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Host Polymorphisms in *TLR9* and *IL10* Are Associated With the Outcomes of Experimental *Haemophilus ducreyi* Infection in Human Volunteers

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Background. In humans inoculated with *Haemophilus ducreyi*, there are host effects on the possible clinical outcomes—pustule formation versus spontaneous resolution of infection. However, the immunogenetic factors that influence these outcomes are unknown. Here we examined the role of 14 single-nucleotide polymorphisms (SNPs) in 7 selected pathogen-recognition pathways and cytokine genes on the gradated outcomes of experimental infection.

Methods. DNAs from 105 volunteers infected with *H. ducreyi* at 3 sites were genotyped for SNPs, using real-time polymerase chain reaction. The participants were classified into 2 cohorts, by race, and into 4 groups, based on whether they formed 0, 1, 2, or 3 pustules. χ^2 tests for trend and logistic regression analyses were performed on the data.

Results. In European Americans, the most significant findings were a protective association of the *TLR9* +2848 GG genotype and a risk-enhancing association of the *TLR9* TA haplotype with pustule formation; logistic regression showed a trend toward protection for the *TLR9* +2848 GG genotype. In African Americans, logistic regression showed a protective effect for the *IL10* –2849 AA genotype and a risk-enhancing effect for the *IL10* AAC haplotype.

Conclusions. Variations in *TLR9* and *IL10* are associated with the outcome of *H. ducreyi* infection.

Keywords. *Haemophilus ducreyi*; chancroid; skin ulcers; immunogenetics; humans; innate immunity.

Haemophilus ducreyi causes chancroid, a sexually transmitted disease that presents as painful genital ulcers and facilitates the transmission and acquisition of the human immunodeficiency virus (HIV) type 1 [1]. Owing to syndromic management of genital ulcers, the global prevalence of chancroid is currently undefined but has declined in many former areas of high endemicity [2, 3]. Recently, *H. ducreyi* was found to be the leading cause of cutaneous ulcers in children in yaws-endemic communities of the South Pacific islands and equatorial Africa [3–7]. Thus, *H. ducreyi* is an important threat to global health.

To study the biology of *H. ducreyi*, we developed a model in which healthy adult volunteers are inoculated at 3 sites on an upper arm with identical doses of the human-passaged (HP) genital ulcer isolate 35000HP [8, 9]. Papules develop at infected sites within 24 hours and either spontaneously resolve or progress into pustules within 2–5 days. Within a person, the outcomes (resolution vs pustule formation) of infected sites tend to be similar, suggesting a host effect on disease progression [10, 11]. When reinfected, volunteers initially classified as "resolvers" or "pustule formers" segregate toward their initial outcomes, confirming a host effect on susceptibility [10].

Experimental pustules and natural ulcers represent a failed immune response. These lesions resemble suppurative granulomas in that they consist of polymorphonuclear leukocytes (PMNs) that form an epidermal abscess, a collar of macrophages admixed with regulatory T cells below the abscess, and a deep dermal infiltrate of memory $CD4^+$, $CD8^+$, and natural killer (NK) cells [12–15]. Unlike most bacteria that cause granulomas, *H. ducreyi* is surrounded by PMNs and macrophages and is extracellular [16, 17]. Thus, evasion of phagocytosis underlies disease progression [18–21]. The mechanism of bacterial clearance in resolvers is unknown but likely involves enhanced phagocytic clearance, which may be shaped by the microenvironment at the infected site [10, 22]. Comparative transcriptional analysis of skin biopsy specimens obtained after a repeat infection showed that, relative to resolvers, the lesional microenvironment of pustule formers is marked by a hyperinflammatory, dysregulated state [22]. When infected with *H. ducreyi*, monocyte-derived myeloid dendritic cells (DCs) obtained from resolvers have a transcriptional response typical of type 1 DCs, while those derived from pustule formers have a mixed response with features of type 1 DCs and regulatory DCs, marked by upregulation of interleukin 10 (IL-10) [22]. In addition, the preinfection microbiome of resolvers shares a similar community structure that significantly differs from the preinfection microbiome of pustule formers, which is more

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diverse [23]. This finding may reflect biases in innate immunity between the 2 groups that drive different compositions of the microbiome [23]. These data led us to hypothesize that there may be an immunogenetic basis for differential innate immune responses to *H. ducreyi* that ultimately determines disease outcome.

Host immunogenetic factors are associated with the outcome of other bacterial sexually transmitted infections [24–27]. For instance, single-nucleotide polymorphisms (SNPs) in the genes encoding Toll-like receptor 4 (TLR4) and TLR9 (*TLR4* and *TLR9*, respectively) affect the susceptibility to and severity of *Chlamydia trachomatis* infections [24, 25]. These polymorphisms affect the ability of the TLRs to detect pathogen-associated molecular patterns, impeding the host immune response to infection.

In this study, we examined whether SNPs in genes that encode pathogen-recognition receptors (PRRs), regulators of innate immune responses, or cytokines correlated with the outcomes of experimental infection in 2 cohorts of experimentally infected European American and African American individuals in the United States. As innate immune responses appear to be important in determining outcome, we analyzed SNPs in TLRs, nucleotide oligomerization domain (NOD)–like receptors, single immunoglobulin interleukin 1 receptor (SIGIRR), and IL–10.

METHODS

Between March 2000 and June 2014, we collected blood specimens from 144 healthy adult volunteers, who had no history of previous *H. ducreyi* infection (Figure 1). Each volunteer was inoculated with strain 35000HP in 1 arm at 3 sites, vertically spaced 3-cm apart on the skin overlying the upper deltoid, via 1.9-mm puncture wounds made with an allergy testing device, which delivers the bacteria to the epidermis and dermis. Each site received identical doses of 35000HP, which was prepared from dedicated freezer lots according to Food and Drug Administration guidelines. Most participants were enrolled in mutant versus parent comparison trials and were also infected on the opposite arm with isogenic mutants derived from 35000HP, which can be attenuated or fully virulent for pustule formation [9]. Resolvers who formed pustules at sites inoculated with virulent mutants were considered capable of pustule formation; 3 such participants were excluded from the analysis.

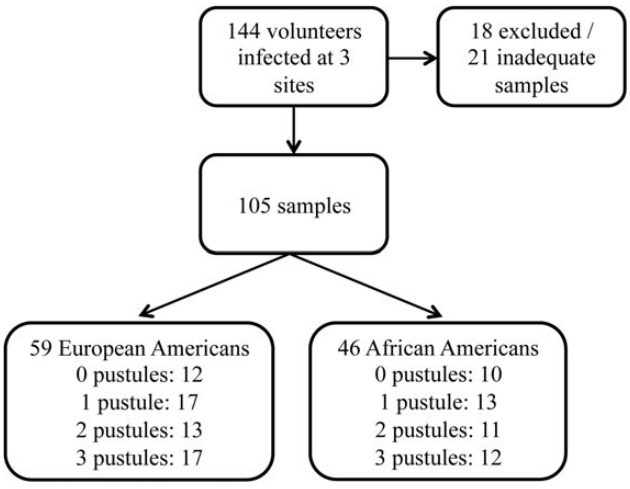

Figure 1. Participant and sample selection flow chart for the European American and the African American cohorts. Data are no. of participants or samples.

In the model, we attempt to deliver a standard dose of approximately 90 colony-forming units (CFU) of 35000HP. However, *H. ducreyi* has a tendency to clump, which causes variation in the actual dose. Data based on infection of 299 participants show a significant effect of dose on pustule formation, which increases by 0.7% per CFU ($P = .001$). To adjust for potential differences in doses between the resolvers and pustule formers, we excluded 15 participants who had been inoculated with 35000HP doses of <34 CFU and ≥130 CFU.

From the remaining 126 persons, 19 samples were lost and 2 samples were not amplifiable; thus, we recovered amplifiable DNA from 105 participants. The participants were divided into European American and African American cohorts on the basis of self-report. Each cohort was divided into 4 groups of individuals, with 0 pustules (resolvers) or 1, 2, or 3 pustules (pustule formers) at 35000HP-inoculated sites. The participants included 59 European Americans (33 males and 26 females; age range, 21–59 years; mean age [±standard deviation {SD}], 36.3 ± 11.8 years) and 46 African Americans (29 males

Table 1. Genetic Characteristics Analyzed in This Study

Gene, SNP(s)	Allele	rs Number	Haplotype Configurations at Select Loci
TLR2			
−16 934	T > A	rs4696480	TG/TA/AG
+2477	G > A	rs5743708	TG/TA/AG
TLR4			
+896	A > G	rs4986790	. . .
TLR9			
−1237	T > C	rs5743836	TA/TG/CA/CG
+2848	A > G	rs352140	TA/TG/CA/CG
NOD1			
+32 656	T > GG	rs6958571	. . .
NOD2			
+2104	C > T	rs2066844	. . .
+3020	C insertion	rs2066847	. . .
SIGIRR			
−146	G > T	rs7396562	GCA/GCG/TTG/TTA
+53	C > T	rs4074794	GCA/GCG/TTG/TTA
+935	G > A	rs3210908	GCA/GCG/TTG/TTA
IL10			
−2849	A > G	rs6703630	AAC/AAT/AGC/GAC/GGC/AGT/GGT
−1082	A > G	rs1800896	AAC/AAT/AGC/GAC/GGC/AGT/GGT
−819	C > T	rs1800871	AAC/AAT/AGC/GAC/GGC/AGT/GGT

Abbreviations: rs, reference SNP cluster identification; SNP, single-nucleotide polymorphism.

and 17 females; age range, 21–64 years; mean age [±SD], 42.3 ± 10.6 years; Figure 1).

Ethics Statement

Study protocols and informed consent statements were approved by the Division of Microbiology and Infectious Diseases of the National Institutes of Allergy and Infectious Diseases and by the Institutional Review Board of Indiana University.

DNA Isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from whole-blood specimens, using the Accuspin System–Histopaque-1077 kit (Sigma-Aldrich). DNA was isolated from PBMCs by using the High Pure PCR Template Preparation Kit (Roche Applied Science).

SNP Determination

The isolated DNA was genotyped for 14 SNPs in 7 genes (Table 1), using real-time PCR assays on the LightCycler 480 (Roche Molecular Diagnostics, Almere, the Netherlands). The PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C for 10 seconds, annealing at 60°C for 60 seconds, and elongation at 72°C for 1 second. For the SNP *IL10* –1082 A > G, annealing was done at 55°C for 1 minute each cycle. The primer and probe sequences used in these assays are specimen in Supplementary File 1.

Statistical Analyses

Statistical analyses were performed using GraphPad Instat 3. Results between sample groups were examined for Hardy-Weinberg equilibrium. χ^2 tests for trends were performed where appropriate to assess differences in genotype distributions between the groups (0, 1, 2, or 3 pustules). Haplotype distribution (Table 1) was inferred using PHASE software and analyzed using χ^2 tests for trends. Carrier trait analyses were performed to examine synergy in protective or risk-enhancing associations of different SNPs and haplotypes. To reduce data complexity, binary logistic regression was performed using SPSS v20.

Analysis of *H. ducreyi* CpG Motifs

To determine the potential immunostimulatory activity of 35000HP DNA, we calculated the CpG index for *H. ducreyi* exactly as described previously [28, 29]. The results for *H. ducreyi* were compared to those calculated previously for *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae.*

RESULTS

Genotyping Results

Amplifiable DNA was recovered from 105 persons who were infected with *H. ducreyi* and met inclusion criteria. The participants included 59 European Americans and 46 African Americans who formed 0, 1, 2, or 3 pustules (Figure 1). In each cohort, there were no significant differences in the doses of *H. ducreyi* among the 4 outcome groups (data not shown). Table 2 shows the overall frequency of the genotypes in each cohort.

We assessed potential links between SNPs and haplotypes and the outcome of infection by using χ^2 tests. Within each

Table 2. Genotype Frequencies, by Study Cohort

		European American Cohort			African American Cohort		
Gene, SNP(s)	Allele	WT	HZ	MT	WT	HZ	MT
TLR2							
−16 934	T > A	6 (10)	27 (48)	26 (44)	23 (50)	20 (43)	3 (7)
+2477	G > A	54 (92)	5 (8)	0 (0)	45 (98)	1 (2)	0 (0)
TLR4							
+896	A > G	52 (88)	7 (12)	0 (0)	39 (85)	7 (15)	0 (0)
TLR9							
−1237	T > C	46 (78)	11 (19)	2 (3)	13 (28)	24 (52)	9 (19)
+2848	A > G	19 (32)	28 (47)	12 (20)	2 (4)	27 (59)	17 (37)
NOD1							
+32 656	T > GG	35 (59)	22 (37)	2 (3)	24 (52)	17 (37)	5 (11)
NOD2							
+2104	C > T	52 (88)	7 (12)	0 (0)	46 (100)	0 (0)	0 (0)
+3020	C insertion	54 (92)	5 (8)	0 (0)	46 (100)	0 (0)	0 (0)
SIGIRR							
−146	G > T	47 (80)	10 (17)	2 (3)	21 (46)	22 (48)	3 (7)
+53	C > T	47 (80)	10 (17)	2 (3)	21 (46)	22 (48)	3 (7)
+935	G > A	27 (46)	25 (42)	7 (12)	41 (89)	4 (9)	1 (2)
IL10							
−2849	A > G	36 (61)	20 (34)	3 (5)	25 (54)	16 (35)	5 (11)
−1082	A > G	20 (34)	25 (42)	14 (24)	15 (33)	23 (50)	8 (17)
−819	C > T	27 (48)	26 (44)	6 (9)	18 (39)	22 (48)	6 (13)

Data represent the number of persons and (their percentage) in each cohort.

Abbreviations: HZ, heterozygous; MT, mutant allele; SNP, single-nucleotide polymorphism; WT, wild type.

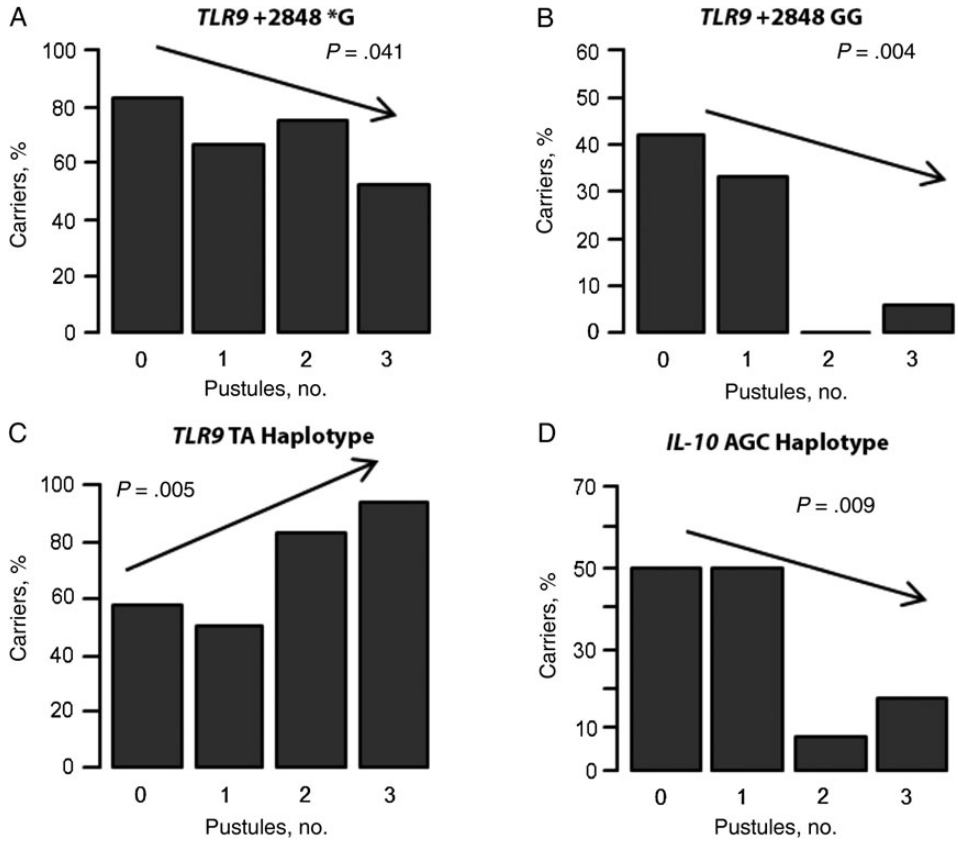

Figure 2. Bar plots and trend lines for single-nucleotide polymorphisms (SNPs) and haplotypes found to have significant effects on the outcome of experimental infection in European Americans, using χ^2 tests for trend. The data show the percentage of volunteers who carried a particular SNP or haplotype in the 4 outcome groups. Analyses are shown for *TLR9*+2848 *G genotype (*A*), *TLR9*+2848 GG genotype (*B*), *TLR9* haplotype TA (*C*), and *IL10* haplotype AGC (*D*). The data in panels *A*, *B*, and *D* show protective effects against pustule formation, while the data in panel *C* show a risk-enhancing effect.

ethnicity, χ^2 tests for trend on the SNPs and haplotypes showed multiple significant results (Figures 2 and 3). There were significant protective associations against pustule formation for the *TLR9* +2848 GG ($P = .004$) and *G ($P = .041$) genotypes and for the *IL10* AGC haplotype ($P = .009$) in the European American cohort. A significant risk-enhancing association for pustule formation was found for the haplotype *TLR9* TA in the European American cohort ($P = .005$); a borderline risk-enhancing association was found for the haplotype *IL10* AAC ($P = .058$) in the African American cohort. No significant results were found for the other analyzed SNPs or haplotypes.

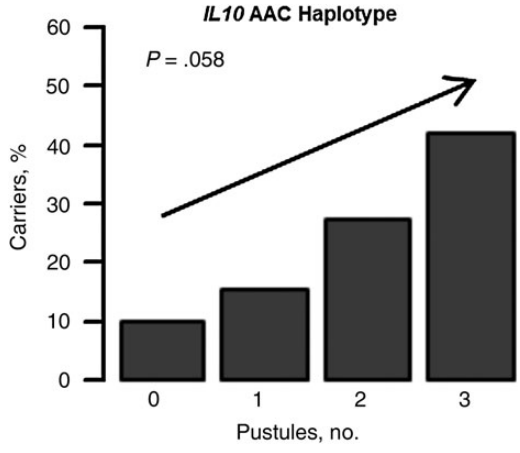

Figure 3. Bar plot and trend line for the *IL10* AAC haplotype, which had a significant risk-enhancing effect on the outcome of experimental infection in African Americans, using χ^2 tests for trend. The data shows the percentage of volunteers who carried this haplotype in the 4 outcome groups.

Carrier Trait Analyses

We assessed the synergy in protective or risk-enhancing associations between combined SNPs or haplotypes and the outcome of infection by χ^2 tests for trends. Two combinations of variables showed a significant association with the severity of *H. ducreyi* infection. In the European American cohort, only the *TLR9* +2848 *G genotype combined with the *IL10* AGC haplotype had an increased significance of a protective effect as compared to any of the single SNPs ($P = .012$). In the African American cohort, the *IL10* –2849 *G genotype combined with the *SIGIRR* TTG haplotype had an increased significance of a protective effect as compared to single SNPs or haplotypes ($P = .02$).

Table 3. Results of Logistic Regression on Probable Association Models

Genotype/Haplotype	Outcome[a]	Cohort	*P*	OR[b] (95% CI)
TLR9 +2848 GG	1, 2, or 3 vs 0	European American	.052	0.42 (.17–1.01)
IL10 –2849 AA	1, 2, or 3 vs 0	African American	.032	0.18 (.04–.86)
IL10 AAC Haplotype	1, 2, or 3 vs 0	African American	.024	3.08 (1.16–8.13)

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Group dichotomization by outcome (0, 1, 2, or 3 pustules).

[b] An OR of <1 indicates a protective effect, and an OR of >1 indicates a risk-enhancing effect.

Logistic Regression

We used forward stepwise binary logistic regression with dichotomized groups of the formed pustules as the dependent variable to produce models for each cohort. Only variables with a *P* value of <.2 in the χ^2 tests for trend were included in the models. In the European American cohort, the model included SNPs at *TLR2* −16934, *TLR9* +2848, and *SIGIRR* +935; the *TLR9* haplotype TG; and the *IL10* haplotypes AGC and GGC. In the African American cohort, the model included SNPs at *IL10* –819 and *IL10* –2849, the *SIGIRR* haplotype TTG, and the *IL10* haplotype AAC. The major results are shown in Table 3. In the European American cohort, there was a trend toward a protective association with the *TLR9* +2848 GG genotype ($P = .052$); in the African American cohort, the *IL10* –2849 AA genotype showed a significant protective association ($P = .032$), and the *IL10* AAC haplotype had a significant risk-enhancing association ($P = .024$). In general, these results were consistent with the trends analysis shown in Figures 2 and 3. No significant results were found for the other analyzed SNPs and haplotypes.

Calculated CpG Index

Since TLR9 is activated by CpG motifs in bacterial DNA, we calculated a CpG index for 35000HP DNA and compared it to results previously described for several other bacterial pathogens [28]. While a CpG index of <1 is considered immunoinhibitory, a CpG index of >1 is regarded as immunostimulatory. The calculated CpG index for *H. ducreyi* was 6.6, which was similar to the indices calculated for *S. pneumoniae* and *H. influenzae* (Table 4).

DISCUSSION

Here we sought to find contributions of host immunogenetic factors on the outcome of experimental *H. ducreyi* infection. Because Hardy-Weinberg equilibrium showed differences in the genotypes of the African Americans and European Americans, these cohorts were analyzed separately. Our cohorts were unique in that the participants had clearly distinguishable phenotypes and could be placed into defined groups (0, 1, 2, or 3 pustules), which allowed us to do a trend analysis. Despite our small sample size, the fact that all our participants were infected with *H. ducreyi* likely permitted us to find significant genetic associations with disease outcomes.

In the European American cohort, we found that the tendency to resolve experimental infection was associated with the *TLR9* +2848 *G and GG genotypes, but the TA haplotype of this gene showed a risk-enhancing effect for pustule formation. In contrast, Sanders et al showed a protective association for *TLR9* +2848 GA or AA alleles in control children versus those with bacterial meningitis in the Netherlands; the protective effect is against *N. meningitidis* but not against *S. pneumoniae* or *H. influenzae* [28]. The *TLR9* +2848 AA genotype is also associated with a decreased incidence of positive blood culture results among children who have meningococcal meningitis, again suggesting that some degree of protection against *N. meningitidis* is conferred by this genotype [30].

One explanation of the different effects of these *TLR9* alleles on susceptibility to bacterial infection could be that the

Table 4. Calculated CpG Indices

	Genome				Consensus CpG Motif[a]		
Bacterium	Size, Mb	G+C, %	CpG Motifs/kb[b]	Total CpG[c]	Stimulatory, %[d]	Inhibitory, %[e]	CpG Index[f]
Haemophilus ducreyi	1.7	38.2	40.9	112.2	124.8	110.4	6.6
Haemophilus influenzae[g]	1.91	38.2	72.8	109.1	105.5	96.4	7.2
Streptococcus pneumoniae[g]	2.22	39.5	78.0	69.5	82.4	66.5	8.6
Neisseria meningitidis[g]	2.27	51.5	132.7	130.6	78.4	140.0	–106.8

[a] Deviations in specified motif occurrence, relative to those expected on the basis of genomic G+C content.

[b] No. of CpG hexamer motifs (NNCGNN) in each genome, normalized to 100 kb of DNA.

[c] Total frequency of CpG hexamer motifs (NNCGNN).

[d] Frequency of stimulatory CpG hexamer motifs (RRCGYY).

[e] Frequency of inhibitory CpG hexamer motifs (NCCGNN and NNCGRN).

[f] Calculated as the difference between stimulatory and inhibitory hexamer motifs, multiplied by the total number of CpG hexamer motifs, normalized to 1 kb.

[g] Data are taken from Table 3 of the article by Sanders et al [28].

activation of TLR9 is triggered by binding of unmethylated bacterial CpG DNA motifs, which lead to the production of inflammatory cytokines [31]. The amount and structure of CpG motifs in bacterial DNA affect its ability to activate TLR9; calculated CpG indices of >1 are proinflammatory, while indices of <1 are antiinflammatory [28, 29]. The calculated CpG index for *N. meningitidis* is very low (−106.8) relative to that for *S. pneumoniae* (8.6) and for *H. influenzae* (7.2). These data led to the hypothesis that the *TLR9* +2848 GA or AA alleles might compensate for the antiinflammatory potential of meningococcal DNA and protect the host against disease [28]. The CpG index of *H. ducreyi* 35000HP DNA, calculated by the same method [28, 29], was 6.6. Since pustule formation is marked by hyperinflammatory responses in tissue and dendritic cells [22], perhaps the *TLR9* +2848 *G and GG alleles counter hyperinflammatory responses to *H. ducreyi* that lead to tissue damage. Similarly, in Ghanaian children with malaria, the *TLR9* +2848 GG genotype is not associated with protection against parasitemia (ie, infection) but is associated with protection from symptomatic disease (ie, inflammation) [32]. In our cohort, the contrasting result found for the *TLR9* TA haplotype may be due to the fact that this haplotype lacks the protective *TLR9* +2848 *G and GG genotypes. Since *TLR9* +2848 G is a synonymous coding SNP, how this SNP affects *TLR9* expression and subsequent activity is unclear.

Variation in *IL10* polymorphisms and IL-10 production are linked to various immunosuppressive or inflammatory conditions. In our study, we found that the *IL10* –2849 AA genotype in the African American cohort had a statistically significant protective effect against pustule formation. Two studies reported an association between *IL10* –2849 AA and low IL–10 production by endotoxin-stimulated whole blood [33, 34]. The finding that *IL10* –2849 AA is associated with resolution is consistent with our previous report showing that DCs derived from resolvers have less IL-10 transcription and secretion than pustule formers in response to *H. ducreyi* [22]. IL-10 is an antiinflammatory cytokine that inhibits the activation and function of T cells, NK cells, and macrophages [35]. Production of high levels of IL-10 by DCs during *H. ducreyi* infection could promote T-helper type 2 (Th2) cell and regulatory T-cell responses and inhibit the activation of Th1 cells and macrophages, leading to impaired clearance of *H. ducreyi* [22].

The *IL10* AGC haplotype had a protective effect on *H. ducreyi* infection in the European American cohort, while the *IL10* AAC haplotype showed a risk-enhancing effect in the African American cohort. Several studies suggest that protection against infection is linked to haplotypes producing low levels of IL-10, while risk enhancement is linked to haplotypes producing high levels of IL-10 [36, 37]. The AAC haplotype has been shown and the ACG haplotype assumed to be producers of low levels of IL-10, owing to the inclusion of genotype *IL10* –2849 A [36]. If this is the case, one would expect both haplotypes to be protective against *H. ducreyi*. However, the levels of IL-10 expression could be influenced by *IL10* –1082 genotypes; PBMCs from European cohorts with the *IL10* –1082 GG genotype secrete more IL-10 than those with the *IL10* –1082 AA genotype in response to *C. trachomatis* [38]. Similarly, *H. pylori*–infected patients with the *IL10* –1082 GG genotype express more IL-10 in mucosal biopsy specimens than those with the AA genotype [37]. Additionally, the general genetic background of the European American and African American cohorts might affect IL-10 expression. As no plasma or peripheral blood samples were available from the *H. ducreyi*–infected cohorts, we were unable to correlate their IL-10 secretion capacity with the 2 IL-10 haplotypes.

In the European American cohort, the *TLR9* +2848 *G genotype combined with the *IL10* AGC haplotype had an increased significance of a protective effect as compared to the single SNPs, which may be due to the potential antiinflammatory effects of both SNPs discussed above. In the African American cohort, the *IL10* –2849 *G genotype combined with the *SIGIRR* TTG haplotype also had an increased significance of a protective effect. The *IL10* –2849 *G genotype is associated with high production of IL-10 [33, 34]. SIGIRR is a negative regulator of the TLR pathways, and SIGIRR deficiency in mice leads to hyperinflammatory response and tissue damage in microbial infections [39]. Currently, there are no other reports on associations of the *SIGIRR* TTG haplotype with any inflammatory conditions. The *SIGIRR* –146TT genotype, which is contained in the TTG haplotype, is significantly associated with the susceptibility to systemic lupus erythematosus [40]. Perhaps hyperinflammatory responses potentially conferred by the *SIGIRR* TTG haplotype are offset by potentially higher levels of IL-10 induced by the *IL10* –2849 *G genotype, leading to a balanced inflammatory response against *H. ducreyi* and effective clearance of the pathogen.

In the human challenge model, there are no effects of race or age on pustule formation, but men form pustules at rates approximately 1.7 fold higher than women, consistent with the high male to female ratio seen in natural chancroid [1]. Men and women were included in this study. Analysis for potential sex-related influences on the results, using Mantel-Haenszel tests in conjunction with the Tarone tests, showed no significant differences between results related to sex.

Since differences in innate immune responses are associated with the outcome of *H. ducreyi* infection, we chose to include genes only from innate immune pathways in this study. One effect of this targeted approach was a reduced need for corrections for multiple comparisons. In addition, the statistical tests used in this study provide a clear picture through both univariate and multivariate testing, while the logistic regression model already accounts for multiple comparisons in its design.

Although we found associations between *TLR9* and *IL10* SNPs with outcome, no significant links were found for other SNPs in several other genes encoding PRRs. Compared to most immunogenetic studies, which usually compare large

groups of infected patients to healthy controls, our cohorts were small; it is possible that the lack of finding other associations was due to our small sample size.

In summary, this is the first study to shed light on the immunogenetic factors affecting the outcome of *H. ducreyi* infection. Our results could be used to predict the risk of susceptibility to *H. ducreyi* infection in future studies. Studies on the effects of the *TLR9*, *IL10,* and *SIGIRR* SNPs on immune responses to *H. ducreyi* are also needed to gain better understanding of differential host susceptibility to the pathogen.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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