

## Is human Dectin-1 Y238X gene polymorphism related to susceptibility to recurrent vulvovaginal candidiasis?

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### Abstract

**Background and Purpose:** Vulvovaginal candidiasis is a frequent disease affecting approximately more than %75 of all childbearing women at least once in their lifetime by overgrowth of opportunistic *Candida* species. Recurrent vulvovaginal candidiasis (RVVC) is common in otherwise healthy individuals. Several risk factors were reported to contribute to RVVC susceptibility. A polymorphism in Dectin-1 (Y238X, rs16910526) was identified in patients with RVVC and hypothesized that genetic factors play an important role in susceptibility to RVVC. Herein, we aimed to survey the polymorphisms in the Dectin-1 gene, linked to susceptibility to RVVC.

**Materials and Methods:** In the current study, blood samples were obtained from 25 patients who had frequent vulvovaginal candidiasis relapses and were diagnosed as RVVC. In addition, blood cultures were obtained from control group comprising of healthy individuals (n=25) with no history of RVVC, vaginal discharge, or itching on the day of examination. Dectin-1 Y238X gene polymorphism was investigated using Bi-PASA and DNA sequencing.

**Results:** The analysis revealed that all of the patients were wild-type homozygous for Dectin-1 Y238X polymorphisms. None of the individuals showed heterozygous or mutant homozygous Dectin-1 polymorphism.

**Conclusion:** No significant correlations were observed between the susceptibility to RVVC and Dectin-1 Y238X polymorphism in the Iranian population, which was not previously studied.

**Keywords:** *Candida* species, Dectin-1 Y238X gene polymorphism, RVVC

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### Introduction

Vulvovaginal candidiasis (VVC) is a frequent disease affecting approximately more than 75% of all childbearing women at least once in their lifetime by overgrowth of opportunistic *Candida* species. This condition is characterized by itching, soreness, bright red rash, and heavy white vaginal discharge [1-4]. It is usually caused by *Candida albicans*, but other species, i.e., *Candida glabrata*, *Candida tropicalis*, and *Candida krusei*, may also be involved [5, 6]. However, recurrent vulvovaginal candidiasis (RVVC) characterized by at least four times infections within a year has had a modest increase in the frequency of attacks due to non-albicans species reaching approximately 10% to 15% [2-4]. It is more common in otherwise healthy individuals. Several risk factors, i.e., prolonged use of antibiotics, pregnancy, intensive immunosuppressive therapy,

diabetes mellitus ketoacidosis, corticosteroid abusers, and stress were reported to contribute to RVVC susceptibility.

However, the majority of patients with RVVC were healthy individuals, suggesting that a genetic component most likely plays an important role in their susceptibility [2, 7]. Several studies proposed that patients with genetic polymorphism and familial history have increased susceptibility to *Candida* colonization, which leads to RVVC [7]. Mannose,  $\beta$ -glucans, and chitin are the main carbohydrates at the surface of cell walls of *Candida* species, which bind to the pattern recognition receptors (PRRs) that are expressed by the innate immune cells as myeloid and epithelial cells.

The first step in the initiation of an immune response is the recognition of conserved structures

of the pathogen (PAMPs) by PRRs [2, 3, 7, 8]. PRRs have several families, e.g., Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), and RIG-I helicases [8]. TLRs and C-type lectin receptors are the main recognition receptors for *C. albicans* [3].

Among the PRRs, C-type lectin receptor Dectin-1 recognizes  $\beta$ -glucans on the cell wall. Ferwerda et al. reported an important link between Dectin-1 Y238X polymorphism and increased predisposition to fungal infections [9]. A polymorphism in the gene for human Dectin-1, Y238X, which results in an early stop codon and leads to abrogated Dectin-1 expression, has been identified in patients with RVVC [7, 10]. In 2009, Plantinga et al. showed that a polymorphism in Dectin-1 (Y238X, rs 16910526) was responsible for recurrent mucocutaneous fungal infections in a Dutch family. This polymorphism resulted in an early stop codon, which led to the loss of the last 10 amino acids of the extracellular domain and a diminished capacity to bind to  $\beta$ -glucans [11].

Studies on Dectin-1 deficient mice demonstrated Dectin-1's protective role during infection with *C. albicans*, *Aspergillus fumigatus*, and *Pneumocystis jirovecii* [10]. Therefore, the aim of the current study was to investigate the impact of single nucleotide polymorphisms (SNPs) in the genes coding Dectin-1 (Y238X) on susceptibility to RVVC in the Iranian population.

## Materials and Methods

### *Patients and control samplings*

One-hundred vaginal swab samples were obtained from patients with history of vaginal discharge. VVC and RVVC were diagnosed based on their clinical manifestation and mycological investigations as previously described [12]. Twenty-five patients who had frequent VVC relapses and were diagnosed as RVVC were involved in this experiment. The control group consisted of healthy individuals (n=25) without any history of RVVC, vaginal discharge, or itching on the day of examination. Blood samples were obtained from the participants and transferred into sterile tubes with sodium-EDTA (0.5 mmol/l) to investigate Dectin-1 Y238X gene polymorphism. We collected the subjects' personal information such as age, pregnancy, delivery type, contraception methods (coitus interruptus, condom, intra-uterine device [IUD], and combined oral contraceptives), broad antibiotic use, chronic disorders, history of familial diabetes mellitus, and RVVC. This study

was approved by the Ethics Committee (nr. 566/92) of Mazandaran University of Medical Sciences, Sari, Iran, and written informed consent was obtained from the patient's next of kin for publication of this report.

### *Genotyping of the SNPs*

Genomic DNA was extracted from peripheral venous blood samples using the YZol pure genomic DNA kit (YTA, Y9204, IR) according to the manufacturer's instructions. DNA extracts were stored at  $-80^{\circ}\text{C}$  prior to use. Genotyping was adjusted using bidirectional polymerase chain reaction (PCR) amplification of specific alleles (Bi-PASA), as previously described by Carvalho et al. [13, 14]. The PCR primers used were as follows: P1 (5'GTAGTCCCAGCTACTTGAGG3'), P2 (5'ACCACTTGAGATTCACAACA3'), P3 (5'ggcggcggggAGTGTGCCCTCATAT3'), and P4 (5'ggcggcggggTTCTTCTCACAATACTC3').

PCR reactions were performed on a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA) in 50  $\mu\text{l}$  volumes containing 25 ng of template DNA, 5  $\mu\text{l}$  of reaction buffer (0.1 M Tris-HCl, pH 8.0, 0.5 M KCl, 15 mM  $\text{MgCl}_2$ , 0.1% gelatin, 1% Triton X-100), 0.2 mM of each dNTP, and 2.0 U Taq DNA polymerase (ITK Diagnostics, Leiden, the Netherlands). Amplification was performed with cycles of 5 min at  $94^{\circ}\text{C}$  for primary denaturation, followed by 35 cycles at  $94^{\circ}\text{C}$  (30 s),  $58^{\circ}\text{C}$  (30 s), and  $72^{\circ}\text{C}$  (45 s), with a final 5 min extension step at  $72^{\circ}\text{C}$ . PCR products were run on 3% agarose gel to confirm the amplification of the genetic locus. Gel was run at 110 V for 15 min and visualized on a UV transilluminator, and the results were compared between the two groups (patient and control) [13].

### *Sequence analysis*

Dectin-1 Y238X polymorphism region was amplified using forward (5-CCAAGAAAACCCA TCTCCAA-3) and reverse (5-CTCCTTCTCCAC CCTTCCTC-3) primers and subsequent sequencing was conducted as previously described [7]. Briefly, amplification was performed with cycles of 1 min at  $96^{\circ}\text{C}$  for primary denaturation, followed by 25 cycles at  $96^{\circ}\text{C}$  (15 s),  $50^{\circ}\text{C}$  (15 s), and  $60^{\circ}\text{C}$  (60 s), with a final 5 min extension step at  $72^{\circ}\text{C}$ . Amplification products were first run on 2.5% agarose gel and visualized with UV after ethidiumbromide staining and then subsequently were purified using GFX PCR DNA (GE Healthcare, Buckinghamshire, UK).

Sequencing was performed as follows:  $95^{\circ}\text{C}$  for 1 min, followed by 35 cycles consisting of  $95^{\circ}\text{C}$

for 10 s, 50°C for 5 s, and 60°C on an ABI 3730xl automatic sequencer (Applied Biosystems, Foster City, CA). The obtained sequence data were adjusted using Lasergene SeqMan software (DNASar, Madison, WI, USA) and the results were evaluated. Dectin-1 genotypes were grouped as wild-type (adenine, adenine), homozygous (cytosine, cytosine), and heterozygous (adenine, cytosine) as previously described [7].

### Statistical analysis

Statistical analysis was performed using SPSS, version 17. Categorical parameters were evaluated using Pearson's correlation coefficient, Chi-square, and Fisher's exact test. *P*-value less than 0.05 was considered statistically significant.

### Results

By use of two outer primers (P & Q) and two inner allele-specific primers (M & W), two or three overlapping fragments were amplified based on genotyping. PQ is always produced as a positive control. PW and MQ are present in a heterozygote individual, but PW is only produced in wild-type homozygote and MQ only in homozygous mutant samples (Table 1).

**Table 1.** Predicted band pattern obtained by Bi-PASA genotyping

	WT	HET	HOM mutant
PQ (675 bp)	—	—	—
PW (423 bp)	—	—	—
MQ (283 pb)	—	—	—

The age range of the experimental and control groups were 20-60 years and 19-58 years, respectively. There was no significant difference in the mean age between these groups ( $P=1$ ). In both groups, the number of cesarean sections was higher than vaginal deliveries. Remarkably, no underlying abnormalities were reported in neither groups. Most of the patients used contraceptives, e.g., coitus interrupts, condom, IUD, and combined oral contraceptives. Pruritus vulvae, pain, itching, and vaginal discharge were observed in the experimental group. Table 2 Summarizes the results of bidirectional PCR amplification of specific alleles (Bi-PASA) among the tested individuals.

Results revealed that 100% of the cases were

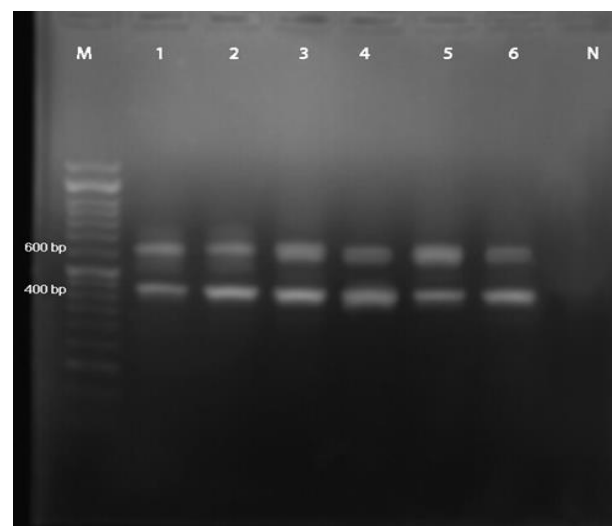
**Table 2.** Distribution of Dectin-1 genotypes

Polymorphism	Group	WT	HET	HOM	In HWE (yes/ no)	<i>P</i>
DECTIN-1 Tyr238Stop	Controls (N = 25)	25(100%)	0 (0%)	0 (0%)	Yes	1
	VVC/RVVC (N =25)	25 (100%)	0 (0%)	0 (0%)	Yes	1

Abbreviations: WT (wild type); HET (Heterozygous); HOM (Homozygous)

\*  $P<0.05$  were considered statistically significant

wild-type homozygous for Y238X polymorphism, and none of the individuals revealed a heterozygous Dectin-1 Y238X polymorphism (Figure 1). Randomly, five experimental groups were sequenced and the results were in concordance. These analyses showed that polymorphism in Dectin-1 Tyr238X, rs16910526 was observed in RVVC patients. When Dectin-1 genotypes were compared, there was no significant difference between the groups ( $P=1$ ; Table 2).



**Figure 1.** Patients' (1-3) and control groups' samples (4-6) M: marker, N: negative control

### Discussion

VVC is one of the most common forms of *Candida* infection that affects up to 75% of women at least once in their lifetime [4, 15]. It is mostly caused by *C. albicans*, but other species including *C. glabrata*, *C. tropicalis*, and *C. krusei* may also be involved. Despite the known risk factors (i.e., host status, diabetes, pregnancy, and immunosuppressive therapy), the majority of the patients are immune-competent individuals.

In the present investigation, we evaluated whether the genetic variation in genes coding (Dectin-1) PRRs involved in *Candida* recognition affects susceptibility to RVVC. This was the first attempt to study this issue in the Iranian population. Our results showed that no obvious polymorphism in Dectin-1 is associated with increased susceptibility to RVVC. Although Dectin-1 plays a significant role in *Candida* defense by TLR2 and

TLR4, which recognize mannan and mannose-binding receptors on the *Candida* cell wall, functional consequences of Dectin-1 deficiency were demonstrated to include impaired induction of both innate and adaptive Th17 immune responses [16]. Based on several reports, Dectin-1 polymorphism affects the immunological response and is a predisposing factor for *Candida* infections in rats. In addition, Dectin-1 plays a crucial role in production of pro-inflammatory cytokines with Toll-like receptors in animal experiments [17, 18].

Interestingly, the previous investigation showed that mutation in genes coding Dectin-1 in murine model causes an increased susceptibility to some opportunistic fungi, while they were not susceptible to candidiasis [19]. However, other researchers believed that individuals with this mutation are susceptible to chronic mucocutaneous candidiasis [9]. In another study, Plantinga et al. showed a link between candidemia and Dectin-1 Y238X polymorphism in cancer patients [11]. However, limited numbers of studies on the relationship between PRR defects and RVVC have demonstrated that genetic diversity in TLRs and CLRs can influence predisposition to RVVC [9, 20]. Moreover, Diana et al. in a study performed in 2014 showed that SNPs in PRRs (TLR1, TLR4, Dectin-1, and CARD9) did not affect susceptibility to RVVC; however, TLR2 increased approximately three times [3]. It seems that variation in TLR2 is caused by decreased induction of mucosal antifungal host defense. In the current study, the correlation between RVVC and mutation in Dectin-1 was investigated and heterozygous and homozygous Dectin-1 polymorphisms were not detected, which implies low incidence of Dectin-1 Y238X polymorphisms in the Iranian population. Nonetheless, it was not possible to correlate the presence of Dectin-1 Y238X polymorphism with RVVC etiology and history of familial RVVC.

## Conclusion

Other genetic mechanisms might play a role in the mucosal defense. In this study, only Dectin-1 Y238X polymorphisms were assessed, whereas the presence of other mutations and epigenetic factors might be responsible. Therefore, further clinical investigations with wide population screenings are required to elucidate the relationship between RVVC and Dectin-1 Y238X polymorphism.

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## Authors' contributions

N.Z. and H.B. designed and managed the study, contributed to data analysis, and wrote the main manuscript. N.Z., H.B., S.A.K., N.A., S.A., and S.M. set up the test and managed the research. Acquisition and interpretation of the data were performed by N.Z., S.A.K., N.A., S.A., and S.M. Critical revision was carried out by H.B.

## Conflicts of interest

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

## Financial disclosure

No financial interests related to the content of this manuscript are declared.

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