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The Journal of Infectious Diseases

PELVIC INFLAMMATORY DISEASE SUPPLEMENT

# Host Genetic Risk Factors for *Chlamydia trachomatis*-Related Infertility in Women

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***Background.*** *Chlamydia trachomatis* (*Ct*) infection ascending to the upper genital tract can cause infertility. Direct association of genetic variants as contributors is challenging because infertility may not be diagnosed until years after infection. Investigating the intermediate trait of ascension bridges this gap.

***Methods.*** We identified infertility genome-wide association study (GWAS) loci using deoxyribonucleic acid from *Ct*-seropositive cisgender women in a tubal factor infertility study and *Ct*-infected cisgender women from a longitudinal pelvic inflammatory disease cohort with known fertility status. Deoxyribonucleic acid and blood messenger ribonucleic acid from 2 additional female cohorts with active *Ct* infection and known endometrial infection status were used to investigate the impact of infertility single-nucleotide polymorphisms (SNPs) on *Ct* ascension. A statistical mediation test examined whether multiple infertility SNPs jointly influenced ascension risk by modulating expression of mediator genes.

***Results.*** We identified 112 candidate infertility GWAS loci, and 31 associated with *Ct* ascension. The SNPs altered chlamydial ascension by modulating expression of 40 mediator genes. Mediator genes identified are involved in innate immune responses including type I interferon production, T-cell function, fibrosis, female reproductive tract health, and protein synthesis and degradation.

***Conclusions.*** We identified *Ct*-related infertility loci and their potential functional effects on *Ct* ascension.

**Keywords.** chlamydia disease; genital tract ascension; GWAS; host genetics; infertility.

Untreated cervical *Chlamydia trachomatis* (*Ct*) infection can ascend to the upper genital tract, eliciting inflammation that results in pelvic inflammatory disease (PID). Pelvic inflammatory disease, clinical or subclinical, can lead to tubal factor infertility (TFI). Tubal factor infertility occurs when a woman's fallopian tube(s) are damaged, preventing fertilization or implantation. A recent Dutch cohort study, which followed over 5500 women for 8 years, found a 4.2-fold increased risk for TFI in women who experienced chlamydial infection versus *Ct*-negative women [1]. Although *Ct* is recognized as an important cause of TFI, most infected women do not develop infertility, possibly because the infection is limited to the cervix. Previous studies of women with *Ct*-related infertility identified host genetic variants associated with altered disease risk but focused on pathophysiological pathways implicated in animal models of chlamydial disease. Direct association of genetic variants as contributors to *Ct* infection-induced infertility is challenging because infertility may not be diagnosed until years after resolution of infection. To bridge this divide, we investigated the intermediate trait of *Ct* ascension because this is a prerequisite for fallopian tube inflammation and TFI.

Genome-wide association studies (GWAS) support broad and unbiased detection of genetic variation. However, applying GWAS to identification of single-nucleotide polymorphism(s) (SNP) associated with ascending *Ct* infection is not feasible, because it would require thousands of participants with endometrial *Ct* infection status diagnosed by an invasive procedure. Furthermore, the complex immunopathology that drives PID and subsequently TFI is likely modulated by multiple genetic variants. Most loci identified by GWAS are not mapped to protein-coding regions, so the mechanism underlying their effect can be difficult to interpret. Nevertheless, when genetic variants affect transcription factor binding sites in the promoter region of a regulated gene to influence its expression, typically within 1 megabyte (Mb) on either side of the transcriptional start site, this can assist in functional annotation [2].

In this study, we attempted to overcome these limitations of GWAS by using multiple relevant patient cohorts (Figure 1). We identified GWAS loci of infertility from 2 studies:

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**The Journal of Infectious Diseases® 2021;224(S2):S64–71**



DOI: 10.1093/infdis/jiab149

(1) in a case-control study, we used deoxyribonucleic acid (DNA) from *Ct*-seropositive cisgender women, with TFI (cases) or with nontubal-factor infertility by other causes (controls); and (2) in a longitudinal cohort study, we included cisgender women with documented PID and *Ct* infection who developed infertility and others who became pregnant. We next explored the functional impact of candidate infertility SNPs on *Ct* ascension from an independent cohort with DNA and messenger ribonucleic acid (mRNA) data from *Ct*-infected women with ($Endo^+$) or without ($Endo^-$) endometrial infection. Finally, we used robust statistical mediation analysis to examine whether multiple simultaneously expressed infertility SNPs influence the risk of *Ct* ascension through modulation of mediator gene expression. These analyses identified important candidates that should be further investigated for their potential roles in *Ct*-induced infertility.

## METHODS

All study participants provided written informed consent before initiation of study procedures. Institutional Review Boards (IRB) for Human Subjects Research at the University of North Carolina, the University of Pittsburgh, and the University of Alabama at Birmingham approved the study. The Centers for Disease Control and Prevention (CDC) human subjects review determined that CDC investigators were not engaged in human subjects research for this study and that CDC IRB approval was not required.

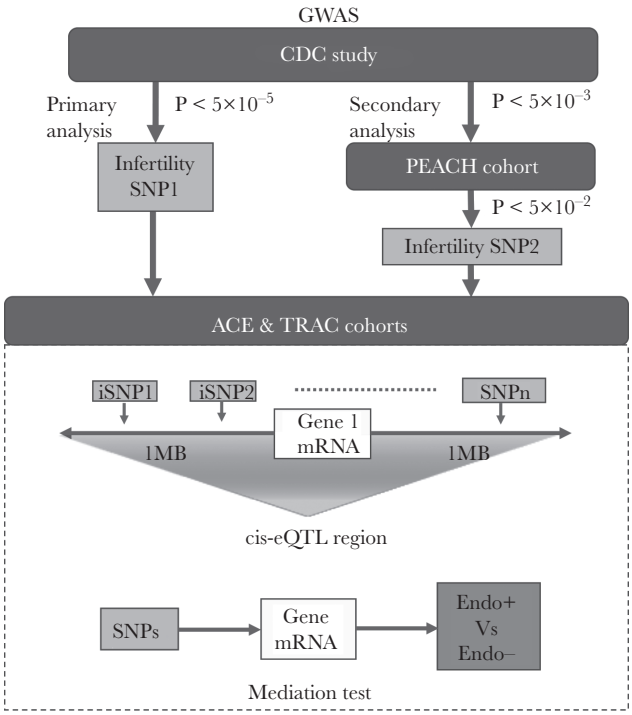


**Figure 1.** Data analysis strategy and selection criteria used in this study. CDC, Centers for Disease Control and Prevention; cis-eQTL, cis-expression quantitative trait loci; GWAS, genome-wide association study; MB, megabyte; mRNA, messenger ribonucleic acid; PEACH, Pelvic Inflammatory Disease Evaluation and Clinical Health cohort; SNP, single-nucleotide polymorphism; TRAC, T cell Response Against Chlamydia study.

### Study Population and Study Design

Whole blood DNA from participants recruited into a CDC-funded case-control study was used for primary analysis to identify potential GWAS loci of *Ct*-related TFI [3]. This discovery dataset included all *Ct*-seropositive cisgender women, tested by anti-immunoglobulin (Ig)G for *Ct* major outer membrane protein (OmpA), anti-IgG1 and IgG3 to *Ct* elementary bodies, or anti-*Ct* HSP60, participating in the CDC-funded study. All participants in the CDC-funded study were infertile, with cases having evidence of tubal blockage as determined by hysterosalpingography or laparoscopy, whereas controls showed tubal patency. A total of 382 females with *Ct*-seropositivity and infertility were genotyped, including 113 with TFI (cases) and 269 with non-TFI infertility (controls).

Cisgender women diagnosed with *Ct*-induced PID with DNA available for analysis from the Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) study were included in a secondary analysis to identify additional infertility loci [4]. A total of 61 women with *Ct*-induced PID, including 15 infertile cases and 46 fertile controls, were genotyped.

THe DNA and RNA data from *Ct*-infected women [5] participating in a randomized clinical trial of enhanced anaerobic treatment for women with PID (The Anaerobes and Clearance of Endometritis, [ACE] trial) [6] or the T cell Response Against Chlamydia (TRAC) study [7] were used to investigate whether infertility genetic loci were also associated with differential risk of endometrial *Ct* infection through alteration of mediator gene expression levels (Figure 1). Blood mRNA and DNA from 143 *Ct*-infected women, 71 who were *Ct* positive only at the cervix, and 72 who were also *Ct* positive on endometrial biopsy, was profiled and genotyped.

Details of these study cohorts are described in Supplementary Methods.

### Genotyping, Imputation, and Data Quality Control in the Centers for Disease Control and Prevention-Funded Study, Pelvic Inflammatory Disease Evaluation and Clinical Health, ACE, and T Cell Response Against Chlamydia Cohorts

Genotyping was performed with Illumina BeadChip arrays, as described in Supplementary Methods.

### Genetic Association Analysis for *Chlamydia trachomatis*-Related Infertility Loci in the Centers for Disease Control and Prevention-Funded Study and Pelvic Inflammatory Disease Evaluation and Clinical Health Cohort

Candidate infertility loci determined by GWAS were defined as (1) loci with $P < 5 \times 10E\text{-}5$ in the CDC-funded study (primary analysis) or (2) by requiring the GWAS-determined loci in the CDC-funded study to meet a less stringent statistical cutoff of $P < 5 \times 10E\text{-}3$ along with a genetic association at $P < .05$ in the PEACH cohort (secondary analysis). Genetic association

analyses were conducted by logistic regression in PLINK (version 1.9). Population stratification was adjusted using the first 15 principal components, which explained over 80% of the total variance.

#### Genetic Association of Infertility Loci With *Chlamydia trachomatis* Ascension in ACE and T Cell Response Against Chlamydia Cohorts

The association of genotypes of candidate infertility loci with *Ct* ascension in ACE and TRAC cohorts was tested in PLINK by logistic regression. Population stratification was adjusted using the first 15 principal components, which explained over 80% of the total variance.

#### Messenger Ribonucleic Acid Array Data Acquisition and Processing in ACE and T Cell Response Against Chlamydia Cohorts

Total RNA was isolated from whole blood of ACE and TRAC participants and profiled by BeadChip microarray [5].

#### Probabilistic Estimation of Expression Residuals Factor Analysis for Confounding Factors in ACE and T Cell Response Against Chlamydia Cohorts

We applied the probabilistic estimation of expression residuals (PEER) method [8] to infer and manage confounding factors affecting gene expression levels, as described in Supplementary Methods.

#### Mediation Test in ACE and T Cell Response Against Chlamydia Cohorts

After examining the association of genotypes at infertility loci with *Ct* ascension in ACE and TRAC cohorts at the individual SNP level, we estimated the joint effect of multiple SNPs on ascension and investigated whether they acted by modulating expression of mediator gene(s). Candidate mediator genes were tested if the infertility locus lay within 1 Mb on either side of the gene's transcriptional start site. To assess the gene mediation effects of our infertility loci on ascension, we used Generalized Multi-SNP Mediation Intersection-Union Test ([GSMUT] [9]) (Supplementary Methods). To determine the directionality of effects of mediator gene expression levels on the risk of ascension, we compared expression levels of mediator genes between Endo$^+$ and Endo$^-$ women in the ACE and TRAC cohorts by logistic regression. For multiple testing correction, the false-discovery rate (FDR) was estimated by using the *fdrtool* package in R. $P < .1$, corresponding to 19.8% FDR, were considered suggestively significant.

## RESULTS

#### Population Characteristics

Participant characteristics from the CDC-funded infertility study and the PEACH cohort are summarized in Supplementary Tables 1 and 2, respectively. In both studies, age, education, insurance, substance use, self-reported infection history, coinfections at enrollment, and contraceptive method use had no statistically significant differences between cases and controls. Potential population stratification in cases and controls was controlled by adjusting the major principal components of genetic ancestry in the statistical model. Characteristics of ACE and TRAC participants used in GSMUT analysis of association with *Ct* ascension are summarized in Supplementary Table 3. Oral contraceptive pill use and *Neisseria gonorrhoeae* coinfection were significantly increased in Endo$^+$ women [7] and adjusted as covariates.

#### Identification of Infertility Loci in Centers for Disease Control and Prevention-Funded and Pelvic Inflammatory Disease Evaluation and Clinical Health Studies and Their Association With *Chlamydia trachomatis* Ascension in ACE and T Cell Response Against Chlamydia

The Manhattan plot of GWAS results from CDC-funded study participants is shown in Figure 2. The GWAS infertility loci determined from primary analysis of the CDC-funded study, predominantly white women without a history of gonorrhea, are detailed in Supplementary Table 4. Twenty-six loci were found, mapping to 15 distinct chromosomes. Infertility loci determined from the secondary analysis incorporating the PEACH cohort, primarily black women with a high rate of gonococcal coinfection, are detailed in Supplementary Table 5. Eighty-six loci on 21 chromosomes were identified. We then determined whether these infertility GWAS loci were also associated with *Ct* ascension in ACE and TRAC; findings are summarized in Supplementary Tables 4 and 5. At the individual SNP level, 48 SNPs from the primary analysis and 65 SNPs from the secondary analysis were modestly associated with ascending infection ($P < .20$). None were statistically significant after multiple-testing correction. Therefore, we formally tested the combined effect of multiple SNPs on the risk of ascending

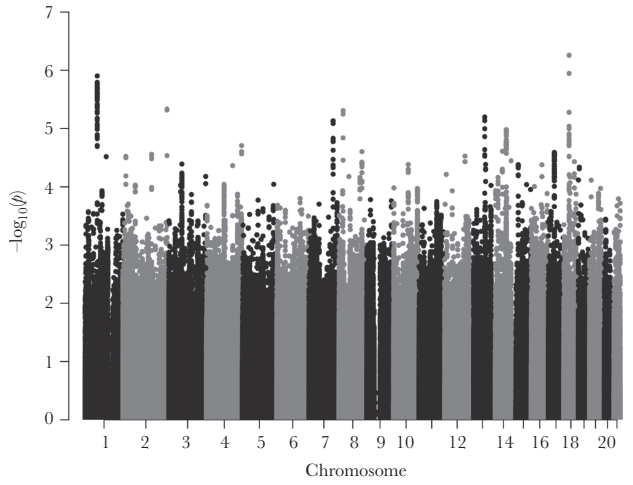


**Figure 2.** Manhattan plot summarizing the infertility genome-wide association study (GWAS) results of the Centers for Disease Control and Prevention-funded study. Each dot represents 1 single-nucleotide polymorphism (SNP). The x-axis indicates the physical base-pair position of each tested SNP; y-axis indicates the –log(10) *P* values from GWAS.

infection through altered expression of a mediator gene by the causal mediation test, GSMUT.

#### Gene Mediation Effects of Infertility Loci on Ascension Identified by Generalized Multi-SNP Mediation Intersection-Union Test

Two loci, *LINC01750* and *SORBS2*, were identified from the primary GWAS analysis of infertility that influenced the risk of ascension in women from the ACE and TRAC cohorts through 5 mediator genes. Secondary analysis using data from both the CDC-funded and PEACH cohorts identified 29 loci that affected the risk of ascension through modulating expression of 36 mediator genes (Supplementary Table 6). All of these mediations were suggestively significant after adjustment for multiple comparisons (FDR <0.2). The association of infertility loci with *Ct* ascension via mediator genes provides potential functional annotation. Because information on directionality is compromised with GSMUT, we directly compared mediator gene expression levels in Endo$^+$ and Endo$^-$ women in ACE and TRAC to determine whether mediator genes were up- or down-regulated with ascension. Table 1 provides general descriptions of mediator gene functionality, as well as directionality of mediator gene expression, in Endo$^+$ versus Endo$^-$ women.

The *LINC01750* locus for infertility was also a locus for ascension mediated through altering *OVGP1* and *CEPT1* expression. *OVGP1* encodes a protein specific to oviduct and endometrial epithelial cells [10]; its transcription was modestly increased in Endo$^+$ women. *CEPT1* encodes a dual specificity enzyme that catalyzes synthesis of 2 major cell membrane phospholipids [11] that control lipid-driven proinflammatory cascades [12]. *CEPT1* expression was modestly decreased in Endo$^+$ women. The *SORBS2* locus for infertility was associated with altered risk for *Ct* ascension through modulation of 3 mediator genes: *LRP2BP*, *HELT*, and *TLR3*. *LRP2BP* encodes lipid receptor-related protein 2 (LRP2) binding protein. THe LRP2, or megalin, is a receptor found in the plasma membrane of many epithelial cells that promotes endocytosis. *HELT* encodes a basic-helix-loop-helix protein belonging to a superfamily of DNA-binding transcription factors that regulate cell cycle, apoptosis, and differentiation [13]. Expression of LRP2BP and HELT was modestly decreased in Endo$^+$ women. *TLR3* was the only mediator gene identified from both primary and secondary analyses, and it was modestly increased in Endo$^+$ women. *TLR3* is an intracellular pattern recognition receptor, detecting double-stranded RNA to induce nuclear factor-κB activation and production of type I interferons (IFNs).

Secondary analysis identified 29 loci influencing the risk of ascension in cisgender women from ACE and TRAC through 36 mediator genes. The mediator genes encode proteins implicated in innate inflammation, T-cell function, wound healing, fibrosis, cell proliferation and apoptosis, protein synthesis and degradation, gene regulation, cell membrane formation and function, cell metabolism, and overall health of the female reproductive tract (Table 1).

## DISCUSSION

We conducted a GWAS for TFI in women from a CDC-funded infertility study who were seropositive for *Ct*. Among loci for infertility from primary analysis, 2 (*LINC01750* and *SORBS2* loci) influenced *Ct* ascension via modulation of 5 mediator genes: *OVGP1*, *CEPT1*, *LR2BP*, *HELT*, and *TLR3*. Our finding that infertility loci modulate expression of an oviduct epithelium-specific gene, *OVGP1*, suggests that genetic polymorphisms affecting production of this protein may alter reproductive tract responses vital to fertility. Animal studies indicate that *OVGP1* aids in sperm capacitation, fertilization, and early embryonic development [14]. *OVGP1* transcription levels were increased in Endo$^+$ versus Endo$^-$ women, possibly reflecting increased production in response to *Ct*-induced inflammation and activation of epithelial repair mechanisms. *CEPT1* is responsible for synthesis of phosphatidylcholine, a constituent of *Ct* membranes that must be acquired from its mammalian host to support chlamydial proliferation. Altered expression could thus control chlamydial ascension by influencing bacterial burden. Furthermore, changes in cell membrane phospholipid composition alter cellular recognition of danger-associated molecular patterns and downstream inflammatory signaling [12], which possibly modulate tubal damage during chlamydial invasion. Changes in cellular endocytosis and trafficking of cargo to lysosomes via differential expression of *LRP2BP* could affect *Ct* ascension via altered uptake or killing of bacteria or through changes in uptake of danger molecules released from damaged infected cells. *HELT* could alter *Ct* ascension through control of reproductive tract epithelial cell cycling, apoptosis, and differentiation [13].

Because *TLR2* and *TLR4* play roles in recognition of *Ct* and are expressed in the human female genital tract, associations between polymorphisms in these TLRs and *Ct* sequelae were investigated in several prior candidate gene studies, which gave inconsistent findings. The relatively small sample size in these studies and/or lack of information related to prior chlamydial infection make their interpretation challenging. Although *TLR3* is present in the female genital tract, we did not find previous studies testing its genetic association with human *Ct* disease. In this study, we identified infertility variants in or near the *TLR3* gene. Activation of *TLR3* and IFN regulatory factor 3 (*IRF3*)-induced production of type I IFNs during chlamydial infection could enhance *Ct* infection and ascension through inhibition of T-cell and IFN-γ responses essential for chlamydial control [15]. We previously reported that women with *Ct*-induced PID and endometritis display elevated type-I IFN-induced gene transcription in peripheral blood [5], and that women with

**Table 1. Mediator Gene Functions and Directionality**

| Overall Function | Gene Name | Specific Function | Expression in Endo$^+$ Versus Endo$^-$ Women | Citation |
|---|---|---|---|---|
| Innate Immune Response | | | | |
| | ABR | Regulation of inflammation | ↑ | [26] |
| | ASB13 | Innate immune response regulator | ↑ | [27] |
| | TLR3[a] | Type I interferon induction | ↑ | [28] |
| | NPR2 | C-type natriuretic peptide receptor which inhibits IFN-γ mediated gene expression | ↑ | [20] |
| | PSG3 | IFN-γ responsiveness | ↑ | [29] |
| | PVR (CD155) | NK cell effector function | ↑ | [30] |
| | Mir620 | Targets CCL2 (MCP-1) | ↑ | [31] |
| | COG6 | Regulation of inflammation | ↓ | [32] |
| | MEIS2 | Myeloid cell differentiation | ↓ | [33] |
| | PSG2 | IDO and TGF-β activity | ↓ | [34] |
| | SLC5A1 | Glucose transport; innate immune cell function | ↓ | [35] |
| Adaptive Immune Response | | | | |
| | PFKP | Glycolysis; T-cell function | ↑ | [23] |
| | TLR3[a] | T-cell activation | ↑ | [36] |
| | LARGE | T-cell development | ↓ | [24] |
| | LGMN | T-cell activation; Class II antigen presentation | ↓ | [25] |
| Fibrosis | | | | |
| | PLOD2 | Fibrotic enzyme | ↑ | [37] |
| | TLR3[a] | Wound healing, fibrosis | ↑ | [38] |
| Proliferation and Apoptosis | | | | |
| | CSE1L | Cell proliferation | ↓ | [39] |
| | HERPUD1 | Apoptosis regulation | ↓ | [40] |
| | TMEM192 | Apoptosis regulation | ↓ | [41] |
| | XRN1 | Mitochondrial function | ↓ | [42] |
| Protein Synthesis and Degradation | | | | |
| | ASB13 | Protein degradation | ↑ | [43] |
| | HERPUD1 | Destruction of misfolded proteins | ↓ | [44] |
| | MRM1 | Ribosomal RNA transferase | ↑ | [45] |
| | MTRF1 | Mitochondrial translation | ↑ | [46] |
| | UROS | Methionine synthesis | ↑ | [47] |
| | WDR33 | Protein synthesis; cell differentiation | ↑ | [48] |
| Gene Regulation | | | | |
| | HELT[b] | Transcriptional repressor | ↓ | [40] |
| | ZNF512 | Transcription regulation | ↑ | [13] |
| Cell Membrane Formation and Function | | | | |
| | PCDH17 | Cell adhesion | ↑ | [49] |
| | PIGO | Cell membrane phospholipid synthesis | ↑ | |
| | SLC26A5 | Membrane anion transporter | ↑ | [50] |
| | CADPS | Vesicle exocytosis | ↓ | [s1] |
| | CEPT1[b] | Cell membrane phospholipid synthesis | ↓ | [s2] |
| | LR2BP[b] | Ligand endocytosis | ↓ | [s3] |
| Intracellular Energy Transfer | | | | |
| | ABR | GTPase | ↑ | [s4] |
| | NDUFB4 | NADH dehydrogenase | ↑ | [s5] |
| Female Reproductive Tract Health | | | | |
| | OVGP1[b] | Fertilization, implantation, embryonic development | ↑ | [10] |
| | NPR2 | Female reproductive tract development | ↑ | [s6] |
| | GAS2L2 | Regulates ciliary orientation | ↑ | [s7] |
| Miscellaneous | EMR4 | Human pseudogene | ↑ | [s8] |
| | RPP38-DT | Unknown function | ↑ | |
| | FAM162B | Unknown function | ↓ | |
| | HEATR9 | Unknown function | ↓ | |
| | KRTAP3-1 | Keratin-associated protein | ↓ | [s9] |

Abbreviations: IDO, indoleamine dioxygenase; IFN, interferon; MCP-1, monocyte chemoattractant protein-1; NK, natural killer; RNA, ribonucleic acid; TGF, transforming growth factor.

[a]TLR3 ($P < .01$) overlapped from primary and secondary analyses coincident with loci for chlamydial ascension.

[b]Mediator genes ($P < .01$) from primary analysis of Centers for Disease Control and Prevention (CDC) cohort ($P < 5 \times 10E\text{-}05$) coincident with loci for chlamydial ascension.

NOTE: The remaining mediator genes ($P < .01$) are from secondary analysis of CDC cohort ($P < 5 \times 10E\text{-}03$) and Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) cohort ($P < 5 \times 10E\text{-}02$) coincident with loci for chlamydial ascension.

endometrial *Ct* infection secrete higher levels of type-I IFN-induced chemokines compared with women with only cervical infection [5]. Mouse models of genital tract chlamydial infection gave conflicting results, finding either no effect of *TLR3* deficiency [16] or altered shedding and pathology [17]. Detecting increased *TLR3* transcripts in Endo$^+$ women supports a deleterious role for this pathogen recognition receptor during chlamydial infection.

*TLR3* was the only gene identified by an additional infertility variant using data from both the CDC-funded and PEACH cohorts. Women in PEACH were often coinfected with gonorrhea, which promotes prominent neutrophil responses and epithelial cell death. It is possible that *TLR3* functions indirectly through sensing self-RNA released from damaged cells [18]. Finally, stimulation of *TLR3* influences wound healing and fibrosis [19], with direct implications for development of TFI.

Using combined data from the CDC-funded and PEACH cohorts, we identified additional infertility GWAS loci mediating *Ct* ascension. *ABR*, *ASB13*, *COG6*, and *SLC5A1* regulate innate inflammatory responses. *NPR2* is the primary receptor for C-type natriuretic peptide, which down-regulates IFN-γ-mediated inflammation and kynurenine generation by IFN-γ-induced indoleamine dioxygenase (IDO) [20]. *PSG3* and *PVR* influence IFN-γ responses and natural killer (NK) cell effector function, respectively, and *PSG2* is linked to changes in IDO activity. Detection of these genes is relevant because IFN-γ-induced IDO is a primary host defense mechanism against *Ct* [21] and NK cell activation drives CD4$^+$ T cells towards IFN-γ-producing Th1 cells that are key to chlamydial control [22]. *PFKP* is essential for T-cell metabolism [23] and *LARGE* for T-cell development [24]. *LGMN* is an upstream activator of the Cathepsin L-Mediated Intracellular C3 Activation (CTSL-C3)-IFN-γ axis in human CD4$^+$ T cells and an important promoter of human Th1 responses [25]. CD4$^+$ T cells isolated from *LGMN*-deficient mice display a specific defect in IFN-γ secretion and Th1 responses [25], so alterations in *LGMN* could influence chlamydial growth and infection duration.

Infertility loci were also associated with mediator genes involved in apoptosis and cell proliferation, protein synthesis and degradation, and overall gene regulation, potentially influencing tissue damage and healing. Our identification of genes involved in the formation and function of cell membranes, endocytosis, exocytosis, and anion transporters is striking because *Ct* invades host epithelial cells by a panoply of mechanisms and resides within a protective vacuole, stealing lipids from the host endoplasmic reticulum-Golgi apparatus.

Our use of GSMUT to test the association of SNPs and ascension mediated by altered gene expression is a strength of our study because this method enables examination of the combined effects of multiple SNPs acting completely through mediator genes, as well as effects partially mediated through these genes. However, a limitation is its inability to determine directionality and magnitude of mediation effects.

Although control participants in the CDC-funded study had patent tubes by hysterosalpingography (HSG) or laparoscopy, they were infertile, which may increase false-negative findings. Mild scarring, undetected by HSG, could have prevented pregnancy. We attempted to overcome sample size limitations by combining results from the CDC-funded and PEACH cohorts and by analyzing tentative loci for their association with alterations in risk for *Ct* ascension in 2 additional patient cohorts. Our mediation test linked coincident infertility and ascension loci to differential expression of mediator genes associated with *Ct* ascension. The biological functions of their gene products suggest that they are plausible candidates for proteins that influence spread of *Ct*.

## CONCLUSIONS

Our study highlights the potential for systematic genetics and mediation testing to dissect a complex disease and identify potential mediation genes involved in its pathogenesis. Data indicate that development of tubal pathology is influenced by multiple inflammatory pathways. A larger, independent cohort with documented prior *Ct* infection and reproductive outcomes (infertility versus fertility) would enable us to validate these findings. Nevertheless, this current study has yielded novel molecular targets that can be investigated in functional studies in vitro or in animal models to better determine their relationship to *Ct* infection and development of tubal disease, with the ultimate goals of determining therapeutics adjunctive to antibiotics and advancing development of biomarkers that indicate risk for ascension and aid in the diagnosis of PID.

### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

***Acknowledgments.*** We thank the women who agreed to participate in this study; Ingrid Macio, Melinda Petrina, Carol Priest, Abi Jett, and Lorna Rabe for their efforts in the clinic and the microbiology laboratory; the staff at the Allegheny County Health Department Sexually Transmitted Disease Clinic for their support; and Centers for Disease Control and Prevention staff Jesse Thomas and Allie Coor for assisting with the consultation process. We also thank Dr. Nicholas A. Cataldo for carefully reviewing this manuscript.

***Disclaimer.*** The study represents the view of the authors and does not necessarily represent the official position of the Centers for Disease Control and Prevention.

***Financial support.*** This work was funded by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health through R01 AI119164 and U19 AI084024 (to T. D.) and U19 AI144181 (to T. D., C. M. C., and X. Z.) and Centers for Disease Control and Prevention, Prevention Research Centers through 5U48DP001915 (to W. M. G.) and 5U48DP001918 to (H. C. W. and C. L. H.).

***Supplement sponsorship.*** This supplement is sponsored by the Centers for Disease Control and Prevention.

***Potential conflicts of interest.*** All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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