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Virulence and antifungal susceptibility of microsatellite genotypes of *Candida albicans* from superficial and deep locations

Running title: Genotyping of *Candida albicans* and infection types

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Abstract

A set of 185 strains of *Candida albicans* from patients with vulvovaginal candidiasis (VVC) and from non-VVC clinical sources in southwest China was analyzed. Strains were subjected to genotyping using CAI microsatellite typing and amplification of an intron-containing region of the 25S rRNA gene. Microsatellite genotypes of strains from non-VVC sources showed high polymorphism, while those of VVC were dominated by few, closely similar genotypes. However, among non-VVC strains, two genotypes were particularly prevalent in patients with lung cancer. 25S rDNA genotype A was dominant in VVC sources (86.7%), while genotypes A, B and C were rather evenly distributed among non-VVC sources; known genotypes D and E were not found. In an experimental mouse model, isolates from lung cancer and AIDS patients proved to have higher virulence than VVC strains. Among 156 mice infected with *C. albicans*, 19 developed non-invasive urothelial carcinoma. No correlation could be established between parameters of virulence, source of infection, and incidence of carcinoma. *C. albicans* strains from VVC were less susceptible to itraconazole than the strains from non-VVC sources, while there was small difference in antifungal susceptibility between different 25S rDNA genotypes of *C. albicans* tested against amphotericin B, itraconazole, fluconazole and flucytosine.

KEY WORDS *Candida albicans*; CAI microsatellite genotype; 25S rDNA genotype; urothelial carcinoma; antifungal susceptibility

Introduction

Candida albicans is involved in a wide variety of infections, ranging from mucosal or vaginal disorders in generally healthy persons to life-threatening, systemic infections in individuals with severely impaired immunity (Achkar & Fries, 2010). Mucocutaneous candidiasis can be divided into genitourinary and nongenital disease. Vulvovaginal candidiasis (VVC) is one of the most frequent manifestations (de Repentigny et al., 2004). *C. albicans* expresses successful adaptation to the human body in response to various stimuli, being able to sustain as a commensal colonizer and as an opportunistic pathogen (Egbe et al., 2015). The host's immune system is a major factor balancing the transition from commensalism to pathogenicity, but also fungal features as adhesion, hyphal formation, phenotypic switching (PS), extracellular enzyme production, and biofilm formation contribute to *C. albicans* virulence. Selective proliferation of some genes of commensal strains may precede the transition of *C. albicans* to infection (Abdulrahim et al., 2013, da Silva-Rocha et al., 2014). The possibility that chronic inflammation, in which *Candida albicans* infection can play a role, may enhance the development of cancer has been brought forward by several authors (e.g. Norgaard et al., 2013, Ramirez-Garcia et al., 2016).

Epidemiological genotyping is relevant in the context of species prevalence, for elucidation of virulence factors and mechanisms of drug resistance, and is of practical importance to support treatment options in candidiasis. Molecular studies have shown that *C. albicans* isolates exhibit a high level of genetic diversity. Therefore, reproducible and precise genotyping methods are required, illustrating possible relationships between strain genotypes, phenotypic properties and host interactions. Strains may differ considerably in virulence, antifungal susceptibility and other clinically relevant parameters, and appropriate genotyping methods may reveal micro-evolution by detecting small genetic variations indicating adaptation to long-term host response. In recent years, short tandem repeats or microsatellites have been used as molecular markers for population genetics and genotyping. Microsatellite loci (e.g. CAI, CAIII, CAV, CAVI and CAVII) located in coding or non-coding regions have been employed for strain typing of *C. albicans* (Sampaio et al., 2003, Sampaio et al., 2005). GeneScan analyses of the CAI microsatellites proved to be particularly powerful (Carvalho-Pereira et al., 2015, Bai, 2014). Previous studies showed promising results when using this method to determine genetic diversity in *C. albicans* with VVC and non-VVC clinical features (Bai, 2014, Ge et al., 2010, Fan et al., 2008). The method proved to be sufficiently sensitive to reveal consistent geographical structuring within the species.

The main aim of our study is to apply genotyping using GenScan analysis of CAI loci aiming to compare genetic diversities of *C. albicans* isolates from vaginal candidiasis with those from other clinical sources in patients from the same region. Experimental mouse infection is used to establish

comparative virulence of strains from particular patient groups, and *in vitro* susceptibility is tested to reveal eventual correlations between virulence, source of infection, drug resistance and genotype.

Materials and methods

Collection and identification of test isolates

A total of 185 independent *C. albicans* strains were isolated from patients treated in the Affiliated Hospital of Guizhou Medical University (Table S1), the Guiyang Women and Children's Healthcare Hospital, the No. 2 Affiliated Hospital of Guiyang College of Traditional Chinese Medicine, the Baiyun No. 1 People's Hospital, and the Guiyang No. 5 People's Hospital. The set included 90 strains from HVS (high vaginal swabs) of female patients with vaginal candidiasis and 95 strains from BAL (bronchoalveolar lavage) fluid, urine and blood from cases of systemic candidiasis of adult male and female patients with various types of candidiasis. *Candida albicans* ATCC 90028 (= CBS 8837) was used as reference. For animal experiments, nine clinical strains from symptomatic patients and three from asymptomatic hosts (Table 1) and reference strain ATCC 10231 (= CBS 6431) were used. All isolates were maintained in cryopreservation in 20% glycerol (wt/wt) at $-AT^{\circ}C$.

DNA extraction and PCR

Nuclear DNA was extracted according to methods described by Kaiser et al. (1994). The rDNA ITS region was amplified using primers ITS1 and ITS4 (Kageyama et al., 2004). Obtained sequence data were compared with those published in GenBank using BLAST. The CAI microsatellite locus was amplified by with primers 5'-ATG CCA TTG AGT GGA ATT GG-3' (fw) and 5'-AGT GGC TTG TGT TGG GTT TT-3' (rv) according to Sampaio et al. (2005). For GeneScan analysis, the forward primer was 5'-fluorescently labeled with 6-carboxyfluorescein. PCR amplification was performed in a thermocycler (iCycler, Bio-Rad, Hercules, CA, U.S.A.) with initial denaturation at 95°C for 5 min, 33 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 1 min, and a final extension step of 7 min at 72°C. Fragment sizes of amplified DNA were determined automatically by GeneScan software v. 3.7. CAI genotypes were designated by the number of trinucleotide repeat units in both alleles (because of the diploid nature of *C. albicans*) of the microsatellite locus, as described by Sampaio et al. (2015). Primer pairs spanning the transposable intron in the 25S rDNA as follows: CA-INT-L (5'-ATA AGG GAA GTC GGC AAA ATA GAT

CCG TAA-3') and CA-INT-R (5'-CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3'). The PCR conditions were: denaturation for 3 min at 94°C prior to 30 cycles of 94°C for 1 min, 65°C for 1 min, and 72°C for 2.5 min and a final extension at 72°C for 10 min.

Antifungal susceptibility

In vitro susceptibilities of *Candida* strains obtained from blood samples were tested against four antifungal agents: fluconazole (0.12–64 µg/ mL), itraconazole (0.015–16 µg/mL), amphotericin B (0.03–16 µg/mL), and flucytosine (0.12–64 µg/ mL). MICs were determined by broth microdilution with a commercial frozen plate for antifungal susceptibility testing (Eiken Chemical Co., Shanghai, China) that complied with Clinical Laboratory and Standards Institute (CLSI) guideline M27-A3, following the manufacturer's instructions. MIC values were interpreted according to the criteria in CLSI guideline M27-S4 and the epidemiological cut-off value (ECV) (Pfaller et al., 2012). *Candida albicans* IFM 40213 (=ATCC 90028) and *C. parapsilosis* ATCC 22019 were used as quality control isolates. We evaluated whether the MIC/MEC values correlated with different genotypes using the Mann-Whitney-Wilcoxon test for skewed distribution.

Mouse model of disseminated candidiasis

Twelve isolates were used in an experimental mouse model of hematogenously disseminated candidiasis as described previously by Sampaio et al. (2010). Nine of these originated from symptomatic, and 3 from healthy individuals; ATCC 10231 = CBS 6431 was applied as reference. The set included genotypes 21-33 and 21-21 which were primarily derived from patients with lung cancer or AIDS, while 30-45, 32-46 and 30-46 were the dominant genotypes of VVC. According to the principle of randomization, control and double-blind, strains and saline were divided into 14 groups (12 mice for each group; Table 1). Strains analyzed (Table S1) originated from unrelated patients in different hospitals. The study was conducted under the guidelines and approval of the Research Ethics Committee of Guizhou Medical University. Briefly, 156 female KM mice (18–22 g body weight; 6 wk, Guizhou Medical University) were injected with 5×10^5 cells of the corresponding strain and via the lateral tail vein (Amorimvaz et al., 2015, Taylor et al., 2000) and 12 mice in the control group were injected with the same volume of saline. All inoculums were confirmed by quantitative culture. For preparation of inoculums, thawed cells from the original stock were grown on PDA medium at 37°C. In each experiment, all isolates were tested simultaneously and inocula were confirmed by CFU counting of the suspensions used to infect the mice. Animal welfare was assessed twice daily during 5 months. The mice were monitored at least two times daily, and dead

mice were dissected immediately. Brain, liver, spleen and kidneys from each mouse were harvested, weighed. After 5 months of infection, all mice were sacrificed for anatomical examination.

Histopathology

Organs excised from all infected mice were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H&E) according to Kumar *et al.* (2015). On the basis of 1000 neoplastic nuclei, Ki-67 labeling index was calculated in each slide as the percentage of immunopositive nuclei (Šteňo et al., 2014).

Statistics

Survival of mice was compared with log-rank test and Gehan-Breslow-Wilcoxon test, using GraphPad Prism 7 for Windows. *P*-values ≤ 0.05 were considered to be significant. 25S rDNA group-I intron genotypes for *C. albicans* from different sources and carcinoma of mice in each group were compared using chi-square (Li, 2010). PowerMarker v3.25 software was used for calculation of polymorphism information content (*PIC*) and Nei's genetic distance of CAI gene. Construction of a distance tree was done using clustering with the Unweighted Pair Group Method with Arithmetic Means (UPGMA). We generated odds ratios for associations between VVC and VVC-prevalent CAI genotypes using generalized estimating unconditional logistic regression.

Results

Genotyping

A total of 185 independent isolates of *Candida* were identified down to species level using the rDNA ITS spacer region; all isolates were confirmed to be *C. albicans*. The strains were analyzed with 25S rDNA amplification, of which 122 were classified as genotype A (65.9%), 35 were classified as genotype B (18.9%), and 28 were classified as genotype C (15.1%). Regarding the 90 strains isolated from VVC, genotype A was preponderant with 78 isolates (86.7%), 7 isolates were genotype B (7.8%) and 5 isolates were genotype C (5.6%). For the strains isolated from non-VVC, there were 44 isolates with genotype A (46.3%), 28 isolates with genotype B (29.5%) and 23 isolates with genotype C (24.2%). Data were inspected with χ^2 ($P < 0.05$) in SPSS v10.0 software. Results showed

significantly different proportions in 25S rDNA group-I intron between the strains from VVC and non-VVC ($P = 0.000$).

Ninety strains isolated from female patients with vaginal candidiasis (VVC) and 95 from other clinical sources were genotyped for the CAI region. In the 95 non-VVC *C. albicans* strains, PCR products revealed 24 separate alleles consisting of fragments with different lengths, varying between 189 bp (11 repeats) and 291 bp (45 repeats) and 40 distinct genotypes were recognized. In the 90 VVC strains we identified a total of 22 separate alleles and 27 distinct genotypes, and the length of CAI fragments varied between 189 bp (11 repeats) and 297 bp (47 repeats). Seven genotypes are reported here for the first time.

The 185 clinical isolates of *C. albicans* were divided into 5 clusters by CAI microsatellite polymorphism analysis (Fig. 1). Most of the genotypes of the VVC strains belong to cluster A (70/90, 77.8%). Prevalent genotypes among VVC strains in cluster A are 30-45 (23 strains, 31.9%), followed by 32-46 (14 strains, 19.4%) and 30-46 (9 strains, 12.5%). These three common genotypes and another 7 similar genotypes account for 63 strains (70.0%) and show a wide distribution.

Unconditional logistic regression analysis showed the risk odds ratio of prevalent CAI genotypes with VVC were 4.3. However, CAI genotypes of non-VVC strains are mainly found widely distributed in clusters B, C, D and E. Among non-VVC strains, ten stains, seven of which belonged to the closely similar genotypes 21-33 and 21-21, were associated with lung cancer.

Antifungal susceptibility

Table 2 summarizes the *in vitro* susceptibilities of 90 VVC and 59 non-VVC isolates of *C. albicans* to amphotericin B, itraconazole, fluconazole and flucytosine. The data are presented as MICs, MIC ranges, median MIC, MIC₅₀ and MIC₉₀. The analysis using CLSI methodology demonstrates that amphotericin B spanned a narrow range of 0.125–1 with GM MIC of 0.70 µg/mL for VVC and a MIC range of 0.25–1 with GM MIC 0.71 µg/mL for non-VVC. The MIC₅₀ and MIC₉₀ for amphotericin B was 1 µg/mL for both VVC and non-VVC. The GM MICs for 5-flucytosine for VVC and non-VVC, respectively, were 1.77 µg/mL and 0.18 µg/mL, the MIC₅₀ for flucytosine were 2 µg/mL and 0.125 µg/mL, and the MIC₉₀ for flucytosine were 4 µg/mL and 0.25 µg/mL. For fluconazole, the GM MIC, MIC range, MIC₅₀ and MIC₉₀ were 1.71, 0.06–64, 2 and 8 µg/mL for VVC and 0.91, 0.125–>64, 0.5 and 4 µg/mL for non-VVC. For itraconazole the GM MIC, MIC range, MIC₅₀ and MIC₉₀ were 0.39, 0.03–8, 0.5 and 2 for VVC and 0.22, 0.015–>8, 0.125 and 8 µg/mL for non-VVC. Overall, all of the strains were susceptible to amphotericin B. There was no significant difference VVC and non-VVC in susceptibility to 5-flucytosine ($P = 0.985$) and fluconazole ($P = 0.272$). However, *C. albicans* from VVC strains were less susceptible to itraconazole than the strains from non-VVC sources ($P = 0.000$).

Table 3 shows the analysis of the MIC ranges, median MIC, MIC₅₀ and MIC₉₀ of *C. albicans* different 25S rDNA genotypes (A, B and C). All strains were susceptible to amphotericin B; there was no significant difference in susceptibility to remaining drugs tested ($P = 0.315$, $P = 0.391$, $P = 0.409$, respectively). Genotypes were differentially susceptible to flucytosine and fluconazole. The *C. albicans* 25S rDNA genotypes of group A were considerably less susceptible to fluconazole, flucytosine and itraconazole than genotypes B and C. MIC₅₀ and MIC₉₀ were 2 and 4 µg/mL for flucytosine and 2 and 8 µg/mL for fluconazole, respectively.

Murine model

Five-month survival rates of all mice are listed in Table 4. Using the sources of isolation as parameter, strains could be divided in: lung cancer, HIV/AIDS, VVC, and asymptomatic individuals. Based on strain sources and analyzed by log-rank test and Gehan-Breslow-Wilcoxon test, overall differences in survival were highly significant ($P < 0.05$). Isolates from lung cancer and AIDS proved to have higher virulence than VVC strains (Fig. 2). Fig. 3A shows histopathology of kidney derived from mice died within 48 days. Flat urothelial hyperplasia comprised an increased number of cell layers with few or no significant cytological abnormalities; fungal cells were predominantly in the hyphal form. Polarity was preserved and none to minimal variation in architectural and nuclear features was noted. Fig. 3B shows histopathology of kidney derived from a mouse that died after 67 days. Inflammation and the atypical epithelial dysplasia were observed in some infected mice; variations in nuclear polarities, sizes, shapes and chromatin patterns occurred. Nuclei were uniformly enlarged with mild differences in shape, contour and chromatin distribution, leading to a diagnosis of non-invasive, low grade (ICD-O code: 8130/21) urothelial carcinoma (John et al., 2004). Mitoses were frequent, sometimes atypical, and occurred at any level including the surface. Fig. 3C shows a predominant pattern of disorder with easily recognizable variations in architectural and cytological features even at low magnification. Marked variations in nuclear polarity, size, and shape and pattern of chromatin were recognized. Nuclei often showed pleomorphism with moderate to marked variation in size and with irregular chromatin distribution. Nucleoli were prominent. This led to a diagnosis of non-invasive urothelial carcinoma, high grade (ICD-O code: 8130/23) (John et al., 2004). It is important to note that linear expression with occasional local interruption was observed. Table 1 shows carcinoma occurrence of mice infected with *C. albicans* strains. Abnormal cells were observed on pathologic sections of murine organs with *C. albicans* infection of 21 days, which was noted by the Ki67 labeling index of 3% (Fig. 4A). Fig. 4B shows *C. albicans* infection after 48 days; the Ki67 labeling index was 40%, leading to a diagnosis of non-invasive urothelial carcinoma, high grade.

Discussion

In the current study, genotyping of *C. albicans* strains from VVC and non-VVC disorders using microsatellite analysis of CAI and 25S rDNA revealed that most VVC-strains possessed specific genotypes. Logistic regression analysis showed that, compared with other genotypes, the prevalent CAI genotypes of VVC have a high risk of odds ratio (OR=4.3). It shows that the risk of VVC in prevalent genotype was 4.3 times higher than that in other genotypes after controlling for age, marital and sexual factors. CAI microsatellite genotyping has repeatedly been proven to be a powerful method for epidemiology of *C. albicans* (Bai, 2014). This approach revealed a significantly larger diversity than classical typing using 25S rDNA introns. The methods are not entirely congruent, indicating that, on the basis of criteria of genealogical concordance, recombination occurs in the analyzed set of strains. However, 63 of the prevalent isolates from VVC (70.0%) with microsatellite types 30-45 and 32-46 and similar genotypes, all belonged to 25S rDNA genotype A. This indicates the occurrence of expansion of closely related genotypes in a particular lineage.

Out of 57 microsatellite genotypes revealed in this study, twelve seemed to have a global distribution, since the same genotypes were reported in Europe (Kageyama et al., 2004). Thirty-six genotypes were shared between Beijing / Tianjing, Guangdong, Guiyang, and Wuhan, representing northern, southern, western and central regions of China, respectively (Bai, 2014, Ge et al., 2010, Fan et al., 2008). Seven novel genotypes were recognized in the current study, which seemed to have a local distribution.

Genotype CAI microsatellite patterns were also structured by association with clinical types. Profiles of systemic infections were found to differ significantly from those of VVC. The majority of randomly taken *C. albicans* strains associated with VVC belonged to the same or highly similar CAI genotypes. Most isolates from patients with lung cancer were recognized as CAI 21-33 and similar genotypes. In contrast, the overall pattern of strains originating from respiratory diseases and from AIDS patients was highly diverse. In respiratory disease, the diversity may be explained by local severe abuse of antibiotics to treat respiratory disorders, which has a negative impact upon the host's immune system and thus predisposes to fungal infection; the number of opportunistic infections in those patients is predicted to be high. In addition, the use of sputum samples rather than BAL fluid or blood may have led to the inclusion of commensal strains with deviating genotypes. AIDS patients, with 16 CAI types occurring in 23 strains, also seem to be infected easily by diverse *C. albicans* genotypes (Fig.1, Table S1).

rDNA genotypes A and B *versus* C differ by absence and presence of a 25S rDNA-based group I intron. *Candida albicans* strains with relatively effective adherence properties are known to have higher virulence than non-*albicans Candida* species such as *C. krusei* (Ferreira et al., 2013).

Genotype A seems to be more virulent than genotypes B and C (Sardiet al., 2012), and is dominant in VVC and in healthy oral mucosa of all age groups (Xu et al., 2008, Qi et al., 2005). In our study, all strains of the preponderant microsatellite genotypes from VVC (mainly 30-45, 32-46, and 30-46) belong to rRNA genotype A, which is consistent with current literature.

The hypothesis that strains differing in predilection for body sites might differ in virulence has been verified with animal inoculation experiments (Li et al., 2015). A progressive reduction of virulence was observed, with systemic isolates being more virulent than VVC strains (Table 2, Fig. 2), judged from the time of death after challenge and from pathologic expression in the infected mice.

We also showed differences in pathologic anatomy of various tissues after prolonged survival of *Candida* yeast cells. The Ki-67 labeling indices of proliferating cells in mice that died after 3 weeks were mostly around 3%. A threshold Ki-67 labeling index of >3% immune-reactivity is termed "atypical adenoma", which suggests a potential stimulus by *Candida* cells (Šteňo et al., 2014, Heaney, 2011). In mice died two months after inoculation, obviously non-invasive urothelial carcinoma was observed (Dalgliesh et al., 2010), a disease associated with tuberous sclerosis that can result in kidney cancer (Ramirez-Garcia et al., 2014, Kapoor et al., 2016). A recent study supports the view that *C. albicans* infection may not just be a consequence of cancer, but is an actively contributing cause as well causative agent (Nørgaard et al., 2016). A significant positive association has been reported of oral *Candida* carriage / infection and oral epithelial dysplasia/neoplasia (Ramirez-Garcia et al., 2014). Since then, several studies have demonstrated that oral cancer and pre-cancer lesions are frequently infected by *Candida* species. The most widely accepted hypothesis is that *C. albicans* produces nitrosamines, which are carcinogens that activate specific proto-oncogenes that could trigger cancerous lesions (Krogh, 1990). In addition, alcohol dehydrogenase (ADH1) produced by *C. albicans* might metabolize alcohol and carbohydrates to acetaldehyde, which is carcinogenic and eventually induces tumor development. Possibly *C. albicans*, *C. tropicalis* and *C. parapsilosis* produce more acetaldehyde than other *Candida* species, often exceeding carcinogenic levels (> 100 μ M) (Nieminen et al., 2009).

Candida albicans yeast cells are transported via the bloodstream into several tissues of the model animal, causing high levels of inflammation. The degree of damage to kidneys in inoculated animals was found to be more severe than to other organs, irrespective of genotype or clinical origin of the inoculated strain. Earlier publications also mentioned that *Candida* infection was less controlled in the kidney than in other organs (Li et al., 2015, Natasha et al., 2014). Yeast cells penetrated through blood vessels into both cortex and medulla, causing an influx of neutrophils. Several authors mentioned an association between inflammation and cancer (Coussens & Werb, 2002, Balkwill & Mantovani, 2001). The inflammatory state enhances maintenance and promotion of tumor progression and the emergence of completely malignant phenotypes through neoplastic tissue remodeling,

angiogenesis, metastasis and inhibition of anticancer innate immune response (Wang et al., 2009). Specifically, IL-18 and TNF- α play a vital role in tumor adhesion and metastasis (Ramirez et al., 2011, Rodríguez-Cuesta et al., 2010).

In the present experiment, non-invasive urothelial carcinoma (high grade) was found in the experiment mice population. The mechanism that caused the appearance of this phenomenon is as yet unclear, but requires close attention. We emphasize that not only there is a need to control *Candida* infection during cancer therapy, especially given the important role of this yeast in nosocomial infections, but also to find new therapeutics to suppress pro-tumor effects by *C. albicans*.

Strains with rDNA genotype A have been shown to have decreased susceptibility to the antifungal agent flucytosine (McCullough et al., 1999). Heterogeneity in resistance between strains is known to exist, but this was ascribed to geographic variation (Fan et al., 2008). Our strains of genotype A originating from VVC showed increased resistance to itraconazole, but neither to flucytosine, nor to other drugs tested. The correlation of the absence of a group I intron in the 25S rRNA with high levels of resistance to flucytosine was therefore not confirmed. The found deviations in drug resistance profiles of *C. albicans* genotype A may thus have been spread by local epidemics. The result of antifungal susceptibility tests showed that all of *C. albicans* strains in our study were susceptible to amphotericin B, and there was a small difference in susceptibility to flucytosine and fluconazole, however, the strains from VVC were less susceptible to itraconazole than strains from non-VVC infections. For some clinicians, the azoles have become the drug of choice for treatment of *Candida* infections, because of high bioavailability, extensive tissue penetration, long half-life, convenient use and good safety. Especially the superficial infection vulvovaginal candidiasis is more diverse than deep infection for local application. But the widespread overuse induces the emergence of drug-resistant strains, resulting in a decline in susceptibility, when clinical medications don't follow the standard guidelines.

Conflict of interest

None of the authors have conflict of interest.

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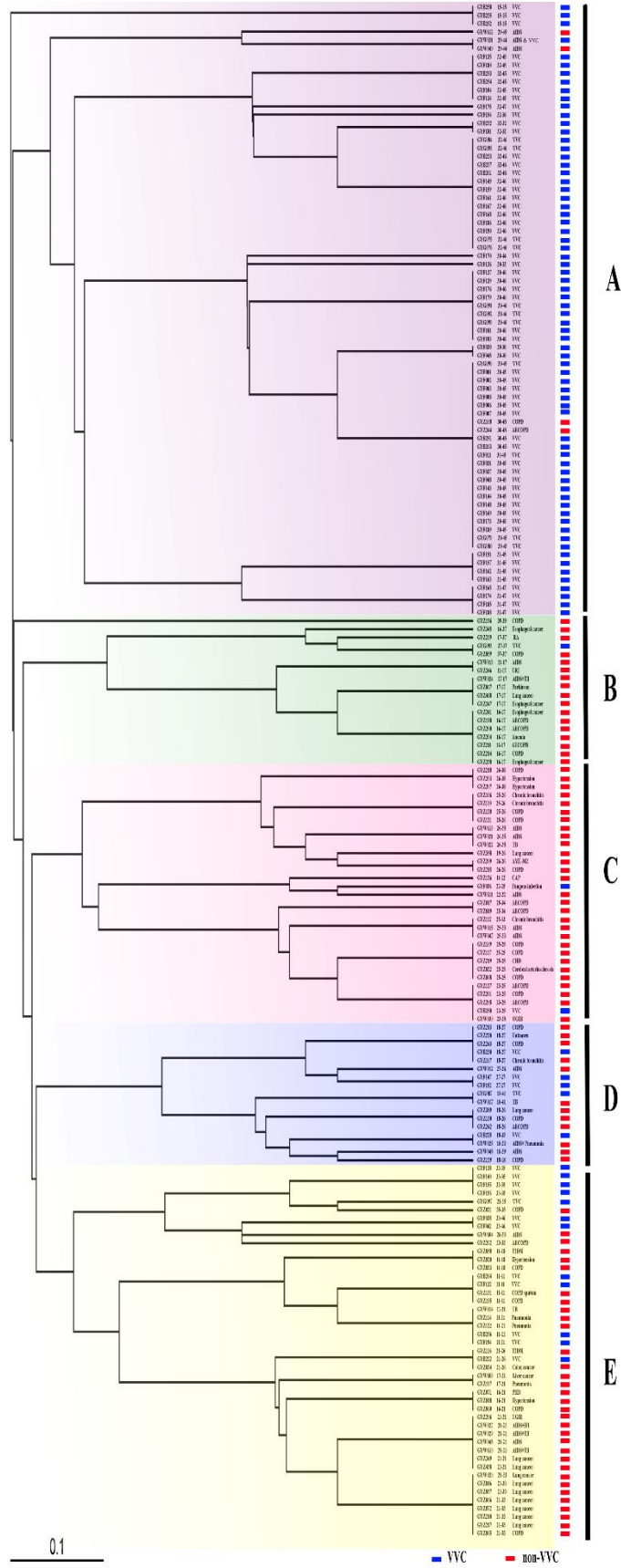


Fig. 1. Construction of a UPGMA distance tree of CAI gene with 185 *C. albicans* strains isolated from VVC and non-VVC. The blue rectangle marked strains are isolated from VVC; and red rectangle marked strains are isolated from non-VVC. COPD: Chronic Obstructive Pulmonary Disease; AECOPD: Acute Exacerbation of Chronic Obstructive Pulmonary Disease; AIDS: Acquired Immunodeficiency Syndrome; RA: Rheumatoid arthritis; URI: Upper respiratory tract infection; TB: Tuberculosis; AML-M2: Acute myeloblastic leukemia with differentiation; CAP: community acquired pneumonia; CHD: Congenital heart defect; UGIB: Upper Gastrointestinal Bleeding; T2DM: Diabetes mellitus type 2.

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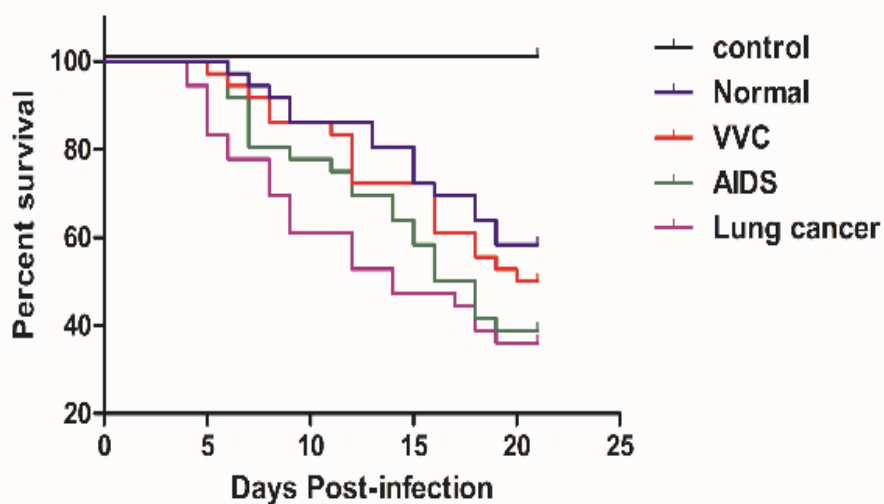


Fig. 2. Survival curves of KM mice following infection with *C. albicans* strains. Mice were inoculated i.v. with 2×10^6 cells of strains from lung cancer, HIV/AIDS, VVC and normal (asymptomatic) individuals; conditions of mice were assessed daily for 21 days.

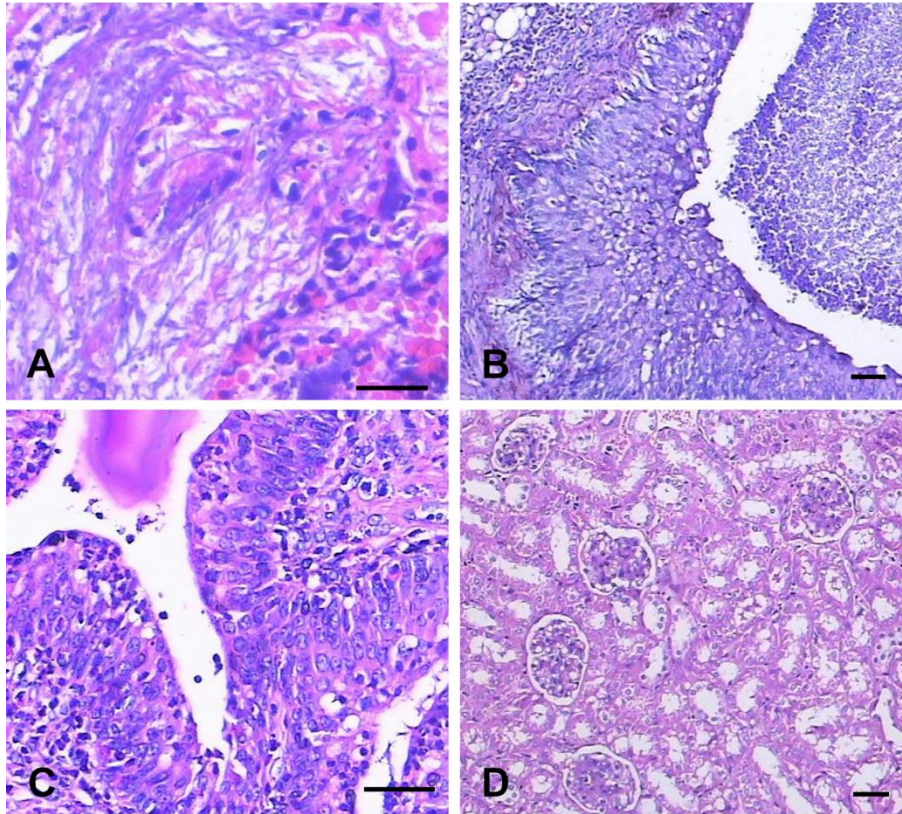


Fig. 3. Pathological changes in the kidney of the mice infected with *C. albicans* (HE staining, A×400, B×200, C×400, D×200). A. Kidney section of mouse (died on day 48) inoculated by GYW045 of genotype 21-21; B. kidney section of mouse (died on day 67) inoculated by GYZ210 of genotype 21-33 ; C. kidney sections of mouse (euthanized on day 168) inoculated by GYZ249 of genotype 21-21; D. kidney section of normal mouse (scale bar = 50 μ m) .

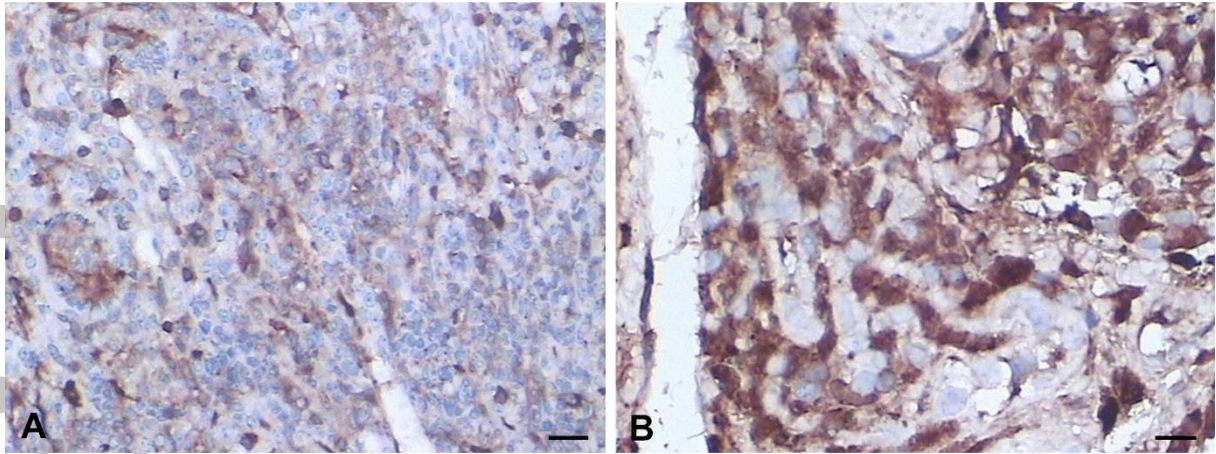


Fig. 4. Immunostained samples for Ki-67 labeling index in the kidney of the mice infected with *C. albicans*. A. Ki-67 labeling index of mouse died within 30 days (3%); B. Ki-67 labeling index of mouse died after 30 days (40%) (scale bar = 50 µm).

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Table 1. Occurrence of carcinoma in mice infected with *C. albicans*.

Group	No.	CAI genotype	Source	30-60 d	61-90 d	91-120 d	121-168 d	Number of carcinomas	Percentage incidence
1	GYF006	30-45	VVC	L(1)				1	8.3
2	GYZ249	21-21	Lung cancer	L(1)			L(1)/H(2)	4	33.3
3	ATCC10231	Untested	Bronchopneumonia			H(1)		1	8.3
4	GYWu27	21-21	HIV/AIDS					0	0.0
5	GYWu45	21-21	HIV/AIDS	L(1)			H(1)	2	16.7
6	GYF76	32-46	VVC				H(1)	1	8.3
7	GYWu20	21-33	HIV/AIDS		H(1)			1	8.3
8	GYF091	30-46	VVC	L(1)/H(1)				2	16.7
9	GYZ210	21-33	Lung cancer			H(1)	H(1)	2	16.7
10	GYZ072	21-33	Lung cancer		H(1)			1	8.3
11	BK60	Untested	Healthy host				H(1)	1	8.3
12	S165	Untested	Healthy host			H(1)	H(1)	2	16.7
13	1107	Untested	Healthy host				H(1)	1	8.3
14	Saline	-	-					0	0.0

Abbreviations used: L = non-invasive urothelial carcinoma, low grade; H = non-invasive urothelial carcinoma, high grade.

Table 2. Results of four antifungal drugs against *C. albicans* from VVC and non-VVC.

Antifungal	MIC ($\mu\text{g/mL}$)	VVC	Non-VVC
Amphotericin B	MIC range	0.125–1	0.25–1
	GM MIC	0.70	0.71
	MIC ₅₀	1	1
	MIC ₉₀	1	1
5-Fluorocytosine	MIC range	0.25–64	<0.125–>64
	GM MIC	1.77	0.18
	MIC ₅₀	2	0.125
	MIC ₉₀	4	0.25
Fluconazole	MIC range	0.06–64	0.125–>64
	GM MIC	1.71	0.91
	MIC ₅₀	2	0.5
	MIC ₉₀	8	4
Itraconazole	MIC range	0.03–8	<0.015–>8
	GM MIC	0.39	0.22
	MIC ₅₀	0.5	0.125
	MIC ₉₀	2	8

Tab.3 Result of antifungal drugs sensitivity test of *C. albicans* different 25S rDNA genotypes

Antifungal Agents	MIC	genotype A	genotype B	genotype C
Amphotericin B	MIC range (ug/mL)	0.125-1	0.25-1	0.125-1
	GM MIC (ug/mL)	0.70	0.76	0.70
	MIC ₅₀	1	1	1
	MIC ₉₀	1	1	1
5-Fluorocytosine	MIC range (ug/mL)	<0.125->64	<0.125-1	<0.125-0.5
	GM MIC (ug/mL)	1.22	0.17	0.17
	MIC ₅₀	2	0.125	0.125
	MIC ₉₀	4	0.5	0.5
Fluconazole	MIC range (ug/mL)	0.06->64	0.125->64	0.25->64
	GM MIC (ug/mL)	1.25	1.06	2.00
	MIC ₅₀	1	0.5	2
	MIC ₉₀	8	16	64
Itraconazole	MIC range (ug/mL)	<0.015->8	0.03->8	0.03->8
	GM MIC (ug/mL)	0.30	0.21	0.50
	MIC ₅₀	0.25	0.125	0.25
	MIC ₉₀	2	8	8

Table 4. Mortality of mice infected with *C. albicans* strains.

Group	Strain	CAI genotype	Source	Mice	Mortality 10 d	Mortality 21 d	Mortality 5 m
1	GYF006	30-45	VVC	12	2 (16.6%)	6 (50.0%)	11 (91.6%)
2	GYZ249	21-21	Lung cancer	12	5 (41.6%)	9 (75.0%)	9 (75.0%)
3	ATCC 10231	Untested	Bronchopneumonia	12	3 (25.0%)	7 (58.3%)	9 (75.0%)
4	GYWu27	21-21	HIV/AIDS	12	3 (25.0%)	7 (58.3%)	12 (100.0%)
5	GYWu45	21-21	HIV/AIDS	12	2 (16.6%)	8 (66.7%)	12 (100.0%)
6	GYF76	32-46	VVC	12	1 (8.3%)	6 (50.0%)	11 (91.6%)
7	GYWu20	21-33	HIV/AIDS	12	3 (25%)	7 (58.3%)	12 (100.0%)
8	GYF091	30-46	VVC	12	2 (16.6%)	6 (50.0%)	12 (100.0%)
9	GYZ210	21-33	Lung cancer	12	4 (33.3%)	8 (66.7%)	11 (91.6%)
10	GYZ072	21-33	Lung cancer	12	5 (41.6%)	6 (50.0%)	11 (91.6%)
11	BK60	Untested	Normal	12	2 (16.6%)	5 (41.6%)	8 (66.7%)
12	S165	Untested	Normal	12	2 (16.6%)	6 (50.0%)	10 (83.3%)
13	1107	Untested	Normal	12	1 (8.3%)	4 (33.3%)	8 (66.7%)
14	Saline	–	–	12	0 (0.0%)	0 (0.0%)	0 (0.0%)