

ORIGINAL PAPER

Interaction between *CD14* –159C>T polymorphism and *Helicobacter pylori* is associated with serum total immunoglobulin E

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Summary

Background Total serum IgE is regulated by both environmental and genetic factors. Association and linkage studies have suggested a role of *CD14* –159C>T polymorphism in the regulation of serum total IgE, but the results have been contradictory. It seems that gene–environment interactions are involved in this regulation.

Objective The aim of this study was to examine the possible gene–environment interactions among *Toxoplasma gondii*, *Helicobacter pylori*, *CD14* –159C>T and Toll-like receptor (*TLR*) 4 +896A>G polymorphism on serum total IgE. For this study, we expanded the scope of our earlier comparison of allergic sensitization and microbial load between Finland and Russian Karelia by studying the *CD14* –159C>T and *TLR4* +896A>G polymorphism in a cohort of Russian Karelian children.

Methods For this study, *CD14* –159C>T and *TLR4* +896A>G polymorphisms were analysed in 264 healthy Russian Karelian children. Serum total IgE levels and *H. pylori* and *T. gondii* antibodies were also measured.

Results We constructed a multiway ANOVA model to analyse the gene–environment interactions among *T. gondii* seropositivity, *H. pylori* seropositivity, *CD14* –159C>T and *TLR4* +896A>G polymorphisms on serum total IgE. The model showed that there was an interaction between the *CD14* –159 allele T carrier status and *H. pylori* antibodies on serum total IgE ($P=0.004$). No other interactions were found.

Conclusion Our results further emphasize the role of gene–environment interaction in the regulation of serum total IgE.

Keywords *CD14* polymorphism, gene–environment interaction, *Helicobacter pylori*, IgE, *Toxoplasma gondii*

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Introduction

IgE is an important mediator in allergic reactions and also contributes to the immune defence against parasites. Serum total IgE is regulated by both environmental and genetic factors, and interaction between the environment and the genetic background of the host seems to be important [1–4]. Environmental factors associated with atopy and serum total IgE include, for example, *Helicobacter pylori* and *Toxoplasma gondii* infections, endotoxin, tobacco smoke, number of siblings, regular contact with stable animals and socio-economic status,

which could reflect the microbial load the population is facing [2–7].

Many linkage studies have indicated that one or more loci on chromosome 5q may control total serum IgE [8–11]. One gene mapping to this area is *CD14*, which is a component of a multi-ligand pattern recognition receptor complex involved in innate immunity reactions [12]. *CD14* is also essential for the T-helper1 (Th1)/Th2 balance and coordinates the adaptive immune responses [13]. *CD14* recognizes, for example, lipopolysaccharide (LPS), lipoteichoic acids, mycobacterial glycolipids and mannan from yeast and enhances the function of Toll-like

receptors (TLR) 2 and 4, which are involved in immune defence against *T. gondii* and *H. pylori* [12–15].

CD14 gene promoter region polymorphism –159C>T has been associated with serum total IgE and atopy, but the results have been contradictory. The same allele has been shown to have opposite effects on IgE [11, 16–21]. The gene–environment interactions in the regulation of serum total IgE detected in recent studies have been postulated to be one explanation for the inconsistent results [1]. Although *CD14* is an important molecule for the endotoxin signalling, other molecules such as *TLR4* are needed for signal transduction [13]. A single A>G base-exchange polymorphism in the *TLR4* gene at the position +896 in the fourth exon has been associated with atopic asthma, but there are also negative findings [22–24].

In our earlier study, we observed that serum total IgE levels were significantly higher in children living in Russian Karelia than in children living in Finland, even though sensitization to allergens was clearly less frequent among Russian Karelian children [6]. The exact reason for this phenomenon is not known, but one explanation for the higher IgE levels could be parasite infections, which are more common in Russian Karelia than in Finland [6, 25]. In this study, we wanted to expand our earlier investigation and analyse possible gene–environment interactions among microbes, *CD14* –159C>T and *TLR4* +896A>G polymorphisms on serum total IgE in a child population from Russian Karelia.

Materials and methods

Study population

Altogether 266 schoolchildren from Petrozavodsk, Russian Karelia, having both parents of either Finnish or Karelian ethnicity participated in the present study. Two children were excluded, because their whole blood samples were accidentally confused in the laboratory. The study population has been described earlier in detail [6]. Finnish schoolchildren from the earlier study were not included in this study due to the low prevalence of seropositive subjects for *H. pylori* and *T. gondii* (5% and 2%, respectively). The collection of samples from Russian Karelia was done as a part of the EU INCO-Copernicus program (contract number IC15-CT98-0316, coordinator Professor Hyöty) during the years 1997–1999. Collection of samples was organized by the Department of Pediatrics, University of Petrozavodsk, and the study protocol was approved by the Ministry of Health in Russian Karelia. There was no local ethics board in Karelia to apply to, and the accepted route for ethical approval for the research studies was via the Ministry of Health. Written consent was obtained from all the children who took part in the study and from their parents. Whole blood and serum samples were collected from each child.

Immunoglobulin E and microbial antibodies

Serum total IgE was measured using the ImmunoCAP® fluoroenzyme immunoassay (Phadia Diagnostics, Uppsala, Sweden). *T. gondii* IgG antibodies were measured by Enzygnost Toxoplasmosis IgG Assay and *H. pylori* IgG antibodies by Enzygnost Anti-*H. pylori*/IgG Assay according to the manufacturer's instructions (Dade Behring, Marburg, Germany). Behring ELISA Processor III (Dade Behring, Marburg, Germany) was used for further processing of the tests and for the calculation of the antibody levels. Measurement of *T. gondii* antibodies succeeded in 263 children of 264 (99.5%), whereas *H. pylori* antibodies were measured among all the 264 (100%) study children.

Genotype analyses

DNA was extracted using standard techniques. Genotyping of *CD14* –159C>T polymorphism (rs 2569190) and *TLR4* +896A>G (rs 4986790) was performed using the ABI PRISM 7000 Sequence Detection System for both PCR and allelic discrimination. The oligonucleotides 5'-CCC TTC CTT TCC TGG AAA TAT TGC A-3' and 5'-CTA GAT GCC CTG CAG AAT CCT T-3' were used as primers for *CD14* –159C>T polymorphism, and the oligonucleotides 5'-TGA CCA TTG AAG AAT TCC GAT TAG CA-3' and 5'-ACA CTC ACC AGG GAA AAT GAA GAA-3' for *TLR4* +896A>G polymorphism.

Statistics

To detect a possible deviation of the genotype frequencies from the Hardy–Weinberg equation, the exact test using the Markov chain algorithm was used (Arlequin program, ver. 2.0. A software for population genetics data analysis; Schneider S, Roessli D, Excoffier L; Genetics and Biometry Laboratory, Geneva, Switzerland). Other statistical analyses were performed with SPSS for Windows version 14.0 (SPSS Inc., Chicago, IL, USA). The statistical method selected is presented in 'Results'. Serum total IgE levels were not normally distributed and therefore non-parametric tests were used when serum total IgE levels were analysed. Serum total IgE values were logarithmically transformed for multiway ANOVA which was used for modelling gene–environment interactions among *CD14* –159 allele T carrier status, *TLR4* +896 allele G carrier status, *T. gondii* seropositivity and *H. pylori* seropositivity on serum total IgE levels. For statistical analysis, we grouped *CD14* –159 genotypes according to the allele T carrier status, because this allele has been shown to be transcriptionally more active in monocytic cell line than allele C [26]. *TLR4* +896 genotypes were grouped according to the allele G carrier status, because the number of GG genotype was too small ($n = 3$) for grouping

according to allele A. *P*-values <0.05 were considered to be significant.

Results

The study cohort of 264 children included 114 boys (43%). The mean age of the children was 11.4 years (range 7.1–15.0). The median total IgE in sera was 76.1 IU/L (interquartile range 30.9–236.0). There were no significant differences in total IgE levels between boys and girls (median 75.8 and 76.4 IU/L, respectively, *P* = 0.8, Mann-Whitney *U*-test). Among the study children, 193 out of 264 (73%) were seropositive for *H. pylori* and 63 out of 263 (24%) for *T. gondii*. Fifty-two (20%) children were seropositive for both the microbes. Only 16% (*n* = 10) of *T. gondii* seropositive children were *H. pylori* seronegative, whereas 72% (*n* = 139) of *H. pylori* seropositive children were *T. gondii* seronegative. Serum total IgE levels were significantly higher in *T. gondii* seropositive children (*P* = 0.009, Mann-Whitney *U*-test), whereas *H. pylori* seropositivity did not have any effect on serum total IgE (Table 1), as reported earlier [6]. Association between *T. gondii* seropositivity and serum total IgE remained statistically significant after Bonferroni correction (*P* = 0.036).

The genotype frequencies of *CD14* –159C>T and *TLR4* +896A>G polymorphisms as well as allele T carrier status of *CD14* –159C>T and allele G carrier status of *TLR4* +896A>G are shown in Table 1. *CD14* –159C>T genotype or allele T carrier status did not show any direct

association with serum total IgE, and there was no association between *TLR4* +896A>G genotype or allele G carrier status and serum total IgE (Table 1). Neither of these polymorphisms was associated with seropositivity for *H. pylori* or *T. gondii* (data not shown). The genotype distributions followed the Hardy-Weinberg equilibrium.

We constructed a multiway ANOVA model to investigate gene-environment interactions among *CD14* –159C>T allele T carrier status, *TLR4* +896A>G allele G carrier status, *H. pylori* seropositivity and *T. gondii* seropositivity on serum total IgE levels. In this model, we found an interaction between *H. pylori* seropositivity and *CD14* –159 allele T carrier status on serum total IgE (*P* = 0.004, multiway ANOVA, Table 2a). The *H. pylori* seronegative children who were allele T non-carriers (i.e. genotype CC) had higher serum total IgE than allele T carriers (i.e. genotypes CT and TT). Among *H. pylori* seropositive children, allele T non-carriers had lower IgE levels than allele T carriers (Table 2a). There was a trend for interaction between *T. gondii* seropositivity and *CD14* –159 allele T carrier status on serum total IgE, but this interaction was not statistically significant (Table 2b). No statistically significant interactions between *TLR4* +896A>G allele G carrier status and *H. pylori* or *TLR4* +896A>G allele G carrier status and *T. gondii* on serum total IgE was found (*P* = 0.95 and 0.3, respectively, multiway ANOVA). We also analysed gene-gene interaction between *CD14* –159C>T allele T carrier status and *TLR4* +896A>G allele G carrier status on serum total IgE, but no

Table 1. Associations between *Toxoplasma gondii* serology, *Helicobacter pylori* serology, *CD14* –159C>T polymorphism, *TLR4* +896A>G polymorphism and serum total IgE

	N (%)	IgE median (quartiles)	<i>P</i> -value	<i>P</i> -value*
<i>T. gondii</i>				
Seropositive	63 (24)	114.0 (44.0–393.0)		
Seronegative	200 (76)	68.5 (28.3–174.3)	0.009 [†]	0.036
<i>H. pylori</i>				
Seropositive	193 (73)	76.8 (32.7–234.0)		
Seronegative	71 (27)	68.2 (30.0–257.0)	0.86 [†]	NS
<i>CD14</i> –159C>T				
CC	88 (33)	66.4 (32.0–238.5)		
CT	139 (53)	80.9 (33.1–203.5)		
TT	37 (14)	62.3 (23.8–262.0)	0.9 [‡]	NS
Allele T carrier (CT and TT)	176 (67)	79.2 (30.8–229.5)		
Allele T non-carrier (CC)	88 (33)	66.4 (32.0–238.5)	0.8 [†]	NS
<i>TLR4</i> +896A>G				
AA	223 (85)	76.4 (34.1–217.0)		
AG	38 (14)	62.0 (21.9–266.0)		
GG	3 (1)	107.0 (14.1–171.0)	0.8 [‡]	NS
Allele G carrier (AG and GG)	41 (15)	67.5 (21.6–262.0)		
Allele G non-carrier (AA)	223 (85)	76.4 (34.1–217.9)	0.5 [†]	NS

*Bonferroni correction.

[†]Mann-Whitney *U*-test.

[‡]Kruskal-Wallis ANOVA.

NS, non-significant.

Table 2. (a) Interaction between *Helicobacter pylori* serology and *CD14* –159C>T allele T carrier status on serum total IgE ($P = 0.004$, multiway ANOVA). (b) Interaction between *Toxoplasma gondii* serology and *CD14* –159C>T allele T carrier status on serum total IgE ($P = 0.06$, multiway ANOVA)

<i>CD14</i> –159 C>T	Seropositive		Seronegative	
	IgE median (quartiles)	N	IgE median (quartiles)	N
(a) <i>H. pylori</i>				
Allele T carrier (CT and TT)	87.4 (36.9–253.8)	130	44.1 (24.2–162.5)	46
Allele T non carrier (CC)	56.0 (29.5–154.0)	63	84.7 (34.1–530.5)	25
(b) <i>T. gondii</i>				
Allele T carrier (CT and TT)	202.5 (59.4–550.8)	42	67.5 (26.8–160.5)	133
Allele T non carrier (CC)	55.3 (39.1–81.7)	21	79.7 (29.5–272.0)	67

interaction was seen ($P = 0.7$, multiway ANOVA). Furthermore, there was no interaction between the environmental factors *T. gondii* and *H. pylori* on serum total IgE ($P = 0.2$, multiway ANOVA).

Discussion

Gene–environment interactions are very complex. It has been speculated that the same genetic variants may be associated with different phenotypes in different environments, but so far, only few human studies have been published [2, 3, 27]. *H. pylori*, *T. gondii* and *CD14* –159C>T polymorphisms have all been independently associated with atopic markers and IgE. In this study, we wanted to further analyse whether there are gene–environment interactions among *H. pylori*, *T. gondii*, *CD14* and *TLR4* polymorphisms that have an effect on serum total IgE. To increase the sensitivity and detect possible interactions, we carried out the study in an environment that is characterized by a high exposure rate to these two microbes.

CD14 is part of the TLR signalling complex that facilitates endotoxin responses through TLR4-MD2 and can also bind with a variety of microbial TLR ligands. *CD14* gene (MIM158120) is located on chromosome 5 (5q31). In many linkage studies, this area has been connected with the regulation of serum total IgE and atopy [8–11]. *CD14* promoter region single base-exchange polymorphism C to T at position –159 has been extensively studied, because it has been associated with the level of protein production allele T being transcriptionally more active in monocytic cell line [17, 26, 28]. An association between *CD14* –159C>T polymorphism and total IgE and other atopic markers has been shown in the earlier studies, but the results have been contradictory. Baldini et al. [17] reported that atopic children homozygous for allele T (i.e. TT genotype) had higher sCD14 levels and lower IgE levels in sera than allele C carriers. An association between high IgE levels or other atopic markers and the *CD14* –159CC genotype has been reported in many other studies [16, 19, 20]. An opposite association has been reported by Ober et al. [11], who showed that the *CD14* –159 allele T was

associated with atopy in a rural population. In some studies, no associations have been found [18, 21].

Environmental differences between study populations may in part explain these conflicting results. In recent studies, exposure to endotoxin and other environmental factors has been studied concomitantly with *CD14* –159C>T polymorphism. Eder et al. [2] reported that the *CD14* –159C>T allele C was associated with lower levels of both total and specific IgE in children in regular contact with stable animals, whereas the result was the opposite in children with regular contact with furry pets. They speculated that one explanation for this difference could be the exposure to different kinds of microbial products [2]. Williams et al. [3] found in an adult female population that the association between *CD14* –159C>T polymorphism and serum total IgE was modified by the level of endotoxin exposure. At lower levels of endotoxin exposure, the CC genotype was associated with the highest serum total IgE levels, whereas at the highest tertile of endotoxin exposure, individuals carrying the TT genotype had the highest IgE levels [3]. On the other hand, in a French study, early-life exposure to a farming environment and the *CD14* –159 TT genotype together significantly reduced the risk of nasal allergy and atopy. In addition, there was a trend among these subjects for lower risk of having increased serum total IgE levels [27].

Eder et al. [2] speculated that the effect of *CD14* –159C>T polymorphism on IgE might be dependent on microbial exposure, but they did not study the specific microbes. In the present study, we found that interaction between *CD14* –159C>T polymorphism and *H. pylori* serology had an effect on serum total IgE. Among *H. pylori* seronegative children, *CD14* –159 allele T non-carriers (i.e. CC genotype) had higher serum total IgE than allele T-carriers (i.e. CT and TT genotypes), whereas among *H. pylori* seropositive children, serum total IgE was lower in allele T non-carriers. There was also a trend of gene–environment interaction between *T. gondii* seropositivity and *CD14* –159C>T polymorphism. Because of our result, one may speculate that *H. pylori*, and possibly also *T. gondii*, could be among the microbes that influence the genetic regulation of serum total IgE, but the exact mechanism is not known.

H. pylori infection has been suggested to influence the development of the immune system by LPS binding with the CD14 receptor which results in increased production of IL-12. This could drive the immune responses towards the Th1 pathway and thereby have a protective effect against the development of Th2 polarized diseases such as atopy [29]. *CD14* -159 allele T carriers have been reported to have higher sCD14 levels than allele T non-carriers and these high sCD14 levels have been associated with low serum total IgE [17, 28]. The exact mechanism is not known, but interaction between sCD14 and B cells has resulted in higher levels of IgG1 and lower levels of IgE production [17, 30]. Karhukorpi et al. [28] have reported that *H. pylori* seropositivity has an effect on sCD14 levels in such a way that *H. pylori* seropositive individuals have higher levels than seronegative subjects, especially if they carry the *CD14* -159 CC genotype. One may speculate that the CC genotype is especially sensitive to environmental factors.

TLR4 plays a role in the same functional pathway as CD14, and both these molecules are needed for endotoxin responsiveness. A single A>G base-exchange polymorphism in the *TLR4* gene at position +896 has been associated with atopic asthma, but the results have been contradictory [22–24]. This base exchange induces an amino acid substitution of glycine for asparagine, which results in a reduction in cell surface expression of TLR4 and subsequent disruption of LPS-mediated signalling [31]. In the present study, *TLR4* +896A>G polymorphism was not associated with serum total IgE and there was no interaction between this polymorphism and *H. pylori* or *T. gondii* on serum total IgE. It has been speculated that the combined effect of the genetic variants of CD14 and TLR4 could strengthen the associations found earlier, but no interaction between *CD14* -159C>T and *TLR4* +896A>G polymorphisms was seen in our study, which is in line with previous findings of Sackesen et al. [24].

The major limitation of our study is the small number of study subjects and therefore these gene-environment analyses should be repeated in a larger population. Another restriction was that we could not use specific IgE in gene-environment interaction analyses, because the number of atopic subjects in the study population ($n = 16$) was too small for statistical analyses. The problem with serum total IgE is that many factors such as allergens and parasite infections influence its levels and therefore the interpretation of the results is challenging. Multiple testing also complicates the interpretation of our results. However, the interaction between *CD14* -159C>T allele T carrier status and *H. pylori* seropositivity on serum total IgE was so significant that it most probably does not disappear even though multiple testings are done (i.e. remaining significant after Bonferroni correction). Caution should also be exercised when interpreting interactions between microbes and *CD14* -159C>T polymorphism on serum total IgE, because both *H. pylori* and

T. gondii could be surrogate markers for poor hygiene and large microbe load. Therefore, the interactions detected could either reflect interaction between the whole microbe load and *CD14* -159 polymorphism or the interaction between specific microbe and the polymorphism.

Our study supports the hypothesis that the same genotype may increase, decrease or have no effect on total IgE depending on the environmental factors, such as microbes, that the population is facing.

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