Single nucleotide polymorphisms in immune response genes in acute Q fever cases with differences in self-reported symptoms

C. C. H. Wielders · V. H. Hackert · B. Schimmer · H. M. Hodemaekers · A. de Klerk · C. J. P. A. Hoebe · P. M. Schneeberger · Y. T. H. P. van Duynhoven · R. Janssen

Abstract Genes involved in human immune response are well recognized to influence the clinical course of infection. The association of host genetics with susceptibility to and severity of clinical symptoms in acute Q fever was investigated. Single nucleotide polymorphisms (SNPs) in the IFNG (rs2430561/rs1861493), STAT1 (rs1914408), and VDR (rs2228570) genes were determined in 85 patients from the 2007 Dutch acute Q fever outbreak, and a symptom score was calculated. IFNG rs1861493 showed a significant association with the symptom score; IFNG rs2430561 showed a similar trend. These SNPs were then used to reproduce results in a 2009 outbreak population (n=123). The median symptom score differed significantly in both populations: 2 versus 7.

The significant association of IFNG rs1861493 with symptom score in the first population was not reproduced in the second population. We hypothesize that individuals in the second outbreak were exposed to a higher Coxiella burnetii dose compared to the first, which overruled the protection conferred by the A-allele of IFNG rs1861493 in the first population.

Introduction

From 2007 through 2010, the Netherlands faced the largest Q fever outbreak recorded to date (>4,000 notified cases) [1, 2]. The causative agent of Q fever is the intracellular bacterium Coxiella burnetii. C. burnetii-infected individuals can remain asymptomatic or develop a flu-like illness, pneumonia, or hepatitis, known as acute Q fever [3]. Approximately 2% of symptomatic acute Q fever cases progress to chronic Q fever [4].

Genes involved in human immune response, and also other genes, are well recognized to influence the clinical course of infection, especially in case of pathogens with cell dependency similar to that of C. burnetii. Significant immunogenetic differences have been found comparing patients suffering from chronic sequelae due to Q fever [Q fever endocarditis or prolonged post-infection fatigue (i.e., Q fever fatigue syndrome)] to patients who had an uncomplicated recovery from acute Q fever or subjects from the general population [5, 6]. Polymorphic variations in individual “candidate genes” [single nucleotide polymorphisms (SNPs)] assumed to be of direct importance in pathogenesis may help clarify the relation between immune response genes and varying degrees of disease severity or susceptibility of subpopulations [7–12].
The aim of this study was to assess whether SNPs in several immune response genes influence susceptibility to and severity of an acute C. burnetii infection, based on self-reported symptoms. We also compared the allelic frequencies observed in these individuals to frequencies in the general population. Four SNPs in genes involved in innate and adaptive immunity were selected to study this association.

Materials and methods

Study design

Study population 1

The first notified cases in the Dutch Q fever outbreak (2007–2010) were residents of a village in the southern parts of the Netherlands (Herpen), where a case–control study was performed for source identification and risk factor analysis in 2007 [13]. This outbreak was likely caused by abortions on a single dairy goat farm in close vicinity to the village. Acute Q fever cases were selected from participants of this study [at least IgM phase II and/or IgG phase II antibodies against C. burnetii antigens ≥1:64 (indirect immunofluorescence assay, IFA; Focus Diagnostics, Cypress, CA, USA)], based on available questionnaire data (self-reported symptoms), including no more than one person per household, and giving consent for the investigation of genetic differences. This study, including our analysis, was approved by the Medical Ethical Committee of the University Medical Center Utrecht (reference number: 07-241).

Study population 2

An abortion wave on a dairy goat farm led to another single-point source outbreak in the southernmost part of the Netherlands in 2009 (Voerendaal). Farm residents, employees, and visitors were serologically screened for C. burnetii and assessed for self-reported symptoms by means of a questionnaire almost identical to the one used in study population 1 and administered immediately following laboratory notification [14]. Laboratory-confirmed acute Q fever cases were defined as: a positive C. burnetii DNA polymerase chain reaction (PCR) test and/or the presence of IgM phase II and/or IgG phase II antibodies [screening by enzyme-linked immunosorbent assay (ELISA; Serion ELISA classic, Institut Virion/Serion GmbH, Würzburg, Germany) and confirmation by IFA ≥1:32 (Fuller Laboratories, Fullerton, CA, USA)] [14]. In addition, laboratory-confirmed community cases of acute Q fever subsequently notified to the regional Public Health Service between March 2009 and April 2010 received a questionnaire with questions on age, sex, and specific symptoms within days following the laboratory diagnosis. In March 2012, all farm and community cases who had earlier returned the questionnaire were selected for participation in our study, following approval by the Medical Ethical Committee of the Maastricht University Medical Centre (reference number: 10-4-034).

SNP selection

Four SNPs in three candidate genes involved in innate and adaptive immunity to infection were selected based on previously published associations with various diseases: two SNPs in the IFNG gene [interferon-γ (IFNγ); rs1861493 and rs2430561], one in STAT1 (signal transducer and activator of transcription 1; rs1914408) involved in the IFNγ-mediated signal transduction, and one in VDR (vitamin D receptor; rs2228570).

The defense against intracellular C. burnetii mainly depends on cell-mediated immunity, including IFNγ-mediated macrophage activation [15–17]. The IFNγ pathway is believed to be crucial for the host defense against this intracellular pathogen [16, 18], as well as other intracellular bacteria, like Mycobacterium spp. [10, 19–21], but has also been associated with outcomes of, for example, severe acute respiratory syndrome (SARS) [11], respiratory syncytial virus infection [22, 23], and gastroenteritis episodes [24]. Consequently, it is reasonable to assume that polymorphisms in the IFNG gene and that of its receptor will modulate the IFNγ host pathogen (C. burnetii) interaction process [6]. A previous study by Vollmer-Conna et al. showed a significant influence of functional polymorphisms in the IFNG loci (rs2430561) on the severity and duration of illness after infection with several pathogens, including C. burnetii [12]. Vitamin D stimulates the innate immune response, but suppresses the adaptive immune response and is important in immunity to tuberculosis, diabetes, and respiratory syncytial virus [25–27].

The association of these SNPs with other diseases increases the chance of selecting SNPs that are actually of functional importance. Allelic frequencies of the four selected SNPs were investigated in study population 1. Only those SNPs that showed a statistically significant association or a trend in association with disease severity were used for reproduction in population 2. The allelic frequencies for a Dutch community control group were also available (“Regenboog” study, a large Dutch population health examination survey in 1998 including randomly selected individuals of all ages [28]).

Sample acquisition, DNA isolation, and genotyping

Blood samples from population 1 were collected and analyzed late 2007 and early 2008 for those patients that gave permission for the genetic analysis. Subjects from population 2, selected for participation based on a returned questionnaire, received a self-administrable buccal swab kit accompanied by
an information folder and an informed consent form in March 2012.

For both populations, DNA was isolated as described by Hoebee et al. [29] using the QIAamp DNA Blood Mini Kit (Qiagen NV, Venlo, the Netherlands). Polymorphisms were genotyped using predesigned or custom TaqMan SNP genotyping assays (Life Technologies, Bleiswijk, the Netherlands). For each sample, 2.5 μL of TaqMan Fast Universal PCR Master Mix (Life Technologies) and 10 ng of genomic DNA were used in a total volume of 5 μL. Primer and probe sequences and assay numbers are as described by Doorduyn et al. [24]. The protocol for amplification was 20 s at 95 °C and 40 cycles of 3 s at 95 °C and 30 s at 60 °C. All genotyping assays were performed on a 7500 Fast Real-Time PCR System (Life Technologies).

Questionnaire

Both questionnaires included demographic information and the following 14 Q fever-related symptoms: fever (>38 °C), malaise, headache, cough, severe fatigue, shortness of breath or respiratory difficulties, pain or pressure on the chest, diarrhea, joint pain, night sweating, loss of weight, itch, clinical diagnosis of jaundice/hepatitis, and clinical diagnosis of pneumonia. Hospitalization at the time of the acute C. burnetii infection was also recorded.

Statistical analysis

Participants with missing self-reported symptom data were excluded. For each participant, a symptom score was calculated as the sum of all reported symptoms (range 0–14). The median score and corresponding interquartile range (IQR) were calculated for both study populations, and the Mann–Whitney U-test was used to assess statistical significance for the difference in the median score between both populations. Multiple regression analysis was used to confirm independence of the symptom score from age and gender.

The genotype data of all tested SNPs were used to estimate Hardy–Weinberg equilibrium by the comparison of genotype frequencies within population 1, population 2, and the community controls by a Chi-square test [30]. To study the association of the determined SNPs with the symptom score, allele frequencies in subjects who indicated no symptoms at all were compared with allele frequencies in subjects who reported at least one symptom (“symptomatic”) by using cross-tabulations and Chi-square tests. Odds ratios (ORs) and 95 % confidence intervals (95 % CIs) were calculated as well. The same type of comparisons were performed for subjects with a symptom score up to and including the median number of symptoms (“symptomatic”) compared with subjects with a symptom score higher than the median score (“severe symptomatic”). Allele frequencies of the “symptomatic” and “severe symptomatic” groups were also compared with the allele frequencies of a Dutch community control group. The distribution of the symptom score for the different genotypes was assessed as well. Data were analyzed using IBM SPSS Statistics version 19.0.0 (SPSS Inc.).

Results

General

All tested polymorphisms were in Hardy–Weinberg equilibrium (p>0.05) in all three groups (population 1, population 2, and community controls). There was no substantial relationship between symptom score and the demographic factors age and sex.

Study population 1

Study population 1 consisted of 85 acute Q fever cases after excluding three subjects with missing symptom data. The median age of the patients was 49 years (IQR: 41–58) and 54 (64 %) were male. The self-reported symptom frequency

| Symptoms                        | Study population 1 (n=85) | Study population 2 (n=123) |
|--------------------------------|--------------------------|---------------------------|
|                                | Total Yes Percentage (%)| Total Yes Percentage (%)  |
| Fever (≥38 °C)                  | 84 23 27                 | 122 98 80                 |
| General malaise                 | 83 35 42                 | 121 104 86               |
| Headache                       | 82 33 40                 | 121 86 71                |
| Cough                          | 84 31 37                 | 123 72 59                |
| Severe fatigue                 | 85 35 41                 | 120 83 69                |
| Shortness of breath of respiratory difficulties | 84 20 24 | 120 43 36 |
| Pain of pressure on the chest  | 84 12 14                 | 122 41 34                |
| Diarrhea                       | 84 19 23                 | 122 42 34                |
| Joint pain                     | 84 23 27                 | 121 63 52                |
| Night sweating                 | 84 28 33                 | 122 80 66                |
| Loss of weight                 | 84 9 11                  | 122 39 32                |
| Itch                           | 83 9 11                  | 120 27 23                |
| Jaundice/hepatita<sup>a</sup>  | 83 1 1                   | 121 4 3                  |
| Pneumonia<sup>a</sup>          | 84 7 8                   | 120 30 25                |
| Hospitalization                | 85 2 2                   | 120 25 21                |

<sup>a</sup> As clinical diagnosis

---

**Table 1**  Frequencies of reported symptoms and hospitalization of study populations 1 and 2

---

---
ranged between 1% for hepatitis/jaundice and 42% for malaise, and 2% of the cases had been hospitalized (Table 1).

The median symptom score was 2 (IQR: 0–6). Table 2 presents the allele distribution of each SNP by using the two different classifications of “symptomatic” (at least one symptom and “severe symptomatic”, i.e., a symptom score above the median score). The Dutch community control group consisted of 1,008 persons, of which 55% was male. A statistically significant difference was observed for both IFNG SNPs when subjects who did not report any symptoms were compared to subjects who reported at least one symptom (Table 2) (IFNG rs1861493: OR: 3.53; 95% CI: 1.39–8.99; p=0.006; IFNG rs2430561: OR: 2.13; 95% CI: 1.09–4.19; p=0.027). A similar result was found for one of the IFNG SNPs (rs1861493) when using the median symptom score as the cut-off (the A-allele was less frequently observed among the participants with the above median symptom score), while the other IFNG SNP (rs2430561) showed a trend in association in the same direction (the T-allele was less frequently observed among the participants with the above median symptom score), though it was no longer statistically significant.

The GG genotype (rs1861493) and AA genotype (rs2430561) seemed to increase the symptom score (Fig.1), though the combination of both genotypes was not significantly associated with the two symptom classifications used. The other SNPs did not show an association with the symptom score.

Table 2: Allele frequencies of four different single nucleotide polymorphisms (SNPs) in study population 1 (n=85; n=170 alleles per SNP) by using two different classifications of the symptom score and a Dutch population control group (n=1,008; n=2,016 alleles per SNP)

| Allele | Symptom score=0, n (%) | Symptom score≥1, n (%) | p-Value | OR (95% CI) | Dutch community controls, n (%) | p-Value | OR (95% CI) |
|--------|------------------------|------------------------|----------|--------------|---------------------------------|----------|--------------|
| IFNG (rs1861493) | A 44 (88) | 81 (68) | 0.006 | Ref | 1,503 (75) | 0.086 | Ref |
| | G 6 (12) | 39 (33) | 3.53 (1.39–8.99) | 513 (25) | 1.41 (0.95–2.09) |
| IFNG (rs2430561) | T 31 (62) | 52 (43) | 0.027 | Ref | 952 (47) | 0.396 | Ref |
| | A 19 (38) | 68 (57) | 2.13 (1.09–4.19) | 1,060 (53) | 1.17 (0.81–1.70) |
| STAT1 | G 36 (72) | 90 (75) | 0.684 | Ref | 1,479 (73) | 0.706 | Ref |
| | A 14 (28) | 30 (25) | 0.86 (0.41–1.80) | 535 (27) | 0.92 (0.60–1.41) |
| VDR | G 27 (54) | 78 (65) | 0.179 | Ref | 1,265 (63) | 0.629 | Ref |
| | A 23 (46) | 42 (35) | 0.63 (0.32–1.24) | 749 (37) | 0.91 (0.62–1.34) |

95% CI 95% confidence interval; OR odds ratio; Ref reference
a Chi-square test
b Symptom score ≥1 vs. Dutch community controls
c 2 subjects/4 alleles missing
d 1 subject/2 alleles missing
e Based on the median symptom score (2) in the study population
f Symptom score ≥3 vs. Dutch community controls
Therefore, the two IFNG SNPs were selected as the most likely candidates for reproduction of our results in study population 2.

Compared with the general Dutch population, the A-allele of the IFNG rs1861493 SNP was more often present in participants with a low symptom score and less often in participants with a high symptom score; a borderline significant result was found for participants with a higher symptom score compared with the community controls (OR: 1.55; 95% CI: 0.96–2.50; \( p = 0.069 \)).

Study population 2

From study population 2, 192 individuals were invited, of whom 129 (67%) submitted a buccal swab sample. Three participants were excluded because only one member per household could be included and another three because of missing symptom data. In the remaining 123 cases, the median age was 48 years (IQR: 40–60) and 70 (57%) were male; the self-reported symptom frequency ranged between 3% for hepatitis/jaundice and 86% for malaise; hospitalization was reported in 21% of the cases (Table 1).

The median symptom score was significantly higher than in population 1: 7 (IQR: 5–8; \( p < 0.001 \)). Non-responders from population 2 did not differ from responders according to age, gender, and median symptom score (data not shown). Figure 2 shows the distribution of the symptom scores in both study populations. As there were only four patients who reported no symptoms in this second population, only the “severe symptomatic” classification with above median symptom score could be used for analysis (Table 3). No statistically significant association between SNP allele frequency and severity of symptoms was observed in this population. There is a less clear distinction between the genotypes and symptom score compared to population 1 (Fig. 1), i.e., all genotypes show moderate to high symptom scores.

We also investigated whether fever in combination with other symptoms, hospitalization, pneumonia, or hepatitis was associated with the allele frequencies of the SNPs, but no clear associations were found.

Discussion

This study investigated the association of four SNPs in several immune response genes with susceptibility to and severity of self-reported symptoms in acute \( C. \ burnetii \) infection. Both IFNG SNPs seemed to be related to the symptom score in population 1. This association remained significant for the IFNG rs1861493 SNP irrespective of the cut-offs for symptom severity chosen (protection conferred by the A-allele, G-allele is the risk allele), while a trend in association was found for the IFNG rs2430561 SNP (protection conferred by the T-allele, A-allele is the risk allele). The other SNPs did not show an association with the symptom score. The associations found in the IFNG SNPs in population 1, however, could not be reproduced in population 2.

The A-allele of the IFNG rs2430561 SNP is associated with low IFN\( \gamma \) production [12, 31]. This A-allele has been reported to significantly increase the susceptibility to develop tuberculosis [10, 19–21] and SARS [11]. A dose-dependent association was found for this A-allele with susceptibility to SARS [11]. Pacheco et al. suggested that the increased levels of IFN\( \gamma \) in the early events during infection could probably control the replication and spread of \( M. \ tuberculosis \) or other intracellular pathogens [20]. With respect to disease severity, however, significant associations of the T-allele with a more severe acute sickness response to infection have been reported previously [12, 32]. This suggests that high or low levels of IFN\( \gamma \) can have different effects on disease, depending on the outcome under investigation.

Fig. 1  Box plots of the IFNG genotype and symptom score in study populations 1 and 2: a IFNG rs1861493, b IFNG rs2430561. The numbers above the bars indicate the number of cases, the horizontal lines within the boxes represent the median symptom score, the lower and upper boundaries of the boxes represent the 25th and 75th percentiles, respectively, and the T-bars represent the 2.5th and 97.5th percentiles. Outliers are indicated by the dots
There are several explanations for our observation that findings from population 1 could not be reproduced in population 2. The first observation is that, surprisingly, symptom severity appeared to be much higher in population 2 compared to population 1, based on symptom scores and hospital admission rates (21 vs. 2%). A possible explanation could be recall bias in study population 1, as participants were asked in September 2007 about their symptoms between 7 May 2007 and 8 July 2007. Nevertheless, the difference in symptom scores seems too large to be solely explained by recall bias in population 1. Furthermore, self-reported hospital admission rates seem highly unlikely to be affected by recall bias.

Higher symptom severity in population 2 could possibly be explained by higher environmental exposure dose, as reflected by the large numbers of abortions in goats in the 2009 outbreak and extremely high attack rates in farm contacts [14]. A high C. burnetii dose in the environment is likely to lead to a higher probability of symptoms (i.e., a higher attack rate), even in less susceptible individuals. A dose-dependent attack rate has earlier been described for Salmonella infections in humans [33, 34]. A similar relationship for C. burnetii is suggested by experimental animal data [35], and a human dose–response model for C. burnetii was recently published as well [36]. Evidence from these studies suggests that higher doses may be likely to overrule the immune system of the exposed person, resulting in an increased probability of illness. This hypothesis, in the light of the findings from our study, leads us to presume that a high dose will not only cause symptoms in the genetically susceptible individuals, but also in those genetically less prone to symptoms. In other words, the effects of heterogeneity in host susceptibility are diminished or even extinguished. Although we cannot exclude some influence of recall bias on our results of population 1, we presume that a higher C. burnetii dose was present in population 2 in 2009 compared to population 1 in 2007. Modeling studies investigating human exposure might give more insight in this hypothesis.

An attempt to rule out dose effects in population 2 by excluding subjects who we assumed had the highest degree of exposure [farm residents, employees, visitors, and people living close (<3 km) to the farm as a measure of exposure dose] still did not enable us to reproduce our results from population 1. This may be due to a loss in statistical power because of excluding a large number of subjects from our analysis. Besides, the question remains as to whether the criteria we used for exclusion was a good measure for high exposure, even though it was the best available option in our study.

Symptoms associated with Q fever are non-specific and are also common in other respiratory tract infections, such as

![Symptom score distribution in study populations 1 and 2. The solid vertical line represents the median score of population 1 and the dashed line represents the median score of population 2](Fig. 2)

### Table 3 Allele frequencies of four different SNPs in study population 2 (n=123; n=246 alleles per SNP) and a Dutch population control group (n=1,008; n=2,016 alleles per SNP)

| Allele   | Symptom score≤7, n (%) | Symptom score≥8, n (%) | p-Value* | OR (95% CI) Dutch community controls, n (%) | p-Value# | OR (95% CI) |
|----------|------------------------|------------------------|----------|------------------------------------------|----------|-------------|
| IFNG (rs1861493) |                        |                        | 0.417    |                                          | 0.309    |             |
| A        | 112 (75)               | 76 (79)                |          | Ref                                      | 1,503 (75) | Ref         |
| G        | 38 (25)                | 20 (21)                | 0.77 (0.41–1.43) | 513 (25) | 0.77 (0.47–1.28) |
| IFNG (rs2430561) |                        |                        | 0.794    |                                          | 0.753    |             |
| T        | 76 (51)                | 47 (49)                |          | Ref                                      | 952 (47) | Ref         |
| A        | 74 (49)                | 49 (51)                | 1.07 (0.64–1.79) | 1,060 (53) | 0.94 (0.62–1.41) |

95% CI 95% confidence interval; OR odds ratio; Ref reference
* Chi-square test
# Based on the median symptom score (7) in the study population
† Symptom score≥8 vs. Dutch community controls
‡ 2 subjects/4 alleles missing
influenza. It can, therefore, be questioned whether the reported symptoms can be ascribed to other respiratory tract infections. Although the two outbreaks took place in different years, both occurred as seasonal peaks in spring (April–June), which corresponds to the main lambing season in goats [37, 38]. Influenza, however, mostly occurs in winter and the incidence in spring was low in both years in the Netherlands [39]. Therefore, it is highly unlikely that other respiratory diseases played a major role in the two populations used for this study.

In conclusion, a significant difference was found for the IFNG rs1861493 SNP between persons with a mild or more severe presentation of acute Q fever, which was not confirmed in a second study population. Such an effect could not be observed in the second outbreak due to the observed high rates of severe symptoms, possibly saturating the effects of host susceptibility factors.

Acknowledgments We would like to thank all participants.

Conflict of interest None declared.

Financial support The Netherlands Organisation for Health Research and Development (ZonMw) financially supported the assessments of the Herpen and Voerendaal outbreaks [Herpen (study population 1), grant number 125050001; Voerendaal (study population 2), grant number 50-50405-98-133].

References

1. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, Wijkmans CJ, Vellema P, Schneeberger PM (2012) The 2007–2010 Q fever epidemic in the Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol 64(1):1–12
2. van der Hoek W, Schneeberger PM, Oomen T, Wegdam-Blans MC, Dijkstra F, Notermans DW, Bijlmer HA, Groeneveld K, Wijkmans CJ, Rietveld A, Kampschreur LM, van Duynhoven Y (2012) Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in the Netherlands: from acute to chronic infection. Euro Surveill 17(3):pii:20059
3. Parker NR, Barralet JH, Bell AM (2006) Q fever. Lancet 367(9511):679–688
4. European Centre for Disease Prevention and Control (ECDC) (2010) Risk assessment on Q fever. ECDC, Stockholm. doi:10.2900/28860
5. Helbig K, Harris R, Ayres J, Dunckley H, Lloyd A, Robson J, Marmion BP (2005) Immune response genes in the post-Q-fever fatigue syndrome. Q fever endocarditis and uncomplicated acute primary Q fever. QJM 98(8):565–574
6. Helbig KJ, Heatley SL, Harris RJ, Mullighan CG, Bardy PG, Marmion BP (2003) Variation in immune response genes and chronic Q fever. Concepts: preliminary test with post-Q-fever fatigue syndrome. Genes Immun 4(1):82–85
7. Schork NJ (1997) Genetics of complex disease: approaches, problems, and solutions. Am J Respir Crit Care Med 156(4 Pt 2):S103–S109
8. Hill AVS (1998) The immunogenetics of human infectious diseases. Annu Rev Immunol 16:593–617
9. McNicholl JM, Downer MV, Udhayakumar V, Alper CA, Swerdlow DL (2000) Host–pathogen interactions in emerging and re-emerging infectious diseases: a genomic perspective of tuberculosis, malaria, human immunodeficiency virus infection, hepatitis B, and cholera. Annu Rev Public Health 21:15–46
10. Tso HW, Ip WK, Chong WP, Tam CM, Chiang AKS, Lau YL (2005) Association of interferon gamma and interleukin 10 genes with tuberculosis in Hong Kong Chinese. Genes Immun 6(4):358–363
11. Chong WP, Ip WKE, Tso GHW, Ng MW, Wong WHS, Law HKW, Yung RWH, Chow EY, Au KL, Chan EYT, Lim W, Peiris JSM, Lau YL (2006) The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. BMC Infect Dis 6:82
12. Vollmer-Conna U, Piraino BF, Cameron B, Davenport T, Hickie I, Wakefield D, Lloyd AR, Dubbo Infection Outcomes Study Group (2008) Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clin Infect Dis 47(11):1418–1425
13. Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, Van Rotterdam B, De Bruin A, Wijkmans C, Rietveld A, Van Duynhoven Y (2009) Investigation of a Q fever outbreak in a rural area of The Netherlands. Epidemiol Infect 137(9):1283–1294
14. Hackert VH, van der Hoek W, Dukers-Muimers N, De Bruin A, Al Dahouk S, Neubauer H, Bruggeman CA, Hoebe CJ (2012) Q fever: single-point source outbreak with high attack rates and massive numbers of undetected infections across an entire region. Clin Infect Dis 55(12):1591–1599
15. Dellacasagrande J, Capo C, Raoul D, Mege JL (1999) IFN-gamma-mediated control of Coxiella burnetii survival in monocytes: the role of cell apoptosis and TNF. J Immunol 162(4):2259–2265
16. Andoh M, Zhang G, Russell-Lodrigue KE, Shive HR, Weeks BR, Samuel JE (2007) T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in Coxiella burnetii infection in mice. Infect Immun 75(7):3245–3255
17. Ghigo E, Pretat L, Desnues B, Capo C, Raoul D, Mege JL (2009) Intracellular life of Coxiella burnetii in macrophages. Ann N Y Acad Sci 1166:55–66
18. Capo C, Mege JL (2012) Role of innate and adaptive immunity in the control of Q fever. Adv Exp Med Biol 984:273–286
19. Rossouw M, Nel HJ, Cooke GS, Van Helden PD, Hoal EG (2003) Association between tuberculosis and a polymorphic NFkappaB binding site in the interferon gamma gene. Lancet 361(9372):1871–1872
20. Pacheco AG, Cardoso CC, Moraes MO (2008) The interferon gamma gene polymorphism +874T/A and cytokine plasma levels are associated with tuberculosis susceptibility: a meta-analysis study. Hum Genet 123(5):477–484
21. Vallinoto I, Machado LFA, Ishak MOG, Ishak R (2010) IFNG +874T/A polymorphism and cytokine plasma levels are associated with susceptibility to Mycobacterium tuberculosis infection and clinical manifestation of tuberculosis. Hum Immunol 71(7):692–696
22. Gentile DA, Doyle WJ, Zeevi A, Piltcher O, Skoner DP (2003) Cytokine gene polymorphisms moderate responses to respiratory syncytial virus infection. Hum Immunol 64(3):338–344
23. Gentile DA, Doyle WJ, Zeevi A, Pitcher O, Skoner DP (2003) Cytokine gene polymorphisms moderate responses to respiratory syncytial virus in adults. Hum Immunol 64(1):93–98
24. Doorduyn Y, Van Pelt W, Siezen CLE, Van Der Horst F, Van Duynhoven YTHP, Hoebee B, Janssen R (2008) Novel insight in the association between salmonellosis or campylobacteriosis and chronic illness, and the role of host genetics in susceptibility to these diseases. Epidemiol Infect 136(9):1225–1234
25. Selvaraj P, Chandra G, Jawahar MS, Rani MV, Rajeshwari DN, Narayanan PR (2004) Regulatory role of vitamin D receptor gene variants of BsmI, ApaI, TaqI, and FokI polymorphisms on macrophage phagocytosis and lymphoproliferative response to Mycobacterium tuberculosis antigen in pulmonary tuberculosis. J Clin Immunol 24(5):523–532

26. Taverna MJ, Selam JL, Slama G (2005) Association between a protein polymorphism in the start codon of the vitamin D receptor gene and severe diabetic retinopathy in C-peptide-negative type 1 diabetes. J Clin Endocrinol Metab 90(8):4803–4808

27. Janssen R, Bont L, Siezen CLE, Hodemaekers HM, Ermers MJ, Doornbos G, van ’t Slot R, Wijmenga C, Goeman JJ, Kimpen JLL, van Houwelingen HC, Kimman TG, Hoebee B (2007) Genetic susceptibility to respiratory syncytial virus bronchiolitis is predominantly associated with innate immune genes. J Infect Dis 196(6):826–834

28. Viet AL, van Gils HWV, de Melker H, Elvers LH, Seidell JC, van den Berg J, van Veldhuizen H (2000) Risk factors and health in the Netherlands, a survey by Municipal Health Centres (Rainbow project); annual report 1998 [in Dutch: Risicofactoren En GezondheidsEvaluatie Nederlandse Bevolking, een Onderzoek Op GGD’en (Regenboog-project); Jaarverslag 1998]. Centraal Bureau voor de Statistiek (CBS-KPE); GGD Nederland; Rijksinstituut voor Volksgezondheid en Milieu (RIVM). RIVM report number 266807003. Available online at: http://www.rivm.nl/bibliotheek/rapporten/266807003.html. Accessed 13 May 2013

29. Hoebee B, Rietveld E, Bont L, Oosten Mv, Hodemaekers HM, Nagelkerke NJD, Neijens HJ, Kimpen JLL, van Duynhoven Y (2010) Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009–2010. BMC Vet Res 7(1):81

30. Rodriguez S, Gaunt TR, Day INM (2009) Hardy–Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 169(4):505–514

31. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV (2000) A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum Immunol 61(9):863–866

32. Mishra N, Arankalle VA (2011) Association of polymorphisms in the promoter regions of TNF-alpha (-308) with susceptibility to hepatitis E virus and TNF-alpha (-1031) and IFN-gamma (+874) genes with clinical outcome of hepatitis E infection in India. J Hepatol 55(6):1227–1234

33. Teunis PFM, Nagelkerke NJD, Haas CN (1999) Dose response models for infectious gastroenteritis. Risk Anal 19(6):1251–1260

34. Teunis PFM, Kasuga F, Fazil A, Ogden ID, Rotaru O, Strachan NJC (2010) Dose–response modeling of Salmonella using outbreak data. Int J Food Microbiol 144(2):243–249

35. Tamrakar SB, Halaska A, Haas CN, Bartrant TA (2011) Dose–response model of Coxiella burnetii (Q fever). Risk Anal 31(1):120–128

36. Brooke RJ, Kretzschmar MEE, Mutters NT, Teunis PFM (2013) Human dose response relation for airborne exposure to Coxiella burnetii. BMC Infect Dis 13(1):488

37. van der Hoek W, Dijkstra F, Schimmer B, Vellema P, van Duynhoven YTHP (2011) Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009–2010. BMC Vet Res 7(1):81

38. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, ter Schegget R, Hackert V, van Duynhoven Y (2010) Q fever in the Netherlands: an update on the epidemiology and control measures. Euro Surveill 15(12):pii:19520

39. Dijkstra F, van ’t Klooster TM, Brandsema P, van Gageldonk-Lafeber AB, Meijer A, van der Hoek W (2010) Annual report on the surveillance of respiratory tract infections 2009 [in Dutch: Jaarrapportage surveillance respiratoire infectieziekten 2009]. National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Bilthoven. RIVM report number 210231006 (http://www.rivm.nl/Documenten_en_publicaties/Wetenschappelijk/Rapporten/2010/september/Jaarrapportage_surveillance_respiratoire_infectieziekten_2009). Accessed 9 April 2014