

## Relation between Recurrent Vulvovaginal Candidiasis, Vaginal Concentrations of Mannose-Binding Lectin, and a Mannose-Binding Lectin Gene Polymorphism in Latvian Women

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**Vaginal concentrations of mannose-binding lectin (MBL) and possession of a polymorphism in codon 54 of the MBL gene were determined in 42 women with recurrent vulvovaginal candidiasis (RVVC) and 43 control subjects. Reduced vaginal MBL levels and an increased occurrence of the polymorphism were present in women with RVVC.**

Mannose-binding lectin (MBL) is a circulating protein and a component of the innate immune defense system. It recognizes and binds to mannose and N-acetyl-glucosamine residues on the surface of microorganisms, resulting in complement activation. In addition, macrophages and dendritic cells contain cell surface receptors that recognize MBL, facilitating opsonization of microorganisms with bound MBL on their surface [1]. Reductions in serum levels of MBL have been associated with opsonization defects [2] and with an increased risk of recurrent infections in young children [3, 4].

The gene coding for MBL is polymorphic, and the decrease in circulating MBL concentrations has been shown to correlate with possession of mutant alleles in exon 1. Most studied is the single nucleotide substitution of an adenine for a guanine in codon 54 that results in replacement of aspartic acid for glycine in the MBL protein [1, 5–7]. As a consequence of this alteration, the assembly and stability of the final MBL protein

is markedly reduced [5, 7]. Serum levels of MBL are ~1.2–1.7  $\mu\text{g}/\text{mL}$  in individuals who are homozygous for the wild-type (guanine) allele, 0.3  $\mu\text{g}/\text{mL}$  in individuals who are heterozygous, and 10  $\text{ng}/\text{mL}$  in individuals homozygous for the variant allele (adenine) [6, 8]. The codon 54 substitution has also been shown to be associated with an increased rate of infection [9, 10].

*Candida* species exhibit strong binding of MBL [11], suggesting that this protein may be involved in immune defense against candidal infection. This prompted us to examine cervicovaginal levels of MBL and MBL genotypes in women with recurrent vulvovaginal candidiasis (RVVC). Women with RVVC experience frequent episodes of a vulvovaginal *Candida* infection, and the underlying mechanism responsible for this occurrence remains unknown in many cases.

**Methods.** The study population consisted of 42 women (age, 18–35 years) who had  $\geq 4$  culture-verified symptomatic episodes of a vulvovaginal *Candida* infection during a 12-month period and who currently had symptoms consistent with a vaginal *Candida* infection (i.e., pruritis, burning, and abnormal discharge) and a positive *Candida* culture. Control subjects were 43 women who had no current gynecologic complaints, who had no history of vaginal *Candida* infection, and who were currently culture-negative for pathogens. All patients and control subjects were seen at the outpatient obstetrics/gynecology department of Riga First Hospital in Riga, Latvia. Patients and control subjects were matched for age, socioeconomic and marital status, and number of children. All were ethnic Latvians. Exclusion criteria for this study were pregnancy, diabetes, known immunodeficiencies, use of immunosuppressive medications, or history of hysterectomy.

Informed consent was obtained from all subjects, and the guidelines for human experimentation of the US Department of Health and Human Services and Riga Stradin's University were followed in the conduct of clinical research.

Cervicovaginal samples were obtained by instilling 3 mL of sterile saline into the posterior vaginal fornix, mixing with a cotton swab, and then withdrawing the solution with a syringe. An aliquot of each sample was cultured for *Candida* species on Sabouraud's medium and speciated by culture on CHROM agar (CHROM Agar *Candida* Co.). Samples were also tested for *Trichomonas vaginalis*, by wet mount; *Chlamydia trachomatis*, by PCR; *Neisseria gonorrhoeae*, by culture; HIV, by antibody testing; and bacterial vaginosis, using the Amsel criteria [12]. The samples were centrifuged to obtain supernatant and pellet fractions that were immediately frozen at  $-20^{\circ}\text{C}$ . Frozen samples were shipped to the Division of Immunology and Infec-

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**Table 1. Demographic and clinical characteristics of patients with recurrent vulvovaginal candidiasis (RVVC) and control subjects.**

Characteristic	Patients with RVVC (n = 42)	Control subjects (n = 43)
Age, mean years (range)	26.8 (18–35)	25.4 (18–35)
University education	21 (50)	16 (37.2)
Salary <\$200 a month, % of subjects	38.1	60.5
Age at first sexual intercourse, mean years	19.6	18.4
Married	20 (47.6)	20 (46.5)
Smoker	12 (28.6)	9 (20.9)
Vaginal or vulvar erythema present	29 (69.0)	0
Abnormal vaginal discharge present	31 (73.8)	0
Vaginal fissures and/or excoriations present	24 (57.1)	0
Mean no. of pregnancies	2.4	2.3
Mean no. of children	1.1	1.0
History of spontaneous abortion	24 (57.1)	23 (53.5)
<i>Candida</i> infection		
<i>C. albicans</i>	38 (90.5)	4 (9.3)
<i>C. tropicalis</i>	3 (7.1)	0
<i>C. krusei</i>	1 (2.4)	0
Bacterial vaginosis	10 (23.8)	0

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated.

tious Diseases, Department of Obstetrics and Gynecology, Cornell (New York) on dry ice for analysis.

The vaginal concentrations of MBL were determined using a commercial ELISA (Cell Sciences) with a 1:5 dilution of the supernatant fractions. The lower limit of sensitivity was 3.1 ng/mL.

DNA was extracted from the pellet fractions as described elsewhere [13]. In brief, the pellets were thawed, washed, and resuspended in a 1% solution of the nonionic detergent, Brij 35 in Tris buffer containing 5 mg/mL proteinase K. Cells were lysed by incubation at 56°C for 60 min, and the proteinase K was then inactivated by increasing the temperature to 95°C for 10 min. The lysed samples were diluted 1:5 in 10 mmol/L Tris-HCl that contained 1.5 mmol/L of MgCl<sub>2</sub>; 50 mmol/L of KCl; 0.2 mmol/L each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and thymidine triphosphate; 1.25 units of Taq DNA polymerase; and 30 pmol of oligonucleotide primers that amplified the codon 54 polymorphic region of the MBL gene [14]. The final volume was 0.05 mL. Samples were incubated in a thermal cycler for 2 min at 94°C, followed by 35 cycles of 94°C for 50 s, 58°C for 1.5 min, and 72°C for 15 s. Lastly, the samples were incubated at 72°C for 5 min.

The PCR amplicons were then digested by incubation at 37°C for 18 h with Ban I endonuclease (New England BioLabs) in buffer provided by the manufacturer. Fragments were analyzed on 2% agarose gels and stained with ethidium bromide. Ban I digestion resulted in either formation of two 260 and 89 bp

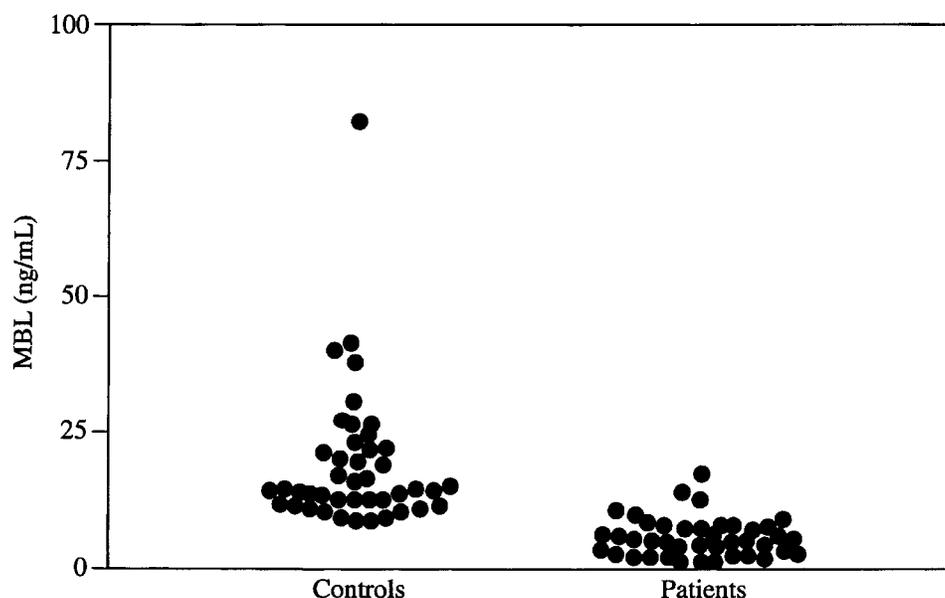
fractions (wild-type; allele A) or a single uncut 349 bp fraction (mutant; allele B).

The relationship between genotype frequencies and diagnosis was analyzed by Fisher's exact test. Differences in median MBL concentrations between patients and control subjects were analyzed using the Mann-Whitney *U* test. The relation between vaginal MBL levels and MBL genotype was analyzed by 1-way analysis of variance and Tukey-Kramer multiple comparisons test. *P* < .05 was considered to be statistically significant.

**Results.** Demographic and clinical characteristics of patients and control subjects are detailed in table 1. *Candida albicans* was detected in 38 (90.5%) of the patients with RVVC, *Candida tropicalis* was detected in 3 (7.1%), and *Candida krusei* was detected in 1 (2.4%). Among the control women, 4 cultures (9.3%) were positive for *C. albicans*. No other *Candida* species were detected in the control subjects. Ten patients with RVVC and none of the control subjects were positive for bacterial vaginosis. All patients and control subjects tested negative for *T. vaginalis*, *C. trachomatis*, *N. gonorrhoea*, and HIV.

The distribution of vaginal MBL protein concentrations in patients with RVVC and control subjects is shown in figure 1. The median MBL concentration in control subjects was 15.9 ng/mL (range, 10.1–84.1 ng/mL, compared with 7.3 ng/mL (range, 2.9–18.7 ng/mL) for women with RVVC (*P* < .0001).

The distribution of MBL alleles was significantly different in control subjects and patients with RVVC (*P* < .0001; table 2). More than 90% of the control subjects were homozygous for



**Figure 1.** Vaginal mannose-binding lectin (MBL) concentrations in women with recurrent vulvovaginal candidiasis (patients) and in control subjects (controls). MBL concentrations in vaginal lavage samples obtained from patients and controls were determined by ELISA.

the wild-type allele (allele A), as opposed to only 31% of patients with RVVC. Conversely, almost 62% of patients with RVVC were allele A/allele B heterozygotes, compared with only 9.3% of control subjects ( $P < .0001$ ). The frequency of allele B was 38.1% in patients with RVVC, as opposed to 4.7% in control subjects ( $P < .0001$ ). Sixty-eight percent of the patients who were culture-positive for *C. albicans* were either allele B heterozygotes or homozygotes, 100% of the women positive for *C. tropicalis* were allele B heterozygotes, and the single woman with *C. krusei* infection was allele A homozygous.

Although the number of subjects is small, there was no relationship between bacterial vaginosis and MBL genotype. Allele A homozygotes, allele A/B heterozygotes, and allele B homozygotes were present in 30%, 50%, and 20% of the patients with bacterial vaginosis, respectively.

The relationship between vaginal MBL concentrations and MBL genotypes is shown in figure 2. The median MBL level in women who were allele A homozygous was 15.6 ng/mL (range, 4.2–84.1 ng/mL). This was significantly greater than the median level of 7.6 ng/mL (range, 4.5–17.3 ng/mL) in the A/B heterozygotes and 2.9 ng/mL (range, 2.9–3.6 ng/mL) in the B/B homozygotes ( $P < .0001$ ).

**Discussion.** Among Latvian women, RVVC was highly associated with carriage of the mutant codon 54 allele of the polymorphic MBL gene. This allele was also associated with a reduction in the vaginal concentration of MBL. Thus, this MBL polymorphism appears to be one determinant of susceptibility to RVVC in this population. The distribution of the different MBL genotypes observed in the control group in the present

study paralleled the distributions noted by other investigators who studied white, Asian, and Eskimo populations [6, 8, 15]. This validates the reliability of our assay and suggests that the findings for these Latvian women may be applicable to other populations of women with RVVC.

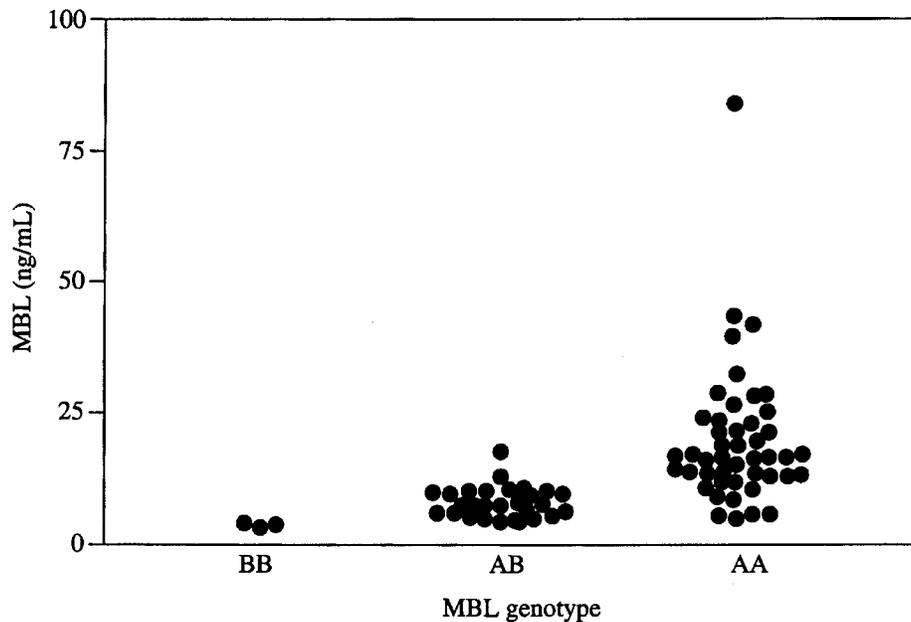
Asymptomatic colonization of the vagina with *Candida* species occurs in ~20%–30% of women of reproductive age [16]. In the present study, 9.3% of the control subjects had positive *C. albicans* culture results. The factors responsible for the conversion of commensal *Candida* into a symptomatic pathogen at this site remain incompletely understood [17]. Th1 lymphocyte-directed cell-mediated immunity is the major defense against *Candida* infections at mucosal sites [18], and IFN- $\gamma$ , a Th1 cytokine, inhibits *C. albicans* yeast cells from undergoing germ tube formation [19]. In healthy women, production of proinflammatory cytokines in response to *Candida* promotes

**Table 2.** Relation between mannose-binding lectin (MBL) gene polymorphism and recurrent vulvovaginal candidiasis (RVVC).

MBL genotype	No. (%) of subjects	
	RVVC group (n = 42)	Control group (n = 43)
AA	13 (31.0) <sup>a</sup>	39 (90.7)
AB	26 (61.9) <sup>b</sup>	4 (9.3)
BB	3 (7.1)	0 (0)

<sup>a</sup>  $P < .0001$ .

<sup>b</sup>  $P < .0001$ .



**Figure 2.** Relation between mannose-binding lectin (MBL) genotype and MBL concentrations in the vagina. A single base pair substitution in codon 54 of the MBL gene in women with recurrent vulvovaginal candidiasis and in control subjects was determined by PCR and endonuclease digestion using primer pairs that spanned the polymorphic region. Vaginal MBL levels in patients and control subjects were determined by ELISA.

the recruitment and activation of phagocytic cells that ingest *Candida* species. A variety of circumstances that result in production of anti-inflammatory Th2-derived cytokines and prostaglandin E<sub>2</sub> in the lower genital tract [20, 21] lead to a diminished capacity of the local cell-mediated immune system to regulate *Candida* proliferation and the subsequent development of clinical symptoms. RVVC results from a chronic or periodic inability to induce a sustained Th1 immune response against *Candida* species in the lower genital tract. If insufficient to effectively promote *Candida* opsonization by phagocytes, deficient vaginal levels of MBL may be a second overlapping mechanism contributing to vaginal *Candida* proliferation in women with RVVC. *C. albicans* was the major, but not the only, *Candida* species isolated from the patients with RVVC. The relative capacity of MBL to interact with these different species remains to be determined. The present study did not include women with a first episode of acute vulvovaginal candidiasis, so we are as yet unable to ascertain whether MBL deficiency is related solely to RVVC or is a general feature of symptomatic *Candida* vulvovaginal infection.

Similar to the situation in serum [6, 8], MBL concentrations in cervicovaginal fluids also appear to vary according to the individual's MBL genotype. Although a direct comparison was not attempted in the present study, it appears that the MBL concentration in the lower genital tract is ~80-fold less than the concentration in serum. Most likely, MBL, which is synthesized primarily in the liver, enters the female genital tract as a transudate from the systemic circulation. MBL production

by murine epithelial cells was recently reported elsewhere [22]. Whether vaginal epithelial cells contributed to the MBL values in the present study remains to be determined.

Not all women in the present study who had RVVC carried the codon 54 B allele, although the level of MBL in their vaginas was reduced. Polymorphisms at other exon 1 codons in the MBL gene have been identified and are also associated with reduced circulating MBL concentrations [5, 7, 14]. The relationship between variations at these other loci and RVVC remains to be determined.

It is interesting to speculate whether periodic administration of exogenous MBL or MBL-containing fluids would be an effective treatment to prevent the recurrence of *Candida* infection in the vagina in women who carry the B allele. The present investigation strongly suggests the potential usefulness of this approach. This would be an improvement over the repeated use of locally applied or systemic *Candida*-static drugs that are currently prescribed for women with RVVC. There are 2 reports on MBL infusions in humans with chronic diseases who were MBL deficient [23, 24]. No adverse effects of exogenous MBL administration were noted, and some clinical benefits were apparent.

## References

1. Babovic-Vuksanovic D, Snow K, Ten RM. Mannose-binding lectin (MBL) deficiency: variant alleles in a Midwestern population of the United States. *Ann Allergy Asthma Immunol* 1999;82:134-43.

2. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannose-binding protein with a common defect in opsonization. *Lancet* **1989**;2:1236–9.
3. Turner MW. Deficiency of mannose binding protein: a new complement deficiency syndrome. *Clin Exp Immunol* **1991**;86(Suppl 1):53–6.
4. Koch A, Melbye M, Sorensen P, et al. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. *JAMA* **2001**;285:1316–21.
5. Sumiya M, Super M, Tabona P, et al. Molecular basis of opsonic defect in immunodeficient children. *Lancet* **1991**;337:1569–70.
6. Garred P, Thiel S, Madsen HO, Ryder LP, Jensenius JC, Svejgaard A. Gene frequency and partial protein characterization of an allelic variant of mannan binding protein associated with low serum concentrations. *Clin Exp Immunol* **1992**;90:517–21.
7. Lipscombe RJ, Sumiya M, Summerfield JA, Turner MW. Distinct physiochemical characteristics of human mannose binding protein expressed by individuals of differing genotype. *Immunology* **1995**;85:660–7.
8. Lipscombe RJ, Sumiya M, Hill AV, et al. High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. *Hum Mol Genet* **1992**;1:709–15.
9. Garred P, Madsen HO, Hofmann B, Svejgaard A. Increased frequency of homozygosity of abnormal mannan-binding protein alleles in patients with suspected immunodeficiency. *Lancet* **1995**;346:941–3.
10. Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infections in consecutive hospital series. *BMJ* **1997**;314:1229–32.
11. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect Immun* **2000**;68:688–93.
12. Amsel R, Totten PA, Spiegel CA, et al. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am J Med* **1983**;74:14–22.
13. Genc MR, Gerber S, Nesin M, Witkin SS. Polymorphism in the interleukin-1 gene complex and spontaneous preterm delivery. *Am J Obstet Gynecol* **2002**;187:157–63.
14. Madsen HO, Garred P, Joergen A, et al. A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics* **1994**;40:37–44.
15. Garred P, Madsen HO, Kurtzhals JA, et al. Diallelic polymorphism may explain variations in blood concentration of mannan-binding protein in Eskimos, but not in black Africans. *Eur J Immunogenet* **1992**;19:403–12.
16. Giraldo P, Von Nowaskonski A, Gomes FA, Linhares I, Neves NA, Witkin SS. Vaginal colonization by *Candida* in asymptomatic women with and without a history of recurrent vulvovaginal candidiasis. *Obstet Gynecol* **2000**;95:413–6.
17. Sobel JD. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. *Am J Obstet Gynecol* **1985**;152:924–35.
18. Santoni G, Boccanera M, Adriani D, et al. Immune cell-mediated protection against vaginal candidiasis: evidence for a major role of vaginal CD4<sup>+</sup> T cells and possible participation of other local lymphocyte effectors. *Infect Immun* **2002**;70:4791–7.
19. Kalo-Klein A, Witkin SS. Prostaglandin E<sub>2</sub> enhances and gamma interferon inhibits germ tube formation in *Candida albicans*. *Infect Immun* **1990**;58:260–2.
20. Witkin SS, Giraldo PC, Linhares I. New insights into the immune pathogenesis of recurrent vulvovaginal candidiasis. *Int J Gynecol Obstet* **2000**;3:114–8.
21. Mardh P-A, Rodrigues AG, Genc M, Novikova N, Martinez-de-Oliveira J, Guaschino S. Facts and myths on recurrent vulvovaginal candidosis—a review on epidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. *Int J STD AIDS* **2002**;13:522–39.
22. Uemura K, Saka M, Nakagawa T, et al. L-MBP is expressed in epithelial cells of mouse small intestine. *J Immunol* **2002**;169:6945–50.
23. Valdimarsson H, Stefansson M, Vikingsdottir T, et al. Reconstitution of opsonizing activity by infusion of mannan-binding lectin (MBL) to MBL-deficient humans. *Scand J Immunol* **1998**;48:116–23.
24. Garred P, Pressler T, Lanng S, et al. Mannose-binding lectin (MBL) therapy in an MBL-deficient patient with severe cystic fibrosis lung disease. *Pediatr Pulmonol* **2002**;33:201–7.