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Probiotic *Lactobacillus acidophilus* Strain INMIA 9602 Er 317/402 Administration Reduces the Numbers of *Candida albicans* and Abundance of Enterobacteria in the Gut Microbiota of Familial Mediterranean Fever Patients

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Intestinal microorganisms play a crucial role in health and disease. The disruption of host–microbiota homeostasis has been reported to occur not only during disease development but also as a result of medication. Familial Mediterranean fever (FMF) is an inflammatory genetic disease characterized by elevated systemic reactivity against the commensal gut microbiota and high levels of *Candida albicans* in the gut. This study's major objective was to investigate the effects of commercial probiotic Narine on the relative abundance of gut bacteria (specifically, enterobacteria, lactobacilli, *Staphylococcus aureus*, and enterococci) of *C. albicans* carrier and non-carrier FMF patients in remission. Our main finding indicates that the probiotic reduces numbers of *C. albicans* and abundance of enterobacteria in male and female patients of *C. albicans* carriers and non-carriers. It has pivotal effect on *Enterococcus faecalis*: increase in male non-carriers and decrease in female ones regardless of *C. albicans* status. No effect was seen for *Lactobacillus* and *S. aureus*. Our data suggest that M694V/V726A pyrin inflammasome mutations leading to FMF disease may contribute to gender-specific differences in microbial community structure in FMF patients. The study’s secondary objective was to elucidate the gender-specific differences in the gut’s microbial community of FMF patients. The tendency was detected for higher counts of enterobacteria in female FMF subjects. However, the small number of patients of these groups preclude from conclusive statements, pointing at the need for additional investigations with appropriate for statistical analysis groups of subjects involved in the study.

**Keywords:** familial Mediterranean fever, M694V/V726A mutations, gut microbiota, gender, *Candida albicans*, *Lactobacillus acidophilus* INMIA 9602 Er 317/402, *Enterococcus faecalis*, Enterobacteriaceae
INTRODUCTION

Candida albicans is an opportunistic pathogen, which often exists as a harmless human commensal microorganism (1). Mutually beneficial associations of C. albicans were reported with several members of the intestinal microbiota (2, 3). In contrast, cooperative interaction of C. albicans and Escherichia coli increased the probability of fungal urinary tract infections due to increased E. coli-induced adhesion of C. albicans to the bladder mucosa (4). Another possibly synergistic interaction was shown between Staphylococcus aureus and C. albicans (5).

The disruption of inflammasomes, intracellular protein complexes with an important role in the sensing of intracellular pathogen- and danger-associated molecular patterns, can lead to susceptibility to infection, gut auto-inflammation, and tumorigenesis (6–8). Disruption of the host immune system—intestinal microbiota’s homeostasis is the main cause of inflammatory bowel disease. In particular, Klebsiella pneumoniae and P. mirabilis were found to be associated with colitis in animals using the T-bet−/− × Rag2−/− ulcerative colitis model (9).

Separate from this is the gene responsible for another inflammatory disease: familial Mediterranean fever (FMF), designated MEFV, which encodes an inflammasome pyrin containing domain of purin (PYD), TRIM, and B30.2 and is activated by bacterial toxins like Clostridium difficile, toxin B (TcdB), and C3 toxins (10). MEFV, in addition to FMF, can cause pyrin-associated auto-inflammation with neutrophilic dermatosis (PAAND) (11). The PYD is detected in more than 20 human proteins related to auto-inflammation with neutrophilic dermatosis (PAAND) (11). It was also shown earlier that the presence of gut enteric bacteria, lactobacilli, S. aureus, and E. faecalis in C. albicans-carrier FMF patients with the MEFV pyrin inflammasome mutation M694V/V726A, which is the prevalent pattern in the Armenian cohort. Main research questions were to show (i) if the changes in gut microbiota of FMF patients, primarily associated with the M694V/V726A pyrin inflammasome mutations, could lead to overgrowth of gut C. albicans of the patients and (ii) if colchicine/probiotic could effect on gut microbiota of patients through the regulation of NLRP inflammasomes.

MATERIALS AND METHODS

Forty healthy volunteers (20 male and 20 female) with less than baseline levels of C. albicans in the gut microbiota and without mutations in MEFV and 48 FMF volunteers (24 males and 24 females) were enrolled in a double-blind, partly randomized, placebo-controlled trial. Out of these participants, only 30 healthy (8 males and 22 females) and 31 (20 males and 11 females; 15 C. albicans non-carriers and 16 C. albicans carriers, see the Table 1) completed the trial. The probiotic Lactobacillus acidophilus INMIA 9602 Er 317/402 strain (Narine, Vitamax-E, Armenia) were prescribed to patients in each Narine FMF group. Remaining patients took placebo (capsule without the probiotic). The study participants took one capsule of placebo or probiotic preparation (150 mg of the probiotic strain contained no less than 1.5 × 10⁶ of viable bacteria) twice a day for 30 days. The datasets generated for this study can be found here: https://www.ncbi.nlm.nih.gov/geo/info/linking.html (GSE111835 study at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111835). Besides, another study on the effect of Narine probiotic on gut C. albicans of 12 FMF patients with C. albicans counts above 10⁶ CFU/g fecal material (7 males and 5 females) were completed during these investigations.

Fecal material was collected twice, before prescribing the probiotic and placebo and immediately after discontinuation of the treatment.

The age range of participants was 18–50 years. All patients’ diagnoses were confirmed by genetic analysis. None of the study participants had been treated with antibiotics, probiotics, hormones, or chemotherapeutic agents during the month leading up to the study. The duration of the colchicine treatment by patients was more than 7 year. During the 30-day period of study, patients with FMF used their regular colchicine medication (1 mg daily).
| Table 1 | Hybridization scores for gut bacteria in Candida albicans-carrier and non-carrier familial Mediterranean fever (FMF) patients after probiotica therapy; average ± SD. |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| **Bacteria**      | **Control FMF group (N = 31)** | **Placebo group† (N = 15)** | **Narine group† (N = 16)** |
|                   | Male (N = 20) | Female (N = 11) | Male (N = 9) | Female (N = 6) | Male (N = 9) | Female (N = 7) |
| C. albicans       | -            | +              | -            | +              | -            | +              |
| Enterobacteriaceae spp. | 5,208 ± 459 | 4,853 ± 378 | 6,417 ± 347 | 8,141 ± 473 | 6,992 ± 568 | 5,267 ± 347 |
|                   | P<0.05       | P<0.05         | P<0.05       | P<0.05         | P<0.05       | P<0.05         |
| C. albicans       | -            | +              | -            | +              | -            | +              |
| Enterococcus faecalis | 4,441 ± 128  | 4,928 ± 144    | 6,405 ± 157  | 4,168 ± 213   | 6,374 ± 169  | 5,689 ± 109   |
|                   | P<0.05       | P<0.05         | P<0.05       | P<0.05         | P<0.05       | P<0.05         |
| Lactobacillus spp. | 3,556 ± 97   | 3,192 ± 107    | 4,112 ± 93   | 2,834 ± 112   | 4,560 ± 299  | 4,360 ± 273   |
|                   | P<0.05       | P<0.05         | P<0.05       | P<0.05         | P<0.05       | P<0.05         |
| C. albicans       | -            | +              | -            | +              | -            | +              |
| C. albicans       | -            | +              | -            | +              | -            | +              |
| **Note:**

- **C. albicans**: carrier or non-carrier.
- **Narine probiotic**: INMIA 9,602 Er-2 strain 317/402 (Narine, Vitarax-E).
- **Placebo** therapy was used as a control.
- **Probiotic treatment** lowered the C. albicans titer in all C. albicans-carriers.

P<0.05 indicates a statistically significant difference compared to the control group.

P<0.05 indicates a statistically significant difference compared to the placebo group.

P<0.05 indicates a statistically significant difference compared to the investigated group.
Study participants collected the fecal materials themselves in sterile plastic bags and transferred them to the laboratory not later than 2 h after collection.

The ZR Fecal DNA MiniPrep (Zymo Research, Irvine, CA, USA) and the UltraClean® Tissue & Cells DNA Isolation Kit (QIAGEN, Germantown, MD, USA) were used for total genomic DNA isolation following the manufacturers’ recommendations.

The primer sequences used for microarrays and 16S rRNA clone libraries were: 27f.jgi (Bacteria-specific) 5′-AGAGTTT GATCCTGGCTCAG-3′ and 1492r.jgi (Bacteria/Archaea-specific) 5′-GGTTACCTTGTTACGACTT-3′.

The fecal bacterial communities were assessed by a third generation, culture-independent, high-density DNA microarray (PhyloChip™; Affymetrix, Santa Clara, CA, USA) analysis as previously described (25). This approach detects and measures the relative abundance of more than 50,000 individual microbial taxa. This approach is based on the analysis of the sequence of 16S ribosomal RNA genes. The PhyloChip™ relies on the analysis of all nine variable regions of the 16S gene and offers deeper taxonomic classification than other general approaches. With 1.2 million probes per chip, the microchip-based hybridization approach ensures that measurements on key low-abundance bacteria are not suppressed by dominant members of the microbial community. Nearly full-length 16S rRNA-gene fragments were amplified using universal bacterial primers. The amplicons were used for PhyloChip™ analysis, assessing the differences in hybridization intensity—reflective of differences in the relative abundance of bacterial taxa (25).

Candida albicans in fecal samples was quantified on Brilliance™ Candida agar (Thermo Scientific, Waltham, MA, USA) and confirmed by a C. albicans PCR kit (DNA-Technology LLC, Russia).

Statistical analyses were performed by the Multibase 2015 Excel Add-in program (NumericalDynamics, Tokyo, Japan).

RESULTS

C. albicans In FMF Patients

Despite the prevalence of men among Armenian FMF patients (16, 26) and the indication of possible associations between C. albicans infections and gender (27), our investigations showed that the number of C. albicans-carrier male FMF patients did not differ from the number of female carriers. Eleven out of 24 male FMF volunteers and 11 out of 24 female FMF volunteers had C. albicans counts above baseline levels in the gut microbiota before the probiotic treatment.

Effects of Probiotic on Gut C. albicans in FMF Patients

Probiotic treatment lowered the C. albicans titer in the FMF patient community. Only one “non-trial” female FMF patient with 10⁷ CFU/g C. albicans in fecal material and one “non-trial” male patient with 10⁶ CFU/g C. albicans in fecal material carried C. albicans at levels 10⁴ CFU/g fecal materials after probiotic treatment.

As expected, no significant differences in C. albicans presence (both yeast numbers and patients’ number) was observed in the placebo-treated group.

Culture-Independent Analysis of Enterobacteriaceae in the Gut Microbiota of FMF Patients

The relative abundance of enterobacteria in the gut microbiota of C. albicans-carrier patients is presented in Figure 1. The results show that female C. albicans-carrier FMF patients differ by their gut microbiota composition from that of male patients. Compared with diseased men, these women carried higher numbers of operational taxonomic units (OTUs) of the genera Salmonella,
Escherichia, Averyella, Cronobacter, Klebsiella, and Serratia (P < 0.00021) (Figure 1A). Differences between the hybridization scores of male/female FMF patients show that the average intensity value of bacteria belonging to Enterobacteriaceae spp. is higher in the gut microbiota of C. albicans-carrier FMF women than in FMF men (8,141 ± 473 vs. 6,417 ± 347; P < 0.05) (Table 1). There was no detectable significant difference between OTUs in C. albicans-carrier male patients as compared to healthy people (P > 0.05) (Figure 1D).

The comparison of OTUs of enteric bacteria in C. albicans-carrier and non-carrier female patients revealed a statistically significant increase in OTUs of Klebsiella and Erwinia spp. in C. albicans-carriers (P = 0.011). On the other hand, the diversity of enterobacteria of C. albicans-carrier female patients significantly differed from that of healthy people (Figure 1B). Compared with the healthy women, C. albicans-carriers contain a high OTUs for the genera Escherichia, Salmonella, Shigella, Klebsiella, and Trabulsiella (P < 10⁻⁵).

Effects of Probiotic on Gut Enterobacteriaceae

Treatment with probiotic Narine led to a decrease in OTUs of the fecal Enterobacteriaceae, especially in the genera Escherichia and Shigella, in C. albicans-carrier FMF women (P = 0.0036) (Figure 1C).

The placebo led to an increase in hybridization scores for gut Enterobacteriaceae, both in male [6,992 ± 558 (C. albicans non-carriers, placebo group) vs. 5,208 ± 459 (C. albicans non-carriers, control group); P < 0.05 and 5,267 ± 325 (C. albicans-carriers, placebo group) vs. 4,853 ± 378 (C. albicans-carriers, control group); P > 0.05] and female [9,590 ± 794 (C. albicans none-carriers, placebo group) vs. 6,417 ± 347 (C. albicans none-carriers, control group); and 9,780 ± 422 (C. albicans-carriers, placebo group) vs. 8,141 ± 473 (C. albicans-carriers, control group); P < 0.05] FMF patients, while the probiotic Narine decreased these scores for gut Enterobacteriaceae in FMF patients, overall (Table 1).

Culture-Independent Analysis of Lactobacilli in the Gut Microbiota of FMF Patients

A statistically significant decrease in lactobacilli was detected in the gut microbiota of FMF C. albicans-carriers (3,192 ± 107 vs. 3,556 ± 97; P < 0.05 and 2,834 ± 112 vs. 4,112 ± 93) (Table 1). The gut microbiota of FMF patients (both male and female) with C. albicans numbers below baseline levels showed a higher abundance of lactobacilli compared to healthy volunteers and C. albicans-carriers (P < 10⁻³) (Figures 2A,B). We detected some changes in hybridization scores for a diversity of lactobacilli in FMF patients after taking placebo (4,560 ± 299 vs. 3,556 ± 97, 4,360 ± 173 vs. 3,192 ± 107, 4,978 ± 138 vs. 4,112 ± 93, and 3,813 ± 201 vs. 2,834 ± 112; P < 0.05). Probiotic uptake had no statistically significant effect on the gut lactobacilli of these patients (4,793 ± 289 vs. 4,560 ± 299, 3,834 ± 263 vs. 4,360 ± 273, 4,873 ± 185 vs. 4,978 ± 138, and 3,941 ± 217 vs. 3,813 ± 201; P > 0.05) (Table 1).

Culture-Independent Analysis of Staphylococcus aureus in the Gut Microbiota of FMF Patients

Changes were detected in S. aureus abundance between C. albicans-carrier and non-carrier FMF women. The female FMF cohort with C. albicans numbers above baseline levels had a low abundance of S. aureus (data not shown). There were no statistically significant

**Figure 2** | Distribution of gut lactobacilli in Candida albicans-carrier familial Mediterranean fever (FMF) patients. Contribution of variables to differentiation between two groups [X axis, average ratio between two groups; Y axis, −Log(P)]. The upper-right and left-down corner are the most significant. (A) FMF women with gut C. albicans numbers above baseline level/FMF women with C. albicans below baseline level; (B) FMF men with C. albicans below baseline level/healthy men; (C) FMF women with C. albicans below baseline level/healthy women; (D) FMF men with gut C. albicans numbers above baseline level/FMF men with C. albicans below baseline level.
changes in the abundance of *S. aureus* between *C. albicans*-carrier and non-carrier FMF men and between FMF and healthy men (*P* > 0.05).

**Effects of Probiotic on Gut *S. aureus***
The investigation also explored if there were any differences in the abundance of gut *S. aureus* in FMF women before and after probiotic administrations. The results showed that the probiotic had no significant effect on *S. aureus* levels in FMF women (*P* > 0.05) (data not shown).

**Culture-Independent Analysis of *Enterococcus faecalis* in the Gut Microbiota of FMF Patients***
The hybridization scores for *E. faecalis* indicate differences between both male and female FMF *C. albicans*-carriers compared with non-carriers (*P* < 0.05) (Table 1). Despite this, these data show no significant differences in the relative abundance of *E. faecalis* between female FMF *C. albicans*-carriers compared with non-carriers (*P* > 0.08), while male FMF *C. albicans*-carriers have increased abundance of *E. faecalis* (*P* = 0.006) in their gut microbiota compared with that of male patients with *C. albicans* below baseline levels (Figures 3A,B). On the other hand, the results showed that both groups of FMF women, *C. albicans*-carriers and non-carriers, differ from healthy volunteers in the abundance of *E. faecalis* in the gut microbiota (*P* < 0.05) (Figures 4A,B). There were no differences between healthy men and FMF men in terms of *E. faecalis* abundance in their gut microbiota (*P* > 0.05) (Figure 5A), while *C. albicans*-carrier FMF men differed from healthy men volunteers (*P* < 0.05) (Figure 5C).

**Effects of Probiotic Administration on Gut *Enterococcus faecalis***
Probiotic-therapy reduced the number of yeast in *C. albicans*-carrier women. However, there were no statistically significant

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**Figure 3** | Distribution of gut *Enterococcus faecalis* in *Candida albicans*-carrier familial Mediterranean fever (FMF) patients. Contribution of variables to differentiation between two groups [X axis, average ratio between two groups; Y axis, −Log (P)]. The upper-right and left-down corner are the most significant. (A) FMF men with gut *C. albicans* numbers above baseline level/FMF men with *C. albicans* below baseline level. (B) FMF women with gut *C. albicans* numbers above baseline level/FMF women with *C. albicans* below baseline level.
differences between \(C. \text{albicans}\)-carrier/healthy women and \(C. \text{albicans}\)-non-carrier/healthy women in their gut \(E. \text{faecalis}\) abundances \((P > 0.05)\) (Figures 4C,D) as well as differences between \(C. \text{albicans}\)-none carrier women before and after the probiotic administration \((P > 0.05)\) (Figure 4E), in spite of this, there were statistically significant differences between \(C. \text{albicans}\)-carrier and \(C. \text{albicans}\)-non-carrier women in their gut \(E. \text{faecalis}\) abundances \((P < 0.05)\) (Figure 4F). The placebo groups showed increase in hybridization scores of gut \(E. \text{faecalis}\) both in male \((6,374 \pm 169 \text{ vs. } 4,441 \pm 128; P < 0.05 \text{ and } 5,689 \pm 109 \text{ vs. } 4,928 \pm 144; P < 0.05)\) and female \((6,534 \pm 205 \text{ vs. } 6,405 \pm 157; \text{and} \ 5,070 \pm 244 \text{ vs. } 4,168 \pm 213; P < 0.05)\) FMF patients. Compared to the placebo effect, the statistically significant increase in scores of gut \(E. \text{faecalis}\) was attributed to male FMF patients not carrying \(C. \text{albicans}\) treated with the probiotic \((7,108 \pm 273 \text{ vs. } 5,689 \pm 109; \ P < 0.05)\), while the probiotic-therapy decreased \(E. \text{faecalis}\) scores in \(C. \text{albicans}\)-carrier and non-carrier female patients \((3,771 \pm 123 \text{ vs. } 5,070 \pm 244 \text{ and } 5,602 \pm 189 \text{ vs. } 6,534 \pm 205; \ P < 0.05)\) (Table 1). After the treatment with commercial probiotic Narine, there were no statistically significant differences between healthy men and FMF men with \(C. \text{albicans}\)-carriers in gut \(E. \text{faecalis}\) (Figure 5B). Statistically significant differences between FMF men with \(C. \text{albicans}\)-carriers before and after the treatment with commercial probiotic Narine were observed (Figure 5D).

DISCUSSION

The differences in the pathogenesis of infectious diseases in male and female patients were previously reported (28), and the significant influences of gender on the development of autoimmune disease have been described (28, 29). It was reported that a female steroid hormone, estrogen, probably playing a role in gut tight junction expression and permeability, might also be the reason for female autoimmune diseases (30). Moreover, the interaction between gender-specific immune differences and the specific immune response to individual microbes was reported previously (31). There are some indications of a possible association between \(C. \text{albicans}\) infections and gender in the literature as well (32, 33).

According to our investigations, the patients’ gender is one of the main factors affecting the composition of gut microbiota in FMF patients despite the lack of previous report on this difference. The male/female ratio of Armenian FMF patients with MEFV mutation patterns M694V/V726A, according to reported data, is 1.16–1.2:1 (16, 26, 34). In addition, our previous studies revealed gender differences in several blood parameters of FMF patients, such as ESR and CRP (35).

Gender-based differences in the relationship of IL-6 signaling and adrenocorticotrophic hormone, which is usually produced in response to biological stress, was reported previously (36),
and the relationships between triggering factors and FMF were reported for Armenian patients (37). The experiments included 177 male and 98 female FMF patients and showed that emotional stress was one of the most common triggering factors for FMF attacks with serositis (49.8%) after cold exposure (59.3%) (38). Moreover, the relationships between triggering factors and both M694V (with starvation) and V726A (with long-duration travel) alleles were revealed (38).

An important role for IL-6 in the immune response to *E. coli* and *C. albicans* was reported earlier using Il6 null mice (Il6−/−) (39, 40). Thus, IL-6 with its pro- and anti-inflammatory characteristics being responsible for the transition of innate-acquired immunity, could participate in “immune dimorphism” in male and female FMF patients. Generally, there should be differences between the specific bacterial OTUs (Enterobacteriaceae/lactobacilli/ *E. faecalis/S. aureus*) of healthy people and *C. albicans*-carrier
FMF patients, as well as in C. albicans-carrier and non-carrier FMF patients. These differences should be expected if the relationship between the investigated gut bacteria and *C. albicans* are critical for the yeast’s increased number in the gut microbiota of FMF patients.

According to our investigations, the diversity of enterobacteria in female *C. albicans*-carrier patients significantly differs from those in healthy participants and from those in female non-carrier patients. In addition, female *C. albicans* non-carrier patients were having a wider diversity of *Enterobacteriaceae* compared with the healthy women. We did not observe differences between the *Enterobacteriaceae* OTUs of healthy men and *C. albicans*-carrier FMF men despite the fact that probiotic treatment decreased the relative abundance of gut *Enterobacteriaceae* in *C. albicans*-carrier FMF men, too.

Possibly, the high diversity of *Enterobacteriaceae* in the gut microbiota of FMF patients may have an effect on the overgrowth of *C. albicans* in FMF women. The tendency of the simultaneous “normalization” of OTUs for enteric bacteria and *C. albicans* in the gut microbiota of female FMF patients after the probiotic Nariñe treatment supports this hypothesis. However, there was no such tendency detected in FMF male subjects.

Based on the effects of probiotic on the relative abundance of fecal-*Enterobacteriaceae*, we suggest that mechanisms of probiotic effects on gut *C. albicans* are different for male and female FMF patients. Similar analysis revealed a potential association between the OTUs of *E. faecalis* and *C. albicans* in the gut microbiota of male FMF patients. In contrast to fecal *Enterobacteriaceae* and *E. faecalis* OTUs, the gut microbiota of FMF patients (both male and female) with *C. albicans* below baseline levels contains a relatively high abundance of lactobacilli compared with *C. albicans*-carriers. However, the probiotic supplementation did not affect the abundance of lactobacilli in *C. albicans*-carrier FMF women and men. The primary comparative analysis on *S. aureus* OTUs in *C. albicans*-carrier/non-carrier FMF and healthy people does not support the hypothesis of potential strong synergistic interaction between *S. aureus* and *C. albicans*.

Although the probiotic *L. acidophilus* INMIA 9602 Er strain 317/402 does not have any effect on *C. albicans* in vitro (16), the number of FMF patients in remission (both male and female) who carried *C. albicans* above baseline levels, decreased after probiotic therapy in the double blind, partly randomized, placebo-controlled trial on 48 volunteer patients with FMF in remission. The abovementioned data show that greater relative abundances of enteric bacteria, especially *Klebsiella*, in *C. albicans*-carrier female FMF patients and *E. faecalis* in *C. albicans*-carrier male FMF patients may contribute to the increase in numbers of *C. albicans* in FMF patients. The report on antagonistic activity of the probiotic against *Klebsiella* in vitro supports this hypothesis (16).

Different inflammasomes are shown to be involved in the response to a *C. albicans* infection, including the NLRP3 inflammasome with a caspase recruitment domain and caspase-1, and the production of interleukin-1 beta (IL-1β) and IL-18 through the NLRP3 inflammasome is crucial for adaptive cellular protection against *C. albicans* (41). Another inflammasome, NLRCA, involved in the response to *C. albicans* infection (42), is able to recognize flagellin and components of the bacterial secretion systems providing host defense to a range of pathogens including *K. pneumoniae* (43) and *Yersinia* (44). NLRP3 and NLRCA, together allow for the recognition of different danger signals from the same pathogenic infection (45). Wild-type pyrin negatively modulates NLRP3, inflammasome-dependent IL-1β release (46).

Our investigations showed that the pyrin inflammasome mutation M694V/V726A, leading to FMF disease, effects the gut microbiota and contributes to differences between male and female patients. The number of *C. albicans*-carrier male FMF patients did not differ from the number of female carriers.
indicating that there was no direct association between the host's genetics and \textit{C. albicans}-carriage. However, the considerable effects of other factors, such as comparatively high concentrations and/or long-term colchicine use (with other environmental factors) may outweigh the effects of the host's genetic background.

The most effective long-term treatment for FMF is the administration of colchicine (47), which inhibits inflammasome activation within macrophages (48, 49). There is no indication of a different effect of the treatment between male and female patients. Most likely, colchicine inhibits NLRC4, inflammasome-dependent IL-1β release, thereby influencing the overgrowth of \textit{C. albicans} in the gut microbiota of FMF patients.

Our investigations indicate that the uptake of \textit{L. acidophilus} INMIA 9,602 Er 317/402 reduces the numbers of yeast in the gut microbiota of FMF patients.

Future microbiological and immunological studies will be aimed at clarification of the functional aspects and the detailed mechanism of action of \textit{Lactobacillus acidophilus} INMIA 9,602 Er 317/402 and its impact on human health in relation to FMF. This will assist in designing “individual diets” for FMF patients.

ETHICS STATEMENT

This study was performed in accordance with institutional ethical guidelines and was approved by the Ethics Committee at the Ministry of Education and Science of Armenia. All investigated patients gave written informed consent prior to the study.

AUTHOR CONTRIBUTIONS

All authors have made a substantial, direct, and intellectual contribution to the work and have approved it for publication. AP and TT conceived and designed the experiments. MB, AM, SP, SPet, LG, and VT performed the experiments. AP analyzed the data and wrote the paper. TT, MC, and SK reviewed data analysis and the manuscript. All authors reviewed the manuscript.

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