

BASIC SCIENCE: GYNECOLOGY

Polymorphism in a gene coding for the inflammasome component NALP3 and recurrent vulvovaginal candidiasis in women with vulvar vestibulitis syndrome

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BACKGROUND AND OBJECTIVE

Vulvar vestibulitis syndrome (VVS) is an enigmatic disorder affecting up to 15% of women. The condition is defined as intense pain confined to the vaginal vestibule when distinct regions are touched with a cotton swab; varying degrees of vestibular erythema; pain upon attempted vaginal penetration with a tampon, during a gynecologic examination, and during sexual intercourse; no readily discernible etiology; and persistence of symptoms for ≥ 6 months.

Many VVS patients report an association between symptom initiation and a vaginal infection, predominantly *Candida albicans*. An increased susceptibility to infection coupled with a decreased capability to eliminate the resulting inflammation may result in the development of a chronic localized inflammatory state and increased contact sensitivity (hyperalgesia), perhaps due to alterations in the sensitivity and/or concentration of peripheral nerves.

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OVERVIEW

Recurrent vulvovaginal candidiasis is associated with a polymorphism in a gene associated with the generation and release of active interleukin-1 in women with vulvar vestibulitis.

Macromolecular structures called inflammasomes regulate the production and release of interleukin (IL)-1 β . A length polymorphism has recently been identified in intron 4 of the *CIAS1* gene that codes for the inflammasome component, NALP3.

Since a vaginal *Candida* infection is frequently reported by patients to be associated with development of their vestibular symptoms, we evaluated VVS patients and controls for the *CIAS1* length polymorphism and its possible relationship to a history of candidal infections. Decreased NALP3 production might result in a diminished capacity to generate biologically active IL-1 β .

MATERIALS AND METHODS

The study population consisted of 143 women with VVS. The control subjects were 182 sexually active women with no history of vulvodynia; they did not undergo a gynecologic examination. All subjects answered a detailed questionnaire in consultation with a gynecologist.

Epithelial cells were obtained from the buccal mucosa with a cotton swab after each subject had rinsed her mouth with water. Cellular DNA was obtained by lysis of the cells and analyzed for the *CIAS1* polymorphism. Allele 12 contained 720 base pairs; allele 9 had 594 base pairs; allele 7 had 520 base pairs; and allele 6 had 468 base pairs.

To verify that NALP3 is expressed in vestibular tissue of women with VVS, tissue specimens were collected from 9 VVS patients who underwent a vestibulectomy after failure of other treatment modalities.

Whole blood was obtained in heparinized tubes from a subset of VVS patients and the ex vivo production of IL-1 β in the presence or absence of *C albicans* was determined.

RESULTS

The 12,12 genotype was found in a higher percentage of controls (47.8%) than in VVS patients (35.7%; $P = .031$). Similarly, the allele 12 frequency was 63.2% in controls as opposed to 54.2% in VVS patients ($P = .024$). Conversely, allele 7 was present in 36.4% of VVS patients and in only 26.9% of controls ($P = .010$).

When the VVS patients were divided according to the self-reported event associated with the onset of their symptoms, the allele 7 frequency was greater than controls only in the 41 women whose VVS began following a vaginal infection ($P = .011$).

When the VVS patients were divided according to whether or not they had a self-reported history of recurrent vulvovaginal candidiasis (RVVC) (Table), the homozygous 7,7 genotype was identified in 30.6% of women with RVVC, 15.4% of patients with no RVVC history, and 15.9% of controls ($P = .048$ vs non-RVVC patients, $P = .025$ vs controls). Similarly, the allele 7 frequency was 43.9% in VVS patients with a history of RVVC, 30.8% in patients without this history, and 26.9% in controls ($P = .035$ vs non-RVVC patients, $P = .001$ vs controls).

TABLE
CIAS1 gene polymorphism and history of recurrent vulvovaginal candidiasis in women with vulvar vestibulitis syndrome

Genotype	No. (%) positive				
	Control N = 182	RVVC N = 49	No RVVC N = 91	Discharge N = 70	No discharge N = 69
12,12	87 (47.8)	18 (36.7)	33 (36.3)	30 (42.9)	21 (30.4)
12,7	35 (19.2)	11 (22.4)	27 (29.7)	19 (27.1)	19 (27.5)
12,9	18 (9.9)	2 (4.1)	13 (14.3)	3 (4.3)	11 (15.9)
7,7	29 (15.9)	15 (30.6) ^{a,b}	14 (15.4)	12 (17.1)	17 (24.6)
9,7	4 (2.2)	2 (4.1)	2 (2.2)	3 (4.3)	1 (1.4)
9,9	4 (2.2)	0	1 (1.1)	1 (1.4)	0
7,6	1 (0.5)	0	1 (1.1)	1 (1.4)	0
6,6	1 (0.5)	1 (2.0)	0	1 (1.4)	0
Allele 12	63.2%	50.0% ^c	58.2%	58.6%	52.2%
Allele 7	26.9%	43.9% ^{d,e}	30.8%	31.4%	38.4%

RVVC, recurrent vulvovaginal candidiasis.

^a $P = .025$ vs control subjects; odds ratio, 2.328; 95% confidence interval, 1.126-4.810; ^b $P = .048$ vs no RVVC; odds ratio, 2.426; 95% confidence interval, 1.055-5.581; ^c $P = .020$ vs control subjects; odds ratio, .582; 95% confidence interval, .371-.913; ^d $P = .001$ vs control subjects; odds ratio, 2.122; 95% confidence interval, 1.338-3.366; ^e $P = .035$ vs no RVVC; odds ratio, 1.759; 95% confidence interval, 1.058-2.924.

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Positive staining for NALP3 was found in the cells at the middle and upper layers of the nonkeratinizing stratified squamous epithelium of the vulvar vestibule, but not in the basal layer, in each subject. The staining was mostly membranous.

Whole blood cultures were performed on peripheral blood samples from 27 VVS patients; 20 were 12,12 homozygous and 7 were 7,7 homozygous. Median (range) IL-1 β production was 21.3 (5.2-87.2) pg/mL in the samples from 12,12 homozygotes as opposed to 15.7 (5.6-24.1) pg/mL in samples from the 7,7 homozygotes ($P = .030$).

COMMENT

Possession of allele 7, which contains 200 fewer base pairs than allele 12, may result in formation of an altered NALP3-containing inflammasome or an inflammasome with reduced biologic activity. In either situation, a consequence of this reduction in inflammasome function would be suboptimal production and release of active IL-1 β . The results of the ex

vivo incubations, demonstrating a reduction in *C albicans*-induced IL-1 β production from individuals carrying the 7,7 genotype, provide strong experimental support for this possibility. It is biologically plausible that recurrent symptomatic vulvovaginal *C albicans* infections would be most frequent in women who are positive for allele 7.

Identification of this protein in the vestibule reinforces the probability that the NALP3 inflammasome is involved in vulvovaginal immune defense and, furthermore, increases the likelihood that a functional polymorphism in the gene coding for NALP3 may influence the response to microorganisms at this site.

A limitation of the present investigation was our inability to independently verify the patients' self-reported history of RVVC. Nevertheless, even if the association is between the variant *CIAS1* allele 7 and a symptom that resembles a vulvovaginal infection, the involvement of this polymorphism in a condition that triggers VVS in a subpopulation of women is still evident.

The present results reinforce that there are subpopulations of VVS patients with distinct etiologies. Clinical trials of VVS treatments must include a genetic component. Otherwise, potentially effective responses in a subgroup of patients may be masked if the group is viewed only as a whole.

CLINICAL IMPLICATIONS

- Susceptibility to develop recurrent vulvovaginal candidiasis may result from the presence of a polymorphism in the gene coding for NALP3, a component of the intracytoplasmic structure (inflammasome) that processes the inactive precursor of interleukin-1 and allows its release from the cell.
- Development of recurrent vulvovaginal infections due to a polymorphism in a gene that influences the immune response to infection may predispose to vulvar vestibulitis syndrome (VVS).
- There is more than 1 etiology for VVS. ■