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TLR2 HAPLOTYPES IN THE SUSCEPTIBILITY TO AND SEVERITY OF *CHLAMYDIA TRACHOMATIS* INFECTIONS IN DUTCH WOMEN

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SUMMARY

Chlamydia trachomatis *infections may cause several disease conditions ranging from asymptomatic infections to severe upper genital tract pathology, thereby causing significant morbidity worldwide. Remarkable interindividual differences in the clinical course of* C. trachomatis *infection have been observed, and are mainly based on variation in genes encoding immune-regulatory and bacteria-sensing proteins. Toll-like receptors (TLRs) are closely involved in*

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pathogen recognition and host defense in C. trachomatis *infections. The aim of this study is to assess the role of* TLR2 *single nucleotide polymorphisms and haplotypes in the susceptibility to, and severity of* C. trachomatis *infections. The study comprised a sexually transmitted disease cohort of 468 Dutch Caucasian women and a control group of 321 women. The subfertility cohort consisted of 56 women with clinically well-defined tubal pathology. The results showed no significant differences in individual* TLR2 *genotype frequencies in the susceptibility for* C. trachomatis *infections between the* C. trachomatis*-positive group and controls. However, haplotype 1 was statistically significant (P = 0.015) and was associated with protection against tubal pathology following* C. trachomatis *infection. The same haplotype was also significantly decreased (P = 0.021) in increasing severity of* C. trachomatis *infections (asymptomatic > symptomatic > tubal pathology) suggesting a protective effect of this haplotype against the development of late complications.*

INTRODUCTION

C. trachomatis is the most important bacterial cause of sexually transmitted infections, and may cause considerable reproductive morbidity with the highest rates in adolescent women. Infection often remains asymptomatic and provides a huge reservoir for transmission of the disease, but identification of these cases by selective screening is difficult and associated costs have to be taken into account when screening programs are considered (1, 2). Repeated infections seem to be associated with severe upper genital tract pathology including pelvic inflammatory disease (PID), ectopic pregnancy and tubal infertility, in decreasing order of occurrence (3). Due to complicated early detection of *C. trachomatis* infection, leading to unrecognized PID, patients may remain untreated. This causes a deterioration in the reproductive health of our society, and entails significant expenditures for the treatment of this pathology.

The clinical course of *C. trachomatis* infection shows remarkable interindividual differences in transmission, symptomatic course, persistence or clearance of infection, and development of late complications. In general, the described differences in clinical course could be explained by the interaction between the host (host factors), pathogen (virulence factors), and environmental factors (such as coinfections). *Chlamydia* consists of different serovars (4, 5), but no specific serovars have been identified that clearly link to the course of infection. However, it is clear that bacterial factors are present that influence the course of infection (6).

Twin studies have advanced the efforts to identify susceptibility genes to infectious diseases. Comparison of concordance rates in monozygotic and dizygotic twins provides an estimate of the size of the genetic component of susceptibility, and for many infectious diseases this is substantial. Recently, Bailey et al. (7) have published the most relevant study in the field of *Chlamydia* immunogenetics. They estimated the relative contribution of host genetics to the total variation in lymphoproliferative responses to *C. trachomatis* antigen by analyzing these responses in 64 Gambian pairs of twins from trachoma-endemic areas. Proliferative responses to serovar A elementary body (EB) antigens were estimated in monozygotic and dizygotic twin pairs. They found a stronger correlation and lower within-pair variability in these responses in monozygotic compared with dizygotic twin pairs. The heritability estimate was 0.39, suggesting that host genetic factors contributed almost 40% of the variation.

As patient-related immunomodulating factors, Toll-like receptors (TLRs) are closely involved in pathogen recognition and host defense in infections, including *Chlamydia*. *Chlamydiae* express a variety of ligands that could serve as potential TLR ligands. TLR2 was previously described as the pattern recognition receptor (PRR) for the *C. trachomatis* component peptidoglycan (8, 9).

Functional single nucleotide polymorphisms (SNPs) in the *TLR2* gene have been described in relation to infection and inflammation (10-12), but no previous studies had been performed in relation to *C. trachomatis* infections. We previously showed that SNPs in the *TLR4* and *TLR9* genes increase the risk of tubal pathology in women with *C. trachomatis* infections (13), but have not yet investigated the role of *TLR2* SNPs in *C. trachomatis* pathogenesis. In knockout mouse studies, TLR2 plays an important role as mediator in the innate immune response to *C. trachomatis* infections as well as in the early production of inflammatory mediators and in the development of chronic inflammatory pathology (14).

We chose two SNPs (+2477, rs5743708 and –16934, rs4696480) that are related to infection. The –16934 polymorphism is associated with sepsis and Gram-positive bacteria (15). In addition, Veltkamp et al. showed a link between carriage of the A allele and increased production of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-12 (16). The +2477 polymorphism results in an amino acid substitution at position 753 (Arg753Gln),

which has been associated with tuberculosis (17) and has been associated with a reduced responsiveness to *Staphylococcus aureus* infections (18).

We aimed to assess the role of variation of these two SNPs in *C. trachomatis*-positive and *C. trachomatis*-negative individuals in general, and *C. trachomatis*-positive individuals with or without symptoms. We also aimed to analyze interindividual genetic differences in the severity of these infections, such as development of tubal pathology post-*C. trachomatis* infection.

MATERIALS AND METHODS

Patient populations

Sexually transmitted disease cohort

Women of Dutch Caucasian (DC) origin (n = 468), under the age of 33 years (range 14–33 years; median 22 years) and consecutively visiting the sexually transmitted disease (STD) outpatient clinic in Amsterdam, the Netherlands from July 2001 to December 2004, were included. *C. trachomatis*-positive cases (n = 147) were defined as immunoglobulin G (IgG) antibody-positive (Medac, Wedel, Germany) and *C. trachomatis* DNA-positive (Roche Diagnostics, Basel, Switzerland). Those without *C. trachomatis* infection, based on negative *C. trachomatis* DNA test and negative *C. trachomatis* serology responses, served as controls (n = 321).

One hundred and eighty-one women were symptomatic and 287 were asymptomatic. Of the symptomatic women 61 were positive and 120 were negative for other microorganisms (*Candida albicans, Neisseria gonorrhoeae, Trichomonas vaginalis* and herpes simplex virus 1 or *2)*.

One hundred and forty-seven women were *C. trachomatis*-positive. In this group 66 women had symptoms, whereas 81 were asymptomatic. Twenty-two women from the symptomatic group were positive and 44 were negative for microorganisms. Of the asymptomatic women 25 patients were positive and 56 were negative for microorganisms. The control group consisted of 321 women.

Participants were asked to sign an informed consent form and fill out a questionnaire regarding their complaints at that moment, varying from increased discharge, bloody discharge during and/or after coitus, recent abdominal pain (not gastrointestinal or menses related) and/or dysuria. A cervical swab was taken for the detection of *C. trachomatis* DNA by polymerase chain reaction (PCR) (19). Peripheral venous blood was collected for the analysis of IgG antibodies against *C. trachomatis* (Medac Diagnostika GmbH, Hamburg, Germany). A titer of ≥ 1:50 was considered positive. Samples with gray-zone values (e.g., cut-off 10% or more) were repeated and considered positive when the result was positive, or again within the gray zone. Infections with *C. albicans, N. gonorrhoeae, T. vaginalis* and herpes simplex virus 1 or *2* may result in symptoms similar to *C. trachomatis* infection. Therefore, an infection status was recorded for these microorganisms. Microorganism detection was performed according to methods described previously (20).

Subfertility cohort

This cohort consisted of Dutch Caucasian women (n = 56) with clinically well-defined tubal pathology visiting the Department of Obstetrics and Gynecology of the Academisch Ziekenhuis Maastricht, the Netherlands, because of subfertility. They were included in the period between December 1990 and November 2000 (21).

A laparoscopy with tubal testing was performed in these women as part of their fertility work-up. Preoperative blood was drawn from all patients for *Chlamydia* IgG antibody testing (CAT) and spare sera were cryopreserved. Two independent investigators, who were unaware of the CAT results, scored the laparoscopy reports to assess the grade of tubal pathology. Tubal pathology was defined as extensive periadnexal adhesions and/or distal occlusion of at least one tube at laparoscopy (22). Subfertile women who had no periadnexal adhesions and had patent tubes at laparoscopy served as negative controls. There were 43 (76.8%) patients with and 13 (23.2%) without tubal pathology. Of 39 *C. trachomatis*-positive women, 26 had tubal pathology. In the *C. trachomatis*-negative group, 17 women had tubal pathology.

IgG antibodies to *C. trachomatis* were detected with a species-specific microimmunofluoresence (MIF) test (AniLabSystems, Finland), as described previously by Land et al. (21), with comparable sensitivity and specificity compared with the IgG ELISA from Medac used for the STD cohort (23). A positive *C. trachomatis* IgG MIF test was defined as a titer ≥ :32.

Immunogenetic analyses

DNA extraction

STD cohort

Eukaryotic DNA from peripheral blood mononuclear cells (PBMCs) was isolated using the isopropanol isola-

tion method. In short, 100 µL PBMC in phosphate buffered saline (PBS) were added to 600 µL L6 (Nuclisens Lysisbuffer, Organon Teknika, Boxtel, the Netherlands) and 1 µL glycogen (Roche Molecular Diagnostics, Almere, the Netherlands). The samples were incubated for 30 min at 65 °C and left to cool at room temperature. An equal volume of cold (–20 °C) isopropanol was added to the samples. The samples were then centrifuged (20 min at 20,000 G). The supernatant was discarded and the pellets were washed twice in 75% ethanol. The pellets were dissolved in T10 overnight at 4 °C and then stored at –20 °C until further analysis.

Subfertility cohort

Genomic DNA was extracted from the cryopreserved sera using High Pure PCR Template Preparation Kit (HPPTP kit, Roche Molecular Biochemicals, Mannheim, Germany).

Gene polymorphisms

TLR2 +2477 G>A Arg753Gln (rs5743708) and *TLR2* –16934 T>A (rs4696480) genotyping was performed with the TaqMan method. The primers and probes are described in Table I.

Statistical analyses

All groups were tested for Hardy-Weinberg equilibrium to check for Mendelian inheritance. Statistical analyses were performed and the Fisher's exact and chi-square tests were used to test for differences in *TLR2* genotype/carrier frequencies between the (sub)groups. P-values < 0.05 were considered statistically significant. *TLR2* haplotypes were inferred using PHASE v2.1.1 and SNPHAP (24-26).

Table I. *Primers and probes of TLR2.*

TLR2 +2477	
Forward primer	CATTCCCCAGCGCTTCTG
Reverse primer	TCCAGGTAGGTCTTGGTGTTCAT
Probe for allele A	AAGCTGCAGAAGAT-MGB
Probe for allele G	AAGCTGCGGAAGAT-MGB
TLR2-16934	
Forward primer	TGGTTCTGGAGTCTGGGAAGTC
Reverse primer	CTCACCATGTGATGCTTTCCAT
Probe for allele T	TCTGGTGAGGGTCAT-MGB
Probe for allele A	ATCTGGAGAGGGTCAT-MGB

RESULTS

Susceptibility to *C. trachomatis* infection

The overall distributions of the *TLR2* SNPs *(TLR2*-16934 T>A and *TLR2* +2477 G>A) are shown in Table II for the genotypes and in Table III for the haplotypes. Both on the genotype and the haplotype level, the two analyzed SNPs showed no statistical significance for susceptibility to *C. trachomatis* infections. Subsequently, we analyzed the effect of coinfections with other microorganisms and symptomatology and found no effect in obtained results. Thus, there is no evidence that these SNPs predispose the development of *C. trachomatis* infection or coinfection with other microorganisms in established *C. trachomatis* infection.

Severity of *C. trachomatis* infection

The overall distributions of the *TLR2* SNPs (*TLR2* –16934 T>A and *TLR2* +2477 G>A) are shown in Table IV

Table II. *Susceptibility analyses: Genotypes of the* TLR2 *gene polymorphisms in subgroups of patients with C. trachomatis infection with and without symptoms and controls.*

TLR2		–16934 T>A						+2477 G>A					
	n	TT	%	TA	%	AA	%	GG	%	GA	%	AA	%
Ct+	147	43	29.3	62	42.2	42	28.6	136	92.5	7	4.8	4	2.7
Ct+ asym	81	28	34.6	31	38.3	22	27.2	78	96.3	0	0.0	3	3.7
Ct+ sym	66	15	22.7	31	47.0	20	30.3	58	87.9	7	20.9	1	1.5
Ct– (controls)	321	89	27.7	152	47.4	80	24.9	287	89.4	30	9.4	4	1.3

Ct, *C. trachomatis;* Sym+, symptom positive; Ct+, Ct DNA and IgG positive; Ct–, Ct DNA and IgG negative.

Table III. *Susceptibility analyses: Frequencies of TLR2 haplotypes formed by –16934 T >A and +2477 G >A SNPs, in the susceptibility to* Chlamydia *infections.*

Haplotypes		I		II		III		IV	
	n	TG	%	AG	%	TA	%	AA	%
Ct+	294	135	45.9	144	49.0	13	4.4	2	0.7
Ct+ asym	162	82	50.6	74	45.7	5	3.1	1	0.6
Ct+ sym	132	53	40.2	70	53.0	8	6.1	1	0.8
Ct– (controls)	642	296	46.1	308	48.0	34	5.3	4	0.6

Ct, *C. trachomatis;* Sym+, symptom positive; Ct+, Ct DNA and IgG positive; Ct–, Ct DNA and IgG negative.

Table IV. *Severity analyses: Genotypes of the* TLR2 *gene polymorphisms in subgroups of patients with* C. trachomatis *infection with or without tubal pathology (TP).*

TLR2		–169394 T>A						+2477 G>A					
	n	TT	%	TA	%	AA	%	GG	%	GA	%	AA	%
Ct–TP+	17	6	35.3	8	47.1	3	17.7	16	94.1	1	5.9	0	0.0
Ct+TP–	13	6	46.2	5	38.5	2	15.4	13	100.0	0	0.0	0	0.0
Ct+TP+	26	7	26.9	9	34.6	10	38.5	21	80.8	5	19.2	0	0.0

Ct, *C. trachomatis;* Ct+, Ct DNA and IgG positive; Ct–, Ct DNA and IgG negative.

Table V. *Severity analyses: Frequencies of TLR2 haplotypes formed by +2477 G>A and –16934 T>A single nucleotide polymorphisms.*

TLR2		I		II		III		IV	
	n	TG	%	AG	%	TA	%	AA	%
Ct–TP+	34	19	55.9	14	41.2	1	3.0	0	0.0
Ct+TP–	26	17	65.4	9	34.6	0	0.0	0	0.0
Ct+TP+	52	18	34.6	29	55.8	5	9.6	0	0.0

Ct, *C. trachomatis;* Ct+, Ct DNA and IgG positive; Ct–, Ct DNA and IgG negative.

for the genotypes and in Table V for the haplotypes. The individually analyzed SNPs showed no statistically significant differences among the different groups in the genotype distribution. However, haplotype 1 was statistically significant (P = 0.015; OR: 0.28; 95% CI: 0.10–0.75) (Fig. 1) and was associated with protection against tubal pathology following *C. trachomatis* infection. The same haplotype was also significantly decreased in increasing severity of *C. trachomatis* infections (asymptomatic > symptomatic > tubal pathology; Fig. 2), suggesting a protective effect of this haplotype against the development of late complications.

DISCUSSION

This study showed no statistically significant differences in *TLR2* genotype distribution in either the susceptibility to or severity of *Chlamydia* infections. However, when the polymorphisms were combined into haplotypes, significant associations were observed, showing a protective effect of *TLR2* polymorphisms against the development of complications after *C. trachomatis* infection.

Haplotype I results in a decreased severity of *C. trachomatis* infection. This haplotype contains the wild-type alleles of both studied polymorphisms. The mutant (A)

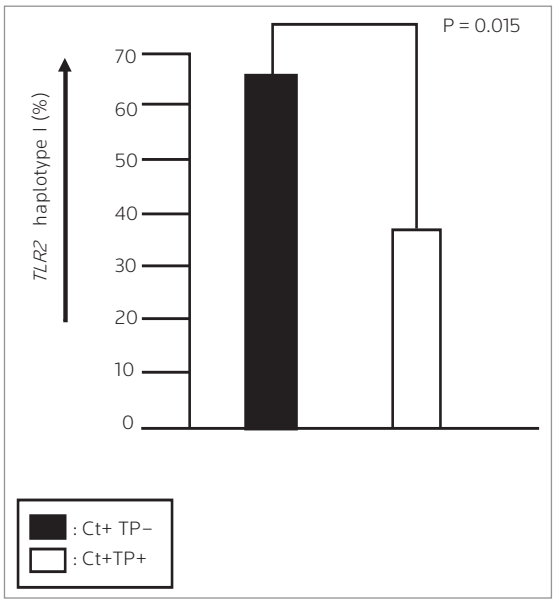

Figure 1. Haplotype I distribution in *C. trachomatis*-positive (Ct+) women with and without tubal pathology (TP+, TP–, respectively) (P = 0.015; OR: 0.28; 95% CI: 0.10–0.75).

allele of the –16934 polymorphism has been linked to increased production of TNF-α, IL-6 and IL-12 (16), although a reduction of IL-6 production has been reported in atopic dermatitis patients (27). Heterozygous carriage of the +2477 polymorphism has been reported to increase TNF-α production, but also to result in reduced IL-8 and IFN-γ production (28, 29).

Bochud et al. observed increased shedding and lesions in genital herpes infections in carriers of two extended *TLR2* haplotypes containing the wild-type alleles of –16934 and +2477 (30). However, other haplotypes containing both wild-type alleles do not show significant differences. It is more likely that the observed effect is caused by other functional polymorphisms within these haplotypes. This is corroborated by other results reported by these authors (30).

TLR2-deficient mice were found to have significantly lower levels of inflammatory mediators in genital tract secretions during the first week of infection, and there was a significant reduction in oviduct and mesosalpinx pathology at late time-points (14). This suggested that TLR2 was the predominant receptor involved in the detection of, and inflammatory response to *C. trachomatis* in the genital tract. Furthermore, TLR2 was found to be tightly associated with the bacteria during the intracellular phase, and that it was recruited to the inclusion membrane together with MyD88. Intracellular TLR2 was suggested to be responsible for the initiation of signal transduction events during infection with *C. trachomatis* (31).

From the published results and the data from this study, one might hypothesize that the increased production of proinflammatory cytokines observed in carriers of the mutant alleles results in a stronger immune response against *C. trachomatis*, which may then result in collateral damage and thus late complications. This is corroborated by the reduced rate of complications in *TLR2*-deficient mice. A similar effect was observed in a previous study where we showed that *CCR5* deficiency results in a delayed clearance of *C. trachomatis* but also in reduced pathology (32).

It is clear that genetic variation in the host immune system can have an impact on the susceptibility to and severity of *C. trachomatis* infections. Combined carriage of polymorphisms may have a more profound impact of *C. trachomatis* pathogenesis as we have shown previously (13).

CONCLUSION

Thus, an adequate recognition of *C. trachomatis* by receptors in the female genital tract is a crucial step in the immune response and may play an important role in the protection of the host against the development of

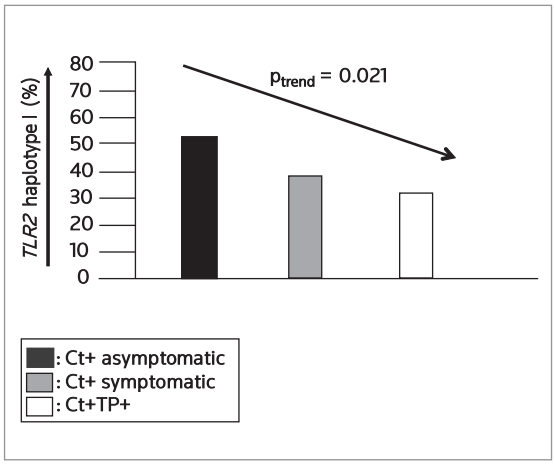

Figure 2. Haplotype I distribution in *C. trachomatis*-positive (Ct+) asymptomatic women, Ct+ symptomatic women, and Ct+ women with positive tubal pathology (TP+)(P_{trend} = 0.021).

the late complications following *C. trachomatis* infections. To confirm our preliminary data, more studies are needed in larger cohorts. Moreover, with regard to earlier studies, we believe that besides large cohort studies, analysis of carriage of multiple SNPs in the *C. trachomatis* infection, related *TLR* pathways may reveal a significant impact on the susceptibility to and severity of *Chlamydia* infections.

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DISCLOSURE

The authors have nothing to disclose.

REFERENCES

1. van Valkengoed, I.G.M., Morre, S.A., van den Brule, A.J.C. et al. *Low diagnostic accuracy of selective screening criteria for asymptomatic Chlamydia trachomatis infections in the general population*. Sex Trans Dis 2000, 76: 375-80.
2. Morre, S.A., Meijer, C.J., Munk, C. et al. *Pooling of urine specimens for detection of asymptomatic Chlamydia trachomatis infections by PCR in a low-prevalence population: Cost-saving strategy for epidemiological studies and screening programs*. J Clin Microbiol 2000, 38: 1679-80.
3. Hillis, S.D., Owens, L.M., Marchbanks, P.A. et al. *Recurrent chlamydial infections increase the risks of hospitalization for ectopic pregnancy and pelvic inflammatory disease*. Am J Obstet Gynecol 1997, 176: 103-7.
4. Morre, S.A., Moes, R., Van Valkengoed, I. et al. *Genotyping of Chlamydia trachomatis in urine specimens will facilitate large epidemiological studies*. J Clin Microbiol 1998, 36: 3077-8.
5. Molano, M., Meijer, C.J., Morre, S.A. et al. *Combination of PCR targeting the VD2 of omp1 and reverse line blot analysis for typing of urogenital Chlamydia trachomatis serovars in cervical scrape specimens*. J Clin Microbiol 2004, 42: 2935-9.
6. Lyons, J.M., Ito, J.I., Jr., Pena, A.S. et al. *Differences in growth characteristics and elementary body associated cytotoxicity between Chlamydia trachomatis oculogenital serovars D and H and Chlamydia muridarum.* J Clin Pathol 2005, 58: 397-401.
7. Bailey, R.L., Natividad-Sancho, A., Fowler, A. et al. *Host genetic contribution to the cellular immune response to Chlamydia trachomatis: Heritability estimate from a Gambian twin study*. Drugs Today (Barc) 2009, 45(Suppl. B): 45-50.
8. Schwandner, R., Dziarski, R., Wesche, H. et al. *Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by Toll-like receptor 2*. J Biol Chem 1999, 274: 17406-9.
9. Yoshimura, A., Lien, E., Ingalls, R.R. et al. *Cutting edge: Recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2*. J Immunol 1999, 163: 1-5.
10. Lorenz, E., Mira, J.P., Cornish, K.L. et al. *A novel polymorphism in the Toll-like receptor 2 gene and its potential association with staphylococcal infection*. Infect Immun 2000, 68: 6398-401.
11. Sutherland, A.M., Walley, K.R., Russell, J.A. *Polymorphisms in CD14, mannose-binding lectin, and Toll-like receptor-2 are associated with increased prevalence of infection in critically ill adults.* Crit Care Med 2005, 33: 638-44.
12. Yim, J.J., Lee, H.W., Lee, H.S. et al. *The association between microsatellite polymorphisms in intron II of the human Toll-like receptor 2 gene and tuberculosis among Koreans.* Genes Immun 2006, 7: 150-5.
13. den Hartog, J.E., Ouburg, S., Land, J.A. et al. *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.
14. Darville, T., O'Neill, J.M., Andrews, C.W., Jr. et al. *Toll-like receptor-2, but not Toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection*. J Immunol 2003, 171: 6187-97.
15. Sutherland, A.M., Walley, K.R., Russell, J.A. *Polymorphisms in CD14, mannose-binding lectin, and Toll-like receptor-2 are associated with increased prevalence of infection in critically ill adults*. Crit Care Med 2005, 33(3): 638-44.
16. Veltkamp, M., Wijnen, P.A., van Moorsel, C.H. et al. *Linkage between Toll-like receptor (TLR) 2 promotor and intron polymorphisms: Functional effects and relevance to sarcoidosis.* Clin Exp Immunol 2007, 149(3): 453-62.
17. Ogus, A.C., Yoldas, B., Ozdemir, T. et al. *The Arg753GLn polymorphism of the human Toll-like receptor 2 gene in tuberculosis disease*. Eur Respir J 2004, 23(2): 219-23.
18. Lorenz, E., Mira, J.P., Cornish, K.L., Arbour, N.C., Schwartz, D.A. *A novel polymorphism in the Toll-like receptor 2 gene and its potential association with staphylococcal infection*. Infect Immun 2000, 68(11): 6398-401.
19. van Doornum, G.J., Schouls, L.M., Pijl, A. et al. *Comparison between the LCx Probe system and the COBAS AMPLICOR system for detection of Chlamydia trachomatis and Neisseria gonorrhoeae infections in patients attending a clinic for treatment of sexually transmitted diseases in Amsterdam, The Netherlands*. J Clin Microbiol 2001, 39: 829-35.

20. Ouburg, S., Spaargaren, J., den Hartog, J.E. et al. *The CD14 functional gene polymorphism -260 C>T is not involved in either the susceptibility to Chlamydia trachomatis infection or the development of tubal pathology.* BMC Infect Dis 2005, 5: 114.
21. Land, J.A., Gijsen, A.P., Kessels, A.G. et al. *Performance of five serological chlamydia antibody tests in subfertile women.* Hum Reprod 2003, 18: 2621-7.
22. Land, J.A., Evers, J.L., Goossens, V.J. *How to use Chlamydia antibody testing in subfertility patients*. Hum Reprod 1998, 13: 1094-8.
23. van Valkengoed, I.G., Morre, S.A., van den Brule, A.J. et al. *Follow-up, treatment, and reinfection rates among asymptomatic chlamydia trachomatis cases in general practice.* Br J Gen Pract 2002, 52: 623-7.
24. Stephens, M., Smith, N.J., Donnelly, P. *A new statistical method for haplotype reconstruction from population data*. Am J Hum Genet 2001, 68: 978-89.
25. Clayton, D.G. SNPHAP 2002. http://www-genecimrca-macuk/clayton/software/2002.
26. Stephens, M., Donnelly, P. *A comparison of bayesian methods for haplotype reconstruction from population genotype data*. Am J Hum Genet 2003, 73: 1162-9.
27. Oh, D.Y., Schumann, R.R., Hamann, L., Neumann, K., Worm, M., Heine, G. *Association of the Toll-like receptor 2 A-16934T promoter polymorphism with severe atopic dermatitis.* Allergy 2009, 64(11): 1608-15.
28. Woehrle, T., Du, W., Goetz, A. et al. *Pathogen specific cytokine release reveals an effect of TLR2 Arg753Gln during Candida sepsis in humans*. Cytokine 2008, 41(3): 322-9.
29. Mrabet-Dahbi, S., Dalpke, A.H., Niebuhr, M. et al. *The Toll-like receptor 2 R753Q mutation modifies cytokine production and Toll-like receptor expression in atopic dermatitis*. J Allergy Clin Immunol 2008, 121(4): 1013-9.
30. Bochud, P.Y., Magaret, A.S., Koelle, D.M., Aderem, A., Wald, A. *Polymorphisms in TLR2 are associated with increased viral shedding and lesional rate in patients with genital herpes simplex virus type 2 infection*. J Infect Dis 2007, 196(4): 505-9.
31. O'Connell, C.M., Ionova, I.A., Quayle, A.J. et al. *Localization of TLR2 and MyD88 to Chlamydia trachomatis inclusions. Evidence for signaling by intracellular TLR2 during infection with an obligate intracellular pathogen.* J Biol Chem 2006, 281: 1652-9.
32. Barr, E.L., Ouburg, S., Igietseme, J. et al. *Host inflammatory response and development of complications of Chlamydia trachomatis genital infection in CCR5-deficient mice and subfertile women with the CCR5delta32 gene deletion*. J Microbiol Immunol Infect 2005, 38(4): 244-54.