

ORIGINAL ARTICLE

Is non-response to fluconazole maintenance therapy for recurrent *Candida* vaginitis related to sensitization to atopic reactions?

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Problem: Is sensitization to atopic reaction related to treatment response of recurrent *Candida* vulvovaginal (RVVC)?

Method of the study: Analysis of ReCiDiF trial data of optimal (OR) and non-responders (NR) to fluconazole maintenance treatment, to explore medical history, physical status, family history, and vaginal immune response for potential sensitization to atopic reaction.

Results: Sociodemographic characteristics of 33 NR women were not different from 38 OR. NR had received higher number of different treatments (mean difference 1.6 different treatments (95% CI: 0.20-2.97), $P = .03$) and had more episodes of disease ($P < .05$). Multivariate regression analysis showed that family history of atopy (OR: 4.9, CI 95%: 1.1-22.2), duration of symptoms (OR: 1.2, CI 95%: 1.02-1.5), and vulvar excoriation (OR: 3.6, CI 95%: 1.4-9.3) were related to non-response. Vulvar excoriation at entry was the only statistically significant predictive factor for non-response in multivariate analysis with specificity 77.8% and sensitivity 51.6%.

Conclusion: Women with RVVC with vulvar excoriation, longer duration of disease, and family history of atopic disease are at increased risk not to respond to maintenance fluconazole treatment.

KEYWORDS

atopy, candidosis, fluconazole, maintenance therapy, recurrent vulvovaginal candidiasis, vulvar excoriation

1 | INTRODUCTION

Recurrent *Candida* vulvovaginitis (RVVC), defined as four or more *Candida* vulvovaginitis episodes per year, decreases quality of life, both physically and mentally.¹⁻³ Despite the recommended treatment of recurrent disease,^{4,5} alternatives are under research,⁶ because recurrences may occur after treatment⁷ and even during treatment.⁵ New genetic findings regarding differences between optimal and poor responders⁸ help to understand RVVC as a non-homogeneous disease and urges for pharmacogenetic research in identifying optimal responders. This is especially important to optimize rational use of antifungals and prevent resistance to

fluconazole. For researchers, it is also relevant to understand the pathogenesis of recurrent *Candida albicans* becoming less responsive to azoles and to develop new treatment strategies. Are genetic variations of the microorganism important to explain resistance to treatment,⁹ are host genetics responsible,⁸ or are both mechanisms important?

In a review, Sobel et al described that atopic disease can present as an allergic triad associated with RVVC: eczema, allergic rhinitis, and asthma.⁴ In addition, type I diabetes mellitus and use of corticosteroids, both linked to autoimmune phenomena, are recognized as risk factors. Autoimmune diseases and atopy are associated with T_{reg}-cell dysfunction, causing an overreaction to antigens.¹⁰

The presence of interleukin (IL)-8 was found to be positively associated with *Candida* infection and negatively to lactobacilli in HIV⁺ women.¹¹ Other researchers found associations between recurrent RVVC and sensitization patterns.¹² IL-1 β was found in higher and IL-1Ra in lower concentrations in the vaginal fluid of RVVC patients when compared to controls.¹³ This constellation of increased IL-1 β and decreased IL-1Ra is linked to a state of vaginal "hyperinflammation".¹⁴ On the other hand, a defect in the MYD88 gene causes an impaired immune response to stimulation with *C. albicans* antigens, characterized by a defective production of IL-17, a condition associated with early onset gastric cancer,¹⁵ but not with RVVC. These findings support the hypothesis that the symptoms of recurrent *Candida* are more likely due to a hypersensitivity reaction than due to immunosuppression.

Still, there is insufficient research disclosing the difference between optimal responders to treatment and non-responders. We previously found that genetic polymorphisms of mannose-binding lectin are more frequent in RVVC patients, but that the presence of this polymorphism is rather associated with better response to fluconazole treatment in these women, as compared to non-responders.⁸ Data from the ReCiDiF study were now analyzed in depth to explore other differences in host immunity between RVVC patients according to their response to fluconazole maintenance treatment.

2 | MATERIALS AND METHODS

We reviewed data on file of a prospective multicentric follow-up study performed in Belgium from May, 2002-August, 2004 (ReCiDiF trial). The entire protocol was reviewed and approved by the central Ethics Committee of the University of Leuven, Belgium (approval Nr. ML1860; file Nr. S19690), and by the local Ethics Committees of all participating centers. The intent of this study was to evaluate effectiveness of individualized decreasing fluconazole maintenance therapy to prevent relapses of *Candida* vulvovaginitis.⁵ After an induction dose of 600 mg fluconazole during the first week, women started maintenance therapy with 200 mg fluconazole weekly (for 2 months, first level), then 200 mg biweekly (for 4 months, second level), and 200 mg monthly (for 6 months, third level).⁵ An exhaustive set of potential risk factors was collected. At the end of the treatment, patients were divided into groups according to their response—optimal responders (no relapses, OR), suboptimal responders (one relapse in the same level, SR), and non-responders (two or more relapses in the same level, NR).⁵ This allowed avoiding bias of evaluation. The first and the last groups were compared in our secondary analysis.

2.1 | Patient characteristics

During treatment and follow-up, the RVVC patients were split into groups of "optimal responders," "suboptimal responders," and "non-responders".⁵ Secondary analysis of medical history, physical

examination, and family history was performed, trying to identify factors that could predict non-response toward fluconazole maintenance treatment. The severity of symptoms has been evaluated through the symptomatic score of Sobel,⁴ which was used in research worldwide.^{16,17} Vaginal (erythema and erosion) and vulvar symptoms (erythema, pruritus, excoriation, and edema) were evaluated according to semiquantitative severity scores from 1 to 4. Scores were evaluated on visual scale: 1 (absent), 2 (light), 3 (moderate), and 4 (severe) (1). A composite "severity score" was composed by adding the points obtained for all the items. The diagnosis of vulvovaginal candidosis was made by confirming positive wet mount microscopy finding (blastospores, pseudohyphae, or hyphae) and by positive vaginal culture and/or PCR demonstrating *Candida*.

2.2 | Wet mount microscopy

At every regular (n = 7) and unscheduled (n = variable) visits, a vaginal speculum examination was performed, and any abnormal signs and symptoms were systematically recorded in a semiquantitative way. From the upper vaginal vault, some fluid was taken with a wooden spatula and spread on three glass slides. Two slides were allowed to dry on the air, one for central laboratory confirmatory wet mount reading after rehydration with saline, and the other was used for Gram stain. The third slide was used for immediate reading after addition of a droplet of saline by the local investigator using a 400 times magnification phase contrast microscope. A scoring system was set up to quantify the lactobacillary grades, diagnose partial or full-blown bacterial vaginosis (BV), and (optional) scoring system for aerobic vaginitis (AV) according to a standardized methodology (Femicare).¹⁸ *Candida* hyphae, pseudohyphae, and blastospores were detected and recorded as positive, doubtful, or negative. Only unequivocally positive samples were included in the analysis.

2.3 | *Candida* confirmation

A double swab was taken from the lateral vaginal vault to culture *Candida* on a Saboroud medium, and for aerobic culture on chocolate agar. A separate dry swab was taken and frozen for later *Candida* PCR in case of positive microscopy and negative culture. Only women with confirmed *Candida*, that is, positive culture or with positive microscopy, or positive microscopy, negative culture and positive PCR at initial visit were included. Cultured *Candida* strains were kept at -80°C for later fluconazole sensitivity testing.

2.4 | Vaginal washing

At the first visit, a vaginal rinsing was performed with 2 mL of saline in a standardized manner as described elsewhere.¹⁴ Vaginal fluid was stored frozen at -80°C for later testing of cytokines and other markers of immune responses that might explain non-responsiveness to the proposed treatment regimen.

2.5 | Cytokine testing

After thawing, vaginal washes were centrifuged for 10 minutes at 647 g. Subsequently, the supernatants were measured for different cytokines (IL-1 β , TNF α , IL-22, IL17, IL-1 α , IL1Ra, IL-8, IL-6, and IFN γ (PeliKine Compact or R&D Systems)) following the manufactures standard protocols and using different dilutions.

2.6 | Statistical analysis

Analysis of the data was performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) with data reported as number (percentage) and mean $\pm$ SD. For analysis of differences between proportions, we used chi-square test or Fisher's exact test for small groups. *T* test for independent samples was used to compare means of group A and group D. Levene test for equality of variances was used to test equality between groups. Spearman's rho and Kendal τ_b were used to calculate correlation between items. Mann-Whitney U test was used to compare two groups. $P < .05$ was interpreted as statistically significant.

To delineate the relative significance of various factors (independent variables) on response to maintenance treatment to fluconazole (binary dependent variable), we built a multivariate logistic regression model.¹⁹ The primary null hypothesis we wanted to test was "the value of the β -coefficient for the various risk factors in the logistic model is zero or its exp(β) is 1.0." To obtain a valid estimate of the association between response to treatment and atopic disease, the analysis was adjusted for the confounding effects of symptoms (vulvar excoriation, eczema, allergy to medications), history of disease severity (symptom duration, family history of atopy) by including them as independent variables in the multivariate logistic regression analysis. Cytokine values have an exponential distribution, so their logarithm (ln) was calculated before introduction in the system. In the model building process, the significance of the independent variables was tested individually and in various combinations. Variables were tested for interaction, and they were analyzed using both nominal and continuous scales of measurement. The assumption of linearity of association was checked for the continuous independent variables using Box-Tidwell test. Cytokines were excluded from independent factor list because

did not meet the requirements. We performed multicollinearity test and found that VIF (variance inflation factor) for all variables was <3 . Cases with missing data were excluded from the multivariable analysis. The model that best predicted the odds of non-response to treatment with the lowest deviance and highest goodness of fit was selected. The Hosmer-Lemeshow test using the "deciles of risk" method was used to assess the goodness of fit.¹⁹

3 | RESULTS

A total 71 women were selected for secondary data analysis of the optimal responders ($n = 38$) versus the non-responders ($n = 33$). Forty-six women (suboptimal responders) were excluded from analysis. Patient's sociodemographic characteristics are presented in Table 1. No differences were observed in most characteristics between groups, but non-responders had more than 4 episodes of recurrence ($P = .02$), received higher number of different treatments (mean difference 1.6 different treatments (95% CI: 0.20-2.97), $P = .03$). Their severity score of clinical symptoms at entry visit of the study was higher insignificantly (mean difference 1.32 (95% CI: 0.05-2.58), $P = .08$). The severity scores of different symptoms were evaluated separately between the groups (Table 2). There was no difference between severity scores of all symptoms except one: The mean score depicting severity of vulvar excoriation was significantly higher in the non-responders than in the responding group (mean difference of score 0.64 ± 0.42). Women, who had a score of 3 on vulvar excoriation or more, had 4 times higher risk of not responding to therapy (OR = 3.99, 95% CI: 1.41-11.23, $P = ns$).

Patients were asked about their history of any disease which could indicate the presence of an (auto)immune or allergic disorders: eczema, allergic rhinitis, asthma, psoriasis, ulcerative colitis, Crohn's disease, autoimmune thyroiditis, rheumatoid arthritis, and known allergy to medicaments. Only few patients had rheumatoid arthritis ($n = 3$), ulcerative colitis ($n = 1$), Crohn's disease ($n = 1$), and none had autoimmune thyroiditis. Other conditions were more prevalent, like allergic rhinitis, allergy to medications, psoriasis, and asthma, but the prevalence of none of them differed between responders and non-responders (Table 3). However, non-responders were more likely to

TABLE 1 Sociodemographic characteristics of RVVC patients with optimal response to fluconazole maintenance therapy and respondents with failure to respond to this therapy

Characteristics	Responder (mean $\pm$ SD)	Non-responder (mean $\pm$ SD)	P value ^a
Age	31.9 $\pm$ 10.6	34.7 $\pm$ 10.6	.2
BMI	22.3 $\pm$ 3.3	22.9 $\pm$ 4.3	.8
Number of pregnancies	1.3 $\pm$ 1.5	1.5 $\pm$ 1.1	.2
Number of children	1.2 $\pm$ 1.2	1.5 $\pm$ 0.8	.1
Duration of symptoms, year	3.7 $\pm$ 3.5	6.5 $\pm$ 7.7	.2
Number of treatments before entry	4.5 $\pm$ 2.5	6.1 $\pm$ 3.3	.03
Debut of symptoms, age	28.2 $\pm$ 10.3	28.2 $\pm$ 8.4	1.0

Bold numbers indicate statistical significance ($P < .05$).

^aMann-Whitney U test.

TABLE 2 Symptom difference between responders and non-responders to maintenance therapy for RVVC

Characteristics	Responder (mean ± SD)	Non-responder (mean ± SD)	P value ^a
Vaginal erythema	3.0 ± 0.7	2.8 ± 0.6	.3
Vulvar erythema	3.1 ± 0.7	3.4 ± 0.6	.09
Vulvar pruritus	3.1 ± 0.8	3.1 ± 0.7	.8
Vulvar excoriation	1.8 ± 0.8	2.5 ± 1.0	.006
Vaginal erosion	1.2 ± 0.4	1.4 ± 0.6	.3
Vulvar edema	2.3 ± 0.8	2.5 ± 0.9	.3

Bold numbers indicate statistical significance ($P < .05$).

^aMann-Whitney U test.

suffer from eczema (OR: 3.6, 95% CI: 1.2-11.2, $P = .03$) and to have relatives who had atopic disease (OR: 5.8, 95% CI: 1.1-29.4, $P = .04$).

Vulvar excoriation was higher among patients with eczema (mean difference 1.03 (95% CI: 0.4-1.6), $P = .02$). However, in multivariate analysis, the risk for non-response to fluconazole maintenance therapy was related to vulvar excoriation (OR: 2.0, 95% CI: 1.1-3.6, $P = .03$), but not with eczema (OR: 0.3, 95% CI: 0.1-1.8, $P = \text{ns}$). Family history of atopic disease was not related with personal history of atopic disease (OR 0.4, 95% CI 0.1-1.2, $P = \text{ns}$), nor with eczema (OR: 0.8, 95% CI: 0.2-3.4, $P = \text{ns}$) or asthma (OR: 0.4, 95% CI: 0.1-1.1 $P = \text{ns}$). The number of RVVC episodes during the year was higher among patients with family (Kendal_tau 0.3, $P = 0.01$), but not personal (Kendal_tau 0.1, $P = \text{ns}$) history of atopy.

Allergy to medications was mostly to penicillin. One patient was allergic to miconazole cream (Daktarin[®]) and one to antitussive medications. There was a correlation between duration of symptoms and allergy to medications (Spearman's rho = $-.246$, $P = 0.04$), number of treatments (Spearman's rho = $.420$, $P < 0.01$). However, allergy to medication was not related to number of treatments (Spearman's rho = $.129$, $P = \text{ns}$). Recent antibiotic use was not associated with non-response to treatment (OR: 1.15 CI 95%: 0.36-3.73, $P = \text{ns}$).

The concentrations of cytokines measured in vaginal fluid in an effort to disclose differences of immune reaction between optimal responders and non-responders failed to show any statistically significant difference between groups (Table 4). However, level of 10 times diluted interferon (IFN)- γ was higher in non-response group (mean

TABLE 4 Means difference of cytokines in vaginal fluid between optimal responders and non-responders

Vaginal fluid cytokine	Responder (mean ± SD)	Non-responder (mean ± SD)	P value
IL-1 β	248.4 ± 375.5	189.1 ± 256.6	.45
IL-6	34.7 ± 68.4	50.6 ± 111.9	.48
TNF- α	67.7 ± 88.6	57.8 ± 96.2	.66
IL-17	42.2 ± 42.4	38.2 ± 43.8	.70
IL-22	96.4 ± 80.2	90.8 ± 83.7	.78
IFN- γ	54.7 ± 61.3	46.9 ± 44.5	.55
IL-8	1785.7 ± 1886.8	3360.4 ± 6655.3	.20
IL-Ra	186 063.7 ± 21 2321.3	131 166.7 ± 130 608.9	.20
IL-1 α	287.7 ± 445.7	325.2 ± 499.6	.74
IL-1 β /IL-Ra ratio	0.003 ± 0.006	0.002 ± 0.002	.26

difference -0.1 (95% CI: -0.3 to 0.2), $P = .03$). Level of IFN- γ did not correlate with vulvar excoriation (Spearman's rho = $.08$, $P = .5$), number of treatments (Spearman's rho = $.1$, $P = \text{ns}$).

In order to assess the independence of the associations between the response to treatment (dependent variable) and risk factors (independent variables), binary regression analysis was carried out. All values with P value lower than $.2$ in univariate analysis were selected for fitting into multivariate logistic regression. From this analysis, it was found that women with vulvar excoriation (OR 3.6, 95% CI 1.4-9.3, $P = .01$), longer duration of symptoms (OR: 1.2, 95% CI: 1.02-1.5, $P = .03$), family history of atopic disease (OR: 4.9, 95% CI: 1.1-22.2, $P = .04$), and no allergic reaction to medication (OR: 13.8 CI: 95% 1.1-166.7, $P = .04$) had a higher probability not to respond to fluconazole maintenance therapy (Table 5).

Receiver operating characteristic curve analysis for the prediction model combining non-respondents to the maintenance treatment, on the presence of vulvar excoriations, duration of symptoms, number of episodes, and no allergy to medication was performed. The cutoff points for the clinical severity score of vulvar excoriation were more than 1 with a sensitivity of 77.4% and specificity of 36.1% and more than 2 with a sensitivity of 51.6% and specificity of 77.8% (ROC area

Characteristic	Responder n (%)	Non-responder n (%)	OR (CI 95%)	P value (Fisher's exact)
Family history atopy	6/36 (9.0)	13/21 (19.4)	3.6 (1.2-11.2)	.03
Eczema	2/38 (2.8)	8/33 (11.8)	5.8 (1.1-29.4)	.04
Asthma	3/38 (4.2)	2/33 (2.8)	0.8 (0.1-4.8)	1.0
Hay fever	4/38 (5.6)	3/33 (4.2)	0.9 (0.2-4.1)	1.0
Allergy to medications	6/38 (8.2)	1/33 (1.4)	6.0 (0.7-52.7)	.1
Autoimmune disease	2/38 (14.1)	3/33 (9.9)	1.8 (0.3-11.5)	.7
Psoriasis	2/38 (2.7)	1/35 (1.4)	0.6 (0.05-6.5)	1.0

Bold numbers indicate statistical significance ($P < .05$).

TABLE 3 Relation between autoimmune diseases and response to treatment in RVVC patients on maintenance therapy

TABLE 5 Multivariate logistic regression model assessing independency for risk factors for non-response to maintenance therapy with fluconazole in RVVC patients

Risk factors	Odds ratio (95% confidence intervals)	P value
Eczema	0.8 (0.1-6.2)	.8
No allergy to medication	13.8 (1.1-166.7)	.04
IFN- γ ($\times 10$)	2.1 (0.7-6.7)	.2
Vulvar excoriation	3.6 (1.4-9.3)	.01
Atopic disease in family	4.9 (1.1-22.2)	.04
Episodes per year	1.6 (0.9-2.6)	.09
Duration of symptoms	1.2 (1.02-1.5)	.03

Bold numbers indicate statistical significance ($P < .05$).

under the curve 0.7 (CI 95%: 0.5-0.8, $P = .02$). Family history of atopic disease (ROC area under the curve 0.6 (CI 95%: 0.4-0.8, $P = .08$) and duration of symptoms (ROC area under the curve 0.6 (CI 95%: 0.4-0.7, $P = ns$) was statistically insignificant in the analysis (Figure 1).

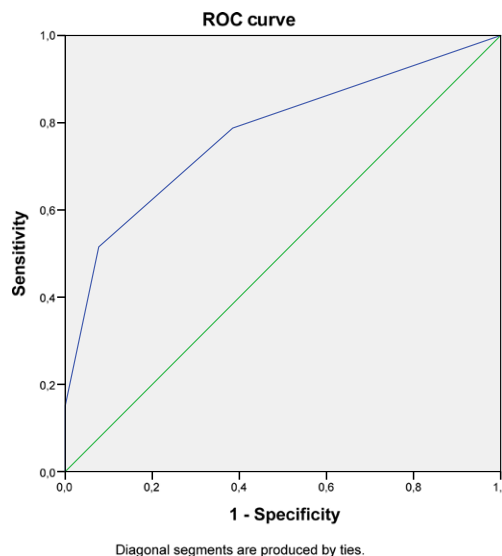
4 | DISCUSSION

4.1 | Main findings

This study provides evidence that the presence of vulvar excoriation as a clinical sign has a prognostic value for non-response to long-term fluconazole treatment in univariate as well as multivariate analysis. The results go in line with previous scientific research, indicating vulvar dermatosis as risk factor for recurrences in RVVC patients.⁴ Although it is not sure that the clinical finding of excoriations on itself is ethio-pathophysiologically related to lower response to therapy, its presence should increase the clinician's awareness, implicating consideration of more intense, combined or alternative treatment. Furthermore, circumstantial evidence calls in family history of atopic disease as a risk factor as well. Cytokine concentrations analysis in vaginal fluid, on the other hand, failed to show any statistically significant differences between optimal responders and non-responders.

4.2 | Strengths and limitations

Our study is one of the few comparing different groups of responders to RVVC therapy. Together with the findings of genetic variation of the innate immune system (8), it could be confirmed that response to the maintenance treatment could also be predicted observing an exuberant host response toward infection, such as the presence of excoriation of the vulva. These findings are especially important for future research analyzing the pathogenesis and treatment of non-response to fluconazole maintenance treatment of RVVC. The study has few limitations. Firstly, we did not analyze immune-active substances associated with desquamation (such as Kallikrein-related (KLK) peptidase). However, our cytokine analysis showed no difference between mean concentration of cytokines in the

**FIGURE 1** Receiver operating characteristic curve analysis for the prediction model combining non-respondents to the maintenance treatment, on the presence of vulvar excoriations

vagina and response to treatment in multivariate analysis. Secondly, we did not perform skin tests to assess allergic reactions, IgE, IL-4 IL-13, nor did we analyze IL-9, suggestive for delayed immune response.²⁰ Vulvar excoriation was subjectively measured. However, our research reveals that the presence of any vulvar excoriation is a risk factor for non-response. Previous research on genetic variation between optimal responders and non-responders was found as important,⁸ so the link with family history of atopy should be analyzed in further genetic research. As is a post hoc study, and the data were not powered for this particular analysis, we did not perform genetic analysis to identify the association between genetic conditions and risk to non-response.

4.3 | Interpretation

An unequivocal answer to explain the lack of association between personal history of atopy and non-response we do not have, but most likely one part of the answer is that our study population is too young (medium age 28 years) to express all the genetic predispositions for atopy and autoimmune diseases. Hence, family history of atopy and autoimmune diseases is present, but demonstration of own disease not yet. In previous studies, more than two-thirds of patients with inhalation allergy had RVVC.^{12,21} However, both researches recruited patients in allergy clinics, while the prevalence of asthma was only 7.0% and allergic rhinitis 9.9% in our sample, which was recruited in an outpatient gynecology setting. Allergic rhinitis or asthma was not related to non-response to fluconazole therapy, but cases were too few. Total prevalence of atopic diseases all together was 25.4%. Although eczema was related to non-response to treatment in univariate analysis, it was not retained as a significant predictive factor in our multivariate logistic regression model. Becker and colleagues found that patients with chronic mucocutaneous candidosis and patients with hyper-IgE syndrome (HIES, with eczema symptoms) both

have deficient cytokine IL-9 production, suggesting T helper (Th)17 activity may be involved.²⁰ However, patients suffering from mucocutaneous *Candida* have functional Th2 defect,²⁰ while HIES patients have a normal Th2 cytokine production, making it unlikely that the same pathogenesis is involved in both situations. IgE sensitization to *Candida* was observed in small number of patients with RVVC,^{12,21} but identification of IgE against *Candida* in blood did not answer to the question why symptomatic *Candida* vaginitis recurred.²² Several genetic mutations with different phenotypes were discovered both manifesting with RVVC and autoimmune diseases.^{23,24} Further research, including precise genetic testing, and testing for IgE, IL4, and IL13 could be interesting to further clarify the link between *Candida* atopy and non-response to therapy.

RVVC can be seen as a disease of an overgrowth of *Candida* or as an alteration of host-protective defense mechanisms.⁴ *C. albicans* yeast produces 10 distinct secreted proteases (Saps), which constitute its most important virulence factors. One of the suggested roles of Saps is their deregulating effect on various proteolytic cascades that constitute the major homeostatic systems in human hosts, including blood coagulation, fibrinolysis, and kallikrein-kinin systems.²⁵ In vitro studies showed that *C. albicans* can be responsible for conversion of plasma prekallikrein to kallikrein (KLK).²⁶ KLK-related peptidase family consists of 15 genes, located on chromosome 19q13.4, encoding secretion of serine peptidases. These enzymes are important for skin desquamation and are elevated in several desquamative conditions, such as psoriasis, atopic dermatitis, desquamative inflammatory vaginitis (DIV, equal to severe aerobic vaginitis (AV)), and peeling skin syndrome.²⁷ Furthermore, KLK is regulated by vaginal epithelial cells, especially under the influence of estrogens and glucocorticoids.²⁷ We found higher excoriation of vulva in the non-respondent group despite no differences in pruritus. Further studies are necessary to clarify whether there are KLK peptidase expression differences in optimal responders and non-responders. Indeed, these findings triggered our interest to see in our ReCiDiF database if moderate/severe AV (DIV) could be related to non-response to maintenance therapy. We will report on these analyses later. A recently described cytolytic toxin, candidalysin, causes epithelial damage and is produced by pseudohyphae of *C. albicans*, thereby discriminating between colonization and infection.²⁸ As activity of this protein is also related to cellular activity of the vaginal epithelium, it would be interesting to study its influence on the local immunity in different genetic settings.

The allergy to medications was more often found in optimal response group. Most patients were allergic to antibiotics, which could shape their antibiotic use pattern. This finding could be more related not to immunity reaction, but differences in patterns of antibiotic use, which could lead to microbiome changes. We will report the results of microbiome analysis later.

Jaeger et al 2016 found that specific alleles in genes responsible for the immune responses are important in RVVC. They report differential production of the inflammasome-associated cytokines between patients and controls, with the patients bearing the risk allele

displaying the highest concentration of IL-1 β in the vaginal fluid.¹³ Also they found decrease of IL1 receptor antagonist (IL-1Ra) in patient samples comparing with controls. However, in our study, we failed to find any differences between concentrations of IL-1 β , IL-1Ra, or several other cytokines in the vaginal fluid.

Exposure to microorganisms induces the immune system to locally produce pro-inflammatory cytokines such as tumour necrosis factor (TNF)- α , IL-6, IFN- γ and IL-8, and anti-inflammatory cytokines (eg, IL-10), which would stimulate and regulate the immune response to limit the spread of infection.²⁹ We did not find any differences in concentrations of these cytokines in vaginal fluid of responders and non-responders, except IFN- γ . Recent research shows the importance of IFN- γ production in host defense against *C. albicans* and could be used as immunotherapy for invasive fungemia.^{4,30} IFN- γ was lower in optimal response group in our research. Decreased level is related to higher susceptibility of cutaneous candidosis.³¹ These findings could raise a hypothesis that women with lower IFN- γ level and higher susceptibility to disease response well to maintenance therapy. However, non-responders should be investigated for other mechanisms.

5 | CONCLUSION

Our study showed that RVVC women with family history of atopy, longer duration of symptoms, and severe vaginal excoriation are at increased risk not to respond to maintenance fluconazole treatment. Despite the current insufficient information about the precise pathogenic pathway, clinicians should be prepared for non-response to fluconazole maintenance therapy among women with family history of atopic disease and/or vulva excoriation as part of their symptoms. Further research is necessary to develop better symptom management strategy for non-responders to RVVC maintenance treatment.

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