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Chlamydia trachomatis OmpA Genotyping As a Tool for Studying the Natural History of Genital Chlamydial Infection

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Abstract

Objective—To investigate the relationship of *Chlamydia trachomatis* (CT) outer membrane protein A (OmpA) type to clearance of CT infection prior to treatment.

Methods—CT OmpA genotyping, with amplification and sequencing of *ompA*, was utilized to study the natural history of CT infection (spontaneous resolution versus persistence) in 102 subjects with chlamydia-positive screening tests returning for treatment.

Results—CT OmpA distribution was associated with spontaneous resolution of CT, most notably with CT OmpA genotype J/Ja detected more often from the initial screening CT test than other genotypes in those who then had spontaneous resolution of CT noted at the time of treatment. Five subjects with presumed persisting CT infection had discordant CT OmpA genotypes at the screening and treatment visits, suggesting possible new interval CT infection.

Conclusions—Clearance of chlamydia by the host prior to treatment may be influenced by the CT OmpA genotype infecting the host. CT OmpA genotyping may be a valuable tool in understanding the natural history of chlamydial infections.

Keywords

chlamydia; genotype; MOMP; OmpA; outcome

INTRODUCTION

The outer membrane protein A (OmpA [i.e. MOMP]) of *Chlamydia trachomatis* (CT) is an antigenically diverse and abundant surface protein. CT strains may be differentiated into individual serovars using microimmunofluorescence typing with anti-OmpA monoclonal antibodies. At least 18 CT serovars have been identified [1–3], with the most common ones isolated from the genital tract being serovars D through K [4]. Serotyping efforts may be limited by the lack of readily available serotyping reagents, difficulty in microimmunofluorescence

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interpretation, and inability to distinguish strain variants within a given serovar. Complete nucleotide sequence analysis of the *ompA* gene (encoding OmpA) is an alternate means for OmpA classification which does not have such limitations, and has additional advantages, such as providing the opportunity to determine OmpA types in specimens collected less invasively (e.g., urine or a self-collected vaginal swab) or those that are CT culture-negative.

CT OmpA typing (serotyping or genotyping) has been utilized to study the relationship of OmpA type distribution to demographical characteristics [4–6], clinical manifestations of uncomplicated or complicated CT infection (e.g. symptoms or signs of lower or upper genital tract infection) [7–12], to CT replication (e.g. CT inclusion-forming unit counts in culture) [13], and in studying sexual networks (e.g., in contact tracing) [14,15]. We are utilizing CT OmpA genotyping in an ongoing study of the natural history of chlamydial infection to investigate the relationship of CT OmpA type to CT outcomes (CT clearance in the interval between screening and treatment). While sparse prior CT natural history studies have investigated the relationship of OmpA type to CT outcome [16,17], they have been limited in not OmpA typing CT strains at follow-up visits to confirm persisting infection with the same OmpA genotype (i.e. identical OmpA sequence); without confirming the same ompA sequences, new CT infections could be misclassified as persisting infection. In this manuscript, we present our findings and discuss possible implications of our findings.

METHODS

Study Participants

In September 2002, we initiated a prospective study of the natural history of CT infection. The study population was comprised of male and female patients ≥16 years of age presenting to the Jefferson County Department of Health (JCDH) Sexually Transmitted Diseases Clinic in Birmingham, Alabama for chlamydia treatment within 60 days of a positive *C. trachomatis* screening test. Patients were not treated at the time of initial chlamydia screening due to lack of chlamydia-associated syndromes (e.g. urethritis or cervicitis) or other treatment indications (e.g. chlamydia contact). We previously published findings from our investigation of the relationship of clinical and epidemiological factors to the natural history of CT infection [18]. In this manuscript, we describe our evaluation of CT OmpA genotypes in those subjects from our cohort who had genital or urine specimens available for CT OmpA genotyping from the initial CT screening visit (comprised of subjects enrolled up until October 1, 2006); all but one of these subjects also had specimens from the follow-up treatment visit available for genotyping. The study was approved by institutional review boards of the University of Alabama at Birmingham (UAB) Institutional Review Board and JCDH.

Specimen Collection and CT Detection

At the CT screening visit, the majority of subjects underwent routine CT testing by CT culture of a genital swab specimen (approximately 25% had CT nucleic acid amplification testing of urine or a genital swab specimen rather than culture as part of a study). At the return treatment visit, CT culture was performed on all patients. Methods for specimen collection, transport, and isolation of CT in cell culture at the UAB Chlamydia Laboratory have been previously described [19]. For subjects with CT culture-negative specimens at the treatment visit, CT PCR (COBAS AMPLICORTM; Roche Diagnostic Systems, Inc., Branchburg, NJ) was performed on residual CT transport medium containing genital swabs following the manufacturer's protocol.

CT OmpA Genotyping

CT OmpA genotyping was performed initially at the Centers for Disease Control and Prevention by reported methods [20], then starting in October 2005 was performed at UAB.

For OmpA genotyping at UAB, CT genomic DNA was extracted and purified from 100–200μl of residual chlamydia transport medium containing the genital swab or from urine (1ml centrifuged at 15,000g for 5 minutes and then the pellet resuspended in PBS) using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's protocol. Nested CT ompA amplification was then performed using High Fidelity PCR Master Mix Kit (Roche Diagnostics) with primer pairs amplifying a DNA fragment containing the entire ompA gene from all CT serovars. In the first amplification, 5 μl of the purified DNA template and primers 60UF (modified to 60UFX: GTGCCGCCAGAAAAAGATAG) and 80DR were utilized [20]. Amplification conditions were: 94°C for 2 min, 35 cycles of 94°C for 30s, 60°C for 30s, 72°C for 1 min, and a final elongation step at 72°C for 7 min. Three μl of the initial PCR was used for the second amplification, using the same conditions as the first amplification, except primers utilized were either 40F and JHC203 or JHC202 and JHC203 and also 40 cycles were used [20,21]. PCR products were visualized on a 1% agarose gel, purified using QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA), quantitated by OD, and sequenced on ABI Automated Capillary DNA Sequencing System (Applied Biosystems, Foster City, CA) using dye terminator chemistry with primers JHC202 [21] and 419F [20]. Sequences were assembled and edited with DNA Sequencher version 4.6 (Genecodes, Ann Arbor, MI), and compared to Chlamydia GenBank sequences for identification.

Statistical Analyses

Analyses were conducted on Stata (Stata Corp. Release 8.0, College Station, TX). The relationship of the CT OmpA genotype at the screening visit with patient characteristics and with CT outcomes (no detection vs. detection of CT by CT culture and PCR) at the follow-up treatment visit were analyzed through parametric or nonparametric methods as appropriate. Subjects with persisting CT who had discordant CT genotypes from the initial screening and follow-up treatment visit were excluded from analyses, although we summarize their characteristics and CT OmpA genotypes in Table 1.

RESULTS

Study Participant Characteristics and CT OmpA Genotype Distribution

Of 105 subjects with genital specimens or urine available from the CT screening visit, OmpA genotyping was unsuccessful in 3 subjects (due to unsuccessful amplification of *ompA*). Of the remaining 102 subjects, 92 (90%) were female, 90 (88%) were African American, and the median age was 22 years (range 17–54). The frequency of CT OmpA genotypes from the CT screening visit in descending order was: E (28%), D/Da (23%), J/Ja (19%), Ia (15%), F (11%), H (3%), G (1%) and K (1%). The CT OmpA genotype distribution did not significantly differ by age or race. CT OmpA genotype distribution significantly differed by gender ($p = 0.009$), with CT OmpA genotype E infecting 8 (80%) of 10 men vs. 21 (23%) of 92 women.

CT Outcomes and Relationship to CT OmpA Genotype

At the follow-up treatment visit, 21 (21%) subjects had a negative CT culture, however, 12 of these CT culture-negative subjects had a positive CT PCR. In the 93 subjects with CT detected at both visits by either culture or PCR (i.e., presumed persisting CT infection), 5 (5%) subjects had discordant CT OmpA genotypes between the initial screening and follow-up treatment visits (Table 1) and were excluded from subsequent analyses of CT outcomes and CT OmpA genotypes; the finding of discordant genotypes suggests the possibility that subjects may have resolved their infections and were re-infected rather than persistently infected. The CT OmpA genotype could not be determined from the follow-up treatment visit in 3 subjects, but they were kept in subsequent analyses as they denied new sexual partners.

After excluding subjects with discordant CT OmpA genotypes detected at the initial screening versus the follow-up treatment visits, we assessed in the remaining subjects the association of CT OmpA genotype detected at the screening visit to CT test results (culture results and PCR results for those culture-negative) at the treatment visit (Table 2); the objective was to assess the relationship of OmpA genotype distribution to CT outcome (clearance of infection by culture or by PCR). We found the screening visit CT OmpA distribution was associated with CT culture results at the follow-up treatment visit ($p = 0.05$), with the most notable findings on analyses of individual OmpA genotypes being that compared with CT culture-positive subjects at the treatment visit, those who were CT culture-negative were more often initially infected with CT OmpA genotype J/Ja (39% vs. 15%; $p = 0.04$) and less often F (0% vs. 13%; $p > 0.1$) and I/Ia (6% vs 18%; $p > 0.1$). When taking into account PCR results in CT culture-negative subjects at the treatment visit, there was no significant association of initial CT OmpA genotype distribution with CT detection (by culture or PCR) versus no CT detection at the treatment visit ($p > 0.1$); however, on analyses of individual genotypes, J/Ja was found more in those who were CT culture and PCR negative (44% vs. 17%; $p = 0.07$).

DISCUSSION

Our study demonstrated that CT OmpA genotyping can be a valuable tool in studying the natural history of CT infection. One application of CT OmpA genotyping with respect to natural history of chlamydia is to study the relationship of CT OmpA genotypes to CT infection clearance prior to treatment (i.e., spontaneous resolution of infection). It is possible that differences in OmpA composition of CT strains or other biological characteristics of different CT OmpA genotypes may influence their susceptibility or resistance to host immune responses. We demonstrated subjects infected with OmpA genotype J/Ja more often cleared CT in the interval between screening and treatment. Eckert et al. previously reported that CT serovar J had the lowest median inclusion-forming unit counts, a surrogate of CT replication, of the genital OmpA genotypes in men and women.[13] Perhaps slower chlamydial growth of OmpA genotype J/Ja facilitates its eradication or alternatively makes it more difficult to detect. Unfortunately, we did not have IFU data from cultures performed at the screening visit so that we could further evaluate the former hypothesis. Gomes et al. previously reported that the probability of being infected with OmpA genotype J was 7.7 fold higher in subjects with prior CT infection [22]. This may suggest that even though some OmpA J strains are more likely to be eradicated by the host, there may be characteristics of OmpA J strains or their hosts that influence susceptibility to infection with OmpA type J in previously infected subjects. In our cohort, there was no difference in reported history of chlamydia in those with OmpA genotype J/Ja versus other genotypes (data not shown).

Another application of CT OmpA genotyping is to help distinguish new infection from persisting infection in subjects who have repeat detection of CT. Without CT OmpA genotyping, subjects with a repeat positive CT test prior to therapy may have been erroneously presumed to have persisting infection. Prior studies evaluating the influence of CT OmpA genotypes on CT natural history, which had longer follow-up periods than in our study, have reported discrepant results [16,17]. This may be due in part to such studies being limited in not typing the CT strain on follow-up to confirm persisting infection with the same CT OmpA genotype, which may have led to misclassification of outcome (CT clearance versus persistence). We demonstrated 5% of subjects had different CT OmpA genotypes at their treatment visit, suggesting they may have resolved their initial infection and now have a new infection with a different genotype; this is a plausible consideration in populations at high risk for chlamydia. We were able to confirm that some subjects did have new sexual partners in the interval between testing and treatment. It is also possible, though less likely, that a mixed infection may have occurred initially, and that the CT OmpA genotype detected with repeat testing may have been present initially but not detected (perhaps due to a low copy number of

the *ompA* gene); for this to occur, the dominant OmpA type detected at the screening visit would have to have been completely or mostly cleared, and the OmpA type not detected at the screening visit would have to be the dominant isolate detected at the treatment visit. Unfortunately, we did not have sufficient sample with which to further investigate for mixed infection (e.g. by plaque purification techniques, etc.)

The smaller sample size of those resolving infection, of men, and of Caucasians was a limitation of our study, and the association of CT OmpA genotype with CT clearance should be confirmed in a larger cohort. A larger cohort is also more ideal for assessing the influence of other factors, such as age, gender, race, and interval between testing and treatment on the relationship of OmpA genotype and CT outcomes. As with most human studies of CT infections, we did not precisely know the duration of CT infection (without knowing when the infection was acquired), and therefore could not fully assess how the impact of duration of infection influenced the relationship of CT OmpA genotype with CT clearance. Because of ethical considerations, we only observed the CT natural history in the interval between screening and treatment visits, and then were obligated to provide treatment at the treatment visits.

In summary, CT OmpA genotyping may be a valuable tool in studying the natural history of CT infections, which remains poorly understood. Improved knowledge of CT natural history could advance our understanding of protective immune responses to CT and impact CT treatment and re-screening recommendations.

KEY MESSAGES

1. Spontaneous clearance of genital chlamydia in humans in the interval between screening and treatment occurs commonly.
2. Clearance of genital chlamydia in humans prior to treatment may be influenced by the *Chlamydia trachomatis* OmpA genotype.
3. *Chlamydia trachomatis* OmpA genotyping may helpful in determining whether repeat positive chlamydia tests represent infection with the same or a new chlamydia strain.

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CONTRIBUTORS: WMG designed the study and carried out collection of clinical data. All authors contributed to collection of laboratory data. WMG performed the statistical analyses. WMG wrote the manuscript with contributions from all authors.

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Table 1

Characteristics of 5 subjects with discordant CT OmpA genotypes between the screening (1st) visit and follow-up treatment (2nd) visit

Sex	Race	Age	New Partner[a]	CT Culture/PCR[b]	1st OmpA[c]	2nd OmpA[c]
Female	AA	22	N/A	+/ND	D/Da	E
Female	AA	17	Yes	−/+	F	E
Female	AA	29	N/A	−/+	H	Ia
Female	AA	28	No	+/ND	E	J/Ja
Female	AA	21	Yes	−/+	D/Da	J/Ja

Notes: Abbreviations – AA, African American; N/A, information not available; ND, not done; CT, *Chlamydia trachomatis*; PCR, polymerase chain reaction; OmpA, outer membrane protein A genotype

[a] Between initial screening visit and follow-up treatment visit

[b] CT test results at treatment visit

[c] Refers to the OmpA type isolated at the 1st or 2nd clinic visit as noted

Table 2

Relationship of *Chlamydia trachomatis* outer membrane protein A genotype at the initial screening visit to subsequent Chlamydia detection at the follow-up treatment visit.

OmpAGenotype[a]	CT Culture[b]		CT Culture and PCR[b]	
	Positive (n=79)	Negative (n=18)	Positive (n=88)	Negative (n=9)
D/Da	18 (23)	3 (17)	20 (23)	1 (11)
E	23 (29)	5 (28)	24 (27)	4 (44)
F	10 (13)	0 (0)	10 (11)	0 (0)
G	1 (1)	0 (0)	1 (1)	0 (0)
H	1 (1)	1 (6)	2 (2)	0 (0)
Ia	14 (18)	1 (6)	15 (17)	0 (0)
J/Ja	12 (15)	7 (39)	15 (17)	4 (44)
K	0 (0)	1 (6)	1 (1)	0 (0)

Notes: Data are presented as number (%). Abbreviations – CT, *Chlamydia trachomatis*; PCR, polymerase chain reaction; OmpA, outer membrane protein A genotype

[a] OmpA genotype detected at the initial screening visit

[b] CT culture was performed at the follow-up treatment visit. CT culture-negative subjects had CT PCR performed.