Impact of host genetics on susceptibility to human *Chlamydia trachomatis* disease

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Accepted: 21 February 2002

ABSTRACT

Evidence that host genetic factors play a major role in susceptibility or resistance to many infectious diseases is increasing, due to major advances in genetic epidemiological methodology. Recent human genome mapping information and the identification of a large number of candidate genes provide the tools for such studies. The information obtained is important for understanding the pathogenesis of disease and for the design of preventive and therapeutic strategies. In the study of *Chlamydia trachomatis* disease pathogenesis, much research focuses on how bacterial factors modulate the immune response and thus contribute to the disease process. It is likely, however, that host factors also play a role, and therefore susceptibility to disease is the result of an environmental effect set against a background of genetic factors. This review outlines the evidence for the contribution of host genetic factors to susceptibility to *C. trachomatis* disease in humans.

KEY WORDS: Chlamydia trachomatis. Disease susceptibility. Genetics. Infection.

Introduction

Chlamydia trachomatis is a Gram-negative obligate intracellular bacterium found in eukaryotic cells, and infection is usually restricted to the epithelial surfaces of the eye and/or the genital tract. *C. trachomatis* is subdivided into 15 serological variants (serovars), due to unique antigenic sequences in the elementary body (EB) outer membrane proteins. Serovars A, B, Ba and C infect the conjunctival epithelium and cause trachoma (a chronic kerato-conjunctivitis), while serovars D to K infect the genital tract epithelium and are the major cause of genital tract infection worldwide. Lymphogranuloma venereum is another sexually transmitted disease caused by *C. trachomatis* serovars L1, L2 and L3, but these serotypes infect lymphatic tissues as well as epithelial cells.

Chlamydial disease usually develops a few days after infection. The typical clinical signs are inflammation and the development of follicles, which can be seen in the eyes in ocular infection. In genital infection, however, pain, fever and discharge are the resulting signs. As the follicles resolve, fibrotic scars appear and, in trachoma and pelvic inflammatory disease (PID), fibrosis causes irreversible late sequelae as a result of deformities to the conjunctiva and pelvic tissues.

The study of trachoma in humans suggests that repeated infection is required for scarring, trichiasis or blinding sequelae to develop,[1,2] and a study of migrants found that scarring did not progress in the absence of continued exposure to infection.[3] It is also likely that persistent infection plays a role in the development of sequelae. Some individuals may not produce an adequate immune response to clear the infection, or inappropriate responses may be produced to some chlamydial antigens, leading to disease sequelae.

Most research into chlamydial disease susceptibility has focused on the organism's virulence factors. However, although virulence and the degree of drug resistance may influence the outcome of infection, host properties also play a role. For instance, studies in The Gambia found that only a very small proportion (less than 1%) of individuals exposed to *C. trachomatis* infection go on to develop severe disease.[4] In addition, there are marked differences in the sequelae among those who develop disease, with conjunctival scarring, fibrosis or corneal opacity in ocular infection, and pelvic scarring and deformity of the pelvic wall in genital infection. This suggests that development of chlamydial disease sequelae probably involves both multiple gene interactions with the organism and environmental factors such as social background, inadequate community sanitation and lack of access to basic medical care for treating early infection.

Methods of identifying susceptibility genes

In humans, identification of genetic markers can be made by studying candidate genes or carrying out random genome screening. In candidate gene studies, which are usually population-based, genes are selected on the grounds of their known function and relevance to disease pathogenesis. Polymorphisms within the candidate genes are tested, in an attempt to demonstrate a relationship between a particular variant of the gene (allele) and the disease.

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Table 1. Examples of human genes associated with susceptibility to infectious diseases

Disease	Allele	R/S*	Population
Malaria	HLA-B53	R	Gambian[6]
Malaria	TNF-α -308	S	Gambian[7]
Leprosy	HLA-DR2	S	Indian[8]
Tuberculosis	HLA-DR2	S	Indian[9]
AIDS	HLA-A1-B8-DR3	S	British[10]
Mucocutaneous leishmaniasis	TNF-α -308	S	Venezuelan[11]
Hepatitis B	TNF-α -238	S	Gambian[12]
Hepatitis B	HLA-DRB1*1302	R	Gambian[13]

** Alleles that confer resistance or susceptibility are denoted as R or S, respectively*

Association studies have been applied to look at polymorphisms in specific candidate genes, and in these studies the frequency of genetic polymorphisms in patients with the disease are compared with matched controls – ideally of the same sex, age, location and ethnic group. The aim is to see whether or not there is a relationship between disease susceptibility and possession of candidate genes. Using this approach, human leucocyte antigen (HLA) genes have been studied extensively in different diseases because of their role in presenting peptide antigens to T cells. Although many associations have been reported, some are questionable as there is little consistency in the associations reported by different workers. Several factors account for this and include the small size of some studies, poor matching of cases and controls, genetic differences between study populations, and molecular diversity of the infectious agent.

It is important to remember that association studies do not demonstrate causation and may be biased by factors such as population mix, linkage disequilibrium with unstudied genes that cause the altered risk of infection, multiple comparisons and improperly matched controls.[5]

Random genome screening identifies novel genes whose structure or function may be unknown. This is done by analysing microsatellites (consisting of di-, tri- or tetra-nucleotide repeats – often cytosine and adenosine) at regular intervals across the whole genome, in an attempt to identify chromosomal regions in which susceptibility or resistance genes are located. Repeat sequences are detected by the polymerase chain reaction (PCR), using oligonucleotide primers that include the microsatellite repeats. Size differences caused by variations in the number of repeats contained in the fragments can be detected for different alleles.

Both candidate gene approach and genome screening are used in linkage studies in which the inheritance of polymorphic markers in relation to disease is studied in families. Linkage is present when the alleles of a marker tend to co-segregate with a disease within a family; however, in instances where the effect is moderate, the power to detect genes that show only modest effect will be limited unless large numbers of families are studied.

Animal studies using 'knock out' mice are also popular because the animals lack specific genes and environmental factors can be controlled. Such mice are bred with targeted gene disruption, based on what is known about the immune response to the infectious organism being studied. Although it can be argued that many of the susceptibility genes identified in murine models are not relevant to humans, they do provide important information about the interaction between genes and the environment.

Evidence that infectious disease may be genetically regulated

Genetic factors involved in disease development could increase either resistance or susceptibility to disease sequelae. Examples of reported associations between infectious diseases and candidate gene polymorphisms are shown in Table 1.[6-13] Evidence that genetically determined host factors are important has been obtained from family, twin and adoption studies.

Family studies looked at the risk of a disease phenotype in the relatives of affected subjects; however, although this may provide some information about the genetic relationship between different phenotypes, it is not easy to eliminate an environmental effect.

Twin studies looked at both monozygotic (identical) and dizygotic (fraternal) twins, and it was assumed that they shared environmental influences. Thus, if monozygotic twins were affected by a disease more often than dizygotic twins then this implied a genetic effect, and evidence of genetic susceptibility to some infectious diseases, such as leprosy and tuberculosis, has been obtained from such studies.[14,15]

Adoption studies, on the other hand, assumed that only genetic factors are transmitted from biological parents, and the aim was to distinguish between genetic and environmental factors by studying whether genetic susceptibility of adopted children to a disease resembles more closely that of the biological or the adopted parents. Indeed, in a study of adoptees in Denmark, it was found that death of a biological parent before the age of 50 from an infectious cause was associated with a six-fold increased risk to the offspring of premature death from an infectious disease.[16] However, this assumption only holds if there are no major differences between the environments in which the biological and adopted parents lived that could increase or decrease the likelihood of exposure to infection.

Genetics of susceptibility to *C. trachomatis* disease sequelae

Trachoma

Ocular chlamydial infection is endemic in many areas of the world and trachoma is the leading cause of infectious blindness.[17] Development of blindness from chlamydial infection progresses through several stages, from inflammation with visible follicles on the conjunctiva, to fibrosis, scarring, trichiasis and finally corneal opacity. The early stages can be treated and, in children, the active disease is often asymptomatic and resolves naturally. It has been suggested that repeated infections are necessary for scarring trachoma and the later stages of the disease to develop.[1,2]

However, even in communities where infection is endemic and people are continually exposed to the organism, not everyone suffers from disease sequelae. A higher proportion of women suffer from scarring and this is thought to be due to the fact that they spend more time with children, who carry the active infection.[18] It is unclear, however, why only a subset of people living in communities where infection is endemic develops adverse sequelae. If we assume that exposure rates and other environmental influences in such populations are the same, then this implies that host factors are important and genetic susceptibility may be a determinant of disease progression.

A case-control study carried out in Kongwa, Tanzania, to assess the risk factors for trichiasis in women showed that subjects with trichiasis were four times more likely to have a mother with trichiasis.[19] Although it could be argued that familial clustering of disease may reflect both environmental and genetic factors, the observation that siblings of active cases showed an increased prevalence does suggests a strong genetic component.[20,21]

The size of the immune response mounted by an individual can vary with the specific homozygous or heterozygous alleles present. Several groups have studied the major histocompatibility complex (MHC) – the HLA genes – because of its role in the control of the immune response and the fact that the genes are highly polymorphic.

In a case-control study in The Gambia in which patients with trachomatous scarring and controls matched for age, sex, ethnic group and location were recruited, the HLA class I antigen HLA-A28 was significantly more common in the trachoma group than in controls.[22] In particular, the HLA-A*6802 allele – a subtype of HLA-A28 – was over-represented among the cases studied, suggesting that immunopathology may be associated with A*6802-restricted cytotoxic T-lymphocyte responses. No individual HLA type was associated with protection from scarring in this study, and the frequency of HLA class II alleles was similar in both groups, suggesting that multiple or complex T-cell responses are involved in protective immunity.

In another case-control study that looked at ocular chlamydial disease in Oman, increased prevalence of the HLA class II allele DR16 and decreased prevalence of DR53 were seen in patients with trachomatous corneal opacity.[23]

Different HLA associations to disease susceptibility were found in the Gambian and Omani studies; however, this is not surprising as different criteria were used to select the cases and controls. In the Gambian study, patients had trachomatous scarring that had progressed to trichiasis in some instances. In the Omani study, the condition had progressed to corneal opacity, which is a later stage of the disease.

Controls were matched in the Gambian study, whereas the Omani study used blood donors as controls and thus were not properly matched with the patients. The use of blood donors as controls is questionable as controls should be exposed equally to the risk of getting the disease, and age and sex have been shown to be important determinants of chlamydial sequelae. In addition, the general genetic differences between the two non-randomly sampled populations means that the general HLA allele frequencies may differ.

Polymorphism in the promoter region of the tumour necrosis factor-α (TNF-α) gene, found in the MHC class III region, also was investigated in the Gambian study. It was found that a G to A nucleotide substitution at position -308 was associated with trachomatous scarring,[24] and this was independent of the HLA class I association found.

Several studies have investigated the importance of TNF-α -308 polymorphism in relation to cytokine production and messenger RNA (mRNA) production. One study found that the G to A polymorphism is associated with increased TNF-α production *in vitro*.[25] However, results from studies that attempted to demonstrate a difference in transcriptional activity between different promoter alleles are conflicting. Two studies were unable to demonstrate a difference[26,27] but a third study found a significant effect on transcriptional activity.[28] If the -308 polymorphism proves to be functional, and the -308A allele is a high TNF-α producer, then this may implicate high TNF-α levels in the development of trachomatous scarring.

Polymorphisms in other candidate genes known to modulate risk of chlamydial disease were also studied in the Gambian case-control study,[29] and a significant association was found between the interleukin-10 (IL-10)-1082G allele and trachomatous scarring in a Gambian ethnic group. This is an important finding because this allele has been associated with higher IL-10 production *in vitro*,[30] and previous studies have shown that T-helper type 2 (Th-2) cell cytokine responses are associated with increased risk of scarring trachoma in humans.[31,32]

In addition, there appears to be a genetic restriction for the 57 kDa chlamydial heat shock protein (cHSP60) antibody response. Antibody to cHSP60 has been implicated in immunopathogenesis[33-35] and correlates with the late tissue-damaging sequelae of *C. trachomatis* infection,[36] although the mechanism responsible is unknown. In the Gambian case-control study of trachoma patients, response to cHSP60 correlated positively with expression of the MHC class II allele HLA DRB1*0701, while the DRB1*0301 and DQB1*0701 alleles were negatively associated.[37] A study of T-cell responses to cHSP60 found that they were more depressed in patients with scarring trachoma than in those who had recovered from infection without disease sequelae.[32] Therefore, the fact that the class II alleles found to be associated with cHSP60 antibody response were not associated with trachomatous scarring may reflect linkage disequilibrium between HLA class II alleles and other immune response genes.

Genital chlamydial diseases

Genital *C. trachomatis* infection is the most common cause of bacterial sexually transmitted infection in the developed world, and, in 1995, the World Health Organisation estimated that 89 million cases occurred worldwide.[38] Transmission occurs through sexual contact and, even though infection can be asymptomatic, serious complications can result.

C. trachomatis commonly causes infection of the urethra in men and the cervix and/or urethra in women, but can extend to the upper genital tract (epididymis, endometrium, salpinx) and into the peritoneum, and, in rare cases, to the rectum. In women, the sequelae of *C. trachomatis* infection include pelvic inflammatory disease (PID) leading to tubal factor infertility (TFI), ectopic pregnancy and chronic pain. In men, infection causes urethritis and may lead to epididymitis. Infection has also been linked to arthritis (sexually acquired reactive arthritis) following non-gonococcal urethritis in 1-2% of men.

HLA genes have been investigated for susceptibility to chlamydial genital disease sequelae. In a study of sex workers in Nairobi, PID was independently associated with the HLA class I allele HLA-A31.[39] Although association of HLA-B27 with reactive arthritis is well known,[40] the role of *C. trachomatis* is implicated by the association of HLA-B27 with Reiter's syndrome that develops following genital chlamydial infection.[41]

There is also evidence to suggest that susceptibility to *C. trachomatis* infection may be HLA class II restricted. Data from a Kenyan study, designed to investigate genetic factors associated with altered susceptibility to *C. trachomatis* infection in women with TFI, showed that HLA-DQA*0102 was associated with resistance and that DQA*1010 and DQB*0501 alleles were associated with *C. trachomatis* TFI.[42]

Recently, the HLA class II alleles DQA1*0401 and DQB1*0402 were shown to be associated with increased prevalence and amount of antibody to cHSP60 in commercial sex workers in Kenya.[43] Furthermore, several studies have provided evidence for a correlation between cHSP60 seroreactivity and development of genital disease sequelae.[44-46]

Polymorphisms in the transporter associated with antigen-processing (TAP) genes also have been studied in relation to *C. trachomatis* genital disease. It was found that TAP1-02011 (Val-333/Gly-637) and TAP2-0201 (Val-379/Ala-565/Ala-665) alleles were more frequent in patients infected with *C. trachomatis*, and joint lesions were associated with the TAP1-02011 allele in this group.[47] TAP proteins are important in controlling the immune response because they transport peptides into the endoplasmic reticulum, where they can bind to MHC class I molecules. Thus, it is possible that the TAP1-02011 allele can affect antigen presentation.

Although this review has examined genetic susceptibility to *C. trachomatis* disease in humans, it is worth noting that genetic susceptibility to chlamydial PID has been studied in mice and in the pig-tailed macaque. In the mouse model, susceptibility to the complications of *C. trachomatis* infection varies with genotype,[48] and both increased and decreased immune responses to cHSP are associated with the expression of specific MHC (H-2) alleles.[49] In the macaque model of chlamydial PID, individual variation in the time it takes for intrapelvic adhesions to develop occurs and correlates with MHC class I allele expression.[50]

Conclusions

As with other infectious diseases, it is likely that several genes are involved in chlamydial pathology. The identification of resistance or susceptibility genes for human chlamydial infection and disease sequelae is important for several reasons. It can help to identify high-risk groups or individuals within a population who would benefit from specific preventive or adjunctive therapy. More importantly, however, as we cannot change a person's genes, it is hoped that such knowledge will allow us to elucidate the immunological pathways, cells and cytokines that are important for protection against the development of sequelae, and thus be in a position to manipulate the type of immune response triggered by designing vaccines to target events during disease progression.

Presently, with the progress that has been made in the analysis of the human genome and the new genetic tools now available, there are real opportunities for the identification of resistance or susceptibility genes to *C. trachomatis* disease sequelae. The whole genome could be screened using microsatellite markers in affected sib-pairs, and, because many polymorphic candidate genes have been identified, case-control studies could be used to test for disease associations with immune response genes that lie both inside and outside the MHC region. □

The author is grateful to the Medical Research Council Laboratories, The Gambia, for support; and to Dr Melanie Newport, Dr Gerald Byrne, Dr Robin Bailey, Dr David Conway, Dr Hilton Whittle and Professor David Mabey for reading the manuscript and making helpful suggestions.

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