Published in final edited form as:
Future Microbiol. 2013 September ; 8(9): 1129–1146. doi:10.2217/fmb.13.80.

Genetic variation in *Chlamydia trachomatis* and their hosts: impact on disease severity and tissue tropism

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Abstract

Chlamydia trachomatis infections are a global health problem. This obligate intracellular bacterial pathogen comprises lymphogranuloma venereum (L1–L3), ocular (A–C) and genital (D–K) serovars. Although genetically similar, each serovar group differs in disease severity and tissue tropism through mechanisms that are not well understood. It is clear that host genetic differences also play a role in chlamydial disease outcome and key host polymorphisms are beginning to emerge from both human and experimental animal studies. In this review, we will highlight pathogen and host genes that link genetic diversity, disease severity and tissue tropism. We will also use this information to provide new insights that may be helpful in developing improved management strategies for these important pathogens.

Keywords

Chlamydia trachomatis; disease severity; gene polymorphisms; genetic variation; genital tract infections; tissue tropism

Chlamydiae are strict obligate intracellular pathogens that depend on eukaryotic host cells to complete their unique biphasic developmental cycle [1]. *Chlamydia trachomatis* is one of several chlamydial species that cause disease in humans. It is by far the most significant in terms of public health concerns, causing hundreds of millions of cases of human genital tract (serovars D–K) or ocular (serovars A–C) diseases throughout the world. *C. trachomatis* genital infections are global and cause substantial morbidity, especially in women [2]. Endemic *C. trachomatis* ocular disease is restricted to the poorest communities, affecting

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Financial & competing interests disclosure
Work on chlamydial immunity and host factors involved in disease severity is supported by an NIH public health service grant, AI 019782 (GI Byrne) and a Department of Defense award W81XWH-09-01-0391 (GI Byrne). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

people with little or no healthcare and resulting in end-stage blinding trachoma [3]. Interestingly, different *C. trachomatis* serovars can infect and survive in diverse host niches represented by different tissue tropism causing a wide spectrum of diseases in humans (Figure 1) [4]. For example, genital serovars favor genital tract epithelial cells while ocular serovars infect conjunctival epithelial cells, and the lymphogranuloma venereum (LGV) serovars infect macrophages and spread systemically through lymph nodes [5]. There are more than 100 completed genome sequences for *C. trachomatis* strains archived and publically available in online databases at the National Center for Biotechno logy Information (NCBI) [201], Sanger Institute [202] or European Molecular Biology Laboratory (EMBL) [203], reflecting each of the three disease wbiovars (serovar groups based on pathotype). All sequenced genomes demonstrated similar size (1.04–1.05 Mb), nucleotide sequence similarity (>99% identical) and nearly identical synteny [4,6,7,201,203]. Given that a 1% difference per 1 million base pairs represents approximately 10 kb of variability, and that differences in genes that define *C. trachomatis* virulence and tissue tropism are disproportionately large [8], it may not be surprising that genetic variability is key to understanding chlamydial virulence differences. In women, lower genital tract infection may or may not be symptomatic and may or may not spread to the upper genital tract. Spread of the infection may or may not result in upper genital tract complications such as pelvic inflammatory diseases [9]. In trachoma endemic areas, the majority of individuals in affected communities becomes infected. Despite this similar exposure rate, only a minority of infected individuals develop severe long-term consequences of acute ocular infection. A number of variables may play roles in defining those at risk for complications, including the presence of different strains circulating within the community, the pathogen burden of each infected individual and polymorphisms in host genetic risk factors [10-19]. The intent of this review is to provide insights for both pathogen virulence factors and host reactivity in defining effectors that contribute to chlamydial disease severity. It is, however, important to note that the full genetic diversity in *C. trachomatis* is not yet adequately described for any regulatory or epigenetic changes that may lead to a change of phenotype or adaptation to a niche. Current technology allows for the effective management of large ‘omics’ data sets that can be manipulated using systems-based approaches to sort through both pathogen and host genetic variation to determine how genetic and gene expression differences impact disease severity and tissue tropism. A number of system-based approaches, including comparative sequencing, cell culture systems, *in vivo* infections, epidemiologic studies and mathematical modeling have been recently reviewed [20]. These types of approaches are required to help understand complex traits and epistatic interactions in order to gain a more comprehensive understanding of host and pathogen factors that impact the outcome of chlamydial infections. In this review, it is our purpose to indicate how current information in these areas might help us understand how genetic variability in both the pathogen and the host contribute to disease severity and tissue tropism with a focus on *C. trachomatis* and the human host.

Genetic variation in *C. trachomatis*

A number of chlamydial virulence factors, such as the chlamydial protease activity factor (CPAF/CT858) and the GroEL (CT110)–GroES (CT111) operon, apparently do not undergo

genetic variability and therefore will not be considered here. However, the fittest genome invariably will be selected in niche-specific ways [21]. This is certainly true for both prokaryotic and eukaryotic pathogens, and their capability for DNA exchange is central to virulence gene and fitness trait acquisition [21,22]. While host genetics may contribute to disease severity [12-17,23-29] the pathogen must also successfully evolve to survive in the hostile host environment (Figure 2), and *C. trachomatis* has developed a number of ways to adapt within host intracellular niches. Prokaryotes acquire beneficial new genetic traits via several standard mechanisms. Point mutations may be selected that encode for effectors with improved functional attributes [30]. Phage transduction, transformation or conjugation enable acquisition by horizontal gene transfer [31]. Gene duplication expands families of related genes with differing functions and expression patterns [32,33]. All of these prominently contribute to bacterial gene variability [22,34].

Certainly, the serovar-defining major outer membrane protein (MOMP) is a prime example of point mutation accumulation resulting in genetic variants, especially in the surface-exposed segments of this molecule [35]. There has also been strong bioinformatics evidence after whole-genome sequencing that chlamydiae have undergone a number of gene duplication events resulting in the creation of several families of proteins important for intracellular survival in cell culture and possibly in disease severity differences [36]. These include the nine poly morphic membrane protein *pmp* loci, and a large family of secreted *inc* loci. *Chlamydia* also undergo genetic recombination [4]. Recombination in the laboratory using mixed infection models occurs readily [37], and several examples of recombined clinical isolates have been described that may impact disease severity. A 30-kb region between *ctl393* and *ctl417* found in the E/SW2 strain (Swedish New Variant) exactly matched the sequence for *C. trachomatis* D/UW3/CX [6,38,39]. In another study, an isolate from rectal epithelia was found to be a recombinant of *C. trachomatis* serovar D and LGV resulting in a hypervirulent strain (L_2C) causing severe hemorrhagic proctitis [40]. Recombination was also reported for the cervical isolate Ds/2923. This isolate was serotyped as a D strain, but otherwise the genome was found to be more similar to serovars E and F [41]. Other reports of recombination include the *rs2* gene (*ct680*) upstream of *ompA* [42], and the mosaic strain B/D, which was isolated from a patient with trachoma, and a B/Da strain isolated from an STD patient [43,44]. Insertion and deletion events have been reported in *Chlamydia* [40,41,45-48]. One example of this is the emergence of the new variant of *C. trachomatis* (*nvCT*) in Sweden, which harbors a 377-bp deletion in addition to a 44-bp duplication in its cryptic plasmid [46-48].

There are a number of *C. trachomatis* gene products that exhibit variability and may modulate disease severity (Figure 3). The major categories of variable chlamydial gene products and their roles in disease severity, if known or suspected, are described below and summarized in Table 1.

Major outer membrane protein

The MOMP is a surface-exposed protein encoded by *ompA* (*ct681*). In cell culture, *ompA* is one of the earliest genes detected (2–8 h postinfection, depending on serovar) and reaches its highest expression level at 18–24 h postinfection. The protein is part of a chlamydial outer

membrane complex. MOMP represents approximately 60% of the total surface-expressed proteins in elementary bodies (EBs). It is postulated that host immune pressure is the trigger for the variability of surface-exposed proteins [20,49]; however, the most successful genital serovars D, E and F carry the least variable *ompA* gene [35,44,50,51] suggesting that other undefined factors are at play in selecting MOMP variants [35]. Millman *et al.* [52] and Andreasen *et al.* [53] proposed that either negative selection removes deleterious mutations by purifying selection or that a selective sweep results in the fixation of a single haplotype. The MOMP-based micro immunofluorescence method for *C. trachomatis* strain classification was established more than 40 years ago by Wang and Grayston [54]. Subsequent *ompA* sequencing studies confirmed the microimmunofluorescence test, and showed that MOMP variability is due to the four surface-exposed variable regions (VRI–VRIV) that define 15 *C. trachomatis* serovars. It is now very clear that MOMP serovars (or more properly MOMP genovars) accurately predict chlamydial disease biovars (A–C: endemic trachoma; D–K: cervicitis/urethritis genital disease; and L1–L3: LGV) [40,49]. Despite this clear MOMP-based disease biovar classification, no correlation between *ompA* differences and disease severity has been demonstrated, although Andreasen *et al.* found an association between an A genotype and clinical symptoms of active trachoma and average ocular infection load [53]. Genovars with the most sequence similarities cross biovar designations. Serum-based cross-reactivity studies have shown that genovars from group B (B, Ba, D, E, L1 and L2) are more cross-reactive, as are genovars from group C (C, J, A, H, I, K and L3) [49]. Thus, classification based on chlamydial pathobiology must involve chlamydial gene products other than MOMP, a supposition that was addressed by Harris *et al.* who demonstrated that variation in *ompA* did not reflect *C. trachomatis* disease severity [6].

If MOMP differences do not reflect disease severity differences, then what does the large amount of genetic variation in *ompA* signify? The *ompA* gene is one of the most highly variable genes in the chlamydial genome. Variability occurs in over 30% of the coding sequence, whereas the gene of another surface protein, *porB/ct713* (porin), exhibits only 1% variability in the gene sequence [55,56]. Several *ompA* sequencing studies have included up- and down-stream contiguous regions from different geographical clinical isolates and reference strains [35,44,50,56-63]. One of the largest studies characterized 795 *C. trachomatis* samples from Lisbon, Portugal [35]. The study demonstrated 80 nucleotide variable sites in all the tested isolates that were distributed randomly throughout the whole gene and included both variable and constant regions. This was confirmed in another study in which *ompA*s from different geographical regions were sequenced [35,64]. More than half of the mutations in *ompA* were found in the constant region, and 50% of these were silent. By contrast, almost 94% of the mutations in variable regions coded for amino acid changes [35,57]. These studies are not surprising, but they do reaffirm that exposed portions of MOMP exhibit a high degree of variability, presumably owing to the immuno genicity of this molecule.

Type III secretion system

The type III secretion system (T3SS) is used by many pathogens to directly deliver effector proteins into a target host cell [65]. The T3SS injectisome is assembled as a molecular

syringe that spans the inner membrane and periplasmic space. *C. trachomatis* contains all genes coding for proteins necessary for a fully functional T3SS. These genes are found in several gene clusters across the chlamydial genome in contrast to the T3SS of other Gram-negative bacteria where T3SS genes are located in pathogenicity islands [66]. The chlamydial T3SS consists of two main sets of proteins: the injectisome and the translocator proteins. The injectisome is built from 20–25 contact-dependent secretion proteins, while the chlamydial outer membrane protein B (CopB/CT578) forms a pore in the host cell membrane or inclusion membrane. The pore allows an entry point for the needle to facilitate the injection of effector proteins into the host cell cytosol or inclusion membrane [67]. A number of highly polymorphic genes (e.g., *CT049* and *CT050*) are predicted to encode T3SS effectors; however, gene products have not been functionally characterized [42,68]. Several polymorphic T3SS effectors have been characterized, including an actin-recruiting effector and a large group of inclusion membrane proteins.

Translocated actin-recruiting phosphoprotein—Studies by Carabeo *et al.* demonstrated that *C. trachomatis* host cell invasion is initiated by cytoskeletal rearrangements forming actin-rich ‘pedestal-like structures’ to facilitate chlamydial entry [69]. The process starts with host-cell attachment of EBs, followed by injection of preloaded effector proteins through the T3SS injectisome [70]. Tarp is one of the very early secreted effector proteins (30 s after host-cell attachment) and induces actin polymerization within 90 s after attachment via the Arp2/3 nucleation site [71] (Figure 3). Biochemical analysis of Tarp revealed N-terminal tyrosine-rich tandem repeats (phosphorylation domain), a proline-rich C-terminal domain and an actin-binding domain [72]. The actin-binding domain is indispensable for actin polymerization since antibodies against this domain inhibit actin nucleation [73].

Tarp genetic variation & disease severity—Sequence analysis of Tarp orthologs from several *Chlamydia* species reveals a number of changes that are thought to be associated with intracellular chlamydial survival and therefore may be linked to disease severity [74]. Tarp amino acid sequence comparisons show proline-rich and actin-binding domain conservation, although the number of actin-binding domains differs between and within the species [73]. Serovar L2 has one actin-binding domain while serovars A and D have four and three, respectively [73]. It is not clear if the number of actin-binding domains play a role in disease severity and tissue tropism. Multiple actin-binding domains theoretically facilitate faster polymerization due to simultaneous binding of several actin monomers. In serovar L2 Tarp, there are six tyrosine-rich repeats, while serovar A and D harbor only three [70]. However, *Chlamydia muridarum*, *Chlamydophila caviae* and *Chlamydophila pneumoniae* are missing the tyrosine repeats, yet are efficiently taken up by host cells suggesting a dispensable role for this domain for internalization or that alternative entry mechanisms exist not requiring Tarp [70]. In *C. trachomatis*, phosphorylation of tyrosinerich repeats was found to be mediated by Src family kinases [75]. The tyrosine-phosphorylated residues then bind PI3K and SHC-1 host-cell adaptor proteins, which in turn affects host-cell growth and survival [76].

Genetic variation of inclusion membrane proteins & correlation with disease severity—Inclusion membrane proteins (Incs) are a family of type III secretion-effector proteins that share a common 40–60 amino acid bilobed hydrophobic secondary motif. In LGV strains several *inc* loci are pseudogenes [77], and although there are 50 or more Incs predicted in *C. trachomatis* [77,78], only 23 members of this family have been detected in the inclusion membrane [79-81], and only a few Incs have been functionally characterized.

IncA: a fusogenic inclusion membrane protein—IncA (CT119) is required for homotypic fusion of intracellular inclusions [67,82] via the formation of SNARE-like fusogenic intermediates (Figure 3) [69,83]. Genetic variation in different members of the Inc family have been reported in several studies [77,84,85]. The early study of Rockey *et al.* demonstrated that *incA* isolated from *C. trachomatis* clinical isolates harbor several types of mutations that lead to truncated forms of IncA affecting immunofluorescence detection of IncA on inclusion membranes [85]. The absence of IncA was correlated with the formation of multilobed nonfusogenic inclusion bodies [84,86]. One study demonstrated a correlation between disease severity and *incA*-negative strains [87]. Here, the authors showed that nonfusogenic clinical isolates have less severe clinical signs of infection with low *Chlamydia* recovery [87]. Variation in *incA* was also reported in a Dutch population-based study of women with documented chlamydial infection [84]; however, a correlation between disease severity and *incA* polymorphisms was not possible owing to the limited number of patients enrolled in the study. On the other hand, LGV strains were found to have amino acid substitutions in Inc proteins specific to LGV that are not found in ocular or genital strains. This work demonstrates an example of genetic variation specific to tissue tropism [77]. The polymorphisms specific for LGV strains, although presently unexplained, might reflect host macrophage and lymph node environments unique to LGV.

IncD: a mediator for sphingolipid transport—The IncD locus (CT115) was found to be one of five key genes that helped to segregate ocular (A–C) from genital (D–K) chlamydial genovars [77]. IncD interacts with CERT, which is required for sphingolipid transport. The Pleckstrin homology domain (PH) in CERT was shown to be critical for binding to IncD and subsequent *Chlamydia* development [88] where knockout of the PH domain resulted in reduced chlamydial growth. Mutations leading to amino acid substitutions specific for ocular and LGV tissue tropism are considered important drivers of *C. trachomatis* evolution and tissue tropism differences; however, none of these mutations affect the binding domain that interacts with either PH or CERT domains required for ceramide binding, this function appears to be conserved across the species [77,89,90].

IncG: an indirect inhibitor of apoptosis—IncG (CT118) is part of the operon IncD–G where expression can be detected 2 h after internalization [91]. It was found that phosphorylated IncG binds directly to the host phosphoserine binding protein 14–3–3β and hence IncG sequesters it from binding to phospho-BAD and consequently inhibits the release of mitochondrial cytochrome C and induction of apoptosis (Figure 3) [92,93]. This might be a mechanism evolved by *Chlamydia* to either establish conditions suitable for completing a productive developmental cycle or a chronic infection where host cell viability is crucial for the long-term intimate host–pathogen relationship [94].

CT229: modulation of inclusion trafficking—CT229 is expressed as early as 1 h postinfection and localizes to the inclusion membrane indicating an important role in the early development of the inclusion [95,96]. Inclusion membrane modifications introduced by insertion of *Chlamydia* proteins occurs as the pathogen-containing vesicle moves to the microtubule organizing center in a dynein-dependent way. Rzomp *et al.* have demonstrated that CT229 binds to the GTPase Rab4A, which interacts with cytoplasmic dynein [97]. Therefore, CT229 potentially plays a role in the regulation of inclusion trafficking or the fusogenicity of the chlamydial inclusion. Although CT229/Rab interactions are clearly functionally important, other Rab family inclusion membrane interactions dictate host cell vesicle-localization events with distinctions between chlamydial strains, the specific host Rab-binding partner and, therefore, the nature of inclusion trafficking activity.

Inclusion-vesicle trafficking is perceived to be important for the intracellular growth and development of chlamydiae; however, direct links to disease severity are still to be determined.

***Other* inc *genes*—**Several *inc* genes (e.g., *ct058, ct192* and *ct214*) are highly expressed in LGV strains and share promoter element motifs, but as with other Incs, their function in disease severity and tissue tropism for LGV strains remains unknown [77].

Pmp autotransporters

In *C. trachomatis*, the *pmps* are a family of nine clustered genes (*pmp A–I*) that encode proteins with molecular weights between 95.5 and 187 kDa [49]. Emergence of this family is likely an example of gene duplications that serve to create functional diversity and possibly environmentally responsive phase variation for chlamydiae. Therefore, this gene family may be significant in contributing to disease severity distinctions between strains. The family members contain two repeated N-terminal motifs, FXXN and GAAL (I, V) [98]. It is likely that Pmps play a pivotal role in *Chlamydia* biology because the genes represent almost 3% of the genome [49,99]. Bioinformatic analysis predicts that Pmps are most likely members of the type V secretion system autotransporters [100]. They share several conserved autotransporter features, including an N-terminal signal sequence for *sec*-dependent secretion, a passenger domain and a C-terminal β-barrel allowing transport of the passenger domain to the surface. Some Pmp members are expressed on the EB surface (PmpsD, E, G and H) [49]. They elicit a strong proinflammatory cytokine response from $CD4^+$ cells (PmpB, D and G) or stimulate cytokine production in endothelial cells in an NfκB-pathway dependent manner (PmpB and D) [20].

Genetic variation in pmps: more than just polymorphisms—Genetic variation in *pmps* has been investigated. Several studies collectively show extensive polymorphisms within this gene family [41,101,102]. For example, *pmpB, E, F* and *H* show the highest nonsynonymous to synonymous base-pair change ratios of all chlamydial genes evaluated, suggesting strong selective pressure to retain gene family diversity [78,102]. Variability for *pmpB, E, G* and *H* also involves deletions and insertions when compared with a reference (serovar D) strain [102]. For instance, PmpD has a single amino acid deletion. In comparison PmpH, has 10-11 amino acids deleted, and PmpB is conserved in all serovars

except Ia, which has an insertion resulting in a protein with the addition of 20 amino acids [102]. A comparison of the *pmpC* sequence from 12 clinical isolates to a reference strain showed that the gene sequence from seven out of the 12 isolates did not match the reference strain sequences. For example, the clinical isolate Ja/10 has a 32-nucleotide difference compared with the Ja reference strain [101]. Thus, the *pmp* gene family demonstrates substantive variability acquired via at least three mechanisms (single nucleotide polymorphisms [SNPs], gene duplication and insertion and deletion mutations) and this variability has the potential of contributing to differences in disease severity for *C. trachomatis*.

Correlation between pmp genetic variation & tissue tropism—Correlation between tissue tropism and genetic variation in *pmps* has been investigated in several studies [41,77,102]. For example, PmpB, C, D and G in LGV strains harbor specific amino acid substitutions that distinguish them from non-LGV strains [102]. In E and F genital serovars, differences in five Pmps (PmpB, C, D, H and I) are distinct from other genital serovars [102]. Also, computational analysis reveals specific regions in *pmpE, F* and *H* associated with high frequency of nucleotide substitutions. These point mutations in *pmps* result in serovar differences. For example, specific regions in *pmpE, F* and *H* segregate ocular serovars from urogenital and LGV serovars. In *pmpE*, a single midgene region differentiates ocular strains from LGV and urogenital strains [102]. Finally, Jeffery *et al.* showed that point mutations in both *pmpE* and *F* are associated with rectal tropism [41]. In conclusion, point mutations in *pmp*-specific regions add another factor for strain distinction, tissue tropism and possibly disease severity; however, the mechanisms that confer these differences are still not clear.

The chlamydial cytotoxin

In *C. trachomatis*, toxin-like genes reside within a 20.3 kb highly polymorphic genomic region called the plasticity zone (PZ) between *ycf* V (*ct152*) and *dbs*B (*ct177*). The *C. trachomatis* toxin shares homology with the enzymatic active site of the *Clostridium difficile* large A and B toxin [45]. Belland *et al.* reported toxin-mediated cytopathic effects of *C. muridarum* and *C.trachomatis* serovar D on HeLa cells [45]. Recently, it has been reported that the *C. trachomatis* serovar D toxin (CT166) glycosylates the small GTPase Rac1, causing actin reorganization in HeLa cells similar to that seen for the clostridial toxin B [103].

Polymorphisms in the cytotoxin locus—Carlson *et al.* used comparative DNA–DNA microarray genomic hybridization to compare the genomes of 15 *C. trachomatis* serovars and found extensive polymorphisms in the cytotoxin locus within the PZ region [104]. The comparison of all serovars demonstrated that each representative strain contained a serovar-specific locus. All genitotropic and oculotropic strains, except serovars J and H, were characterized by a central deletion, while serovars J and H have an apparently intact gene. Furthermore, genital serovars (D–K) possess the N-terminal domains for glucosyltransferase activity and uridine diphosphate–glucose binding, while serovars A and C harbor only the uridine diphosphate–glucose binding domain. LGV serovars (L1–L3) lack both domains [104]. Somboonna *et al.* showed that the hypervirulent recombinant L_2C strain acquired the

functional cytotoxin from the D strain, which may account for its reported high level of disease severity [40]. Taken together, these studies provide evidence that cytotoxin polymorphisms may be crucial for biovar classification and likely play a role in disease severity.

Tryptophan synthase genes, an operon that differentiates between ocular & genital strains

C. trachomatis strains possess a partial tryptophan operon (*trpRBA; ct169–ct171*) [105]. A novel tryptophan synthesis operon (*trpRBAFCD*) is found in *C. caviae*, *C. felis* and *C. pecorum*, but is absent in other chlamydial species (*C. muridarum*, *C. pneumoniae* and *C. abortus)* [105-109]. A potential role for the tryptophan operon was described in *C. trachomatis* [105]. In tryptophan-deficient medium, growth of *C. trachomatis* was inhibited. Growth of genital, but not ocular serovars could be restored after the addition of indole. In addition, the growth inhibition of *C. trachomatis* after tryptophan depletion by IFN-γ, which induces the tryptophan decyclizing enzyme indoleamine-2,3-dioxygenase, was rescued by addition of indole in genital, but not ocular serovars [110]. Since *C. trachomatis* carries a truncated version of the operon (*trpRBA*), containing only the genes for the α- and β-subunit of the tryptophan synthase encoded in the PZ it requires indole to produce tryptophan (Figure 3) [20]. Ocular serovars (A, Ba and C) uniformly contain a frame-shift mutation (nucleotide position 531) in *trpA* resulting in an early stop codon and a truncated, nonfunctional protein [110]. Thus, *trpA* (*ct171*) mutations differentiate ocular from genital serovars. It is well established that unless a genomic function is actively protected by selection, it will accumulate deleterious mutations and will cease to be functional. It is proposed by Caldwell and colleagues that genital serovars retain a functional partial *trp* operon because indole compounds are available (probably due to the genital microbiota) and tryptophan, an essential amino acid for chlamydiae, is required for productive growth [110]. Thus, retaining the capacity to generate tryptophan from indole possibly helps to ensure successful transmission [111-115]. Ocular *C. trachomatis* serovars are similar to genital serovars in that they require tryptophan to complete their developmental cycle [110,115]. Since ocular strains uniformly have *trp* operon mutations resulting in loss of function (Figures 3 & 4) these genes therefore must be nonessential for *Chlamydia* survival in the conjunctival epithelium. This could be because tryptophan is plentiful in the conjunctival microenvironment, indole-producing flora, although perhaps present [116,117], are in a separate niche from chlamydiae, or that indole is otherwise unavailable.

Arginine decarboxylase

Chlamydia import arginine from the host cell by the *aaxABC* gene products. *aaxA* (*ct372*) encodes an outer-membrane transporter, *aaxB* (*ct373*) encodes arginine decarboxylase pro-enzyme catalyzing the decarboxylation of arginine to agmantine, and *aaxC* (*ct374*) encodes a cytoplasmic membrane arginine:agmatine antiporter [118,119]. Some *C. trachomatis* clinical isolates express a functional arginine decarboxylase [41], but a number of loss-of-function SNPs have been reported in this operon [120]. *C. trachomatis* serovar L2/434 has a nonsense point mutation in *aaxB* resulting in an early stop codon. Replacement of the stop codon with its original amino acid restores the full-length protein and normal function when expressed in *Escherichia coli* [120]. *C. trachomatis* serovar D/UW-3 possesses a full length but nonfunctional AaxB protein. Protein function is restored in surrogates by replacing

arginine 115 with the original glycine [120]. *Chlamydia* also have a putative ABC arginine transporter; therefore, it has been suggested that the Aax system may inhibit host cell polyamine synthesis, elevate the intracellular pH or inhibit host nitric oxide synthesis [119]. In this case, it is possible that *C. trachomatis* D/UW-3 and L2/434 have either developed alternative pathways or are deficient in the perceived role for this operon in promoting chlamydial intracellular survival.

Role of host genetics in *C. trachomatis* disease severity

Role of host genetics in genital *C. trachomatis* disease severity

Although pathogen genetic variability has a large impact on disease outcome, host genetics also likely play a role in the interaction between the pathogen and the host. The additive, synergistic or antagonistic effects of bacterial and host factors often determine pathogenicity, disease severity and outcome of infections, including those caused by *C. trachomatis* [12-17,23-28,62]. For example, not all women infected with genital *C. trachomatis* develop clinical complications such as tubal factor infertility (TFI).

Pathogen burden and reinfection status are undoubtedly contributing variables to disease outcomes; however, several studies have investigated the correlation between host genetic factors and chlamydial disease severity. Kinnunen *et al.* investigated the correlation between cytokine polymorphisms and *C. trachomatis*-associated TFI (Figure 5) [23]. These investigators demonstrated that Finnish women attending an infertility clinic and diagnosed with TFI harbored specific HLA DQ alleles (*HLA DQA1*0102* and *HLA DQB1*0602*) as well as SNPs in the IL-10 promoter (IL-10-1082AA genotype) more frequently than control subjects. In another study with a larger cohort of patients, both the IL-10-1082AA promoter allele and the TNF-α-308 promoter allele were found to be associated with a high risk of severe TFI [27]. Furthermore, Ohman *et al.* found that an IL-10 promoter with an adenosine residue at position 1082 (1082A) was associated not only with decreased IL-10 production, but also with an increase in IFN-γ and TNF-α production from peripheral blood mononuclear cells stimulated *in vitro* with *C. trachomatis*, compared with individuals with the 1082G version of the IL-10 promoter [26]. In other studies of genital tract infections with *C. trachomatis* in women, no association was found between TFI and polymorphisms in TLR4 and its coreceptor CD14, IL-1β and IL-1RN [121,122]. However, women diagnosed with TFI were found to express the variant allele B of mannose-binding lectin-2, while the wildtype allele A was associated with an absence of disease complications [29].

Role of host genetics in ocular *C. trachomatis* disease severity

Host genetic factors also likely play a role in trachoma, the leading cause of infectious preventable blindness worldwide [13-17]. In endemic areas, the infection starts early in life, individuals are re-infected multiple times and ultimately develop conjuctival scarring and trichiasis (in-turning of eye lashes) later in life. Although this complex multifactorial disease undoutedly has multiple host and pathogen risk factors associated with severity, several interesting studies have provided links to severity and host inflammatory response genes. Natividad *et al*. found a specific haplotype (H-RISK) associated with high risk of trichiasis and scarring trachoma in a Gambian population [17]. The haplotype consists of four SNPs

(*IL10 −3575A*, *IL10 −1082C*, *IL10 −592G* and *IL10 +5009*) spanning upstream and downstream regions of the *IL-10* gene [17]. Furthermore, this allele was found to be associated with a relative increase in *IL-10* gene expression [15]. A previous study showed that TNF-α in tears is important in the pathogenesis of scarring trachoma [123] The study also showed an association between trachoma and the promotor alleles *TNFA-308A* and *TNFA-238A*. MMP-9 is another factor that plays a role in trachoma pathogenesis. A nonsynonymous mutation resulted in a single nucleotide polymorphism in MMP-9 (Q279R) and was found to be associated with low risk of trachoma sequelae [13].

Experimental studies of host genetics & disease severity

It is clear that key events controlling disease outcome for chlamydial infections involve more than simply the repertoire of pathogen virulence gene variants and the nature of the host response to infection. Live-attenuated chlamydial strains, cured of the 8 kb plasmid, have been developed. These strains can elicit host protective responses in the absence of disease pathology, presumably due to downregulation of virulence gene expression [124,125]. However, for chlamydial strains circulating in communities, population-based studies suggest that polymorphisms involving the inflammatory response to infection may be key host determinants of disease severity, irrespective of the pathogen strain and the repertoire of virulence factor genes encoded by the pathogen. Qualitative and quantitative differences in host inflammation also seem to be important in experimental infection of animal models of chlamydial disease [121]. Genome-wide association studies in humans have provided some key insights for disease-provoking host responses; however, these types of investigations also have limitations. These studies require large cohorts of patients, large sequence data sets and lack causal conclusions.

The use of genetically modified animal models have been useful in assessing host responses that modulate chlamydial disease severity. Most published studies have focused on single selected host genes to investigate disease severity outcomes. The use of knockout mice, for example, has helped to provide insights concerning the importance of immune-regulated cytokines (e.g., IFN-γ), a variety of inflammatory proteins (e.g., MMPs), chemokines (e.g., CXCRs) and elements of the adaptive response important for resolving infections [126-132]. In some cases, (e.g., type I $IFN^{-/-}$ mice) the absence of an immune-regulated cytokine promoted improved adaptive immunity [133]. This targeted approach is sometimes limited by host response redundancy and compensating mechanisms to overcome the targeted deficiency, and can also be considered biased in the sense that host factors are specifically selected by the investigator, which may limit the relevance based on the deficiency selected and whether or not it actually plays a role in development of severe chlamydial disease. Any approach chosen to sort out host factors important to disease outcome has limitations; however, the use of sets of genetically diverse advanced recombinant inbred mice, have proven to be valuable tools in forward genetic studies to help relate host susceptibility, resistance and disease severity phenotypes to specific genetic loci with a high degree of penetrance. The mouse genome, although 95% similar to the human genome [134], exhibits critical differences in innate and acquired responses. This fact may limit the degree of correlation for inflammatory responses in mice and humans, although the cited study was only on the use of endotoxin and therefore may have its own set of interpretation limitations

[135]. Other types of differences exist between responses in mice and in humans. For example, in a *C. psittaci 6BC* systemic mouse infection model, forward genetics approaches identified a locus on chromosome 11 (1.5 Mb) responsible for susceptibility and resistance to the infection. This locus comprised 18 genes including p47 GTPases (*Irgb10*, *Irgm2* and *Iigp2*) [136]. These GTPases exhibit cell autonomous effects on chlamydial growth, influence inflammatory cell recruitment to the site of disease [137], and are important in resolving LGV [138,139] and *C. muridarum* infections in mice [140]. However, this family of interferon-induced GTPases is not present in humans and therefore a host genetic correlation with direct translational potential was lacking in these studies. Despite limitations of animal models regarding reproducing results that correlate with human disease, use of advanced recombinant inbred mice have been shown to be of benefit and relevance to human disease [136,141,142]. These genetically diverse murine strain sets may prove to be of value in the study of genital, respiratory and ocular chlamydial infections.

Therapeutics against *C. trachomatis* infection

There are several avenues for development of therapeutics and management of chlamydial infections. One way is to target the pathogen arm such as the usage of antibiotics or targeting virulence factors such as type III secretion effectors or the cytotoxin with small inhibitors especially designed for these proteins. The other way is to target the host side by dampening the inflammatory response or by upregulating protective immunity via vaccination. Although antibiotic resistance is problematic in *C. suis* [143,144], the emergence of antibiotic resistance in *C. trachomatis* is not yet a threat to limit the effectiveness of postexposure antibiotic use.

There are two concerns related to antibiotics and the management of chlamydial infections. The first is accurate identification of those with chlamydial infections, who are often asymptomatic [145-147]. There is equal risk of developing untoward complications of infection, especially genital tract infection in women, irrespective of whether acute infection is symptomatic or asymptomatic [148]. Therefore, to identify all of those infected with *Chlamydia* and at risk of complications, blanket screening programs – although costly and manageable in only a limited number of countries – need to be effectively implemented when feasible. The second concern is that in areas where screening and treatment are most effective, the incidence of infection is not diminishing, but rather is increasing [204]. The ‘arrested immunity’ hypothesis is one possibility that has been posited to account for this observation [149], although if arrested immunity is important, it apparently does not impact disease complications, which continue to fall even with increasing prevalence of lower genital tract infection [150,151].

If arrested immunity contributes to the maintenance of a high level of susceptible individuals in the population, then other intervention strategies must continue to be considered. Certainly, development of a safe and effective vaccine is first on the list of alternatives; however, chlamydiae are highly successful pathogens and development of testable candidate vaccines has yet to occur, and safety due to untoward complications of vaccination as a result of heightened re-exposure pathology may be a concern.

One additional alternative management strategy might be considered, especially if host reactivity can be convincingly established as a contributing factor to disease severity. The development and use of compounds that can safely and effectively reduce host responses that contribute to development of disease might prove to be beneficial in limiting the severity of chlamydial infection complications. This strategy could be as simple as developing management strategies that take maximum advantage of beneficial side effects of antibiotic regimens including interfering with MMPs [152] and dampening NFκB-mediated inflammation [153], or as sophisticated as targeting more individual host-specific responses as personalized medicine becomes a reality.

Conclusion

Genetic variation is a method by which pathogens have evolved new phenotypes to evade host responses in a dynamic and niche-specific manner. Several types of genetic variation are found in *C. trachomatis* that impact variability and expression of virulence factors, as described in this review. These strategies have been shown to promote chlamydial intracellular survival, greatly impact disease severity and are the basis for distinct chlamydial disease biovars and differences in host tissue tropism. Host genetic factors also play a role in disease severity where there is genetic variation in a wide range of pathogen-elicited response genes ranging from MMPs, TLRs, cytokines, cytokine promoters and receptors, to MHCs; all of which have been shown to contribute to disease severity and its control. Therefore, the overall picture of chlamydial disease severity is the outcome of the interaction between host and pathogen factors, which must be considered in a systems-based manner to gain a complete understanding of the process of chlamydial pathogenesis and the management of chlamydial infectious diseases.

Future perspective

The advent of genome sequencing, systems biology and bioinformatics has advanced the field of *Chlamydia* research to enable identification and function of chlamydial virulence factors. These approaches have also provided refined insights of host responses that are linked to either beneficial or detrimental outcomes. The identification of signature chlamydial genetic variants and key host polymorphisms is likely to provide highly specific diagnostic tools for identifying highly virulent strains of chlamydiae and individuals who are at risk for developing complications of their chlamydial infections. This type of information may also provide a basis for improved management of chlamydial infections in the era of personalized medicine.

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Executive summary

Background

- *Chlamydia trachomatis* is a major health burden worldwide in humans.
- The two *C. trachomatis* biovars show large differences in disease severity and tissue tropism despite less than 1% genetic differences.

Genetic variation in Chlamydia

- Genetic variations by gene duplication, insertion/deletions and point mutations in the form of single nucleotide polymorphisms in pathogen factors are the means of evading the immune response and adaptation of *C. trachomatis* to diverse environments.
- A high degree of mutations in *ompA* altering the variable region in major outer membrane protein and mutations in *pmp* genes causes differences in the immune response to these surface proteins.
- Gene duplication of *inc* genes results in different functions of the Inc proteins within the Inc family.
- The presence of the chlamydial cytotoxin and the type III secretion effector Tarp allow invasion of serovar/biovar-specific eukaryotic cells as a means of tissue tropism.
- Metabolic enzymes such as gene products of the tryptophan operon and the arginine decarboxylase allow a response to environmental changes triggered by the immune response.

Role of host genetics in C. trachomatis *disease severity*

- Differences in host genetics affect susceptibility and resistance to *C. trachomatis.*
- Genetic variability in pathways and proteins important for the immune system, such as antigen-presenting proteins, cytokines and metalloproteinases, affect host response to *C. trachomatis*.
- The utilization of recombinant inbred mice allows identification of important host genes that affect susceptibility to *C. trachomatis* and the study of their role during infection.

Therapeutics

- The use of effective therapeutics is limited by the proper identification of infected individuals.
- Arrested immunity in populations with effective screening and treatment programs leads to an increase in the number of susceptible individuals providing impetus for vaccine development.

- Interfering with host responses that contribute to disease severity opens new research opportunities for investigating chlamydial infections.

Conclusion

- Genetic variations in *C. trachomatis* enable the pathogen to evade and survive the host immune response, compete with other bacteria or grow in a suitable environment within the host; while genetic variations in the host alter susceptibility to *C. trachomatis* and the ability of the host to clear the pathogen.
- Understanding the connection between genetic variation on the pathogen and host side will lead to a greater understanding of the pathogenicity of *C. trachomatis* and provide new targets to develop specific and novel therapeutics for *C. trachomatis* treatment.

Future perspective

- The identification of genetic variations in the host and pathogen as well as understanding their role in disease severity during *C. trachomatis* infections will be important in the future to develop novel therapeutics, to understand pathogenicity of *C. trachomatis* and to design new diagnostic methods.

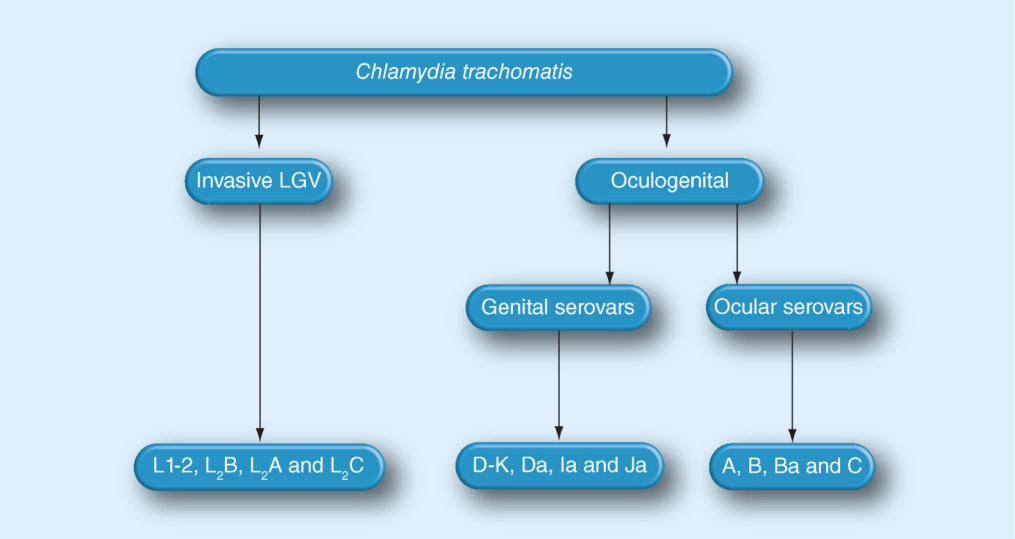

Figure 1. Classification of *Chlamydia trachomatis* based on tissue tropism

Chlamydia trachomatis is divided into the oculogenital and LGV biovars. The ocular (A–C) and genital (D–K) serovars infect conjuctival and genital epithelia, respectively, while LGV (L1–L3) spreads systemically in macrophages and other host cell types via lymph nodes. LGV: Lymphogranuloma venerum.

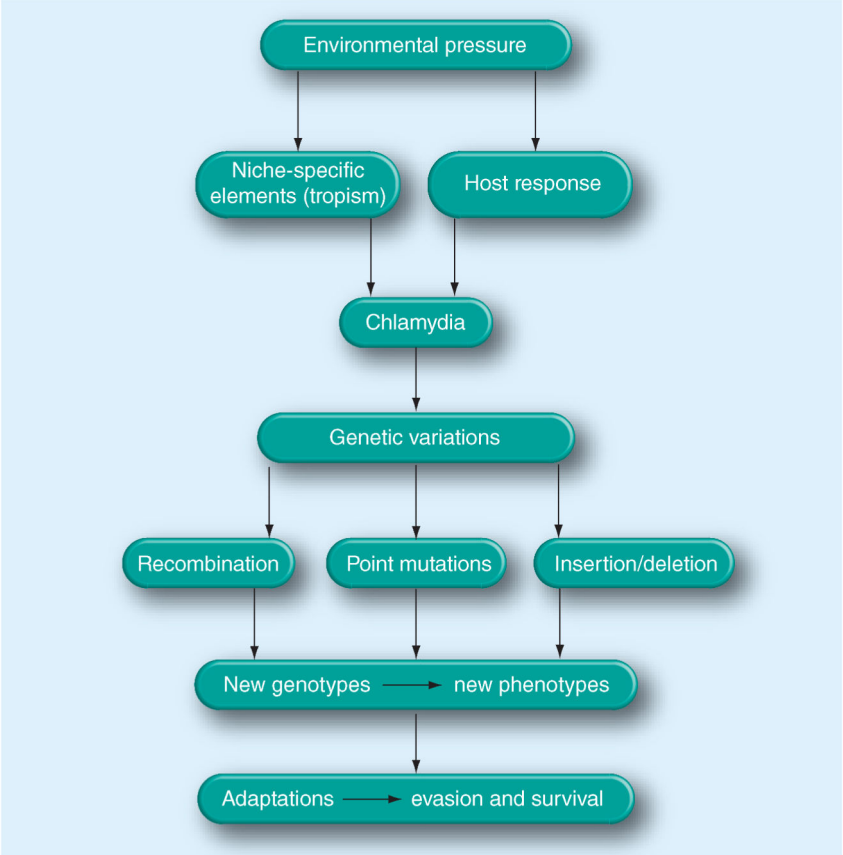

Figure 2. Genetic variation as an adjustment to environmental changes

Genetic variation allows *Chlamydia trachomatis* to exploit diverse niches within a host (tissue tropism) and to avoid, escape or resist host responses. Consequently, new *Chlamydia* genotypes arise within the population and the potential for strain selection with increased virulence is possible.

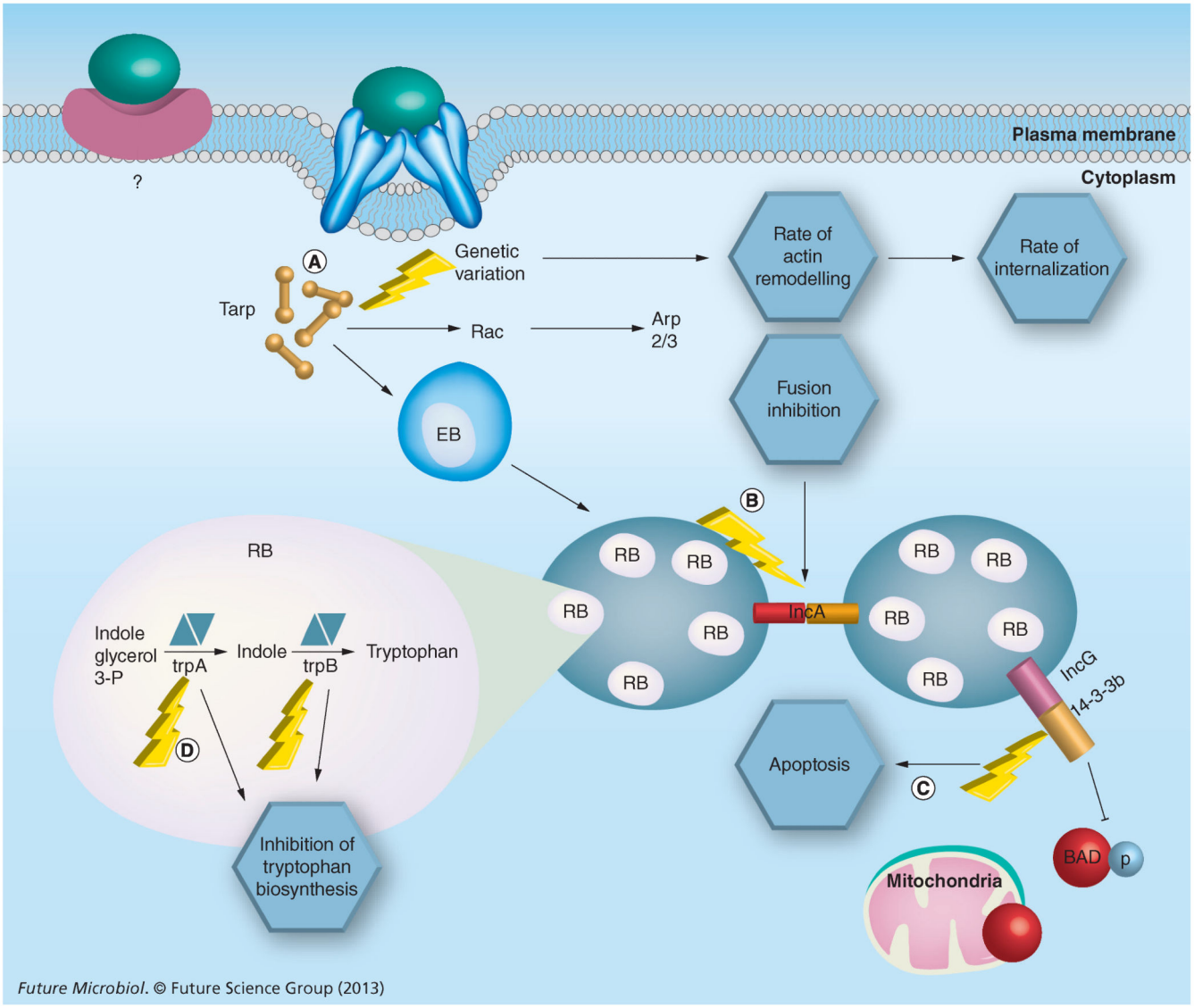

Figure 3. Consequences of genetic variation for key *Chlamydia trachomatis* virulence factors

Genetic variation (lightning bolts) results in serovar-specific differences in invasiveness, evasion of host responses and tissue tropism. **(A)** Tarp variation alters the number of actin-binding domains and the rate of internalization. **(B)** Variation in IncA results in nonfusogenic inclusions. **(C)** IncG, a result of gene duplication within the Inc-family, interacts with the host protein 14-3-3β preventing initiation of apoptosis by the host cell. **(D)** Mutation in the partial *trp* operon results in nonfunctional tryptophan synthase unique to ocular *Chlamydia trachomatis* isolates. '?' indicates that a 'receptor' or binding partner of *Chlamydia* EBs is not fully known and may be different between different chlamydial species. EB: Elementary body; RB: Reticulate body.

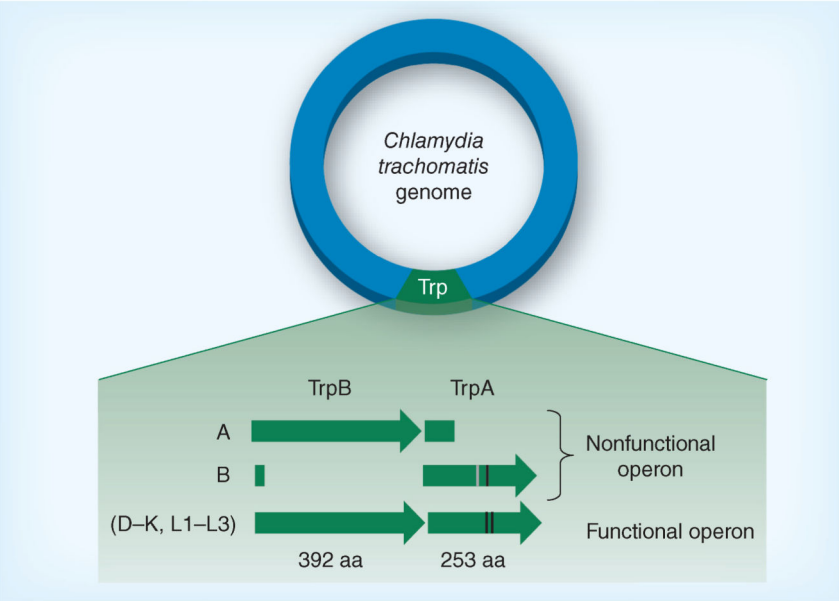

Figure 4. Trp synthase in different *Chlamydia trachomatis* serovars

All ocular serovars sequenced thus far harbor a nonfunctional *trpRBA* operon, whereas *trpRBA* is functional for all sequenced genital serovars. For ocular serovars, mutations may result either in a truncated TrpA or in a nonsynonymous point mutation that results in incorporation of an amino acid that inhibits function. Point mutations have been described for serovars E, F, G and Ia for *trpA* (e.g., change at C177Y) and serovars L1–L3 (change at Q178E); however, these mutations do not impact TrpA function. Nonsynonymous point mutations are represented by black lines while deletion mutations and their effect on TrpA are represented by the gray line. Not drawn to scale.

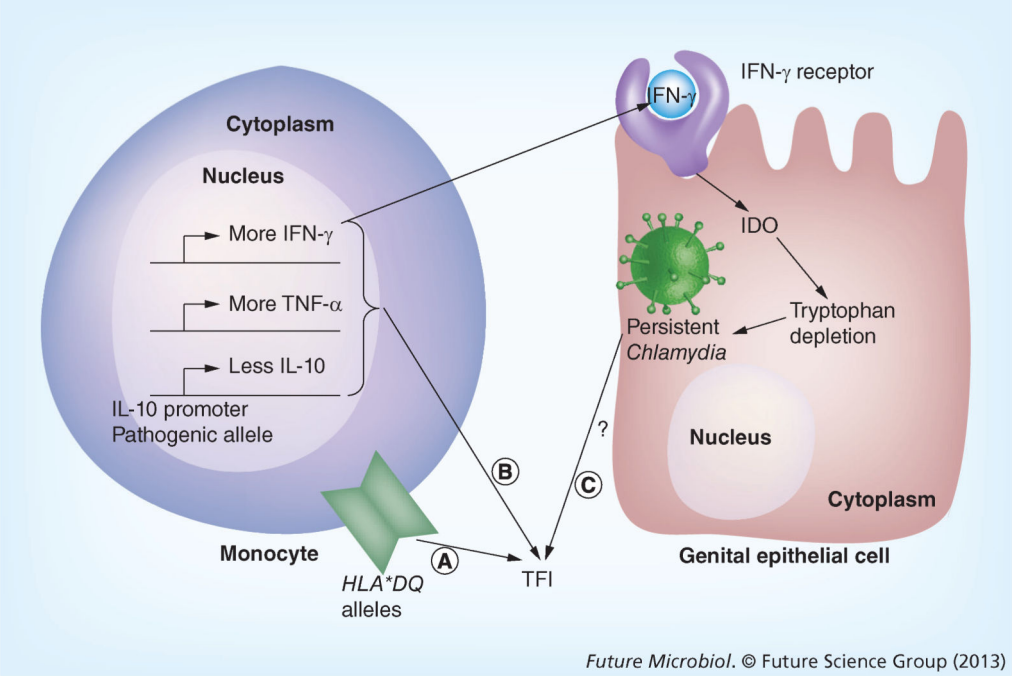

Figure 5. Contribution of host factors to *Chlamydia trachomatis* disease severity

Influence of genetic variation in host epithelial and immune cells on *Chlamydia trachomatis* infection and potential TFI induction. **(A)** Specific *HLA*DQ* alleles are correlated to TFI induction. **(B)** TFI patients carry an IL-10 promoter polymorphism. Cells from these individuals tend to produce less IL-10, more IFNγ and more TNF-α when leukocytes are stimulated *in vitro*. **(C)** IFN-γ induces IDO, which degrades tryptophan, an essential amino acid for both *Chlamydia* and the host cell. This may lead to dampening of the immune system, from a productive to a nonproductive chlamydial growth phenotype, which has been suggested to be associated with chronic infection and increased disease severity. IDO: Indoleamine 2,3-dioxygenase; TFI: Tubal factor infertility.

Future Microbiol. Author manuscript; available in PMC 2014 May 05.

Table 1

Effects of gene diversity in chlamydial proteins on disease severity and tissue tropism.

Gene family	Gene name	Locus	Function/role	Effect on tissue tropism	Potential effect on disease severity
Omp family	*omp A*	*ct681*	Most prominent outer membrane protein; porin; antigen-variants	Classification of *Chlamydia trachomatis* serovars into A–K and LGV	No correlation between *ompA* and disease severity demonstrated
Pmp family	*pmp A*	*ct412*	Type V secreted proteins: some members (D, E, G and H) are expressed on the EB surface. PmpD, D and G elicit a strong inflammatory response. All of these proteins likely play a role in phase variation	Unknown	High variability of *pmp* genes by SNPs, gene duplication and indel mutations may be significant in contributing to disease severity
	pmpB	*ct413*		Specific amino acid substitutions distinguish LGV from non-LGV; difference in *C. trachomatis* E and F to other genital serovars	
	pmpC	*ct414*		Specific amino acid substitutions distinguish LGV from non-LGV; difference in *C. trachomatis* E and F to other genital serovars	
	pmpD	*ct812*		Specific amino acid substitutions distinguish LGV from non-LGV	
	pmpE	*ct869*		Specific nucleotide substitutions segregate ocular from urogenital and LGV; difference in *C. trachomatis* E and F to other genital serovars	
	pmpF	*ct870*		Specific nucleotide substitutions segregate ocular from urogenital and LGV	
	pmpG	*ct871*		Specific amino acid substitutions distinguish LGV from non-LGV	
	pmpH	*ct872*		Specific nucleotide substitutions segregate ocular from urogenital and LGV; difference in *C. trachomatis* E and F to other genital serovars	
	pmpl	*ct874*		Difference in *C. trachomatis* E and F to other genital serovars	
	tarp	*ct456*	Binds to actin, phosphorylated by host kinases; cytoskeleton rearrangement; involved in internalization	Number of actin-binding sites differentiate LGV and non-LGV; number of actin-binding sites important for tropism?	Number of actin-binding sites important for disease severity?
Inc proteins	*incD*	*ctll 5*	Sphingolipid transport; interacts with CERT	Mutations in *incD* specific to ocular and LGV tissue tropism	Unknown
	incE	*ct116*	Unknown	Unknown	Unknown
	incF	*ct117*	Unknown	Unknown	Unknown
	incG	*ctll8*	Blocks release of cytochrome C and inhibits apoptosis	Unknown	Unknown
	incA	*ct119*	Homotypic fusion of intracellular inclusions	In LGV the genetic variation of *incA* may reflect tissue tropism	Nonfusogenic clinical isolates have less severe clinical signs
		ct229f †	Modulation of inclusion trafficking; binds to Rab4A	Unknown	Unknown

Gene family	Gene name	Locus	Function/role	Effect on tissue tropism	Potential effect on disease severity
	incB	*ct232*	Unknown	Unknown	Unknown
	incC	*ct233*	Unknown	Unknown	Unknown
	aaxB	*ct373*	AAX: arginine decarboxylase proenzyme; inhibits host cell polyamine synthesis, or inhibition of nitric oxide synthesis?	Unknown	Unknown
	aaxC	*ct374*	AAX: cytoplasmic arginine:agmatine antiporter; inhibits host cell polyamine synthesis, or inhibition of nitric oxide synthesis?	Unknown	Unknown
	trpA	*ct171*	Subunit of Trp synthase, which produces Trp from indole	Mutations in *trpA* segregate ocular from genital isolates	Unknown
		ct166f	Toxin-like protein; glycosylates Rac1; causing actin reorganization	Contains serovar specific loci, missing in LGV strains; potential role in invasiveness?	Potentially plays a role in disease severity (LGV strains lack the cytotoxin)

AAX: Arginine:agmatine exchange system; EB: Elementary body; Indel: Insertion and deletion; LGV: Lymphogranuloma venereum; SNP: Single nucleotide polymorphism.

[†]These loci have not, as yet, been named.