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Brief Report

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# High variability within *Candida albicans* transcription factor *RLM1*: Isolates from vulvovaginal infections show a clear bias toward high molecular weight alleles

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## Abstract

Previous studies have correlated the severity of recurrent vulvovaginal *Candida* infections (VVC) and balanitis in patients from China with the presence of some dominant genotypes at the ORF *RLM1*. Here we tested VVC vs non-VVC isolates from Portugal, Brazil and Greece and, although the same genotypes were identified in VVC isolates, they were present in only five out of 150 strains. However, this analysis showed that VVC isolates presented a higher percentage of genotypes with similar high molecular weight alleles, in comparison with strains isolated from other biological sources.

**Key words:** *Candida albicans*, *RLM1*, vulvovaginal candidiasis, alleles, stress resistance.

Vulvovaginal candidiasis (VVC) is the second most common gynecologic infection and affects over 75% of women. It is known that about 40–50% of these women experience a recurrence, and up to 5% suffer more than four episodes during 1 year.[1,2] VVC decreases women's life quality, and the high cost of constant medical visits stimulates the use of un-prescribed therapies, which may increase antifungal resistance. The commensal yeast *Candida albicans* is the most common etiological agent of VVC, being responsible for 70–80% of all cases.[3] The success of this pathogenic yeast depends on their dynamic interactions with the competitive microbiota and with the host that enable it to adjust to a changing environment. Microorganisms developed molecular mechanisms for increasing genetic variations, such as the addition or deletion of repeat units through slipped-strand mispairing or gene conversion, in loci that are involved in critical interaction with the host.[4] The genome of the human pathogen *C. albicans* contains approximately 2600 repeat-containing open reading frames (ORFs), three and 10 times more, respectively, than those of the ascomycete yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.[5,6] To date, only a few of these genes have been

characterized, including *RLM1* that encodes for a transcription factor important in the cell wall integrity (CWI) pathway. Rlm1p presents great variability at its C-terminus, conferred by the CAI microsatellite with around 35 different alleles identified.[7,8] More than 70% of the CAI alleles identified previously had between 17 and 28 (CAA/G) repetitions, being the most frequent the alleles with 21 and 25 repetitions.[7,8] Curiously, several studies identified specific CAI genotypes in Chinese isolates from vulvovaginal infections and balanitis that were correlated with severe recurrent infection.[9–12] These genotypes were composed of alleles with 30, 32, 36, 45, 46, and 47 (CAA/G) repetitions, high molecular weight alleles. Phenotypic analysis of strains harboring alleles with higher number of (CAA/G) repetitions, higher than 30, showed that they displayed higher tolerance to combined cell wall and oxidative stress[8] and a high ability to undergo phenotypic switching.[13] This information suggested that these alleles, with higher number of repetitions, may be correlate with the strain capacity to resist and rapidly adjust to this particular niche. In this context, we addressed the question if these genotypes were also present in *C. albicans* strains from VVC infection from other geographic regions, in comparison with isolated from other biological sources.

A total of 329 *C. albicans* strains from Portugal, Greece, and Brazil of different body locations, including 150 strains from vulvovaginal infections and 179 strains from other different sources (79 from the oral cavity, 27 form the urine, 43 from the respiratory tract, and 30 from blood cultures) were genotyped in order to identify the alleles from ORF *RLM1* by amplifying CAI microsatellite. Strain SC5314 (ATCC MYA-2876) was used to correlate genotypes. Yeast cells were grown at 30°C for 48 hours on YPD-agar medium and CAI amplification was performed by colony PCR as previously described,[14] using the primers reported by Sampaio et al.[7] Automatic allele size determination was carried out in an ABI310 Genetic Analyser (Applied Biosystems Inc., Foster City, California), using the GeneScan 3.5 Analysis Software. Allelic and genotypic frequencies, as well as genetic and genotypic population differentiation tests were performed using GENEPOP (version 4.0.7).[15]

Genotyping of all 329 *C. albicans* isolates within ORF *RLM1* identified a total of 39 alleles with fragments varying from 183 bp (11 CAA/G repeat units) to 306 bp (52 repeat units), four new alleles than those reported previously. The most frequent CAI genotype was 21–25 (35 strains, 10.6%), followed by 21–22, 21–26, and 25-25 (17 strains, 4.0%). Once more, the most frequent alleles were the ones that presented between 17 and 28 repetitions. In order to search for the VVCs specific CAI genotypes described by Li et al.[9] in our analysis, we first correlated and adjusted the molecular weight of both alleles from strain SC5314 presented in Li et al.[9] with the GeneScan values obtained in our study for the same strain. This correlation showed that the molecular weights obtained were identical so, all other alleles could also be directly correlated.

Li et al.[9] described that *C. albicans* strains isolated from vulvovaginal candidiasis (VVC) and balanitis showed mainly four CAI genotypes (30-45, 32–46, 30–36, and 30–47), when compared with strains isolated from asymptomatic women. Our analysis showed that only five out of 150 strains, all isolated from VVC, presented the VVCs specific Chinese genotypes, namely two strains with the genotype 30–45, two strains with the genotype 30–47 and one strain with the genotype 32–46. Since this was a very low frequency in comparison with the Chinese studies, we decided to compare the VVC CAI genotypes with the ones obtained in the non-VVC strains. The differentiation tests, concerning allelic/genotypic distribution between the group of VVC isolates and the group of non-VVC isolates showed highly significant differences ($P < .0001$), both in the genetic and genotypic distribution. In order to identify these differences, CAI genotype frequencies of VVC versus non-VVC strains were visually compared (Fig. 1). This analysis showed a clear bias toward high molecular weight alleles in strains from VVCs in comparison with strains from other biological sources. If we consider the frequency of strains presenting genotypes with one allele with 30 or more repetitions (molecular weight of 240 bp or higher) the VVCs group has 46.7%, while the other group has only 20.1% ($\chi^2 = 16.3618$, $P = .00052$). Furthermore, if we consider both alleles with 30 or more repetitions a higher difference is observed, the VVCs group having 21.3%, while to the other group belonging only 4.5% of the strains ($\chi^2 = 11.1272$, $P = .00851$).

In the present study we gathered VVC and non-VVC isolates from Brazil, Portugal, and Greece to test for the CAI genotypes that were previously correlated with the severity of vulvovaginal candidiasis and balanitis in the Chinese population. However, those specific genotypes were found only in 3.33% of strains from VVCs. What we observed was that the VVC isolates presented a bias in CAI genotypes with higher molecular weight alleles, such as 46–48, 47-47 or 49–52, similar to the Chinese genotypes.[9,10]

Our previous studies demonstrated a relationship between genotype and phenotype at ORF *RLM1*, in which strains with higher molecular weight alleles presented a higher resistance to combined stress conditions including lower pH and ROS (Reactive Oxygen Species) inducing agents.[8] Recently, a correlation between higher white–opaque switching in strains with longer CAA/G repeats in both CAI alleles was also identified.[13] Additionally, we have also demonstrated that *RLM1* is involved in *C. albicans* cell wall biogenesis and its deletion greatly reduces

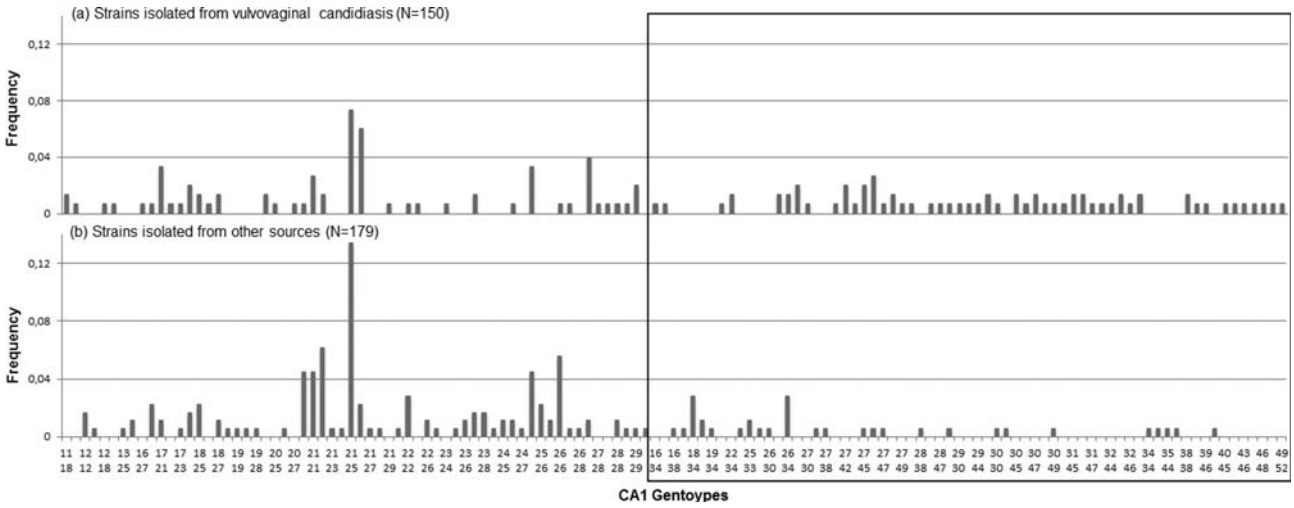


**Figure 1.** Genotypic frequencies based on CAI microsatellite analysis of *C. albicans* strains from (**a**) vulvovaginal infections and (**b**) from nonvaginal sources. Highlighted with the square are the genotypes with higher molecular weight.

the yeast virulence in the murine model disseminated infection.[16] In this view, and considering that the vagina has a characteristic environment, with a low pH, which is known to induce phenotypic switching, and specific competing microbiota that secretes cell wall damaging factors, the higher molecular weight CAI alleles in ORF *RLM1*, may confer a higher fitness to this environment however, further studies are needed.

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### Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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