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Original article

Population-based genetic epidemiologic analysis of *Chlamydia trachomatis* serotypes and lack of association between *ompA* polymorphisms and clinical phenotypes

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Abstract

Chlamydia trachomatis is the leading cause of bacterial sexually transmitted diseases worldwide. Urogenital strains are classified into serotypes and genotypes based on the major outer membrane protein and its gene, *ompA*, respectively. Studies of the association of serotypes with clinical signs and symptoms have produced conflicting results while no studies have evaluated associations with *ompA* polymorphisms. We designed a population-based cross-sectional study of 344 men and women with urogenital chlamydial infections (excluding co-pathogen infections) presenting to clinics serving five U.S. cities from 1995 to 1997. Signs, symptoms and sequelae of chlamydial infection (mucopurulent cervicitis, vaginal or urethral discharge; dysuria; lower abdominal pain; abnormal vaginal bleeding; and pelvic inflammatory disease) were analyzed for associations with serotype and *ompA* polymorphisms. One hundred and fifty-three (44.5%) of 344 patients had symptoms consistent with urogenital chlamydial infection. Gender, reason for visit and city were significant independent predictors of symptom status. Men were 2.2 times more likely than women to report any symptoms ($P = 0.03$) and 2.8 times more likely to report a urethral discharge than women were to report a vaginal discharge in adjusted analyses ($P = 0.007$). Differences in serotype or *ompA* were not predictive except for an association between serotype F and pelvic inflammatory disease ($P = 0.046$); however, the number of these cases was small. While there was no clinically prognostic value associated with serotype or *ompA* polymorphism for urogenital chlamydial infections except for serotype F, future studies might utilize multilocus genomic typing to identify chlamydial strains associated with clinical phenotypes.

Keywords: *Chlamydia trachomatis*; Molecular epidemiology; Population-based; Genotype; Phenotype; Genetic polymorphism

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1. Introduction

Chlamydia trachomatis (CT) is the leading bacterial cause of sexually transmitted diseases (STD) worldwide. The sequelae of these infections, including pelvic inflammatory disease (PID), ectopic pregnancy and infertility, account for ~80% of the ~US$2.5 billion annual cost of STDs in the United States [1]. There has been considerable interest in identifying a precise CT strain typing system that is clinically prognostic to guide therapeutic algorithms.

CT strain identification involves serotyping the major outer membrane protein (MOMP) [2] and genotyping *ompA*, which encodes MOMP. Serotypes A, B, Ba and C cause trachoma, a chronic ocular disease found in developing countries. Ba has also been isolated from the genital tract, and *ompA* sequences of urogenital and ocular Ba strains have identified nucleotide changes that divide Ba into distinct tissue-tropic groups [3,4]. Serotypes D-K, Da, Ia, L1–3 and L2a are responsible for urogenital disease. Lymphogranuloma venereum (LGV) serotypes L1, L2, L2a and L3 cause more invasive disease. These latter serotypes as well as D, Da, and G are more frequently associated with proctitis than any of the other urogenital serotypes [5–7].

ompA allelic polymorphisms have been found in 39–66% of trachoma [8–10] and urogenital [3,11–14] samples worldwide. Differences within *ompA* can encode MOMP epitopes that are immunologically distinct from reference serotypes [8,13,15,16]. Serotyping and genotyping classifications differentiate the more virulent LGV strains from all others and, in some cases, predict ocular vs. urogenital tissue specificity. However, research directed at extending clinical disease correlates of MOMP and *ompA* with non-LGV strains has been limited, and the results of previous studies have been inconsistent.

Multiple studies in Europe found associations between serotypes and genital tract signs and symptoms that were not reproducible even though patient populations were similar. These associations were likely due to chance since significance levels were not corrected for multiple comparisons [6,17–21]. A recent U.S. study found no association between serotype and disease manifestations, but the authors did not exclude patients with co-pathogens as potential confounders in the analyses [22]. Two studies have evaluated the relationship between *ompA* genotypes and disease severity: one found an association of specific genotypes with upper or lower genital tract disease [12] while the other found no such association [23]. The inconsistency of these findings is multifactorial, including differences in study design, insufficient control for confounding, lack of multiple comparison correction for significance levels, and small sample sizes. Additionally, insufficient sequencing of and failure to identify *ompA* polymorphisms has resulted in imprecise assignment of serotypes/genotypes for many studies.

Because past studies lacked precise CT strain typing, we recently sequenced over 280,000 base pairs of *ompA* and identified nucleotide and amino acid polymorphisms for serotypes of 507 urogenital CT samples from five cities in the U.S. [4]. In the present study, we applied this new strain differentiation scheme for correlating CT with clinical disease. We analyzed 344 urogenital samples from men and women with CT infections from the aforementioned study [4]. Serotype based on *ompA* genotype and *ompA* polymorphisms were correlated with clinical and demographic data to investigate associations with clinical signs and symptoms and outcome, such as PID, that might predict virulence determinants and clinical phenotype.

2. Methods

2.1. Study design and population

This cross-sectional case series was conducted in 1995 to 1997 in collaboration with the Centers for Disease Control (CDC), Atlanta, GA. The CDC conducted a large parent study that was designed to (1) evaluate diagnostics for urogenital chlamydial infection for females and asymptomatic males and (2) determine risk factors for urogenital chlamydial infections [24]. As part of this larger study, a sentinel surveillance network was established utilizing family planning, STD and community health clinics in and around the Birmingham, Indianapolis, New Orleans, San Francisco and Seattle metropolitan areas [4]. Clinicians were chosen from these clinics to collect the medical, STD, reproductive and treatment histories, demographic information and clinical samples from consented individuals using sample collection procedures as described previously [4]. Ethnicity was self-reported with categories as defined by the standard NIH guidelines as per the NIH enrollment form. For all participants, clinical samples were obtained to detect CT and *Neisseria gonorrhoeae*. If clinical concern warranted it, additional samples were obtained to test for *Candida albicans, Trichomonas vaginalis, Herpes simplex*, HPV and *Gardnerella* (causative for bacterial vaginosis) using methods that were considered standard microbiologic technique for that organism. Patients were excluded from the parent study if they had taken antibiotics within the past 30 days, were pregnant, had had a hysterectomy, or had not received a pelvic examination.

Cases for the genotyping study were defined as those consented patients who did not meet exclusion criteria of the parent study diagnosed with CT urogenital infection by ligase chain reaction, Amplicor polymerase chain reaction and/or culture as described previously [4]. From the thousands of cases available, approximately one hundred cases from each metropolitan site with roughly equal distribution of gender were randomly allocated for inclusion into the genotyping study [4]. Given that co-pathogen infection might confound the relationship between *C. trachomatis* genotype and clinical phenotype, we chose to exclude patients from the genotyping study if they had diagnostic evidence of STD co-pathogen infection (e.g. *C. albicans, T. vaginalis, H. simplex*, HPV, *Gardnerella*, etc.) or had insufficient laboratory testing to determine the co-pathogen status. From the 507 patients included in the genotyping study, we excluded 163 patients who had diagnostic evidence of co-pathogen infection or who had insufficient information on co-pathogen status. Thus, 344 consented patients aged 14–49 years of age with documented CT infection and

without exclusion criteria for the parent study or this study remained in the analysis. For the 344 patients included in the analysis, all clinical and demographic data were checked by study staff for accuracy. Patient confidentiality was maintained by using identification numbers. Chlamydial strain differentiation based on *ompA* genotyping and estimation of serotype was performed as described previously [4].

2.2. Statistical analysis

Six dichotomous outcome variables were analyzed: (1) CT-probable symptoms including any in #2–5; (2) mucopurulent cervicitis, abnormal vaginal or urethral discharge; (3) dysuria; (4) lower abdominal pain; (5) abnormal vaginal bleeding; and (6) PID. Patient complaint was used except that PID was evaluated by a physician and defined as lower abdominal pain with >2 of the following: adnexal tenderness, cervical motion tenderness and uterine tenderness. For mucopurulent cervicitis, vaginal or urethral discharge, physician findings were used when available as they were considered more accurate than patient reporting; clinician assessments were available for all (58/58) individuals in Birmingham, 81 (94.2%) in Seattle, 50 (70.4%) in Indianapolis, 9 (22.0%) in San Francisco, and 0 (0%) in New Orleans. Covariates that were analyzed included gender, race/ethnicity, age, metropolitan area, reason for visit, clinic type, usual (majority of time) contraceptive method in last 60 days, *ompA* genotype (as defined in [4]), and *ompA* nucleotide positions that varied when comparing the entire sequence set.

For outcome variables, unadjusted analysis of each main variable was performed using unconditional logistic regression with significance determined using the likelihood ratio test (alpha of 0.05). In the unadjusted analysis of serotype, data were too sparse to determine valid parameter estimates. Thus, the variable was fit to a serotype without including the two rare serotypes Ba and H. Covariates considered for inclusion in multiple regression models included the main variable of interest and any potential confounder that, when included in the model, resulted in at least a 10% change of the regression coefficient from its baseline value. For the outcome variables that were rare (abnormal vaginal bleeding, lower abdominal pain and PID), Fisher's two-tailed exact test was used. All analyses were performed using SAS version 7.

For nondichotomous main variables, including *ompA* serotype and genotype, the individual components were analyzed further when the global analysis was significant using a Bonferroni adjustment to account for the number of comparisons involved. As an exception to this, we tested individual serotype associations found in previous studies including: (a) Ia with asymptomatic infections [17]; (b) D with asymptomatic infections [18]; (c) G/Ga with symptomatic infections among women [17]; (d) Ga with symptomatic infections overall and among men [17]; and (e) F with PID [12] using Fisher's two-tailed exact test. Since these were a priori hypotheses, no corrections for multiple comparisons were applied to these individual pairwise tests. Any *ompA* polymorphism was considered an additional main variable of interest. To determine if nucleotide position was associated with outcome variable, a Chi-square test of homogeneity was performed using nucleotide character states as categories, both overall and by gender. Significance levels were corrected with the Bonferroni method for the total number of positions tested (5 comparisons × number of polymorphisms).

3. Results

3.1. Nucleotide sequence data

As described previously [4], 810 base pairs of the *C. trachomatis ompA* gene obtained from clinical specimens were sequenced for all participants in the study. These 810 base pairs spanned from nucleotide 244 to 1053, which corresponds to the region upstream of variable segment one (VS1; nt 256–324) without interruption through the end of variable segment four (VS4; nt 949–1053). Within the region sequenced, there were 329 nucleotide polymorphisms, with nonsynonymous and synonymous mutation rates within serotype groups being similar to that already published [4].

3.2. Demographics

There were 180 (52%) males and 164 (48%) females, 72% of whom were African American, 17% Caucasian, 4% Hispanic, 4% Asian/Pacific Islander, 3% other, and <1% unknown; 75% were <25 years of age (Table 1).

3.3. Risk factors for CT urogenital symptoms and outcomes

Gender, reason for visit, and metropolitan area were significant independent predictors of symptoms consistent with

Table 1
Demographic distribution for the study population from five metropolitan areas in the United States

Gender	$n = 344$ (%)
Male	180 (52.3)
Female	164 (47.7)
Race/ethnicity	
Caucasian	57 (16.6)
African American	246 (71.5)
Hispanic	16 (4.7)
Asian/Pacific Islander	16 (4.7)
Other	8 (2.3)
Unknown	1 (0.3)
Age	
<15 years	5 (1.5)
15–20 years	143 (41.6)
20–25 years	116 (33.7)
25–30 years	48 (14.0)
30–35 years	21 (6.1)
>35 years	11 (3.2)
Birmingham	58 (16.9)
Indianapolis	69 (20.1)
New Orleans	90 (26.2)
Seattle	86 (25.0)
San Francisco	41 (11.9)

CT; overall, 153 (44.5%) of 344 study subjects (99 males; 54 females) reported CT-probable symptoms; 141 (41%) complained of vaginal or urethral discharge, 39 (11.3%) complained of dysuria, 12 (7.3%) of 164 women complained of lower abdominal pain, 3 (1.8%) of 164 women complained of abnormal vaginal bleeding, and 2 (1.2%) of 164 women were clinically diagnosed with PID. The relative risk for men to report any symptom was 2.2 times more than for women ($P = 0.03$) and 2.8 times more for urethral discharge than women for a vaginal discharge ($P = 0.007$) in adjusted analyses (Table 2). Those who had contact with an individual with nongonococcal urethritis (NGU) or CT, or who presented because of symptoms, were 14.9 ($P = 0.004$), 4.4 ($P = 0.0001$) and 6.8 ($P = 0.0001$) times more likely to report symptoms, respectively, than those who presented for STD evaluation. In contrast, those who presented for family planning evaluation were 0.11 times less likely to report symptoms than those who presented for STD evaluation ($P = 0.04$; Table 2).

3.4. Serotype and symptoms consistent with CT infection

The serotype distribution was: E (30%); F (20.6%); Ia (14.5%); D (13.5%); J (9.9%); G (4.4%); K (4.4%); Ja (1.5%); Ba (0.6%) and H (0.6%). None of the covariates analyzed was significantly associated with serotype except for metropolitan area, where Ba and G predominated in San Francisco [4].

Serotype was not significantly associated with CT-probable symptoms both overall and stratified by gender for the composite variable. Details are given in Table 3. This was the case in unadjusted analysis and after adjusting for metropolitan area. When analyzing the four most prevalent serotypes (D, E, F and Ia) alone, there was still no significant association between serotype and CT-probable symptom ($P = 0.69$). Similarly, seroclass (defined as B: Ba, D, Da and E; Intermediate: F and G; and C: A, C, H, I, Ia, J, Ja and K) or individual serotypes D, Ia and G (those reported in other studies to have associations) were not significantly associated with CT-probable symptoms.

There were no significant serotype differences for dysuria, abnormal vaginal bleeding or lower abdominal pain after adjusting for confounders. When evaluating for serotype differences for vaginal or urethral discharge and after stratifying by gender, women were 4.5 (95% confidence interval (CI): 1.01–20.0) times less likely to have a vaginal discharge when infected with serotype D compared to others ($P = 0.03$). Both women with clinically diagnosed PID (2 (1.4%) of 147) were infected with F and were significantly more likely to be infected with F than any other serotype ($P = 0.046$; odds ratio (OR) = infinity). Table 4 reveals the number and distribution of serovars for each specific symptom.

3.5. ompA polymorphisms and symptom status

To determine whether *ompA* polymorphisms (distinct from serotype) were associated with outcome variables, each of the 329 *ompA* polymorphisms identified was tested. For women, five polymorphisms were significantly associated

Table 2
Adjusted odds ratios for risk of chlamydial-probable symptoms and for risk of having mucopurulent cervicitis, vaginal or urethral discharge according to the significant independent predictors measured in the population-based study

Covariate	Adjusted OR (95% CI) symptom status	*P* value symptom status	Adjusted OR (95% CI) cervicitis or discharge	*P* value cervicitis or discharge
Gender				
Female	1.0[a]		1.0[b]	
Male	2.2 (1.1–4.3)[a]	0.03	2.8 (1.4–5.6)[b]	0.007
Reason for visit[c]				
STD check	1.0[b]		1.0[c]	
Contact CT	4.4 (1.6–11.9)[d]	<0.001	5.1 (1.8–14.1)[d]	0.002
Contact NGU	14.9 (1.7–131.1)[d]	0.004	8.2 (1.5–45.4)[d]	0.02
Contact GC	1.28 (0.3–5.5)[d]	0.74	1.6 (0.4–7.0)[d]	0.55
Contact other STD	0.76 (0.3–2.3)[d]	0.63	0.70 (0.2–2.2)[d]	0.52
Symptomatic	6.8 (2.8–16.5)[d]	<0.001	5.0 (2.1–11.8)[d]	<0.001
Family planning	0.11 (0.01–0.88)[d]	0.04	0.12 (0.02–1.1)[d]	0.06
Prenatal	0.46 (0.08–2.5)[d]	0.36	0.2 (0.02–2.0)[d]	0.18
Other reason	0.59 (0.2–1.8)[d]	0.34	0.7 (0.2–2.1)[d]	0.52
Race/ethnicity				
African American		>0.05	1.0[e]	
Caucasian		>0.05	1.28 (0.5–3.0)[e]	0.56
Asian/Pacific Islander		>0.05	0.11 (0.02–0.65)[e]	0.02
Hispanic		>0.05	0.22 (0.05–1.0)[e]	0.05
Other race/ethnicity		>0.05	0.75 (0.1–4.5)[e]	0.80

OR, odds ratio; CI, confidence interval; STD, sexually transmitted disease; CT, *Chlamydia trachomatis*; NGU, nongonococcal urethritis; GC, gonorrhea.
[a] Adjusted for age, metropolitan area and reason for visit.
[b] Adjusted for metropolitan area and reason for visit.
[c] Adjusted for "reason for visit".
[d] Adjusted for metropolitan area.
[e] Adjusted for reason for visit, metropolitan area and gender.

Table 3
Chlamydial-probable symptom status across all chlamydial serotypes and stratified by gender

Serotype	*n*	Overall		Men		Women	
		Asymptomatic (%)	Symptomatic (%)	Asymptomatic (%)	Symptomatic (%)	Asymptomatic (%)	Symptomatic (%)
Ba	2	2 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
D	47	29 (61.7)	18 (38.3)	11 (44.0)	14 (56.0)	18 (81.8)	4 (18.2)
E	103	56 (54.4)	47 (45.6)	21 (42.0)	29 (58.0)	35 (66.0)	18 (34.0)
F	71	36 (50.7)	35 (49.3)	17 (46.0)	20 (54.0)	19 (55.9)	15 (44.1)
G	15	9 (60.0)	6 (40.0)	5 (62.5)	3 (37.5)	4 (57.1)	3 (42.9)
H	2	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
Ia	50	28 (56.0)	22 (44.0)	13 (46.4)	15 (53.6)	15 (68.2)	7 (31.8)
J	34	21 (61.8)	13 (38.2)	10 (50.0)	10 (50.0)	11 (78.6)	3 (21.4)
Ja	5	2 (40.0)	3 (60.0)	1 (25.0)	3 (75.0)	1 (100.0)	0 (0.0)
K	15	8 (53.3)	7 (46.7)	2 (28.6)	5 (71.4)	6 (75.0)	2 (25.0)
Total	344	191 (55.5)	153 (44.5)	81 (45.0)	99 (55.0)	110 (67.1)	54 (32.9)

with abnormal vaginal bleeding after adjusting for multiple comparisons (Table 5). When stratified by serotype, these five positions and two additional positions (nt 289 and nt 534; Table 5) for woman infected with D were significantly associated with abnormal vaginal bleeding (Table 5).

4. Discussion

This is the first study that couples a large gene sequencing effort with clinical data to investigate the genetic epidemiology of CT STDs in the U.S. population. We provided enhanced genetic and statistical testing to examine whether serotype as defined by *ompA* sequence or specific *ompA* polymorphisms comprise virulence determinants and can predict clinical phenotype. Further, since previous studies included patients with more than one STD, which biases the OR, this study provides a more accurate measure of clinical signs and symptoms that can be attributed solely to CT.

We first evaluated risk factors for symptoms consistent with urogenital infection among CT-positive individuals. Gender, reason for visit and metropolitan area were significant independent predictors. The difference in reported OR between this (OR, 2.2) and other studies (OR, 3.0) [25–27] where men were more symptomatic than women is explained by the fact that only asymptomatic men were included in the first component of the parent study, which comprised 44% of the study data. This represented a selection bias for analysis of gender and symptoms.

We tested whether the MOMP-based serotype classification is predictive of clinical manifestations. We supposed that if there was no difference in clinical presentation for different serotypes that virulence determinants could still be present at individual amino acid positions that do not correlate with serotype. This latter point is supported by the fact that new chlamydial serotypes have been identified based on *ompA* polymorphisms where the corresponding epitope was subsequently recognized by new monoclonal antibodies (MAbs) or a combination of existing MAbs [15,16]. For this reason, we also tested whether specific *ompA* polymorphisms were associated with clinical signs or symptoms, gender or serotype differentiation.

In multiple logistic regression, we found no association of serotype with outcome variable that was a composite of chlamydial probable symptoms, which disagrees with serotype-based studies [6,12,17–21]. For example, associations were found between serotype Ia and asymptomatic individuals; Ga and symptomatic individuals; and G/Ga and symptomatic women [17]. In contrast, Lan et al. found an association only between serotype D and asymptomatic individuals [18]. Both studies utilized Dutch populations and measured serotype by RFLP. However, serotype was tested separately without correction for multiple comparisons. If such corrections had been made, most if not all of the associations would likely have been explained by chance. In contrast, we tested this relationship globally with logistic regression. Since there was no association by the global test, we did not test individual serotype associations except for those reported in other studies (D, F, G, Ga and Ia).

We found a significant association between serotype D and absence of vaginal discharge, albeit the CI was wide. We also found a strong and significant association between PID and serotype F. Despite the rarity of PID, the probability of finding only one serotype in both cases out of 10 possible serotypes that could cause PID is low and would be unexpected due to chance. While there may be an association between F and PID supported by this and our earlier work [12], there are inherent difficulties in measuring PID as clinical assessments vary between observers and silent PID is prevalent and unmeasured by noninvasive techniques. Moreover, PID is associated with prior infection and possibly persistence. Confirmation of the association would require prospective measurement of outcome and serotype as well as invasive confirmation of PID, a study that would be impossible to perform.

We also tested whether *ompA* polymorphism(s) predicts clinical findings. Five polymorphisms among women with any serotype, including D, and seven polymorphisms among women infected with D were significantly associated with abnormal vaginal bleeding (Table 5). These seven polymorphisms represented a unique D sequence divergent from its most similar reference serotype. Thus, the association was solely driven by one sequence. Two women with abnormal vaginal bleeding who were infected with serotypes other than D (i.e., E and J)

Table 4
Number and distribution of serovars for each specific symptom

Serotype	*n*	Vaginal or urethral discharge		Dysuria		Lower abdominal pain		*n*	Women			
									Vaginal bleeding		PID	
		No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)		No (%)	Yes (%)	No (%)	Yes (%)
Ba	2	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	1	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
D	47	31 (66.0)	16 (34.0)	41 (87.2)	6 (12.8)	44 (93.6)	3 (6.4)	22	21 (95.5)	1 (4.5)	22 (100.0)	0 (0.0)
E	103	59 (57.3)	44 (42.7)	89 (86.4)	14 (13.6)	101 (98.0)	2 (2.0)	53	52 (98.1)	1 (1.9)	53 (34.0)	0 (0.0)
F	71	38 (50.7)	33 (49.3)	64 (90.1)	7 (9.9)	68 (95.8)	3 (4.2)	34	34 (100.0)	0 (0.0)	32 (94.1)	2 (5.9)
G	15	9 (60.0)	6 (40.0)	14 (93.3)	1 (6.7)	15 (100.0)	0 (0.0)	7	7 (100.0)	0 (0.0)	7 (100.0)	0 (0.0)
H	2	0 (0.0)	2 (100.0)	1 (50.0)	1 (50.0)	2 (100.0)	0 (0.0)	2	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
Ia	50	30 (60.0)	20 (40.0)	43 (86.0)	7 (14.0)	49 (98.0)	1 (2.0)	22	22 (100.0)	0 (0.0)	22 (100.0)	0 (0.0)
J	34	23 (67.6)	11 (32.3)	32 (94.1)	2 (5.9)	33 (97.0)	1 (3.0)	14	13 (92.9)	1 (7.1)	14 (100.0)	0 (0.0)
Ja	5	2 (40.0)	3 (60.0)	5 (100.0)	0 (0.0)	5 (100.0)	0 (0.0)	1	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
K	15	9 (60.0)	6 (40.0)	14 (93.3)	1 (6.7)	13 (86.7)	2 (13.3)	8	8 (100.0)	0 (0.0)	8 (100.0)	0 (0.0)
Total	344	203 (59.0)	141 (41.0)	305 (88.7)	39 (11.3)	332 (96.5)	12 (3.5)	164	161 (98.2)	3 (1.8)	162 (98.8)	2 (1.2)

Both men and women were included in the first three symptom categories. PID, pelvic inflammatory disease.

Table 5
Odds ratios for abnormal vaginal bleeding according to nucleotide character states at specific variable *ompA* positions

Nucleotide position	Character state with increased risk	Character state with decreased risk	OR	*P* value
Women infected with any serotype, including D				
241	C	G	Infinity	<0.001
295	A	T/G	Infinity	<0.001
399	A	G	Infinity	<0.001
591	G	C/T	Infinity	<0.001
607	C	G	Infinity	<0.001
Women infected with only serotype D				
289	A	G	Infinity	<0.001
534	A	G	Infinity	<0.001

OR, odds ratio.

did not have these polymorphisms. While it is possible that these polymorphisms result in abnormal vaginal bleeding only when the infecting serotype is D, we think it unlikely.

Multiple theoretical explanations for the inability to detect a global association between clinical findings and serotype or genotype, if one exists, include lack of power, nondifferential misclassification, inadequate measurement of confounding, and limiting the search for associated polymorphisms to a single nucleotide. This study was sufficiently powered for prevalent serotypes (D, E, F and Ia) for which we had 80% power to detect a relative risk as low as 2.2. However, the power was insufficient for rare serotypes Ba and H (1100 and 8600 CT-infected individuals, respectively, would be needed for similar power and RR) or PID. Thus, we cannot rule out a difference for rare serotypes or for rare outcomes including PID.

There was essentially no misclassification in exposure variables since the definition of serotype and polymorphism was objective [4]. We think it unlikely that nondifferential misclassification of outcome variables explains the inability to find an association, except for PID where there is considerable misclassification as the diagnosis is based on clinical criteria. Since dysuria and lower abdominal pain are subjective variables, there is minimal chance for misclassification despite wide differences in pain perception. For vaginal or urethral discharge, physician finding was used 58% of the time. Vaginitis due to *C. albicans, T. vaginalis* and *Gardnerella* was not included since each was excluded based on microbiologic testing. While there is some interobserver variation in diagnosing vaginal or urethral discharge, physician finding (along with microbiologic testing) was considered to be more accurate than patient reporting and was used, when available, as the gold standard. For data where both physician finding and patient complaint were available, we found patient reporting to be highly specific (94.1%) but not sensitive (45.6%); patients tended to underreport the complaint compared to physician findings.

It is likely that other outcome variables such as abnormal vaginal bleeding were also underreported when present (i.e., underreported if attributed to menses, spotting between periods or hormonal therapy). The estimate of specificity for abnormal discharge in this study was high, and we expect the same for abnormal vaginal bleeding. Thus, it is unlikely that nondifferential

misclassification biased the risk ratio substantially because a lack in sensitivity in the response variable produces no bias in the risk ratio while a lack in specificity does [28].

Co-pathogen infection was an exclusion criterion. However, co-pathogens such as *Mycoplasma genitalium* were not routinely measured unless there was clinical suspicion, which would result in some misclassification of confounder status. Further, sensitivities of most STD assays are not 100%, which would also contribute some misclassification and confounding. Also, genetic variation in the immunomodulating genes such as the interleukins and TLR4 as well as HLA types could affect the susceptibility and severity of *C. trachomatis* infection and thus could confound the relationship between genotype and phenotype.

While various MOMP determinants and *ompA* genetic differences have been reported to be important in host immune responses [29,30] and tissue tropism [31], they do not appear to contribute to clinical outcomes except perhaps for PID and vaginal bleeding. One shortcoming of our analysis was limiting our search of associated polymorphisms to single nucleotides. We did not search for strings of polymorphisms that could be associated with clinical symptoms because of the difficulty in developing a statistically appropriate searching strategy. Thus, there may be strings of *ompA* polymorphisms that portend clinical phenotype that we were unable to detect. The addition of serology to this study may have been beneficial in this regard. However, there may also be strings of polymorphisms associated with phenotype not expressed by serological determinants. In addition, there may be single or multiple polymorphisms that are related to phenotype in regions not sequenced. This may especially be true upstream of VS1 since this region seems to provide some differentiation for serotype B in terms of tissue tropism [3]. Additional studies may be needed to further explore these possibilities.

Other chlamydial determinants may significantly contribute to enhancing our understanding of clinical phenotypes. These include heat shock protein 60, which has been associated with immunopathogenic responses in human fallopian tissues [32–34], cytotoxin genes [35], partial tryptophan operon proteins that may allow for resistance to interferon gamma through indole rescue [36], Type III secretion system proteins that disrupt signal transduction pathways [37], and chlamydial protease- or proteasome-like activity factor [38]. These areas of research will facilitate development of a multilocus CT classification system and enhanced strain typing for predicting virulence determinants and clinical phenotype. If a new typing system was able to predict which strains were associated with recurrent infection, sequelae such as PID or HIV transmission, a longer treatment period or other therapeutic strategies may be indicated for disease prevention.

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