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SHORT COMMUNICATION

Functional interferon-epsilon gene polymorphisms and sexually transmitted infections of the endometrium

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Abstract

Problem: Interferon-epsilon (IFNε) is the only type I IFN constitutively expressed in the female reproductive tract and fluctuates across the menstrual cycle in humans. Mouse models show that IFNε protects against *Chlamydia trachomatis*, Herpes Simplex Virus, HIV, and Zika in mice, but human studies are limited. Bacterial sexually transmitted infections (STI) can ascend to the upper genital tract and cause pelvic inflammatory disease (PID) and subsequent infertility. However, the host immunological mechanisms that play a role in the ascension and infection of the endometrium in individuals with clinically suspected PID are not elucidated.

Method of study: This pilot investigation determined if IFNε gene variants are associated with bacterial vaginosis (BV) and endometrial infection with *C. trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium* using biospecimens from 154 self-report Black individuals who participated in the PID Evaluation and Clinical Health (PEACH) study.

Results: The T allele for rs2039381 was associated with endometrial STI infection (OR 2.7, 95% CI: 1.0-7.1) and the C allele for rs1125488 was inversely associated with BV (OR: .2, 95% CI: .05-.8).

Conclusions: Few studies have examined IFNε gene variants, our study raises the possibility that IFNε gene variants may be a potential host contributor to STI pathogenesis.

KEYWORDS
endometritis, genetics, interferons, sexually transmitted diseases

1 | INTRODUCTION

Increased expression of type I interferon-epsilon (IFNε), a recently characterized type I IFN, may be required in the reproductive tract to form innate immune defense against genital infections.[1,2] Similar to other type I IFNs such as alpha and beta, IFNε signals through traditional IFNAR1/2 receptors, activates JAK-STAT pathway, and modulates IFN regulated genes.[3] IFNε has immune regulatory functions and anti-viral and anti-bacterial effects, but is not robustly upregulated following infection like other type I IFNs.[3,4] Instead, IFNε is the

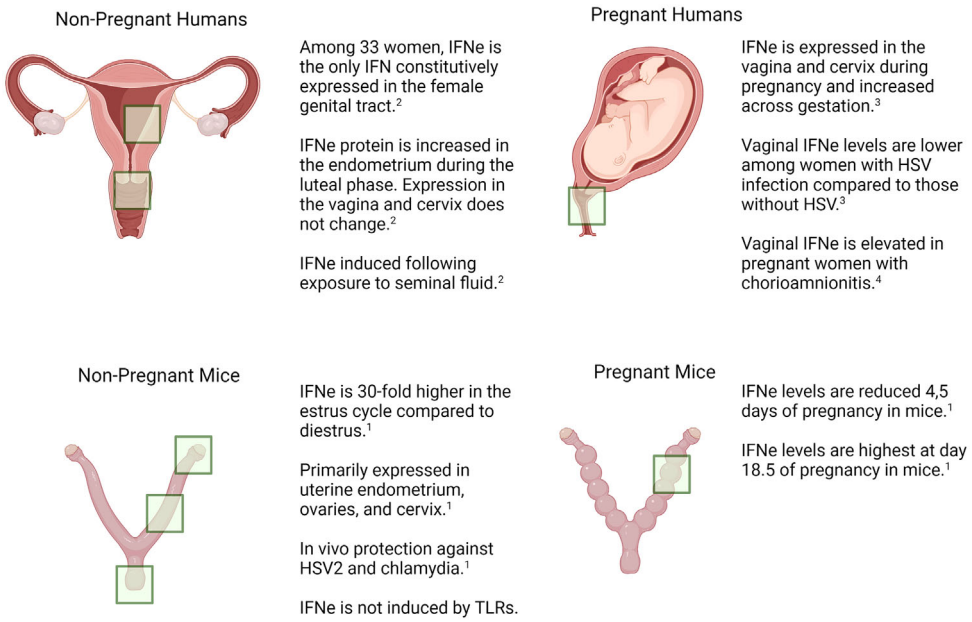

FIGURE 1 Summary of studies that have measured interferon epsilon in the genital tract of mice and humans. Each green square represents the site where interferon epsilon was detectable either in tissue or vaginal/cervical fluid samples. The image was created in Biorender.

only type I IFN with constitutive expression in epithelial cells of the vagina, cervix, and endometrium.[1,2] Another unique biological feature of IFNε is that it is not regulated by Toll-like receptors rather, it is regulated by fluctuating concentrations of reproductive hormones across reproductive cycles in mice, horses, non-human primates, and humans (Figure 1).[1,2,5,6] For example, in equines endometrial IFNε appears to be progesterone dependent and is elevated in diestrus phase.[6] In mice, endometrial expression is highest in estrus phase.[1] While similar estrogen-driven patterns were initially found in humans,[1] a subsequent study reported endometrial expression was highest in the luteal phase.[5] Overall, IFNε is understudied in comparison to other type I IFNs, and researchers are still learning about its biological functions and potential clinical importance.

Given the constitutive expression, it has been theorized that IFNε has distinct immune regulatory mechanisms for mucosal protection against genital tract infections, unlike IFN α/β that have low baseline levels and are upregulated after infection.[5,7] For example, IFNε null mice have increased susceptibility to herpes simplex virus (HSV) and *Chlamydia muridarum*.[1] IFNε can block HIV-replication in human macrophages,[7] and protect against Zika virus even in the absence of other type I IFN signaling.[8] In the mouse uterus, IFNε can assist with recruiting natural killer cells, for epithelial barrier immunity, functions that are eliminated in IFNε null mice.[2] During pregnancy, IFNε is reduced in the vagina and cervix of individuals with herpes simplex virus[9] and may be elevated among individuals with infection and inflammation of the chorio-amnion.[10,11] This unique type one IFN may play critical roles in the female reproductive tract but studies directly examining IFNε and sexually transmitted infections (STI) in humans are lacking.

Pelvic inflammatory disease (PID) is infection and inflammation of the female upper genital tract and typically occurs when microorganisms ascend past the cervix and infect the endometrium, fallopian tubes and ovaries, often leading to subsequent infertility and ectopic pregnancy.[12] *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium*, are associated with PID.[12] In addition, bacterial vaginosis (BV), an alteration of the vaginal microbiome may enhance STI ascension into the upper genital tract.[12] It is unclear why some individuals develop upper genital tract pathology following infection and other individuals do not. Although reproductive tract immunity is known to be vital for reproductive function and health, the role of host immunity in bacterial ascension to the upper genital tract is not well established in human populations.

We previously reported that Toll-like receptor variants are associated with *C. trachomatis*, upper genital tract infection, and reduced pregnancy rates among individuals with PID.[13,14] This led to the hypothesis that a combination of host genetics and environmental factors explain the variability in outcomes following lower genital infection. Thus, genetic variations in the IFNε gene may be a novel mechanism that alters host response to genital tract infection leading to increased susceptibility or complications. However, no studies have examined polymorphisms in IFNε to determine if there is an association with PID-associated microbes and confirmed upper genital tract pathology. This pilot study determined if variants which lead to amino acid substitutions in the IFNε gene are associated with evidence of lower or upper genital tract infection among women with clinically suspected PID.

2 | MATERIALS AND METHODS

This analysis used data and biospecimens from the PID Evaluation and Clinical Health (PEACH) study, which has been described

previously.[15] Briefly, PEACH was a randomized clinical trial conducted in the 1990's comparing inpatient and outpatient treatment among 831 individuals, ages 14–37 years, with clinically suspected PID. Within this study, we previously conducted a genetic sub-study in 2010 among 234 study participants who had stored buffy coats genotyped for innate immune genes, primarily Toll-like receptors.[14] There were 154 participants of self-reported Black/African American ancestry included in this study. All participants with stored buffy coats had provided informed consent for future use of biospecimens. The University of Pittsburgh Institutional Review Board approved the PEACH study and genetic sub-study. The University of Texas Medical Branch Institutional Review Board approved the current study.

Participants were followed for a mean of 84 months and at enrollment demographic and clinical information was collected. Baseline information was obtained on pain history, history of PID and STIs, contraceptive practices, new sexual partners in past 30 days, lifetime sexual partners, medical history, and lifestyle habits. All participants were then followed in-person at 5 and 30-days then interviewed by telephone every 3–4 months for 5 years. The follow-up data included information on fertility, pregnancy, pain, and complications from PID. At enrollment a standardized screening pelvic examination included obtaining cervical swabs for *N. gonorrhoeae* culture and *C. trachomatis* polymerase chain reaction (PCR). These pathogens were also measured at 5-, and 30-days post-treatment during gynecological exams following standardized protocols. Only baseline infection data was used for this study. Vaginal smears were gram stained for BV using methods described by Nugent et al in a central laboratory.[16] PEACH collected endometrial biopsy specimens for histological examination and chlamydial PCR and gonococcal cultures were performed. *M. genitalium* was measured later on stored cervical swabs and endometrial specimens using a microwell-plate-based PCR assay (MgPa-IMW) targeting the MgPa gene.[17]

We had identified two functional IFNε SNPs (rs1125488 and rs2039381) which were genotyped by fluorescence polarization.[18] Briefly, amplification was performed using a Peltier Thermal Cycler (MJ Research) and the PCR products were resolved by electrophoresis in a 3% agarose gel and visualized under UV light after ethidium bromide staining. Genotypes were assigned by direct comparison to controls of sequence confirmed genotypes, and a 5% random resample was included for consistency of the genotyping. All SNPs were tested for deviations from the Hardy-Weinberg equilibrium (HWE) using a p-value <.05.

As altered vaginal flora may increase susceptibility to other pathogens, we first compared IFNε SNP allele frequencies between individuals with BV or intermediate flora (Nugent score 4–10) and normal flora (Nugent score 0–3). We then examined SNP allele frequencies among individuals with evidence of upper genital tract infection (confirmed endometrial *C. trachomatis*, *N. gonorrhoeae*, or *M. genitalium*) and inflammation (histologic endometritis determined by Kiviat's criteria: ≥5 neutrophils and ≥1 plasma cells). Odds ratios (OR), 95% confidence intervals were calculated using logistic regression with Firth's penalized likelihood method, when appropriate. Crude models are presented. All analyses were performed using SAS/Genetics v9.1.3 (Cary, NC).

3 | RESULTS AND DISCUSSION

At enrollment, 64.5% of participants were under the age of 25, 43.4% smoked, 93.1% were single, 43.4% had less than a high school education, 8.3% reported new sexual partner in past month, 60.6% reported condom use, and 31.7% reported illicit drug use (Figure 2). Among our cohort, 84.9% had abnormal vaginal flora (BV or intermediate), 39.3% had confirmed endometrial infection with one or more sexually transmitted pathogens, and 53.1% had histologically confirmed endometritis (Figure 3).

The IFNε rs1125488 C allele had a frequency of 3% and the IFNe rs2039381 T allele had a frequency of 4%. Individuals who carried one or more of the rs2039381 T allele were more likely to have endometrial infection with *C. trachomatis*, *N. gonorrhoeae*, or *M. genitalium* (59.1% vs. 40.9%; OR: 2.7, 95% CI: 1.0-7.1) (Table 1). Individuals who carried the rs1125488 C allele were less likely to have abnormal vaginal flora compared to individuals with normal flora (55.6% vs. 86.9%; OR: .2, 95% CI: .05-.8) but there were no associations with endometrial infection. IFNε may have a unique role in protecting the female genital tract against infection. Challenging IFNε knockout mice with *Chlamydia muridarum* and HSV results in more severe disease than observed for wild type mice[2] and IFNε null mice are also more susceptible to Zika virus, even in absence of other type I IFN signaling.[8] This suggests that the constitutive expression of IFNε may play a role in protection against viral and bacterial STIs. However, it is not known if IFNε has any association with *N. gonorrhoeae*, *M. genitalium* or bacterial vaginosis. Research on IFNε in human populations is limited and very few studies have examined IFNε genital tract expression or gene variants.

Variant rs2039381 is in the stop codon and causes a Gln71Thr substitution. This nonsense polymorphism has been linked to intracerebral hemorrhage in a Korean population, of which inflammation is a key contributor.[19] However, studies have not determined if rs203981 has any association with viral or bacterial infections. In general, it is not entirely clear how IFNε protects against genital tract infections. Type I IFNs α/β are well known to have anti-viral effects and despite low baseline levels are rapidly upregulated during infection.[20] However, while traditional type I IFN signaling is beneficial it can also exacerbate disease such as HIV progression[21] and is detrimental to the host following bacterial infections such as *Chlamydia muridarum*.[21,22] Both *C. trachomatis* and *N. gonorrhoeae* induce production of type I IFNs but this subsequently limits IFN-γ-mediated CD4-T cell recognition of infected cells, a method of immune evasion.[23] Interestingly, Zika virus is also known to evade traditional type I IFN responses post-infection but is susceptible to IFNε, furthermore IFNε null mice are rescued by IFNε prophylactic treatment.[24] In the mouse uterus, IFNε null mice cannot effectively recruit natural killer cells but cell populations are restored after intravaginal administration of recombinant IFNε.[2] We know that natural killer cells are important for defense against *C. trachomatis* through the induction of IFNγ and Th1 cell response, pathways also

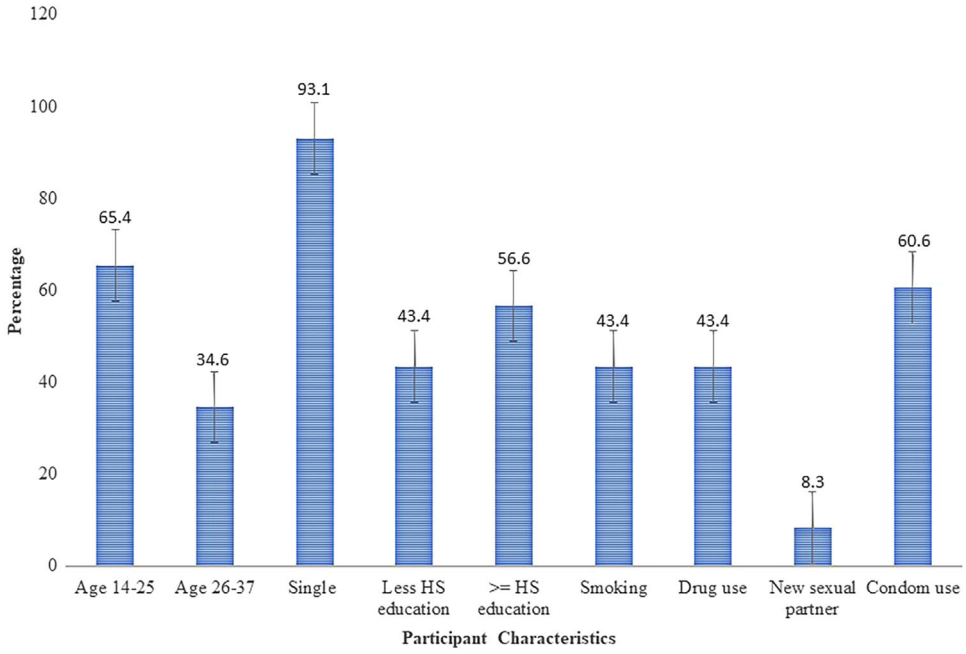

FIGURE 2 Describes the patient population and characteristic. Percentages are provided for each participant demographic and behavioral variables. HS = high school.

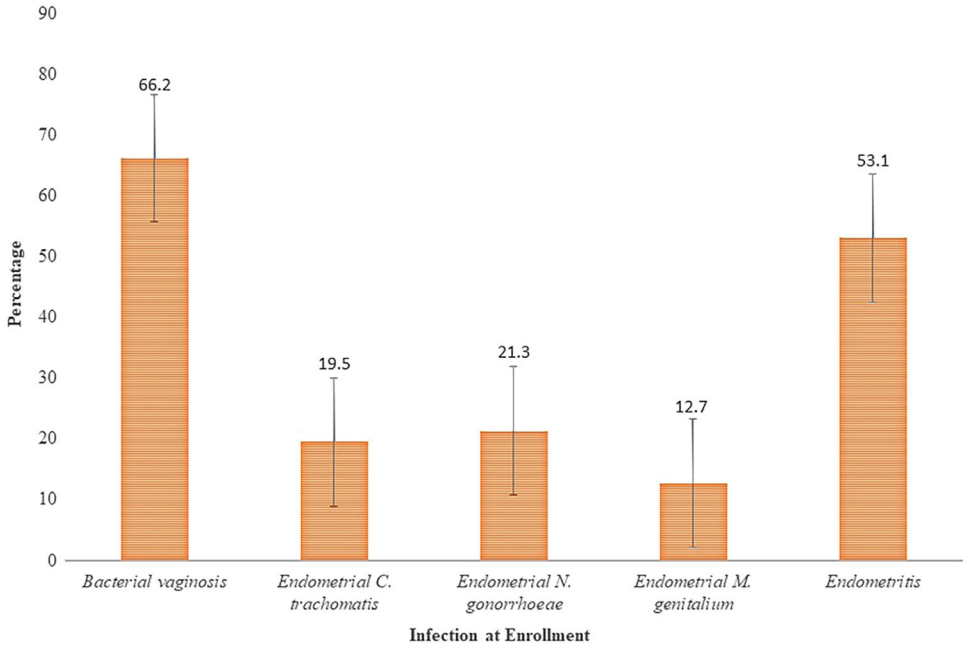

FIGURE 3 Describes the frequency of bacterial vaginosis and endometrial infection with bacterial sexually transmitted infections among the population.

important in *N. gonorrhoeae* infection.[23] These data support the leading theory that constitutive expression of IFNε results in unique mucosal immunity that may protect against infection. It is possible that variants in rs203981 could alter basal IFNε levels, but the function is not known.

Variant rs1125488 is a missense mutation and causes a Gln46His substitution. This variant was found to be associated with heart rate in the Strong Heart Family Study[25] but again has not be linked directly to bacterial infections and the function is not known. It is not entirely clear why this variant was associated with bacterial vaginosis but not endometrial infection. The effect estimates were in the same direction and therefore we were likely underpowered to examine endometrial infection. Bacterial vaginosis is polymicrobial and some bacterial

TABLE 1 Comparison of IFN allele frequencies by BV/intermediate flora, upper genital tract infection, and endometritis.

	[a]BV or intermediate flora			Histologic endometritis			[b]Any endometrial STI			
	No N(%)	Yes N(%)	*p*-Value	OR (95% CI)	No N(%)	Yes N(%)	*p*-Value	OR (95% CI)	No N(%)	Yes N(%)	*p*-Value	OR (95% CI)
rs2039381 allele												
C	18 (15.6)	97 (84.4)	.23	1.1 (.3–3.3)	49 (49.5)	50 (50.5)	.34	1.5 (.6–3.5)	58 (65.9)	30 (34.1)	.04	2.7 (1.0–7.1)
T	4 (13.8)	25 (86.2)			11 (39.3)	17 (60.7)			9 (40.9)	13 (59.1)		
rs1125488 allele												
A	18 (13.1)	119 (86.9)	.01	.2 (.05–.8)	54 (44.6)	67 (55.4)	.08	.2 (.03–1.1)	62 (59.1)	43 (40.9)	.24	.3 (.1–2.3)
C	4 (44.4)	5 (55.6)			6 (85.7)	1 (14.3)			6 (85.7)	1 (14.3)		

Note: OR, 95% CI, and *p*-values were calculated using logistic regression with Firth's penalized likelihood method. Crude results are presented as adjustment did not alter our results.

[a]BV and intermediate flora determine by Nugent score.

[b]Endometrial infection with chlamydia, gonorrhea, or *Mycoplasma genitalium*.

vaginosis-associated bacteria (e.g., *Atopobium vaginae*, BVAB-1, *Gardnerella vaginalis*) have been linked to endometritis and infertility.[26] However, we did not have data on endometrial infection with BV-associated bacteria. We did have data on cervical BV-associated bacteria, but the sample size was too small to separate by individual pathogens. As these bacterial have species specific innate immune responses and immune evasion techniques,[27–29] it is not known how this variant would protect against bacterial vaginosis.

As all individuals in the parent PEACH study had clinically suspected PID, comparisons among individuals with histologically confirmed endometritis and endometrial infection to internal comparison groups of individuals testing negative for each of these may have biased our comparisons toward the null. Further, our pilot study was limited by the small sample size which resulted in imprecise estimates and wide confidence intervals. Another limitation is that we were not able to conduct functional studies to understand how IFNε gene variants may influence risk of genital tract infections. Still, the intriguing trends reported from our exploratory study adds to the current literature suggesting the IFNε may have important roles in the female reproductive tract.

The function of IFNe in human reproduction and pregnancy remains to be fully delineated despite knowledge of its constitutive expression in the female genital tract and unique hormonal control. Our results suggest that IFNe variants may alter the odds of upper genital tract infection and abnormal vaginal flora among individuals with clinically suspected PID. As significant gaps remain in our understanding of the biological functions of IFNe, further studies in humans may be warranted, especially studies of IFNe expression in the genital tract.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors: The data that support the findings of this study are available from CLH upon reasonable request.

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