomyces cerevisiae. *Microbiol Mol Biol Rev.* 2006;**70**(1):253–82. doi: 10.1128/MMBR.70.1.253-282.2006. [PubMed: 16524925]

8. Miwa T, Takagi Y, Shinozaki M, Yun CW, Schell WA, Perfect JR, et al. Gpr1, a putative G-protein-coupled receptor, regulates morphogenesis and hypha formation in the pathogenic fungus Candida albicans. *Eukaryot Cell.* 2004;**3**(4):919–31. doi: 10.1128/EC.3.4.919-931.2004. [PubMed: 15302825]

9. Sabina J, Brown V. Glucose sensing network in Candida albicans: a sweet spot for fungal morphogenesis. *Eukaryot Cell.* 2009;**8**(9):1314–20. doi: 10.1128/EC.00138-09. [PubMed: 19617394]

10. Bialková A, Šubík J. Biology of the pathogenic yeastCandida glabrata. *Folia Microbiologica.* 2006;**51**(1):3–20. doi: 10.1007/bf02931443. [PubMed: 16821705]

11. Barns SM, Lane DJ, Sogin ML, Bibeau C, Weisburg WG. Evolutionary relationships among pathogenic Candida species and relatives. *J Bacteriol.* 1991;**173**(7):2250–5. [PubMed: 2007550]

12. Wong S, Fares MA, Zimmermann W, Butler G, Wolfe KH. Evidence from comparative genomics for a complete sexual cycle in the 'asexual' pathogenic yeast Candida glabrata. *Genome Biol.* 2003;**4**(2):R10. [PubMed: 12620120]

13. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, et al. Genome evolution in yeasts. *Nature.* 2004;**430**(6995):35–44. doi: 10.1038/nature02579. [PubMed: 15229592]

14. Palma M, Seret M, Baret PV. Combined phylogenetic and neighbourhood analysis of the hexose transporters and glucose sensors in yeasts. *FEMS Yeast Res.* 2009;**9**(4):526–34. doi: 10.1111/j.1567-1364.2009.00511.x. [PubMed: 19459981]

15. Yin Z, Wilson S, Hauser NC, Tournu H, Hoheisel JD, Brown AJ. Glucose triggers different global responses in yeast, depending on the strength of the signal, and transiently stabilizes ribosomal protein mRNAs. *Mol Microbiol.* 2003;**48**(3):713–24. [PubMed: 12694616]

16. Rodaki A, Bohovych IM, Enjalbert B, Young T, Odds FC, Gow NA, et al. Glucose promotes stress resistance in the fungal pathogen Candida albicans. *Mol Biol Cell.* 2009;**20**(22):4845–55. doi: 10.1091/mbc.E09-01-0002. [PubMed: 19759180]

17. Fan J, Chaturvedi V, Shen SH. Identification and phylogenetic analysis of a glucose transporter gene family from the human pathogenic yeast Candida albicans. *J Mol Evol.* 2002;**55**(3):336–46. doi: 10.1007/s00239-002-2330-4. [PubMed: 12187386]

18. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol.* 2011;**7**:539. doi: 10.1038/msb.2011.75. [PubMed: 21988835]

19. Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J, et al. A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res.* 2010;**38**(Web Server issue):W695–9. doi: 10.1093/nar/gkq313. [PubMed: 20439314]

20. McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, et al. Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Res.* 2013;**41**(Web Server issue):W597–600. doi: 10.1093/nar/gkt376. [PubMed: 23671338]

21. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol.* 2011;**28**(10):2731–9. doi: 10.1093/molbev/msr121. [PubMed: 21546353]

22. Zuckerkandl E, Pauling L. Evolutionary Divergence and Convergence in Proteins. *Evolv Genes Proteins.* 1965;**97**:97–166. doi: 10.1016/b978-1-4832-2734-4.50017-6.

23. Felsenstein J. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution.* 1985;**39**(4):783. doi: 10.2307/2408678.

24. Sherman F. Getting started with yeast. *Methods Enzymol.* 2002;**350**:3–41. doi: 10.1016/s0076-6879(02)50954-x. [PubMed: 12073320]

25. Brown V, Sexton JA, Johnston M. A glucose sensor in Candida albicans. *Eukaryot Cell.* 2006;**5**(10):1726–37. doi: 10.1128/EC.00186-06. [PubMed: 17030998]

26. Collart MA, Oliviero S. Preparation of yeast RNA. *Curr Protoc Mol Biol.* 2001:1–13. doi: 10.1002/0471142727.mb1312s23.

27. Resuehr D, Spiess AN. A real-time polymerase chain reaction-based evaluation of cDNA synthesis priming methods. *Anal Biochem.* 2003;**322**(2):287–91. [PubMed: 14596842]

28. Bader O, Schwarz A, Kraneveld EA, Tangwattanachuleeporn M, Schmidt P, Jacobsen MD, et al. Gross karyotypic and phenotypic alterations among different progenies of the Candida glabrata CBS138/ATCC2001 reference strain. *PLoS One.* 2012;**7**(12):e52218. doi: 10.1371/journal.pone.0052218. [PubMed: 23284942]

29. Inglis DO, Arnaud MB, Binkley J, Shah P, Skrzypek MS, Wymore F, et al. The Candida genome database incorporates multiple Candida species: multispecies search and analysis tools with curated gene and protein information for Candida albicans and Candida glabrata. *Nucleic Acids Res.* 2012;**40**(Database issue):D667–74. doi: 10.1093/nar/gkr945. [PubMed: 22064862]

30. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 2001;**29**(9):e45. [PubMed: 11328886]

31. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res.* 2002;**30**(9):e36. [PubMed: 11972351]

32. Ozcan S, Johnston M. Function and regulation of yeast hexose transporters. *Microbiol Mol Biol Rev.* 1999;**63**(3):554–69. [PubMed: 10477308]

33. Ozcan S, Dover J, Rosenwald AG, Wolfl S, Johnston M. Two glucose transporters in Saccharomyces cerevisiae are glucose sensors that generate a signal for induction of gene expression. *Proc Natl Acad Sci U S A.* 1996;**93**(22):12428–32. [PubMed: 8901598]

34. Ozcan S, Dover J, Johnston M. Glucose sensing and signaling by two glucose receptors in the yeast Saccharomyces cerevisiae. *EMBO J.* 1998;**17**(9):2566–73. doi: 10.1093/emboj/17.9.2566. [PubMed: 9564039]

35. Kim JH, Polish J, Johnston M. Specificity and regulation of DNA binding by the yeast glucose transporter gene repressor Rgt1. *Mol Cell Biol.* 2003;**23**(15):5208–16. [PubMed: 12861007]

36. Sexton JA, Brown V, Johnston M. Regulation of sugar transport and metabolism by the Candida albicans Rgt1 transcriptional repressor. *Yeast.* 2007;**24**(10):847–60. doi: 10.1002/yea.1514. [PubMed: 17605131]

37. Sickmann A, Reinders J, Wagner Y, Joppich C, Zahedi R, Meyer HE, et al. The proteome of Saccharomyces cerevisiae mitochondria. *Proc Natl Acad Sci U S A.* 2003;**100**(23):13207–12. doi: 10.1073/pnas.2135385100. [PubMed: 14576278]

38. Nourani A, Wesolowski-Louvel M, Delaveau T, Jacq C, Delahodde A. Multiple-drug-resistance phenomenon in the yeast Saccharomyces cerevisiae: involvement of two hexose transporters. *Mol Cell Biol.* 1997;**17**(9):5453–60. [PubMed: 9271421]