

## Original article

Opposite gene by environment interactions in Karelia for *CD14* and *CC16* single nucleotide polymorphisms and allergy

**Background:** Finnish Karelians have a higher prevalence of allergic disease than Russian Karelians. As both populations are generally from the same ethnic group, the Karelian population offers a unique opportunity to analyse genetic and allergic disease interactions between 'Western' and 'Eastern' environments.

**Objectives:** We investigated associations between allergic diseases and *CD14* and *CC16* polymorphisms in Finnish vs Russian Karelian women.

**Methods:** Adult female Karelians (330 Finnish and 274 Russian) were recruited, examined for a range of symptoms and conditions including rhinitis, itchy rash, asthma and atopy and genotyped for *CD14* C-159T and *CC16* A38G.

**Results:** For both *CD14* C-159T and *CC16* A38G, the risk allele for atopic phenotypes in Finnish Karelia was the protective allele in Russian Karelia. For *CD14* C-159T, an interactive effect on ever itchy rash ( $P_{\text{interaction}} = 0.004$ ), itchy rash < 12 mo ( $P_{\text{interaction}} = 0.001$ ) and dry cough at night in the past 12 months (< 12 months) ( $P_{\text{interaction}} = 0.011$ ) was found; the risk allele was C in Russians and T in Finns. For *CC16* A38G, an interaction was significant for ever rhinitis ( $P_{\text{interaction}} = 0.006$ ), rhinitis < 12 mo ( $P_{\text{interaction}} = 0.004$ ), and marginally significant for ever hayfever ( $P_{\text{interaction}} = 0.07$ ), allergic eye symptoms < 12 mo ( $P_{\text{interaction}} = 0.09$ ); their risk allele was G in Russians and A in Finns.

**Conclusion:** An Eastern vs Western environment appears to exert an effect via opposite alleles on risk of allergic diseases in adult women.

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Interactions between inherited and environmental risk factors are accepted as the cause of complex diseases, including asthma and allergy (1). The exact aetiologies of most of these diseases, however, are still not well understood as they involve complicated interactions between multiple environmental risk factors and genetic variants (2). For asthma/allergy, numerous studies have shown consistent associations with genetic predisposition, urban environment and Western lifestyle (3). However, there have been many inconsistent findings with replication of results for specific risk factors, particularly with associations with particular alleles (2, 4). These discrepancies may result from variable exposures to certain environmental risk factors resulting in differential effects on individuals susceptible to these stimuli because of their genetic make-up (5). In this regard, several authorities have suggested that opposite effects of genetic elements on asthma and atopic diseases may occur in populations with high exposure or low exposure to specific environmental risk factors (2, 5, 6). For example, in a recent study comparing CC

with TT homozygotes of the *CD14* C-159T single nucleotide polymorphism (SNP), with low exposure to endotoxin those with CC exhibited increased risk of atopy relative to TT homozygotes, while in contrast, with high exposure to endotoxin the CC homozygotes were at markedly decreased risk for atopy compared to those with TT (7).

Considering the prevalence of asthma/allergic diseases, an East-West gradient has been consistently confirmed between 'Western' affluent countries and 'Eastern' developing countries (8), with, e.g. atopic diseases being more prevalent in Western than Eastern Europe (9). Finnish Karelians ('Western environment') have previously been shown to have a higher prevalence of allergic disease than Russian Karelians ('Eastern environment') (10, 11). These two areas are geographically adjacent and are expected to have similar outdoor air pollution (11). The Karelians were one ethnic group until they were artificially divided by a new Finnish/Russian border during the Second World War, after which changes have occurred mainly on the Russian

side of the border with an influx of 'white Russians' (12). However, the genetic make up of the two populations should still be similar and, indeed, any differences can be readily detected as allele distribution differences between the populations on each side of the border. The major differences between Finnish and Russian Karelians therefore are likely to be in the cultural, economic and lifestyle conditions with which they lived since their separation at the time of the Second World War (11). These genetically similar populations therefore represent model cohorts for analysing contrasting 'Western' or 'Eastern' environmental exposures on genetic variants associated with asthma/allergy.

This study analysed two asthma/atopy-related genes, *CD14* and *CC16*, which were chosen due to strong evidence of gene by environment interactions (13–17). Many studies have analysed the *CD14* C-159T polymorphism for associations with asthma and atopy (13–18). The promoter polymorphism of the *CD14* gene has been inconsistently associated with asthma-related phenotypes, such as atopy (14), IgE levels (16), rhinitis (19), eczema (20) and soluble CD14 levels (21). Three independent studies showed that the strength of exposure to a specific environmental factor strongly influenced the direction of the association between *CD14* C-159T and asthma-associated phenotypes (6, 7, 22). We have previously found that the *CC16* A38G polymorphism was associated with an increased risk of asthma-related phenotypes in Caucasian populations (23–25). These two genes, *CD14*, coding for the pattern recognition receptor which plays a pivotal role in innate immune responses, and *CC16*, coding for the Clara cell 16-kDa protein which has anti-inflammatory effects and is secreted by a multifunctional epithelial cell in the lung, are promising candidates for an inherited susceptibility to asthma and atopic diseases. In this study, the primary hypothesis was that the associations of allergic conditions with the two SNPs, C-159T in the *CD14* gene and A38G in the *CC16* gene, were dependent on each population's domiciliary environments ('Western' and 'Eastern'), with opposite relationships with particular alleles. The aim of this study was therefore to delineate these complex genes by environment interactions with respect to the pathogenesis of allergic diseases.

## Methodology

### Study population

As described in detail elsewhere (10, 26), a Karelian study was conducted in 2003. The Karelian population was from two bordering areas: the Finnish study area, North Karelia, and Russian area, the Republic of Karelia. There were fundamental differences between the two areas with respect to their current cultural, political and socio-economic conditions. This study included 604 adult women (330 Finnish and 274 Russian) from the

Karelian population with mean age of 40.9 years and 35.7 years in Finnish and Russians, respectively. Informed consent was obtained from all the participants and the study was approved by the Ethics Committee of the Helsinki University Central Hospital.

### Questionnaire and phenotype definitions

The International Study of Asthma and Allergies in Childhood questionnaire was employed for this study (26). Ten conditions or symptoms were defined based on the questionnaire survey and included: ever asthma, wheezing in the past 12 months (<12 months), dry cough at night <12 months, ever rhinitis, rhinitis <12 months, ever hayfever, allergic eye symptoms <12 months, ever itchy rash, itchy rash <12 months and ever eczema. Skin prick tests (SPT) were conducted during the study visit as previously described (26). Thirteen common allergens (birch, timothy grass, mugwort, cat, dog, horse, cow, *Dermatophagoides pteronyssinus*, wheat, hen's egg, peanut, fish (cod) and cow's milk) were selected for SPT. Atopy to SPT was defined as one or more SPT wheals of size  $\geq 3$  mm.

### Genotyping

Genomic DNA was extracted by an automated DNA extraction instrument (Autopure LS; Qiagen, Hilden, Germany). The *CD14* C-159T and *CC16* A38G genotypes were determined by using iPLEX assay on a MALDI-TOF MassARRAY platform (Sequenom, San Diego, CA, USA) according to manufacturer's instructions (<http://www.sequenom.com>).

### Statistical analysis

Independent sample *T* test and chi-square test, wherever appropriate, were conducted to examine the difference in the demographic characteristics and genotype frequencies between Finnish and Russian Karelians. The genetic effects of the two SNPs were analysed in the two populations separately. Pearson's chi-square test, unless specified otherwise, was employed to compare the difference in the prevalence of atopic diseases and symptoms between genotypes. Logistic regression was further conducted to estimate the odds ratios (OR) of the specific genotype for those diseases and symptoms.  $P_{\text{linear}}$  values were estimated by fitting the number of allele (0, 1 or 2) into the logistic regression model as a continuous variable.

For those conditions defined from the questionnaire, interactive effects of the two SNPs with the 'Western' or 'Eastern' environment were investigated using the Likelihood-ratio test after estimation in a logistic regression in STATA. Both the unrestricted (with interaction) and the restricted (without interaction) models were fitted using the maximum likelihood method and the two models were compared with to identify the significant interactions ( $P_{\text{interaction}} < 0.05$ ) (27). In the logistic regression analyses, we also adjusted for confounders of interest.

## Results

The frequencies of the *CD14* C-159T and *CC16* A38G genotypes and their minor alleles are shown in Table 1. The two SNPs were in Hardy–Weinberg Equilibrium in Finnish and Russian Karelians, with no significant differences in genotype frequencies between Finland and Russia.

Table 1. Frequencies of genotypes and minor alleles of *CD14* (C-159T) and *CC16* (G38A) in Finnish and Russian Karelians

| <i>CD14</i>   | Finnish  |      |      |     | Russian  |      |      |     |
|---------------|----------|------|------|-----|----------|------|------|-----|
|               | <i>n</i> | %    | T%   | HWE | <i>n</i> | %    | T%   | HWE |
| CC            | 121      | 36.7 | 39.7 | NS  | 91       | 33.2 | 41.2 | NS  |
| CT            | 156      | 47.3 |      |     | 140      | 51.1 |      |     |
| TT            | 53       | 16.1 |      |     | 43       | 15.7 |      |     |
| <i>CC16</i> * |          |      | A%   |     |          |      | A%   |     |
| AA            | 45       | 13.7 | 35.1 | NS  | 37       | 13.5 | 37.0 | NS  |
| AG            | 141      | 42.9 |      |     | 129      | 47.1 |      |     |
| GG            | 143      | 43.5 |      |     | 108      | 39.4 |      |     |

HWE, Hardy-Weinberg Equilibrium; NS, not significant; T%, T allele frequency for *CD14* (C-159T); A%, A allele frequency for *CC16* (G38A).

\*One missing value for *CC16* genotyping.

### *CD14* C-159T

The *CD14* C-159T had an opposite effect on the prevalences of the allergic conditions in Finnish and Russian adult women. Russian women with the T allele appeared to have a lower rate of these phenotypes except for atopy (SPT) (Table 2). The associations between *CD14* C-159T and atopic phenotypes were significant or approaching borderline significance for dry cough at night < 12 months ( $P_{\text{linear}} = 0.089$ ), allergic eye symptoms < 12 months ( $P_{\text{linear}} = 0.076$ ), itchy rash < 12 months ( $P_{\text{linear}} = 0.010$ ) and ever itchy rash ( $P_{\text{linear}} = 0.002$ ), with the C allele being a risk in Russian women. An interactive effect of the SNP on ever itchy rash ( $P_{\text{interaction}} = 0.001$ ; data not shown) and itchy rash < 12 months ( $P_{\text{interaction}} = 0.004$ ; Fig. 1) and dry cough at night < 12 months ( $P_{\text{interaction}} = 0.011$ ; Fig. 1), was found with C being a risk allele in Russians and, in contrast, T being a risk allele in Finnish. For other phenotypes, no significant interactions were found.

### *CC16* A38G

Table 3 shows prevalences and OR of the atopic diseases and symptoms in Russian and Finnish women stratified by genotypes of the *CC16* A38G polymorphism. Notably, Russians with the G allele of the SNP appeared to have higher rates of these allergic diseases and symptoms, relative to those with the A allele. The individuals with GG had OR of 2 or more for ever rhinitis ( $P = 0.022$ ), rhinitis < 12 months ( $P = 0.08$ ), allergic eye symptoms < 12 months ( $P = 0.03$ ), itchy rash < 12 months ( $P = 0.07$ ), ever itchy rash ( $P = 0.12$ ), compared to those with the AA genotype. In contrast, in Finnish the GG genotype was associated with a lower risk for ever rhinitis ( $P = 0.045$ ), rhinitis < 12 months ( $P = 0.013$ ). Figure 2 shows several prevalence rates of those symptoms or conditions in Finnish and Russian women stratified by the genotypes of *CC16* A38G. The interaction between genotypes of *CC16* A38G and the 'Western' or 'Eastern'

environment was significant or marginally significant for ever rhinitis ( $P_{\text{interaction}} = 0.006$ ; data not shown), rhinitis < 12 months ( $P_{\text{interaction}} = 0.004$ ; Fig. 2), ever hayfever ( $P_{\text{interaction}} = 0.07$ ; data not shown) and allergic eye symptoms < 12 months ( $P_{\text{interaction}} = 0.09$ ; Fig. 2).

We further examined the interactive effects between the two SNPs and Finnish and Russian Karelian domiciliary environments on atopic phenotypes, after adjusting for several potential confounders, such as age, smoking and number of pregnancies. The significant interactions reported above remained with slight changes in their significance (data not shown). We also investigated the associations of the two SNPs with atopic phenotypes in the whole population (not stratified by Finnish and Russians) and no associations were found except that the GG homozygotes of the *CC16* A38G SNP were at increased risk for allergic eye symptoms < 12 months (data not shown).

### Discussion

This study allowed a unique investigation into 'Western' vs 'Eastern' influences on gene by environment interactions of two key asthma/allergy candidate genes. This was carried out by comparing two genetically similar populations, the Finnish and Russian Karelians, who live on either side of the boundary between Finland and Russia. We demonstrated gene by environment interactions on asthma-related phenotypes with opposite effects for specific alleles of both *CD14* C-159T and *CC16* A38G in the Karelian women. This study has provided valuable data for understanding the complex interactions between genes and environment on asthma/atopy. The data further emphasize that in association studies on complex diseases environmental risk factors must be accounted for separately in populations with differing environments to fully evaluate the genetic effects.

As almost all of the relationships, we found were not apparent when both populations were combined, our findings also demonstrate that attempting to improve statistical power with larger populations can miss important gene by environmental interactions that are opposite in direction in sub-populations. Clearly, the demonstration that the same SNP can produce opposite effects in different environments highlights the importance of investigation of environmental exposures in genetic association studies.

Our findings in the Finnish population were consistent with numerous previous reports associating the two SNPs with the allergic conditions (13, 16, 23, 24, 28). In addition, our data on the comparison of Finnish versus Russian Karelians were also consistent with the literature. A reasonable expectation would be that microbial components, associated with reduced allergy (29) but not wheezing (7), would be present in higher concentrations on the Russian side of the border. Indeed, an up to a

Table 2. Prevalences and odds ratio (OR) of atopic diseases and symptoms in Karelian women stratified by genotypes of the CD14C-260T polymorphism

|                                               | Yes, Russia |      |      |                 |                            | Yes, Finland |      |      |                 |                            |
|-----------------------------------------------|-------------|------|------|-----------------|----------------------------|--------------|------|------|-----------------|----------------------------|
|                                               | <i>n</i>    | %    | OR   | <i>P</i> -value | <i>P</i> <sub>linear</sub> | <i>n</i>     | %    | OR   | <i>P</i> -value | <i>P</i> <sub>linear</sub> |
| Asthma (ever)                                 |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 91          | 1.1  | 2.65 | 0.39            | 0.93                       | 121          | 13.2 | 0.65 | 0.26            | 0.73                       |
| CT                                            | 140         | 2.9  |      |                 |                            | 156          | 9.0  |      |                 |                            |
| TT                                            | 43          | 0    |      |                 |                            | 53           | 13.2 |      |                 |                            |
| Wheezing (in the past 12 months)              |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 91          | 17.6 | 0.56 | 0.14            | 0.12                       | 121          | 14.0 | 1.34 | 0.38            | 0.25                       |
| CT                                            | 140         | 10.7 |      |                 |                            | 156          | 17.9 |      |                 |                            |
| TT                                            | 43          | 9.3  |      |                 |                            | 53           | 20.8 |      |                 |                            |
| Dry cough at night (in the past 12 months)    |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 90          | 24.4 | 0.58 | 0.10            | 0.089                      | 121          | 10.7 | 2.40 | <b>0.012</b>    | 0.17                       |
| CT                                            | 140         | 15.7 |      |                 |                            | 156          | 22.4 |      |                 |                            |
| TT                                            | 43          | 14.0 |      |                 |                            | 53           | 15.1 |      |                 |                            |
| Rhinitis (ever)                               |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 91          | 42.9 | 0.76 | 0.33            | 0.63                       | 121          | 68.6 | 1.00 | 1.00            | 0.63                       |
| CT                                            | 140         | 36.4 |      |                 |                            | 156          | 68.6 |      |                 |                            |
| TT                                            | 43          | 51.2 |      |                 |                            | 52           | 73.1 |      |                 |                            |
| Rhinitis (in the past 12 months)              |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 91          | 33.0 | 0.94 | 0.84            | 0.31                       | 120          | 60.8 | 1.04 | 0.82            | 0.35                       |
| CT                                            | 139         | 31.7 |      |                 |                            | 156          | 62.2 |      |                 |                            |
| TT                                            | 43          | 44.2 |      |                 |                            | 52           | 69.2 |      |                 |                            |
| Hay fever (ever)                              |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 90          | 3.3  | 1.31 | 0.71            | 0.90                       | 121          | 21.5 | 0.91 | 0.74            | 0.67                       |
| CT                                            | 139         | 4.3  |      |                 |                            | 156          | 19.9 |      |                 |                            |
| TT                                            | 43          | 2.3  |      |                 |                            | 53           | 18.9 |      |                 |                            |
| Allergic eye symptoms (in the past 12 months) |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 91          | 28.6 | 0.63 | 0.13            | 0.076                      | 120          | 35.8 | 1.11 | 0.67            | 0.55                       |
| CT                                            | 140         | 20.0 |      |                 |                            | 154          | 38.3 |      |                 |                            |
| TT                                            | 43          | 16.3 |      |                 |                            | 52           | 40.4 |      |                 |                            |
| Itchy rash (in the past 12 months)            |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 89          | 19.1 | 0.62 | 0.20            | <b>0.028</b>               | 99           | 28.3 | 0.86 | 0.63            | 0.29                       |
| CT                                            | 140         | 12.9 |      |                 |                            | 126          | 25.4 |      |                 |                            |
| TT                                            | 43          | 2.3  |      |                 |                            | 45           | 40.0 |      |                 |                            |
| Itchy rash (ever)                             |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 89          | 23.6 | 0.54 | 0.076           | <b>0.014</b>               | 121          | 31.4 | 1.09 | 0.73            | 0.24                       |
| CT                                            | 140         | 14.3 |      |                 |                            | 156          | 33.3 |      |                 |                            |
| TT                                            | 43          | 2.3  |      |                 |                            | 53           | 41.5 |      |                 |                            |
| Eczema (ever)                                 |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 90          | 10.0 | 0.69 | 0.44            | 0.76                       | 121          | 24.0 | 0.92 | 0.76            | 0.20                       |
| CT                                            | 140         | 7.1  |      |                 |                            | 156          | 22.4 |      |                 |                            |
| TT                                            | 42          | 9.5  |      |                 |                            | 53           | 35.8 |      |                 |                            |
| Atopy*                                        |             |      |      |                 |                            |              |      |      |                 |                            |
| CC                                            | 90          | 14.4 | 1.43 | 0.33            | 0.12                       | 117          | 37.6 | 0.88 | 0.62            | 0.61                       |
| CT                                            | 139         | 19.4 |      |                 |                            | 153          | 34.6 |      |                 |                            |
| TT                                            | 43          | 25.6 |      |                 |                            | 52           | 36.5 |      |                 |                            |

\*By skin prick test. Significant values are in bold.

20-fold higher concentration of muramic acid, particularly abundant in gram-positive bacteria, was recently found in Russian as compared with Finnish house dust (30). As can be seen from Fig. 1, which relates CD14 genotype with risk of various phenotypes in the two populations, this was indeed the direction found as the

risk of allergy was less in Russians, but risk of wheezing was not.

Of particular interest was the finding of paradoxical gene by environment responses for several allergy phenotypes. For some of these, itchy rash in particular, the CD14 TT genotype conferred the greatest risk among

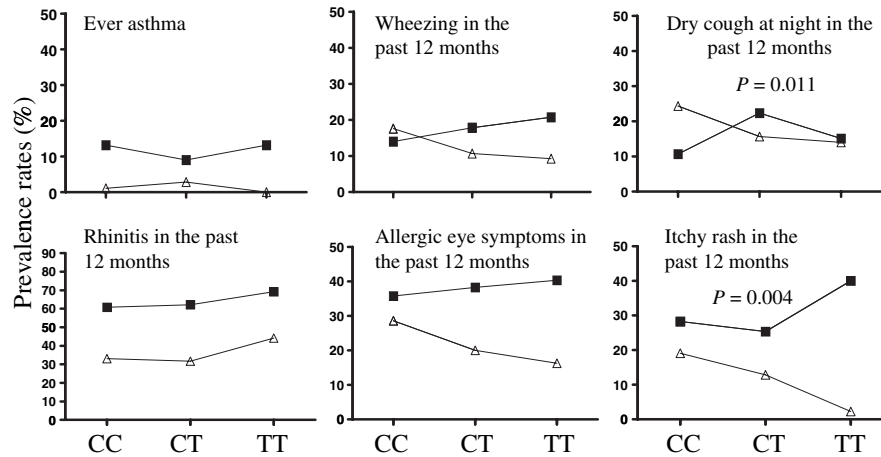


Figure 1. Prevalence rates (%) of asthma/allergy symptoms or conditions in Finnish (closed square) and Russians (open triangle); stratified by the genotypes of *CD14* C-159T; significant *P* values for interaction are presented.

those in Finland, but the TT genotype was associated with the least risk in Russians. A paradox was also found for *CC16* as the AA genotype was associated with the greatest risk of rhinitis and allergic eye symptoms in Finnish subjects, but the least risk for these phenotypes in Russians. Opposite effects of a specific genetic variant in individuals exposed to different environmental risk factors have been reported previously (31), particularly for the *CD14* gene (6, 7) but ours is the first study finding such differences between countries. The mechanisms underlying this discordance are poorly understood. Considering the bidirectional capacity of antigens to modulate allergy and asthma (32) and accumulating evidence for gene by environment interactions (1, 33), the finding of opposite effects of the two SNPs on the asthma/allergy phenotypes in Karelian adult women is biologically plausible. The question remains as to the nature of the risk factors in the 'Western' or 'Eastern' environments that are interacting with the two SNPs in relation to asthma/allergy diseases.

*CD14* plays a pivotal role in innate immune maturation and is thus a prime candidate for influencing gene by environment interactions in allergic diseases (34). The T allele of the functional SNP (*CD14* C-159T) in the *CD14* promoter has been reported to be associated with higher transcription rates than the C allele (35), increased levels of soluble CD14 (21, 28) and decreased total serum IgE (28). Carriers of the T allele were also associated with increased expression of membrane CD14 in the surface of antigen-presenting cells (36). Consistent with its higher gene expression levels, the T allele in combination with endotoxin conferred protection to atopy, which was supported by the findings in the Tucson Children Respiratory Study (CRS) (28). However, no such association was found in the Multicenter Allergy Study (MAS) (37). Furthermore, contrary to the finding in the CRS, a study in a rural population in the northern US

found that the C allele of *CD14* C-159T was protective against allergic sensitization (38).

Gene by environment interactions have been suggested to explain the inconsistencies in the associations of *CD14* C-159T with atopic phenotypes and an endotoxin switch model has been postulated, in which the *CD14* promoter polymorphism changes the threshold at which environmental endotoxin stimulation leads to a TH2 immune response (36). Consistent with this, several population-based and family-based studies (6, 37, 38) showed that the *CD14* C-159T polymorphism had an interactive effect with endotoxin exposure on atopic phenotypes. The T allele of the *CD14* C-159T SNP, with possibly higher expression of CD14 (35), exhibited protective effects on atopy with low exposure to endotoxin, in contrast, being a risk factor with high exposure to endotoxin (7).

In this study, Russian Karelian women with the T allele appeared to be at low risk for allergic eye symptoms and itchy rash < 12 months, contrasting with Finnish women where the T allele was associated with being at increased risk (although not significantly so). As noted above, Russian Karelians in the 'Eastern' environment may have had higher levels of endotoxin exposure, relative to Finnish Karelians in the 'Western' environment. Indeed, recent studies found that Finnish Karelian children had a lower prevalence of microbial antibodies (39), and less exposure to microbial loads in drinking water (40), compared with Russian Karelian children. With regard to the endotoxin switch model, Russian women with the T allele of *CD14* C-159T, potentially exposed to higher levels of endotoxin relative to Finnish women should have an increased risk for atopic phenotypes. However, our study found that in Russian women the T allele was protective against atopic conditions, which is inconsistent with the switch model and indicates the complexity of interactions between environmental exposure to endotoxin and genotypes of *CD14*. Further conflicting with

Table 3. Prevalences and odds ratios (OR) of atopic diseases and symptoms in Karelian women stratified by genotypes of the CC16A38G polymorphism

|                                               | Yes, Russia |      |      |                 |                            | Yes, Finland |      |      |                 |                            |
|-----------------------------------------------|-------------|------|------|-----------------|----------------------------|--------------|------|------|-----------------|----------------------------|
|                                               | <i>n</i>    | %    | OR   | <i>P</i> -value | <i>P</i> <sub>linear</sub> | <i>n</i>     | %    | OR   | <i>P</i> -value | <i>P</i> <sub>linear</sub> |
| Asthma (ever)                                 |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 37          | 0    |      |                 |                            | 45           | 11.1 |      |                 |                            |
| AG                                            | 129         | 3.1  | —    | —               |                            | 141          | 12.1 | 1.10 | 0.86            |                            |
| GG                                            | 108         | 0.9  | —    | —               | —                          | 143          | 10.5 | 0.94 | 0.91            | 0.80                       |
| Wheezing (in the past 12 months)              |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 37          | 16.2 |      |                 |                            | 45           | 17.8 |      |                 |                            |
| AG                                            | 129         | 14.0 | 0.84 | 0.73            |                            | 141          | 18.4 | 1.05 | 0.92            |                            |
| GG                                            | 108         | 10.2 | 0.59 | 0.33            | 0.28                       | 143          | 14.7 | 0.80 | 0.62            | 0.47                       |
| Dry cough at night (in the past 12 months)    |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 37          | 18.9 |      |                 |                            | 45           | 17.8 |      |                 |                            |
| AG                                            | 128         | 18.8 | 0.99 | 0.98            |                            | 141          | 13.5 | 0.72 | 0.48            |                            |
| GG                                            | 108         | 17.6 | 0.91 | 0.86            | 0.82                       | 143          | 20.3 | 1.18 | 0.71            | 0.36                       |
| Rhinitis (ever)                               |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 37          | 27.0 |      |                 |                            | 45           | 82.2 |      |                 |                            |
| AG                                            | 129         | 38.0 | 1.65 | 0.22            |                            | 141          | 68.1 | 0.46 | 0.072           |                            |
| GG                                            | 108         | 49.1 | 2.60 | <b>0.022</b>    | <b>0.012</b>               | 142          | 66.2 | 0.42 | <b>0.045</b>    | 0.082                      |
| Rhinitis (in the past 12 months)              |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 36          | 25.0 |      |                 |                            | 45           | 77.8 |      |                 |                            |
| AG                                            | 129         | 30.2 | 1.30 | 0.54            |                            | 141          | 63.8 | 0.50 | 0.086           |                            |
| GG                                            | 108         | 41.7 | 2.14 | 0.08            | <b>0.032</b>               | 142          | 56.7 | 0.37 | <b>0.013</b>    | <b>0.013</b>               |
| Hay fever (ever)                              |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 36          | 0    |      |                 |                            | 45           | 22.2 |      |                 |                            |
| AG                                            | 128         | 3.9  | —    | —               |                            | 141          | 21.3 | 0.95 | 0.89            |                            |
| GG                                            | 108         | 4.6  | —    | —               | —                          | 143          | 18.2 | 0.78 | 0.55            | 0.47                       |
| Allergic eye symptoms (in the past 12 months) |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 37          | 10.8 |      |                 |                            | 45           | 40.0 |      |                 |                            |
| AG                                            | 129         | 19.4 | 1.98 | 0.23            |                            | 138          | 36.2 | 0.85 | 0.65            |                            |
| GG                                            | 108         | 29.6 | 3.47 | <b>0.03</b>     | <b>0.010</b>               | 142          | 38.0 | 0.92 | 0.81            | 0.95                       |
| Itchy rash (in the past 12 months)            |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 37          | 5.4  |      |                 |                            | 39           | 23.1 |      |                 |                            |
| AG                                            | 128         | 10.9 | 2.15 | 0.33            |                            | 110          | 29.1 | 1.37 | 0.47            |                            |
| GG                                            | 107         | 18.7 | 4.02 | 0.07            | <b>0.024</b>               | 120          | 30.0 | 1.43 | 0.41            | 0.47                       |
| Itchy rash (ever)                             |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 37          | 8.1  |      |                 |                            | 45           | 31.1 |      |                 |                            |
| AG                                            | 128         | 14.1 | 1.86 | 0.35            |                            | 141          | 34.8 | 1.18 | 0.65            |                            |
| GG                                            | 107         | 19.6 | 2.77 | 0.12            | 0.079                      | 143          | 33.6 | 1.12 | 0.76            | 0.88                       |
| Eczema (ever)                                 |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 36          | 5.6  |      |                 |                            | 45           | 22.2 |      |                 |                            |
| AG                                            | 128         | 7.8  | 1.44 | 0.65            |                            | 141          | 27.0 | 1.29 | 0.53            |                            |
| GG                                            | 108         | 10.2 | 1.93 | 0.41            | 0.35                       | 143          | 23.8 | 1.09 | 0.83            | 0.94                       |
| Atopy*                                        |             |      |      |                 |                            |              |      |      |                 |                            |
| AA                                            | 37          | 16.2 |      |                 |                            | 43           | 39.5 |      |                 |                            |
| AG                                            | 129         | 21.7 | 1.43 | 0.47            |                            | 138          | 35.5 | 0.84 | 0.63            |                            |
| GG                                            | 106         | 16.0 | 0.99 | 0.98            | 0.66                       | 140          | 35.0 | 0.82 | 0.59            | 0.64                       |

\*By skin prick test. Significant values are in bold.

our findings, a recent study (41) in adult women was consistent with the switch model and reported that the T allele may be a risk allele with high exposure to endotoxin. However, in this study, the adult women subjects were living in metropolitan Detroit and Michigan and their Western lifestyle environments would be

significantly different from those of the Russian Karelian women.

As previously reported (1, 2, 5, 42), complex interactions between genetic and environmental factors on asthma/allergy are also present in other genes. A study reported an interaction between day care attendance and

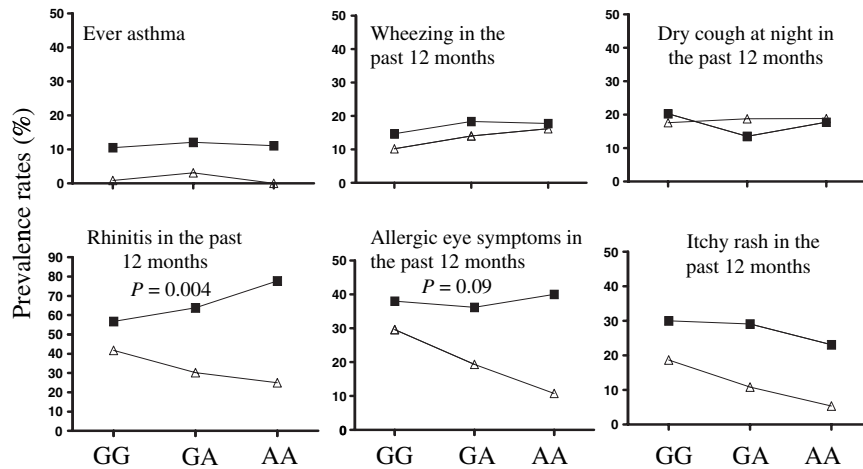


Figure 2. Prevalence rates (%) of asthma/allergy symptoms or conditions in Finnish (closed square) and Russians (open triangle); stratified by the genotypes of *CC16A38G*; significant or marginally significant *P* values for interaction are presented.

several SNPs (*NOS3* Glu298Asp, *FCER1B* Glu237Gly and *IL4RA* Ile50Val) in different genes on immune responses in children (28). In this study, we also found that there was an interaction between *CC16* A38G and the 'Western' or 'Eastern' environment on several phenotypes in Karelian women (Fig. 2). *CC16*, which codes for one of the major secretory proteins of Clara cells, has been associated with airway responsiveness (43), asthma (23) and rhinitis (44). Our data supported the primary hypothesis that the *CC16* gene would interact with the 'Western' or 'Eastern' environment for the asthma/allergy conditions. However, because of a paucity of studies on interactions with *CC16*, the complex mechanism of these interactions still needs to be clarified.

The Karelians living in the province of North Karelia, Finland, had higher prevalence rates of atopic diseases than those in the Pitkäranta district, the Republic of Karelia, Russia (10, 26). As we did not find significant differences in frequencies of the two genotypes between Finnish and Russians, gene by environment interactions, rather than genetic differences, are far more likely to account for the huge gradient in the prevalence rates of atopic diseases between the two populations. On the basis of this study, the interaction effects of the two SNPs of *CD14* and *CC16* genes would explain a relatively small part of the difference and other asthma/allergy candidate genes should also be investigated.

The 'flip-flop' associations between SNPs have been suggested to explain the opposite effects of the given allele at the same locus (45). For complex diseases, with multi-locus genetic effects, the direction of allelic association may flip when the target risk allele is either inversely correlated with another risk allele or positively associated with a protective allele at a different locus (45). In this

study, however, the 'flip-flop' explanation is less likely as there were no significant differences in allele frequencies between the Finnish and Russian Karelians. Although Finnish women were older than the Russian women, the significant *P* values for interaction terms between the environment and *CD14* and *CC16* genes changed only slightly and remained significant (data not shown) after the adjustment for women's age.

Population stratification is a potential limitation of studies comparing different populations. However, Finnish and Russian Karelians subjects were chosen randomly from the general populations and we did not find significant differences in frequencies of the two SNPs. We therefore think it unlikely our results are attributable to population admixture. Another potential limitation is that corrections were not made for multiple testing. However, we have described all statistical analyses carried out and agree with Rothman (46) that there is little to be gained by adjustment, provided that all the analyses are described when the study is reported. In this study, we consistently found significant gene by environment interactions for the two SNPs with several allergic phenotypes in the Karelian population ( $n = 604$ ). Considering that demonstration of interactive effects needs a large sample size (47), our findings are unlikely to be due to multi-comparison.

In summary, this study showed that 'Western' or 'Eastern' environments, together with the two functional SNPs in the *CD14* and *CC16* genes, influenced the development of asthma/allergy in Karelian women, but many of the genetic effects were opposite in the two populations. These findings have major implications for the interpretation of the results of genetic studies from varying environments.

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