Association of MICA gene polymorphisms with *Chlamydia trachomatis* infection and related tubal pathology in infertile women

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**BACKGROUND:** The course and morbidity of *Chlamydia trachomatis* infections are determined by host genetic factors, virulence of the micro-organism and environmental factors. Major histocompatibility complex class I chain-related A (MICA) gene is highly polymorphic as a potential host genetic candidate. The aim of this study was to investigate the association of polymorphic extracellular domains of MICA with *C. trachomatis* infection and related tubal factor infertility.

**METHODS:** Effect of MICA on the susceptibility to *C. trachomatis* infection and its association with tubal pathology were investigated in 214 infertile women recruited during the period from 2004 to 2007. Subjects were tested for *C. trachomatis* antibodies, and were further divided into two groups: those with (*n* = 42) and without (*n* = 59) tubal pathology based on laparoscopy results. The relationship between prevalence of *C. trachomatis*, tubal pathology and MICA allele polymorphisms was analysed.

**RESULTS:** Women with tubal infertility more often had antibodies to *C. trachomatis* [66.7 versus 39.1%; odds ratio (OR): 3.12, 95% CI: 1.68–5.78, *P* = 0.004] than infertile women without tubal pathology. Moreover, allele 008 had a highly negative correlation with *C. trachomatis* infection (*P* = 0.0036, OR: 2.14), while other allele polymorphisms showed no significant association with the disease. No statistically significant differences were found in the MICA allele frequencies of *C. trachomatis*-positive women with or without tubal pathology.

**CONCLUSIONS:** The association of a specific MICA allele with *C. trachomatis* IgG antibodies among women with infertility suggests that the MICA locus might modify host susceptibility to *C. trachomatis* infection.

**Key words:** *Chlamydia trachomatis* / MICA / infertility / gene polymorphism

**Introduction**

*Chlamydia trachomatis* is an obligate intracellular gram-negative like bacterium and an important cause of sexually transmitted diseases worldwide (de Muylder et al., 1990; Ville et al., 1991; den Hartog et al., 2005). Many chlamydial infections are asymptomatic, and re-infections are common. If left untreated, *Chlamydia* has a high tendency to remain persistent in inflamed tissues of the upper genital tract of patients with pelvic inflammatory disease (Cohen and Brunham, 1999; den Hartog et al., 2006). Prolonged inflammation may lead to tissue scarring and occlusion of Fallopian tubes. Although many women are infected with *C. trachomatis*, only a minority will develop tubal factor infertility. Moreover, a clearance rate of 44.7% has been reported in asymptomatic and untreated women after 1 year follow-up (Morre et al., 2002). These results imply that host genetic factors play an important role in modulating the immune defence mechanisms and thereby determining the pathogenesis of chlamydial diseases. However, the genetic basis underlying this phenomenon has remained unclear.

Genes involved in the immune response appear ideal candidates for further study, given their function and polymorphism, as well as data from previous studies (Cohen et al., 2000, 2003; Kinnunen et al., 2002). It has been found that, after *C. trachomatis* infection, down-regulation of the major histocompatibility complex (MHC) class I is one of the mechanisms *C. trachomatis*-infected cells use to evade an immune response involving recognition and destruction by cytotoxic T lymphocytes (Zhong et al., 2000). However, the down-regulation of MHC class I leads to activation of natural killer (NK) cells because of a decreasing inhibitory signal generated by the binding of inhibitory receptors to their ligands (Fig. 1). Simultaneously, the NK cell-activating ligands, such as major histocompatibility complex class I chain-related A (MICA), may be up-regulated. Through the
interaction between the activating ligands and their corresponding receptors, the activating signals of the NK cells increase. Moreover, one of the most relevant cytokines involved in the response against *C. trachomatis* is interferon-γ (IFN-γ) (Srivastava et al., 2008). One of the most important sources of IFN-γ is from NK cells. Stimulation of NK cells by the NKG2D receptor by means of its ligand, MICA, results in the release of the cytokine IFN-γ (Bauer et al., 1999) (Fig. 1). We therefore believe that the relationship between polymorphism of the MICA ligand and susceptibility to *C. trachomatis* infection deserves further study.

The MICA genes are situated 46 kb upstream of human leucocyte antigen B and encode a stress-inducible molecule with three extracellular domains (α1, α2 and α3), a transmembrane region and a cytoplasmic tail. Its domain structure is similar to MHC class I antigens without β2-microglobulin (Bahram et al., 1994; Tieng et al., 2002). Like MHC class I genes, the MICA locus is highly polymorphic, with at least 53 alleles having been described.

At present, the role of MICA polymorphisms has not been investigated either in the susceptibility to the development of tubal pathology or in *C. trachomatis*-associated tubal factor infertility. Therefore, the first objective of our study was to assess whether polymorphisms of MICA genes are associated with tubal pathology by comparing women with confirmed severe tubal pathology and without tubal pathology. Secondly, we investigated the group of women with a probable history of clinical *C. trachomatis* infection (as suggested by positive *C. trachomatis* serology) to evaluate the possibility that these gene polymorphisms might identify those women at risk of infection.

**Materials and Methods**

**Subjects**

Infertility was defined as not becoming pregnant after trying for 1 year. The study was performed in women who sought treatment at the infertility Clinic of Jingzhou Central Hospital, China, during the period from June 2004 and October 2007. Laparoscopy with tubal testing was part of the fertility workup. Only patients who had undergone a laparoscopy and tubal testing were included in the present study. Tubal pathology was defined as extensive periadnexal adhesions with severe distal occlusions of the tubal continuity of one or both tubes. Of the 230 women who underwent laparoscopy, women with severe tubal pathology (*n* = 63) and ones without tubal pathology with laparoscopy (*n* = 151) were enrolled in the present study in order to compare the most well-defined cohorts. Other women (*n* = 16) with only minor upper genital tract pathology or having undergone pelvic surgery previously were excluded. The study was approved by the

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**Figure 1** Model for self-tolerance and activation of NK cells. NK cell function is regulated by a balance between signals transmitted via stimulatory and inhibitory receptors. MHC class I molecules generally inhibit NK cell function by binding to inhibitory receptors, resulting in phosphorylation and recruitment of SHP1/SHP2. The stimulatory signals are transmitted via DAP10-associated receptor NKG2D through binding to its ligands, such as MICA/MICB. The signaling cascade induced by DAP10 involves PI3K/Grb2. **(A)** The inhibitory receptor signal counters the stimulatory receptor signal so that the NK cell maintains a steady-state condition and self-tolerance. **(B)** MHC class I molecules are down-regulated on infected cell, and the balance of signals transmitted via stimulatory and inhibitory receptors is impaired. The NK cell becomes activated (cytotoxicity and cytokine secretion). 176 × 125 mm (600 × 600 DPI).
Medical Ethical Committee of the Jingzhou Central Hospital, China. Written informed consent was obtained from each participant in the study.

Serology
Chlamydial antibody testing is a routine procedure in the fertility workup. Peripheral venous blood was collected for the analysis of IgG antibodies against C. trachomatis using commercially available enzyme immunoassay kits (Immuno-Biological Laboratories, Minnesota, USA). The results were obtained in terms of the mean absorbance (optical density, OD) at 450 nm. The antibody index of each determination was calculated by dividing the OD value of each sample by the cut-off value. Positive C. trachomatis specific IgG results were defined as antibody index >1.1, as recommended by the manufacturer. Samples with grey zone values, e.g. 1.0 ± 0.1, were repeated and considered positive when the result of antibody index was >1.1.

Analysis of MICA alleles
Genomic DNA was isolated from EDTA anticoagulated peripheral venous blood by a conventional proteinase K digestion/salt-out extraction method. MICA genotyping was performed through the PCR-SSP (sequence-specific primer) method with minor changes (Rees et al., 2005). The primers (sense 5'-GCTTTCACCAACATTCCCTTA-3' and anti-sense 5'-GAGAAAGGCGTTAGGATTCC-3'), which amplify an 834-bp portion of the human growth hormone gene, were used as an internal positive control. PCR amplifications were performed in 10-μl reaction volumes. The reaction mixtures comprise 100 ng of genomic DNA, 1× PCR buffer (MBI Fermentas, Vilnius, Lithuania), 200 μmol/l of each deoxy-nucleoside triphosphates (MBI Fermentas), 1.75 mmol/l MgCl₂ (MBI Fermentas), 0.25 units of Taq polymerase (MBI Fermentas), 1.5–2.0 μmol/l allele or group-specific primers, and 0.115 μmol/l internal control primers. Amplification consisted of initial denaturation for 4 min at 94°C followed by 10 cycles of denaturing at 94°C for 30 s, annealing at 64.5°C for 50 s, and elongation at 72°C for 20 s; followed by 10 cycles of denaturing at 94°C for 30 s, annealing at 61.5°C for 50 s, and elongation at 72°C for 30 s; followed by 10 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 50 s, and elongation at 72°C for 40 s; then cooling to 4°C. From each PCR reaction, 8 μl was transferred to a 2% agarose gel and electrophoresed. Results were interpreted using the worksheet.

Statistical analysis
The MICA distribution was tested for Hardy–Weinberg equilibrium to assess Mendelian inheritance. Statistical analysis was performed with SPSS 11.5 statistical software (SPSS, Inc., Chicago, IL, USA). Allelic frequencies were calculated by direct counting and statistical comparisons between groups which, depending on the number involved, were performed by the chi-square test with Yates’ correction or Fisher’s exact test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for the disease risk in carriers of the specific alleles. Bonferroni multiple correction was used for the corrected P (Pc) by multiplying the P-value by the number of the statistical tests. The two-sided P < 0.05 was considered to be statistically significant.

Results

C. trachomatis IgG antibodies in relation to tubal pathology
Serological test results for C. trachomatis antibody levels in infertile women with or without tubal pathology were elucidated in detail (Table I). Of the infertile women, 66.7% (42/63) with severe tubal pathology and 39.1% (59/151) without tubal pathology were C. trachomatis IgG positive (OR: 3.12, 95% CI: 1.68–5.78, P = 0.004). The antibody index is proportional to the level of IgG specific antibody in the sample. In order to observe the association between the levels of IgG antibody against C. trachomatis and tubal pathology, the infertile women with C. trachomatis antibodies were further divided into two groups (low and high level) according to their calculated antibody index. A high level of C. trachomatis IgG (antibody index >1.5) increased the risk of severe tubal pathology (OR: 6.88, 95% CI: 3.34–14.20, P = 0.001).

Effect of MICA on the susceptibility to C. trachomatis infection
The prevalence of MICA alleles were assessed and compared between infertile women with and without C. trachomatis IgG antibodies. The results from this cohort are shown in Table II. There were nine MICA alleles found in the two groups. Among them, the frequency of the MICA*008 allele was significantly higher in infertile patients with C. trachomatis IgG antibodies than those with C. trachomatis IgG antibodies (38.1 versus 22.3%, P = 0.0004, Pc = 0.00367, OR: 2.14, 95% CI: 1.4–3.28).

MICA in relation to tubal pathology
The comparison of the MICA allele was done between infertile women with and without tubal pathology. The allele distribution in infertile women with severe tubal pathology was similar to the distribution in women without tubal pathology (Table III). No statistical significant differences were found in the MICA allele frequencies for tubal pathology.

MICA polymorphisms in C. trachomatis antibody-positive women with and without tubal pathology
A significant association was clearly observed between high levels of antibodies against C. trachomatis and tubal pathology. However, no significant differences were found in the MICA allele frequencies for the presence or absence of tubal pathology in C. trachomatis antibody-positive women (Table IV).
**Discussion**

This was the first study to assess the role of MICA extracellular domain polymorphisms in tubal pathology and its association with *C. trachomatis* infections. The presence and high levels of IgG against *C. trachomatis* were more associated with severe tubal pathology compared with the absence of tubal pathology ($P = 0.004$, OR: 3.12, $P = 0.001$, OR: 6.88, respectively). *C. trachomatis* antibodies were therefore important risk indicators for tubal infertility in this study. This is in accordance with previous findings elucidating that tubal pathology is associated with *C. trachomatis* infections in infertile women (Murillo et al., 2003; den Hartog et al., 2004).

At the same time, our results showed that only 41.6% (42/101) of women with *C. trachomatis* IgG antibodies had developed severe tubal infertility. Moreover, clearance of *C. trachomatis* has been reported in some untreated women after follow-up (Morre et al., 2002), suggesting that, besides the factor of *C. trachomatis* infection, the host immunity affects the susceptibility to and severity of infection.

As an example, a specific immunological response to the Chlamydial Heat Shock Protein (CHSP60) as a subgroup of chlamydial serology positive patients has shown a correlation with tubal disease (Claman et al., 1997). Genetic factors play an important role in modulating the immune defence mechanisms and thereby determining the pathogenesis of chlamydial diseases. Genes involved in the immune response, for example, HLA, have been investigated. Kinnunen et al. (2002) found an association between cellular immune response with HLA class II alleles and patients with chlamydia-induced tubal pathology. HLA-DQA1*0102 and HLA-DQB1*0602 alleles are present significantly more frequently in the *C. trachomatis*-associated tubal factor subfertility cases than in controls.

On the basis of the present study, associations between MICA alleles and clinical features were suggested. Allele MICA*008 had a high negative correlation with *C. trachomatis* IgG antibodies ($P_c = 0.0036$, OR: 2.14). Among the known MICA alleles, allele MICA*008 is distinct from the other forms, in having an insertion of a guanine at position 952, resulting in the expression of an MICA...
protein with a truncated transmembrane region and no cytoplasmic tail (Gaudieri et al., 1997; Visser et al., 1999). Population genetics research results have shown that this mutation of MICA with shortened intracellular elements is the most common allele in most populations studied (Petersdorf et al., 1999; Zhang et al., 2001; Zhang et al., 2003). Previous research on the relationship between MICA polymorphisms and other micro-organisms may interpret possible underlying immune mechanisms of our findings. Zou et al. (2005) have found that, after HCMV infection in vitro, MICA*008 appeared to be stably expressed on the surface of cells, while proteins coded by other MICA alleles were down-regulated by virus. Moreover, NK cytotoxicity experiments have shown that this allele, MICA*008, which resists down-regulation, is functionally relevant and may aid in the elimination of HCMV-infected cells (Zou et al., 2005). Whether the same innate immune mechanisms against C. trachomatis have happened is not known. The associations between C. trachomatis infection and the MICA allele 008 warrant further investigation to elucidate the biological mechanism of action of the MICA protein and its role in the disease process.

Because antibody IgG against C. trachomatis was associated with tubal pathology and MICA*008 had a negative correlation with C. trachomatis IgG antibodies in our study, we hypothesized that MICA alleles might play an important role in the development of tubal pathology. However, in the infertile women of our study, we could not establish an association between MICA alleles and tubal pathology with or without C. trachomatis IgG antibodies. It seems that these specific polymorphisms are not associated with the inflammatory condition inherent in tubal pathology. Considering several limitations, such as the relatively small study cohort, existed in our study, further work need to be done.

In conclusion, our findings suggest possible specific MICA allele restriction of susceptibility to C. trachomatis tubal infertility. Further in vitro studies will provide more insights into the underlying immunopathological mechanisms associated with C. trachomatis tubal disease. In future studies, we would like to use C. trachomatis to infect cell lines with different homozygous MICA genotypes (including MICA*008, etc.). The discrepant effects of C. trachomatis on the expression of MICA encoded by different alleles will be analysed at mRNA and protein level. Susceptibility of these infected cell lines to lysis by NK cells will also be assessed in order to confirm the role of MICA in C. trachomatis infection.

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**References**


den Hartog JE, Land JA, Stassen FR, Slobbe-van Drunen ME, Kessels AG, Bruggeman CA. The role of Chlamydia genus-specific and
den Hartog JE, Ouburg S, Land JA, Lyons JM, Ito JI, Pena AS, Morre SA. Do host genetic traits in the bacterial sensing system play a role in the development of *Chlamydia trachomatis*-associated tubal pathology in subfertile women? *BMC Infect Dis* **2006;6**:122.
Murillo LS, Land JA, Pleijster J, Bruggeman CA, Pena AS, Morre SA. Interleukin-1B (IL-1B) and interleukin-1 receptor antagonist (IL-1RN) gene polymorphisms are not associated with tubal pathology and *Chlamydia trachomatis*-related tubal factor subfertility. *Hum Reprod* **2003;18**:2309–2314.
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