White-opaque Switching in Different Mating Type-like Locus Gene Types of Clinical Candida albicans Isolates

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Abstract

Background: Candida albicans (C. albicans) can become a pathogen causing superficial as well as life-threatening systemic infections, especially in immunocompromised patients. Many phenotypic attributes contribute to its capacity to colonize human organs. In our study, 93 C. albicans isolates from patients of various candidiasis in a hospital of China were surveyed. We aimed to investigate the white-opaque (WO) switching competence, drug sensitivity, and virulence of mating type-like (MTL) a/α isolates.

Methods: Internal transcribed spacer (ITS) gene and the MTL configuration were detected in all the isolates by reverse transcription-polymerase chain reaction. White/opaque phenotype and doubling time of cell growth were determined. The minimum inhibitory concentrations of antifungal agent were measured using broth microdilution method.

Results: Sixty-four isolates (69.6%) were classified to serotype A, 19 (20.6%) to serotype B, and 9 (9.8%) to serotype C. Moreover, phylogenetic analysis showed that these isolates were divided into four different subgroups of ITS genotypes. Most of our clinical isolates were MTLα/α type, while 6.8% remained MTLα or MTLα type. The frequency of opaque phenotype was 71.0% (66 isolates). Following the guidelines of Clinical and Laboratory Standards Institute M27-A3, all isolates were susceptible to caspofungin and a few (0.6–3.2%) of them showed resistance against amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole.

Conclusions: From these analyses, there were comparatively more C. albicans strains classified into serotype B, and the frequency of opaque phase strains was significant in the clinical isolates from China. Genetic, phenotypic, or drug susceptibility patterns were not significantly different from previous studies. MTLα/α isolates could also undergo WO switching which facilitates their survival.

Key words: Candida albicans; Drug Susceptibility; Genotype; Mating Type-like; White-opaque

INTRODUCTION

Candida albicans (C. albicans) is a commensal microorganism living on the gastrointestinal and urogenital mucosa in healthy individuals.[1] However, it can become a pathogen causing superficial as well as life-threatening systemic infections, especially in immunocompromised patients.[1] Many phenotypic attributes, such as the yeast and filamentous forms, contribute to its capacity to colonize all body organs virtually.[2,3] C. albicans is once considered to be an imperfectible fungal species, lacking a sexual cycle. However, this paradigm was challenged when mating type-like (MTL) loci, MTLα and MTLα, were identified, which is orthologous of MATα and MATα in Saccharomyces cerevisiae.[4]

In 1987, a spontaneously and reversibly switch termed white-opaque (WO) switching from the normal, round-to-oval yeast form to an elongate cell form was found in C. albicans WO regulator-1 strain.[5] All strains undergoing WO switching were considered to be homozygous at the MTL locus.[6] The homeodomain protein MTLa1-α2 complex represses WO switching in MTLα/α cells.[7,8] This is why only about 3% of naturally occurring strains are homozygous at the MTL locus, and most clinical isolates produced white colonies.[6] Recent studies also found that these two cell
types had different pathogenic traits. In 2013, Xie et al. discovered that a number of natural $MTL\alpha$ strains were capable of WO switching under condition mimicking aspects of the host environment.

In this context, we surveyed the microbiological characteristics including genetic, phenotypic, and drug susceptibility patterns of 93 $C.\text{albicans}$ isolates from patients in a hospital of China and found some intriguing phenomenon of natural strains.

**Methods**

**Fungal isolates**

Ninety-three clinical $C.\text{albicans}$ strains isolated from candidiasis patients in Peking University First Hospital were used in this study. Isolates were collected from vagina (67, 72.0%), glans penis (16, 17.2%), skin (5, 5.4%), and oral cavity (5, 5.4%). All the isolates were from immunocompetent patients and were sampled by swap. These clinical isolates were purified to single colony on yeast peptone dextrose agar (PDA, Becton, Dickinson and Company, USA) slants and incubated at 25°C overnight. The experiments were done in Medical Mycology Research Center, Chiba University, Japan (as IFM 61638–61730). When these clinical isolates were purified, we isolated two colonies LH770-1 and LH770-2 which have different colony sizes compared with the LH770 strain.

The standard strain $Candida\ parapsilosis$ (American Type Culture Collection [ATCC] 22019), $Candida\ krusei$ (ATCC 6258), and $C.\text{albicans}$ (ATCC 90028) were included in antifungal susceptibility assays as quality control. The isolates were streaked onto potato dextrose agar (PDA, Becton, Dickinson and Company, USA) slants and incubated at 25°C overnight. The experiments were done in Medical Mycology Research Center, Chiba University, Japan.

**Genotyping**

Primers CA-INT-L and CA-INT-R were used for serotype determination of $C.\text{albicans}$ on the basis of 25S rDNA. Internal transcribed spacer (ITS) gene was detected in these isolates. ITS5 (forward) and ITS4 (reverse) primers were used to amplify the ITS1, 5.8S, and ITS2 regions according to the results of reverse transcription-polymerase chain reaction (RT-PCR).

**Analysis of the mating type-like configuration**

The $MTL$ configuration (heterozygous or homozygous) was determined by RT-PCR using primers specific for $MTLa$ and $MTL\alpha$. RT-PCR reactions were carried out on Bio-Rad T100 thermocycler (Bio-Rad, USA) using a standard program: 94°C incubation 5 min, and then 35 cycles of 94°C for 60 s, 55°C for 60 s, 72°C for 60 s, followed by a final extension step at 72°C for 10 min. Primer sequences were as follows: $MTLa1$ forward, 5'-TTGAGGCTGAGGCGAGGCAG-3' and $MTLa1$ reverse, 5'-GATTAGGGCTGGTTCTTCTCG-3'; and $MTL\alpha2$ forward, 5'-CATGATACGTAGTTGAGGCAC-3' and $MTL\alpha2$ reverse, 5'-AAGCAGCCAAACTCAGTAC-3'.

**Determination of white/opaque phenotype**

Yeast peptone dextrose agar (YPD, 1% yeast extract, 2% peptone, 2% dextrose, and 2% agar) supplemented with 50 μg/ml phloxine B (Wako Pure Chemical Industries, Ltd., Japan) was used for white/opaque phenotype detection. Cells were streaked onto YPD-phloxine B plates and incubated at room temperature for 2 weeks. The phenotype of colonies was observed under a stereoscopic dissecting microscope (Leica M125, Leica, Germany), and the phenotype of cells was observed under a scanning electron microscope (JSM-7200F, JEOL, Japan).

**Determination of doubling time of cell growth**

Cells were inoculated into liquid YPD medium and incubated overnight at 25°C at 20 ×g. Culture was diluted by 200 folds to 5 ml of fresh liquid YPD medium, and growth at 25°C at 20 ×g was automatically recorded as $A_{600nm}$ using the TVS062CA Bio-photorrecorder (Advantec, Tokyo, Japan).

**Fluorescence-activated cell sorting analysis**

Strain was precultured in liquid YPD medium at 25°C, and a stationary phase culture was diluted 100 folds to 10 ml of a new YPD. Cell suspension was diluted 10 folds to normal saline buffer containing 10 μg/ml of propidium iodide (Wako Pure Chemical Industries, Japan) and 1 mg/ml of RNase (Wako Pure Chemical Industries, Japan) and incubated at 37°C for 2 h. Stained cells were diluted 10 folds to sterilized distilled water and applied to a flow cytometry, On-chip Sort (FISHMAN, On-Chip Biotechnologies, Japan) according to the procedure manuals.

**Antifungal susceptibility**

The minimum inhibitory concentrations (MICs) of antifungal agents; amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, and caspofungin against the tested strains were measured using broth microdilution methods following the guidelines of the Clinical and Laboratory Standards Institute M27-A3. Microtiter plates (Dry Plate; Eiken Chemical Co., Ltd., Japan) were used in the assay. $C.\ parapsilosis$ (ATCC 22019), $C.\ krusei$ (ATCC 6258), and $C.\text{albicans}$ (ATCC 90028) were used as controls.

**Statistical analysis**

Statistical analysis was performed using Excel 2010 software (Microsoft Corporation, USA). Student's $t$-test was applied in the analysis of the correlation between WO switching and cellular growth rates. A value of $P < 0.05$ was considered statistically significant.

**Results**

**Genotyping**

In 93 isolates, 64 isolates (68.8%) were classified into serotype A, 19 (20.4%) to serotype B, and 10 (10.8%) to serotype C [Tables 1 and 2]. This result showed no significant difference in the genotyping with previous studies with the other sources of $C.\text{albicans}$ isolates. It was also showed that 70.0% of isolates from oral cavity and 50.0% of isolates from sputum were serotype B [Table 2], which were higher than
Table 1: Summarized information of the source, MTL type, ABC serotype, and phenotype of *Candida albicans* isolates

| Strain | No. | Source                  | MTL type | ABC genotype | ITS phylogenetic analysis | White/opaque phenotype |
|--------|-----|-------------------------|----------|---------------|---------------------------|------------------------|
| LH495  | 61638 | Glans penis discharge | a/α      | C             | 3                         | 100% opaque phenotype  |
| LH496  | 61639 | Vaginal discharge       | a/a      | A             | 1                         | 98% pink               |
| LH498  | 61640 | Vaginal discharge       | a/a      | A             | 1                         | 84% pink               |
| LH502  | 61641 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH521  | 61642 | Vaginal discharge       | a/a      | A             | 1                         | White                  |
| LH527  | 61643 | Vaginal discharge       | a/a      | A             | 1                         | Almost 100% pink       |
| LH529  | 61644 | Vaginal discharge       | a/a      | A             | 1                         | Almost 100% pink       |
| LH532  | 61645 | Vaginal discharge       | a/a      | A             | 1                         | White                  |
| LH533  | 61646 | Vaginal discharge       | a/α      | A             | 1                         | White                  |
| LH534  | 61647 | Sputum                  | a/a      | A             | 3                         | Pink                   |
| LH537  | 61648 | Sputum                  | a/α      | B             | –                         | Pink                   |
| LH538  | 61649 | Sputum                  | a/a      | B             | 2                         | Few pink, under 50%    |
| LH544  | 61650 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH565  | 61651 | Sputum                  | a/a      | B             | 1                         | Pink                   |
| LH566  | 61652 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH567  | 61653 | Vaginal discharge       | a/a      | B             | 3                         | Few pink, under 50%    |
| LH568  | 61654 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH569  | 61655 | Vaginal discharge       | a/a      | A             | 1                         | 21.6% pink             |
| LH570  | 61656 | Vaginal discharge       | a/α      | A             | 1                         | Few pink, under 50%    |
| LH573  | 61657 | Vaginal discharge       | a/α      | A             | 1                         | Few pink, under 50%    |
| LH574  | 61658 | Vaginal discharge       | a/a      | A             | 1                         | White                  |
| LH575  | 61659 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH576  | 61660 | Vaginal discharge       | a/a      | B             | 3                         | Few pink, under 50%    |
| LH577  | 61661 | Vaginal discharge       | a/a      | C             | 1                         | White                  |
| LH602  | 61662 | Vaginal discharge       | a/a      | A             | 1                         | 78% pink               |
| LH603  | 61663 | Vaginal discharge       | a/a      | A             | 1                         | White                  |
| LH605  | 61664 | Vaginal discharge       | a/a      | A             | 1                         | White                  |
| LH606  | 61665 | Vaginal discharge       | a/a      | A             | 1                         | White                  |
| LH607  | 61666 | Vaginal discharge       | a        | A             | 1                         | 46.8% pink             |
| LH613  | 61667 | Unknown                 | a/a      | A             | 1                         | White                  |
| LH623  | 61668 | Sputum                  | a/a      | A             | 1                         | 13.7% pink             |
| LH685  | 61669 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH729  | 61670 | Vaginal discharge       | a/a      | B             | 3                         | White                  |
| LH730  | 61671 | Vaginal discharge       | a/a      | A             | 1                         | White                  |
| LH731  | 61672 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH732  | 61673 | Oral cavity             | a/a      | B             | 1                         | White                  |
| LH735  | 61674 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH737  | 61675 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH739  | 61676 | Unknown                 | a/a      | A             | 3                         | White                  |
| LH740  | 61677 | Oral cavity             | a/a      | B             | 1                         | Few pink, under 50%    |
| LH742  | 61678 | Glans penis discharge   | a        | B             | 4                         | Few pink, under 50%    |
| LH743  | 61679 | Unknown                 | a/a      | A             | 2                         | 55.8% pink             |
| LH744  | 61680 | Unknown                 | a/a      | C             | 3                         | Few pink, under 50%    |
| LH549  | 61681 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH610  | 61682 | Vaginal discharge       | a/a      | A             | 1                         | White                  |
| LH734  | 61683 | Vaginal discharge       | a/a      | A             | 1                         | Few pink, under 50%    |
| LH738  | 61684 | Vaginal discharge       | a/a      | A             | 3                         | White                  |
| LH745  | 61685 | Unknown                 | a/a      | A             | 1                         | White                  |
| LH746  | 61686 | Onychomycosis           | a/a      | A             | 1                         | Few pink, under 50%    |
| LH747  | 61687 | Unknown                 | a/a      | C             | 3                         | White                  |
| LH748  | 61688 | Sputum                  | a/a      | C             | 3                         | White                  |
| LH750  | 61689 | Tinea corporis          | a/a      | A             | 1                         | 45.4% pink             |
| LH751  | 61690 | Unknown                 | a/α      | A             | 3                         | White                  |

Contd...
11.1% of isolates from vaginal discharge. Phylogenetic analyses divided the 91 clinical isolates from China into four different subgroups. In 52 isolates from vaginal discharge, most were subgroup I (86.5%), seven isolates (13.5%) were subgroup III, and no strain was subgroup II or IV [Table 3]. From other sources, strains of subgroups I, II, III, and IV could be isolated although there was no strain of subgroup III from oral cavity.

**Analysis of the mating type-like configuration**

MTL analysis showed that 86 strains (92.5%) were a/α type, while only seven strains (6.5%) were considered to be aa or αα type [Table 1]. We discovered that minimal a/a or α/α single colonies can be isolated from a/α isolates. As shown in Figure 1, from LH770 a/α isolates, we isolated single colonies LH770-1 and LH770-2. The LH770-1 remained a/α mating type, but LH770-2 was α/α mating type. This demonstrates that a/α clinical isolate may transform to a/a or α/α strain in host as well as in nature.

**Determination of white/opaque phenotype**

WO phenotype in these isolates was also observed by phloxine B staining. Cells from white isolates were smaller in size and had a smooth surface and while cells from pink isolates were larger in size and had a rough surface [Figure 2], which

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**Table 1: Contd...**

| Strain | No. | Source | **MTL** type | ABC genotype | **ITS phylogenetic analysis** | White/opaque phenotype |
|--------|-----|--------|--------------|--------------|-----------------------------|------------------------|
| LH752  | 61691| Vaginal discharge  | a/α | A | 1 | Few pink, under 50% |
| LH753  | 61692| Oral cavity | a/α | A | 3 | White |
| LH756  | 61693| Vaginal discharge | a/α | A | 1 | 14.5% |
| LH759  | 61694| Unknown | a/α | A | 1 | Few pink, under 50% |
| LH760  | 61695| Onychomycosis | a/α | A | 1 | 98% pink |
| LH761  | 61696| Vaginal discharge | a/α | A | 1 | 99% pink |
| LH766  | 61697| Vaginal discharge | a/α | A | 1 | White |
| LH767  | 61698| Vaginal discharge | a/α | A | 1 | Few pink, under 50% |
| LH768  | 61699| Vaginal discharge | a/α | A | 1 | All white |
| LH769  | 61700| Glans penis discharge | a/α | C | 3 | All white |
| LH770-1 | 61701| Vaginal discharge | a/α | C | – | 100% pink |
| LH770-2 | 61702| Vaginal discharge | a | C | – | Few pink, under 50% |
| LH771  | 61703| Vaginal discharge | a/α | A | 1 | Few pink, under 50% |
| LH772  | 61704| Glans penis discharge | a/α | A | 3 | Few pink, under 50% |
| LH773  | 61705| Vaginal discharge | a | A | 1 | Few pink, under 50% |
| LH775  | 61706| Vaginal discharge | a/α | B | 1 | White |
| LH776  | 61707| Vaginal discharge | a/α | A | 2 | Almost 100% pink |
| LH777  | 61708| Vaginal discharge | a/α | A | 1 | Few pink, under 50% |
| LH778  | 61709| Unknown | a | A | 1 | 38.8% pink |
| LH791  | 61710| Vaginal discharge | a/α | A | 3 | White |
| LH795  | 61711| Vaginal discharge | a/α | A | 3 | White |
| LH804  | 61712| Vaginal discharge | a/α | A | 1 | White |
| LH805  | 61713| Vaginal discharge | a/α | B | 1 | Almost 100% pink |
| LH806  | 61714| Vaginal discharge | a/α | A | 1 | Few pink, under 50% |
| LH807  | 61715| Vaginal discharge | a/α | A | 4 | Few pink, under 50% |
| LH808  | 61716| Vaginal discharge | a/α | B | 1 | White |
| LH852  | 61717| Sputum | a/α | A | 1 | Few pink, under 50% |
| LH855  | 61718| Sputum | a/α | B | 3 | Few pink, under 50% |
| LH856  | 61719| Sputum | a/α | B | 3 | White |
| LH857  | 61720| Oral cavity (AIDS) | a/α | B | 3 | White |
| LH858  | 61721| Sputum | a/α | A | 1 | White |
| LH864  | 61722| Glans penis discharge | a/α | A | 1 | Few pink, under 50% |
| LH865  | 61723| Oral cavity (AIDS) | a/α | B | 4 | Few pink, under 50% |
| LH866  | 61724| Oral cavity (AIDS) | a/α | B | 3 | Few pink, under 50% |
| LH867  | 61725| Oral cavity (AIDS) | a/α | C | 1 | Almost 100% pink |
| LH868  | 61726| Blood culture | a | A | 1 | Almost 100% pink |
| LH869  | 61727| Oral cavity (AIDS) | a/α | B | 1 | All white |
| LH871  | 61728| Urine culture | a/α | B | 2 | All white |
| LH874  | 61729| Oral cavity | a/α | B | 3 | Almost 100% pink |
| LH875  | 61730| Oral cavity (AIDS) | a/α | C | 3 | All white |

—: Not applicable; **MTL**: Mating type-like; **ITS**: Internal transcribed spacer; **AIDS**: Acquired immune deficiency syndrome.
we identified as opaque phase. In 93 isolates, about 29.0% isolates (27 strains) showed all white colonies on the solid medium and the remaining isolates (71.0%, 66 isolates) showed some pink colonies on the solid medium [Tables 1 and 4]. In these 66 strains producing opaque colony, 59 strains were heterozygous at the mating-type locus. We determined MTL DNA sequences of 10 MTLa/α and WO switchable strains and found that the three strains (498, 874, and 805 strains) had a point mutagenesis in their MTLa1 sequence. DNA sequence changed thymine to cytosine at the 139 position and amino acid sequence changed serine to lysine [Figure 3].

**Correlation between white-opaque switching and cellular growth rates or chromosomal ploidy**

WO switching has been previously described to be controlled by mating-type locus homeodomain proteins, to be induced by N-acetyl glucosamine, 5% CO₂, or to be related with cell growth. In the 59 WO switchable and a/α strains, the switching was neither rigidly related with MTL homozygotes nor induced by N-acetyl glucosamine and CO₂. Growth

| Source of isolates | ABC genotypes, n (%) | Total, n |
|--------------------|----------------------|---------|
|                    | A        | B        | C        |         |
| All isolates       | 64 (68.8)| 19 (20.4)| 10 (10.8)| 93      |
| Oral cavity        | 1        | 7        | 2        | 10      |
| Sputum             | 4        | 5        | 1        | 10      |
| Vaginal discharge  | 45 (83.3)| 6 (11.1) | 3 (5.6)  | 54      |
| Others             | 14       | 1        | 4        | 19      |

**Table 3: Distribution of Candida albicans isolates in four phylogenetic groups**

| Source of isolates | ITS Subgroups, n (%) | Total, n |
|--------------------|----------------------|---------|
|                    | I        | II       | III      | IV       |         |
| All isolates       | 61 (67.8)| 4 (4.4)  | 22 (24.4)| 3 (3.3)  | 90      |
| Oral cavity        | 4        | 0        | 5        | 1        | 10      |
| Sputum             | 3        | 1        | 4        | 1        | 9       |
| Vaginal discharge  | 45 (86.5)| 0        | 7 (13.5) | 0        | 52      |
| Others             | 9        | 3        | 6        | 1        | 19      |

*ITS: Internal transcribed spacer.

**Table 4: White-opaque phenotype in Candida albicans isolates from different sources**

| Source of isolates | Phenytoypes, n (%) | Total |
|--------------------|--------------------|-------|
|                    | Only white | White-opaque |     |
| All isolates       | 27 (29.0) | 66 (71.0) | 93   |
| Oral cavity        | 6        | 4        | 10   |
| Sputum             | 3        | 7        | 10   |
| Vaginal discharge  | 11 (32.1)| 43 (67.9)| 54   |
| Others             | 7        | 12       | 19   |

Figure 1: Mating type-like locus gene type of Candida albicans: (a) Type (heterozygous or homozygous) was determined by reverse transcription-polymerase chain reaction using primers specific for MTLa and MTLα. (b) We isolated different single colonies from LH770 a/α isolate. LH770-1 remained a/α mating type and LH770-2 was α/α mating type. M: Molecular marker. MTL: Mating type-like.

Figure 2: Determination of white/opaque phenotype of Candida albicans. Phenotypes were stained with phloxine B. (a) Stereomicroscope image showed all white phenotypes. (b and c) Stereomicroscope images showed white-opaque phenotype. (d) Stereomicroscope image showed all opaque phenotypes. (e and f) Scanning electron microscope images showed that white cells have smaller size and smooth surface. (g and h) Scanning electron microscope images showed that opaque cells have larger size and rough surface. Original magnification: a–d, ×8; e–h, ×20,000.
We discovered that the LH770 (a/α) strain produced the colony MTLα/α (770-2). This led to the hypothesis that some WO switchable and a/α strains occurred parasexual reproduction and changed from diploid to tetraploid. Fluorescence-activated cell sorting analysis using DNA propidium iodide staining was conducted for a sample of 45 WO transition strains and 9 white phase strains picked out in a random manner. From the pattern of fluorescence intensity, we classified three groups; pattern I, pattern II, and pattern III [Figure 4]. Strains of the pattern I containing fifty strains were diploid. In the pattern II or pattern III, fluorescence intensity was lower or higher than that of the Pattern I, and strains classified into these patterns seemed to be an irregular ploidy.

**Antifungal susceptibility and resistance**

The 93 isolates were tested for antifungal susceptibilities [Table 5]. All were susceptible to micafungin with MIC between 0.015–0.250 µg/ml. The MIC of amphotericin B against *C. albicans* was 0.250–4.000 mg/ml. Most of the isolates were susceptible with only four isolates (4.3%) resistant to this medicine. The MIC of flucytosine was between 0.125 and 64.000 mg/ml. Two isolates were resistance to this antifungal drug and three isolates showed intermediate with MIC, higher than susceptible and lower than resistance. In our study, the MICs of miconazole, fluconazole, itraconazole, and voriconazole were between 0.030–4.000, 0.125–64.000, 0.015–4.000, and 0.015–0.500 mg/ml, respectively. Most of the isolates showed high susceptibility to these drugs. In the commonly used azole drugs such as fluconazole, itraconazole, and voriconazole, two isolates and one isolate showed resistance to fluconazole and itraconazole, respectively, but all isolates were susceptible to voriconazole.

**Discussion**

In recent years, the sexual mating and WO switching of *C. albicans* have aroused a great interest. In this context, we compared the genetic and phenotypic heterogeneities of clinical strains isolated from China and previously described articles. It is previously considered
that before mating, *C. albicans* strains must first undergo homoyzogosis in *MTL* allele, and then switch from white to opaque. White cells are round or oval which form smooth colonies that hardly be dyed by phloxine B, while opaque cells are long shaped which form rough colonies that can be dyed into pink by phloxine B. In our research, we found a higher frequency of *a/α* opaque phenotype [71.0%, 66 isolates, Table 4]. Therefore, we hypothesize that even *a/α* isolates can also switch to opaque phenotype and mate in *in vivo* host environment. Of course, in natural *a/α* strains, they have been induced to WO switching by using N-acetyl glucosamine as the sole carbon source and incubation in 5% CO₂. However, in our experiments, these isolates could have WO transition when they grew onYPD solid medium at room temperature. Pendrak et al.[15] found that decreased expression of hemoglobin response gene 1 (*HBR1*) has been shown to alter the expression of the *MTL*. Our finding suggested that *a/α* cells may mate in *in vivo* which coincides with the hypothesis of Pendrak et al.[15] Moreover, we found a point mutagenesis in *MTLα1* DNA sequences of *MTLα/α* WO-switchable strains. Some mutations like this might have effect on WO transition.

Currently, the most effective drugs for *C. albicans* include azoles and echinocandins.[12] However, repeated exposure to triazole drugs is a major risk factor for drug resistance, and *in vitro* resistance to fluconazole and itraconazole in *C. albicans* had been reported. Opaque cells reported to be more resistance to amphotericin B, nystatin, 5-fluorocytosine, and miconazole nitrate than white cells.[16] In our results, fluconazole and itraconazole resistance ratio was under 3%. No voriconazole resistance was seen. This may be related to the duration the antifungal drug has been available clinically. We detected the susceptibility of micafungin and found no resistance. Moreover, most of the clinical isolates were sensitive to amphotericin B with 4.3% resistance ratio. Although an appearance of WO switching was remarkable in the clinical isolates, we could not find a correlation between WO switching and resistance to amphotericin B, 5-fluorocytosine, and miconazole nitrate.

There was no significant difference between *ITS* sequence and ABC typing in previous studies as well as the other sources of *C. albicans* isolates.[13] However, variation of genotype was significantly observed in isolates from oral cavity or sputum against isolates from vaginal discharge in our results [Tables 2 and 3]. High frequency of *a/α* opaque phenotype was not associated with the variation of genotypes. We will clarify why the isolates from China occurred significantly WO transition. Furthermore, the mechanism of WO switching in *MTLα/α* isolates has not been clearly explained yet, although stress-activated protein kinase pathway and *WOR1* gene have been reported to play roles in the process of switching.[17] Further investigations are still needed.

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Conflicts of interest
There are no conflicts of interest.

Table 5: Antifungal susceptibility of *Candida albicans* isolates, *n* = 93

| Antifungals          | MIC (mg/ml) | Susceptible, *n* (%) | Susceptible-dose dependent, *n* (%) | Intermediate, *n* (%) | Resistant, *n* (%) | Nonsusceptible, *n* (%) |
|----------------------|-------------|----------------------|-------------------------------------|-----------------------|---------------------|-------------------------|
| Amphotericin B       | 0.250–4.000 | 89 (95.7)            | –                                   | –                     | 4 (4.3)             | –                       |
| Fluconazole          | 0.125–64.000| 88 (94.6)            | –                                   | 3 (3.2)               | 2 (2.2)             | 0                       |
| Micafungin           | 0.015–0.250 | 93 (100)             | –                                   | 2 (2.2)               | –                   | –                       |
| Fluconazole          | 0.125–64.000| 90 (96.7)            | 1 (1.1)                             | –                     | 1 (1.1)             | –                       |
| Itraconazole         | 0.015–4.000 | 89 (95.7)            | 3 (3.2)                             | –                     | 0                   | –                       |
| Voriconazole         | 0.015–0.500 | 93 (100)             | 0                                   | –                     | –                   | –                       |

*: Not applicable; MIC: Minimum inhibitory concentration.

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