Review (Narrative)

Molecular Epidemiology of *Chlamydia Trachomatis* Infection in the Genitourinary Tract

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SUMMARY
The genitourinary *Chlamydia trachomatis* infection is a crucial part of sexually transmitted disease worldwide. In recent years, the incidence rate has increased dramatically, and the proportion of asymptomatic infections is particularly large. Therefore the burden for preventing and controlling its epidemic is severe. With the development of laboratory technology and genomics, the understanding on the molecular epidemiology of genital *Chlamydia trachomatis* is gradually clearer than ever, and the monitor on its epidemic trends and strain variation is becoming more effective.■

KEYWORDS *Chlamydia trachomatis*; Genitourinary infection; Epidemiology; Molecular classification

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CHLAMYDIA trachomatis (CT) is a Gram-negative pathogen with strict intracellular parasitic property. CT infection is prevalent worldwide. Approximately 3.0 million visual injuries and over 1.5 million cases of blindness worldwide are caused by CT infection (1). CT sexual transmission can also cause genitourinary tract infection, which increases the risk of infection with other sexually transmitted diseases (2). A survey of female sex workers shows that CT infection is an independent risk factor for HIV infection (3).

CLASSIFICATION OF *CHLAMYDIA TRACHOMATIS*

Laboratory CT serum cell typing technology, although applied earlier, is gradually replaced by molecular typing technology because its cell culture process is time-consuming and requires a large number of monoclonal antibodies. The most widely used method is the typing method based on the ompA gene (4). The ompA genotyping method is mainly restriction endonuclease fragment length polymorphism analysis (RFLP) and direct sequencing. RFLP uses restriction endonucleases to produce DNA fragments of different numbers and lengths. After electrophoresis separation, a characteristic restriction map is generated for genotyping; direct sequencing is to directly measure the ompA nucleic acid sequence of the DNA to be detected by a sequencer. Although the sequencing method is more expensive and time-consuming than RELF, it is more accurate and can distinguish the difference of one nucleotide. At the same time, the sequencing method can further establish a phylogenetic tree, suggesting the evolutionary relationship between the clinical strain and the target strain, to some new ones. Although the ompA typing method has many advantages, in recent years some scholars have suggested that the ompA gene is often exchanged between different strains to produce an exchange-type strain, and the conventional ompA typing method is difficult to distinguish. At the same time, because the ompA variant strain can escape the body immunity, it has more advantages in the population. If the ompA typing method is used, it will miss important epidemiological information.

Therefore, new typing methods have emerged: CT multi-site sequence typing (MLST) and multi-site variable number tandem repeat analysis (MLVA). MLST compares the alleles of the strains by comparing the nucleic acid sequences of the internal fragments of ≥ 5 CT housekeeping genes, and has high resolution compared to the ompA typing. MLST typing was used in male-male contact (MSM) and heterosexual populations, and the same two mpA genotypes were found to have different MLST aggregation characteristics (5); while the same MLST clusters may come from Different ompA genotypes of MSM population and heterosexual populations of CT infected individuals showed significantly different MLST aggregation characteristics. Therefore, MLST typing technology can be used as an effective tool to study the characteristics of CT transmission among high-risk groups. MLVA analyzed the characteristics of variable number tandem repeats (VNTR) in the CT genome. The number of repeats among different species was highly variable, and cluster analysis was used to classify the strains. In 2008, Pederson and others first applied MLVA screening to describe three VNTR sites (MLVA3), which were classified by CT (6). On this basis, in 2012, Peuchant et al selected five VNTR points (CT5I, CT531, CT719, CT1025, CT1035), which showed that MLVA5 has higher resolution than MLVA3 and ompA, and can be directly applied to clinical CT positive samples (7). Therefore, using MLVA typing, it is possible to more accurately reveal epidemiological information on CT aggregation characteristics, strain origin and variation.

DIFFERENT GENES OF *CHLAMYDIA TRACHOMATIS* AND GENITOURINARY TRACT DISEASES

Many studies have found that the ompA genotype is related to disease invasiveness and clinical manifestations. Globally, E, D and F are the dominant genotypes of CT; E-type CT female infected patients have relatively mild clinical symptoms, mainly manifested as purulent discharge of cervical cat liquid and F type although cervical mucus (8). The symptoms of purulent secretions are mild, but they are prone to endometrial lesions and are associated with lower abdominal pain in women. G and F CT are prone to infection of married women and cause abnormal vaginal discharge: D and G type CT are more susceptible to MSM. H and J type CT male infections often have dysuria and urinary incontinence. Although the ompA genotype is confirmed, there are many literatures with clinical manifestations, but reports that are inconsistent with this are not uncommon. No defi-

nite correlation was found between 132 women and 101 male patients with CT infection, and so it was believed that due to the existence of genetic recombination (9). The view that mpA gene polymorphism is associated with disease severity is not the thing we can rely on, the association between genetic polymorphism and disease need to be explored further. Therefore, to study this field, it is necessary to use genome-wide data analysis instead of being limited to individual causal points.

In addition to the ompA gene, other genes and gene families, such as inclusion body proteins (Inc), PmP, and tarP, are thought to affect CT-related disease through a variety of mechanisms. The type III secretion-regulating gene TarP affects disease by modulating a tyrosine-rich repeat region and an Actin-binding domain. Inclusion body membrane egg from (Incs) is a type III secretion effect egg from one of the family members (10). Study found that LGV and inflammatory symptoms caused by CT infection not expressed by ineA were mild, and incG was closely related to clinical chronic infection (11). The pmp gene family (pmpA-I) encodes a polymorphic outer membrane protein that binds to and stimulates the production of pro-inflammatory cytokines and regulates gene replication and mutation of CT, thereby affecting the body's immune level to surface proteins. The immunogenicity of different pmp proteins is different. PmpA, PmpE, PmpF and PmpH are weaker, while PmpB, PmpC, PmpD and PmpI are stronger. The expression of PmpB and PmpI is more likely to cause pelvic inflammatory disease (12).

LABORATORY TEST METHOD FOR *CHLAMYDIA TRACHOMATIS* INFECTION

Laboratory tests for CT infection include direct smear, CT cell culture, immunological detection, and molecular biology methods. Although the colloidal gold method is applied more, its main advantage is that it is fast, simple and inexpensive, and the disadvantage is low sensitivity (~30%-50%) and is not suitable for screening for atypical CT infection (13). Point of care diagnostics (POC) detection technology is highly valued by disease control professionals because of its fast detection speed, portability and easy operation. The POC detection technology is not only fast, but also can be analyzed on site, eliminating the complicated pre-processing process of the sample, analyzing it immediately at the sampling site, and quickly obtaining the test result. Currently, the most commonly used POC technologies for CT are immunochromatographic assays (ICTs) and optical immunoassays (OIAs), all based on antigen-antibody reactions, with specificity up to 95%, but low sensitivity (39%-79%), too low a degree of flexibility makes it impossible to meet the epidemiological needs of "multiple discoveries" of infected population, so the specificity is limited (14).

The current nucleic acid expansion test (NAAT) is considered to be the gold standard for the diagnosis of CT infection (15). NAAT has the advantages of high sensitivity (90%-97%) and high specificity (99%-100%). It not only detects 20% to 30% of positive samples more than traditional detection methods, but also has a relatively short detection period and sensitivity. High, easy to operate, can use male, female urine, cervical swab, urethral swab and other samples. There are a variety of commercial NAATs available on the market, and the detection loci are usually multiple copies of the cryptic plasmid, 16S RNA and ompA. Although NAAT is the primary basis for laboratory diagnosis, such techniques are limited by the speed of detection and equipment requirements, and cannot be quickly screened for a large number of target populations. An X-pert technology developed in recent years, combined with microfluidic technology and real-time PCR, can achieve ideal sensitivity and specificity in various target populations (15). X-pert technology allows patients to sample themselves and report results within 90 minutes (16). According to a study in Australia, if this test covers 44% of the target population each year, the Australian CT infection can be reduced from 11.9% to 8.9%; if 60%-80% of the population is covered, the infection rate will drop to 1.5% (17).

CONCLUSIONS

Traditional research strategies relying on ompA genotyping are being questioned, and current research on the relationship between CT gene polymorphism and genitourinary disease is still not systematic enough to draw definitive conclusions. The recently proposed multi-copy concealed plasmid typing and genome-wide data analysis possess a promising value. Therefore, relevant databases containing various target populations should be established as soon as possible in order to monitor CT trends and strain variations in a more effective way.■

ARTICLE INFORMATION

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Acquisition, analysis, or interpretation of data: Landonishi.
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