Gene Polymorphisms of 22 Cytokines in Macedonian Children with Hyperimmunoglobulinemia E

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Abstract

INTRODUCTION: For some time it is known that cytokines and their receptors are encoded by highly polymorphic genes. These polymorphisms can be responsible for differences in the production of cytokines between individuals. Large number of the polymorphisms within the regulatory regions of the cytokine genes is in correlation with the production and there are variations among populations.

AIM: The aim of this study was to analyze association between polymorphisms in the IFN-gamma, IL-1alpha, IL-1beta, IL-1R, IL-2, IL-4, IL-4R alpha, IL-6, IL-10, IL-12B, TGF-beta1, and TNF-alpha and hyperimmunoglobulinemia E.

MATERIAL AND METHODS: The study included 28 unrelated patients with high IgE levels in serum and the control group consisted of 301 unrelated healthy individuals. Cytokine genotyping was performed with PCR-SSP method. We analyzed the allele frequencies, genotypes, haplotypes and diplotypes of the cytokine genes. The differences were analyzed using \( \chi^2 \) test, odds ratio and Confidence Interval.

RESULTS: Susceptible association with hyperimmunoglobulinemia E was found for four different cytokine alleles (IL-4 -33/T, TGF-beta1 cdn25/C, IL-1 alpha -889/T and TNF-alpha -238/A), ten different genotypes (IL4 -1098/G/G, IL4 -33/T:T, IL-1 alpha -889/C:T, IFN gamma utr5544/A:T, TGF-beta1 cdn25/C:G, IL-6 -174/G:G, IL-1 beta -511/C:T, IL-10 -1082/A:G, TNF alpha -238/A:G and IL-1 beta +3962/C:T) and five different combinations of haplotypes (IL-4-GTT, IL-4/TCT, IL-6/TCC, TNF-alpha/GA and TGF-beta1/CC). Protective association with hyperimmunoglobulinemia E was found in four cytokine alleles (IL-4 -33/C, TGF-beta1 cdn25/G, IL-1 alpha -889/C and TNF-alpha -238/G), three genotypes (IL-10 -1082/A:A, IL-1 alpha -889/C:C and IL-4 -33/C:C) and for only one haplotype (IL-4/GCC).

CONCLUSION: Several susceptible and protective associations between cytokine gene polymorphisms and hyperimmunoglobulinemia E were found. However, it is still speculative whether these polymorphisms contribute to susceptibility/protection from hyperimmunoglobulinemia E or they might be in significant linkage disequilibrium with some unknown gene responsible for the disease. It is also possible that different ethnic groups show different association with cytokine polymorphisms.

Introduction

Hyperimmunoglobulinemia E Syndrome (HIES) was described by Davis and Wedgwood in 1966 [1, 2]. Through the years different groups further characterized the syndrome by reporting immunological and clinical features of Hyperimmunoglobulinemia E and established two forms of the Syndrome: a dominant and a recessive form [3–13].

Minegishi et al. in 2007 found that eight out of fifteen unrelated non-familial HIES patients had heterozygous STAT3 mutations, but their parents and siblings did not have the mutant STAT3 alleles, suggesting that these were de novo mutations. Five different mutations were established, all of which were located in the STAT3 DNA-binding domain. In the patients’ peripheral blood cells they found impaired responses to cytokines, including IL-6 and IL-10. They also discovered that the DNA-binding ability of STAT3 in these cells was very moderate. All of them demonstrated dominant-negative effects when they were co-expressed with wild-type STAT3. These
results emphasize the multiple roles played by STAT3 in humans, and indicate the involvement of multiple cytokine pathways in the pathogenesis of HIES [14]. Also in 2007, Holland et al. reported clinical data from HIES patients and their families. They measured the cytokine levels exuded by stimulated leukocytes. They also measured the gene expression in resting and stimulated cells. In the HIES patients the levels of proinflammatory gene transcripts in neutrophils and mononuclear cells from the peripheral blood were increased and they suggested that there is a defect in the IL-6 signaling pathway. The defect was in the signaling through the downstream mediators, one of which is STAT3. They sequenced the STAT3 gene from the HIES patients and found missense mutations and single-codon in-frame deletions within STAT3. They suggested that eighteen discrete mutations, five of which were hot spots, are involved in affecting DNA binding and SRC homology 2 (SH2) domains. They found that STAT3 mutations are present in sporadic and dominant forms of the hyper-IgE syndrome [15]. From this, the conclusion was made that a dominant form is caused by mutations in STAT3.

In 2009, Zhang et al. reported 11 patients with the AR form of HIES and discovered homozygosity or compound heterozygosity for deletions or mutations in the DOCK8 gene. The protein function was lost and they could not detect the DOCK8 protein itself in primary T-cell cultures or in transformed lymphocyte lines [16, 17]. In continued investigations by Alsum et al., 25 patients with the AR form of HIES were described and three novel DOCK8 mutations and two large deletions in thirteen patients were identified [18].

It is known that cytokines and their receptors are coded by highly polymorphic genes. These polymorphisms are responsible for individual differences which appear in the production of the cytokines and maybe this represents one of the mechanisms which are responsible for the defective Th1/Th2 imbalance. Because of this, in the last years there is an increase of the studies which analyzed polymorphic genes responsible for the cytokines and their receptors [19-21]. Therefore we can expect that cytokine gene polymorphisms will have a great impact in the pathogenesis of the Hyperimmunoglobulinemia E Syndrome.

The aim of this study was to determine if there is an association between genetic polymorphisms of the cytokine genes and Hyperimmunoglobulinemia E.

Healthy individuals. All of the healthy individuals included in this study attended the Institute of Immunobiology and Human Genetics for donation of DNA and signed written consent to participate in the study. There were 301 unrelated individuals. Individuals with family history of allergies and atopy were excluded from the investigation.

Hyperimmunoglobulinemia E. Twenty eight hyperimmunoglobulinemia E patients aged 4 months to 21 years participated in this study that took place during 2006 - 2010 at the Institute of Immunobiology and Human Genetics. The 20 male and 8 female patients exhibited at least three-fold higher IgE levels than normal.

All individuals were of Macedonian origin and nationality, and residents of different regions of the Republic of Macedonia. Each individual was interviewed on a one-to-one basis, his/her genealogy was recorded for the past three generations, and a signed consent was obtained. All of the patients and normal individuals included in this study signed a written consent to participate in the study which was approved by the Committee of the Ministry of Education and Science from Republic of Macedonia (No 087405), and Ethical Committee of the Medical Faculty in Skopje (No 03-5325/2).

Genomic DNA Isolation and Storage

Ten millilitres of blood samples were collected after the signing of the written consent and with the usage of phenol-chloroform extraction method or BioRobot EZ1 workstation (QIAGEN) the DNA was isolated [22]. The quality and quantity of DNA were analyzed by GeneQuant (Pharmacia Biotech, Uppsala, Sweden). The DNA samples were stored in the anthropology project field of the Macedonian Human DNA Bank (hDNAMKD) [23].

Typing Methods

For cytokine genotyping we used commercially available PCR-SSP kit (Heidelberg kit, Cytokine genotyping Tray, Invitrogen, GmbH, Karlsruhe, Germany). Fourteen cytokine genes with 22 single nucleotide polymorphisms (SNP) were typed: IL-1alpha -889, IL-1beta -511, IL-1beta +3962, IL-1R psit1970, IL-1RA mspa11100, IL-4Ralpha +1902, IL-12 -1188, IFNGamma utr5644, TGF-beta1 cdn10, TGF-beta1 cdn25, TNF-alpha -308, TNF-alpha -238, IL-2 -330, IL-2 +166, IL-4 -1098, IL-4 -590, IL-4 -33, IL-6 -174, IL-6 565, IL-10 -1082, IL-10 -819, and IL-10 -592. Briefly, PCR-SSP typing Heidelberg kit consists of 48 PCR primer mixes aliquoted in 96-well PCR trays (two typing per tray). Master mix, which was supplied along with the reagents and consisted of MgCl2, buffer, dNTP’s, and glycerol was mixed with 1.2-3.0 μg DNA and 20 U Taq polymerase and dispensed in 48 wells [24]. Agarose gel

Patients and Methods

Groups

The total studied sample consisted of 329 examinees, divided into healthy individuals, and patients with Hyperimmunoglobulinemia E.
electrophoresis on a 2% gel revealed a positive or negative signal for specific amplification in each well. Subsequently, the results were analysed according to the interpretation scheme provided with the kit.

**Statistical Analysis**

For analysis of the data we used the population genetics analysis package, PyPop, developed by the Biostatistics Core for the Workshop [25]. Allele frequencies and expected Hardy Weinberg proportions (HWP) for each single nucleotide polymorphism (SNP) were determined [25]. The exact test for genotype frequency deviation from HWP was calculated using the Arlequin implementation accessed via PyPop [26]. Those SNPs that did not fit HWP were evaluated to determine whether there was an excess of homozygotes or heterozygotes, or if any particular genotypes significantly differed from the expected frequencies by the chi square test [27]. Comparisons of frequencies for two groups were tested by the χ² test. Crude odds ratios (OR) (as estimates of the relative risk) were calculated with 95% confidence interval (CI).

**Results**

**Cytokine Alleles**

Cytokine allele frequency, Pearson’s p-value, Odds ratio and Wald’s 95% confidence interval in patients with Hyperimmunoglobulinemia E and normal Macedonian population are shown in Table 1. In the region of IL-1 gene cluster, IL-1 alpha -889/T (P=0.002, OR=2.835, Wald’s 95% CI between 1.596 and 5.038) we found positive (susceptible) association with Hyperimmunoglobulinemia E, and negative (protective) association for HIES was found in IL-1 alpha -889/C (P=0.006, OR=0.352, Wald’s 95% CI between 0.199 and 0.627).

In the group of proinflammatory cytokines positive (susceptible) association was found also for TNF-alpha -238/G (P=0.041, OR=0.391, Wald’s 95% CI between 0.154 and 0.992). This means that patients with TNF-alpha -238/A have 2.556 times higher risk (possibility) to develop Hyperimmunoglobulinemia E (P=0.041, Wald’s 95% CI between 1.007 and 6.481) in comparison with those who have TNF-alpha -238/A.

In the group of anti-inflammatory cytokines, positive (susceptible) association with HIES was found for IL-4 -33/T (P=0.001, OR=0.087, Wald’s 95% CI between 2.646 and 14.00), whereas IL-4 -33/G has shown negative (protective) association (P=0.001, OR=0.164, Wald’s 95% CI between 0.071 and 0.377).

### Table 1: Cytokine allele frequency, Pearson’s p-value, Odds ratio and Wald’s 95% confidence interval in patients with HIES and normal Macedonian population.

<table>
<thead>
<tr>
<th>Cytokine Polymorphism</th>
<th>A/G</th>
<th>HIES (n=24)</th>
<th>Control (n=330)</th>
<th>Pearson’s p-value</th>
<th>Odds ratio</th>
<th>Wald 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1 alpha -889</td>
<td>C</td>
<td>56 0.077  462 0.014</td>
<td>0.002</td>
<td>0.352</td>
<td>0.199-0.627</td>
<td></td>
</tr>
<tr>
<td>IL-1 beta -511</td>
<td>T</td>
<td>20 0.335 110 0.185</td>
<td>0.067</td>
<td>2.835</td>
<td>1.596-5.038</td>
<td></td>
</tr>
<tr>
<td>IL-1 beta +3962</td>
<td>C</td>
<td>40 0.075 439 0.012</td>
<td>0.164</td>
<td>0.365</td>
<td>0.199-0.627</td>
<td></td>
</tr>
<tr>
<td>IL-12 +1588</td>
<td>A</td>
<td>33 0.375 145 0.242</td>
<td>0.041</td>
<td>1.332</td>
<td>0.795-2.238</td>
<td></td>
</tr>
<tr>
<td>IL-17A masp1/1100</td>
<td>C</td>
<td>35 0.065 420 0.096</td>
<td>0.020</td>
<td>1.977</td>
<td>1.292-3.049</td>
<td></td>
</tr>
<tr>
<td>TNF -857</td>
<td>G</td>
<td>47 0.135 323 0.512</td>
<td>0.010</td>
<td>1.617</td>
<td>0.929-2.810</td>
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<tr>
<td>TGF-beta1 cdn10</td>
<td>G</td>
<td>33 0.675 185 0.320</td>
<td>0.044</td>
<td>1.811</td>
<td>0.929-3.498</td>
<td></td>
</tr>
<tr>
<td>TGF-beta1 cdn25</td>
<td>C</td>
<td>40 0.075 339 0.012</td>
<td>0.032</td>
<td>1.332</td>
<td>0.795-2.238</td>
<td></td>
</tr>
<tr>
<td>TGF-beta1 +3962</td>
<td>T</td>
<td>20 0.335 110 0.185</td>
<td>0.067</td>
<td>2.835</td>
<td>1.596-5.038</td>
<td></td>
</tr>
</tbody>
</table>

### Cytokine Genotypes

Cytokine genotype frequency in patients with HIES and normal Macedonian population are shown in Table 2. From all genotypes which belonging to IL-1 gene cluster we found that only homozygous C:C genotype in IL-1 alpha -889 is with negative (protective) association with HIES (P<0.001, OR=0.185, Wald’s 95% CI between 0.065 and 0.385), while the heterozygous genotype showed positive association (P=0.001, OR=7.668, Wald’s 95% CI between 3.242 and 18.13). We found positive (susceptible) association between patients with HIES and heterozygous genotypes of IL-1 beta -511 and IL-1 beta +3962 (P=0.009, OR=3.877, Wald’s 95% CI between 1.653 and 9.088, and P=0.031, OR=2.307, Wald’s 95% CI between 1.057 and 5.037). Apart from that, in the IL12/IFN gamma axis, we found that only heterozygous genotypes of IFN gammautr5644 showed positive association with HIES (P=0.005, OR=5.991, Wald’s 95% CI between 1.924 and 16.85).
Table 2: Cytokine genotype frequency, Pearson’s p-value, Odds ratio and Wald’s 95% confidence interval in patients with Hyperimmunoglobulinemia E and normal Macedonian population.

<table>
<thead>
<tr>
<th>Cytokine polymorphism</th>
<th>Gene</th>
<th>HIES (n=28)</th>
<th>Control (n=301)</th>
<th>Pearson’s p-value</th>
<th>Odds ratio</th>
<th>Wald’s 95% CI</th>
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</thead>
<tbody>
<tr>
<td>IL-1beta -314</td>
<td>C/C</td>
<td>8</td>
<td>5</td>
<td>0.074*</td>
<td>0.889</td>
<td>2.019-7.589</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>11</td>
<td>12</td>
<td>0.830</td>
<td>1.770</td>
<td>0.944-3.340</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>19</td>
<td>22</td>
<td>0.623</td>
<td>1.326</td>
<td>0.765-2.327</td>
</tr>
</tbody>
</table>

IL-1β -592

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HIES (n=28)</th>
<th>Control (n=301)</th>
<th>Pearson’s p-value</th>
<th>Odds ratio</th>
<th>Wald’s 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>6</td>
<td>5</td>
<td>0.623</td>
<td>1.326</td>
<td>0.765-2.327</td>
</tr>
<tr>
<td>C/T</td>
<td>6</td>
<td>9</td>
<td>0.623</td>
<td>1.326</td>
<td>0.765-2.327</td>
</tr>
<tr>
<td>T/T</td>
<td>6</td>
<td>16</td>
<td>0.623</td>
<td>1.326</td>
<td>0.765-2.327</td>
</tr>
</tbody>
</table>

IL-1β rs156626

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HIES (n=28)</th>
<th>Control (n=301)</th>
<th>Pearson’s p-value</th>
<th>Odds ratio</th>
<th>Wald’s 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>10</td>
<td>17</td>
<td>0.623</td>
<td>1.326</td>
<td>0.765-2.327</td>
</tr>
<tr>
<td>C/T</td>
<td>6</td>
<td>13</td>
<td>0.623</td>
<td>1.326</td>
<td>0.765-2.327</td>
</tr>
<tr>
<td>T/T</td>
<td>2</td>
<td>11</td>
<td>0.623</td>
<td>1.326</td>
<td>0.765-2.327</td>
</tr>
</tbody>
</table>

Cytokine Haplotypes

Cytokine haplotypes frequency, Pearson’s p-value, crude odds ratio and Wald’s 95% confidence interval in the patients with hyperimmunoglobulinemia E and normal Macedonian population are presented in Table 3. With the Heidelberg kit it was possible to analyze for TGFB1, TGFalpha, IL-2, IL-4, IL-6 and IL-10.

Table 3: Haplotype frequency, Pearson’s p-value, Odds ratio and Wald’s 95% confidence interval in patients with hyperimmunoglobulinemia E and normal Macedonian population.

For the IL-4 gene, we found positive association for the homozygous genotypes IL-4 -1098/G:G (P=0.006, OR=27.272, Wald’s 95% CI between 1.600 and 465.1) and IL-4 -33/T (P<0.001, OR=17.812, Wald’s 95% CI between 5.161 and 61.46) with HIES, and for the homozygous variant of IL-4 -33/C:C (P=0.042, OR=0.314, Wald’s 95% CI between 0.097 and 1.016) we found negative association with HIES.

We found that patients with IL-10 -1082/A:G have positive association with HIES (P=0.007, OR=3.706, Wald’s 95% CI between 1.333 and 10.305), while IL-10 -1082/G:G genotype is with negative association (P=0.033, OR=0.303, Wald’s 95% CI between 1.117 and 5.831) in comparison to patients who have the rest of the IL-10 genotypes. We also found that patients with genotype IL-6 -174/G have 4.633 times higher possibility to develop HIES (P=0.033, OR=2.552, Wald’s 95% CI between 1.523-14.09) than the patients with the others IL-6 genotypes.

Positive association with HIES was also found for the heterozygous genotypes of TGFB1 cdn25 and TGF-alpha -238 (P=0.001, OR=5.560, Wald’s 95% CI between 2.132 and 14.49, P=0.013, OR=3.296, Wald’s 95% CI between 1.215-8.941).

Susceptible and protective cytokine polymorphisms for hyperimmunoglobulinemia E

Summary of all susceptible and protective cytokine polymorphisms for hyperimmunoglobulinemia E in Macedonian population are presented in Table 4.
We can see that most of the cytokine genotypes (ten of them) and cytokine haplotypes (five of them) have shown positive association with hyperimmunoglobulinemia E, but only three cytokine genotypes and only one cytokine haplotype have negative association. Positive association was found for four cytokines alleles. Negative association was also found in four cytokines alleles.

**Table 4: Summary of all susceptible and protective cytokine polymorphisms for hyperimmunoglobulinemia E in Macedonian population.**

<table>
<thead>
<tr>
<th></th>
<th>Susceptible Polymorphisms</th>
<th>Protective Polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine Alleles</td>
<td>IL-10-1082A/G</td>
<td>IL-10-1T/1T</td>
</tr>
<tr>
<td>Polymorphism</td>
<td>-0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>E-Data Rate</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td>Cytokine Genotypes</td>
<td>IFN-gamma +874 A: T</td>
<td>IL-12 +238 G: C</td>
</tr>
<tr>
<td>Polymorphism</td>
<td>-0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>E-Data Rate</td>
<td>0.016</td>
<td>0.016</td>
</tr>
</tbody>
</table>

**Discussion**

Previous studies of the cytokine polymorphisms in Macedonian population have shown association with a number of diseases [28-32]. In this paper we present our results of 22 cytokine polymorphisms in patients with HIES and in normal Macedonian population. To our knowledge, these are first results about the cytokine polymorphism in the world.

We examined the role of two single nucleotide polymorphisms of the IL-1 beta gene (-511 C/T and +3962 C/T), one polymorphism of IL-1 alpha gene (-889 C/T), one polymorphism of IL-1R gene (pgs1970 C/T) and one polymorphism of the IL-1RA gene (mspa11100 T/C) in the pathogenesis of hyperimmunoglobulinemia E. The results have shown that the genes from the IL-1 gene cluster are associated with the hyperimmunoglobulinemia E, meaning that persons who have IL-1 alpha -889/T allele have three times higher susceptibility for hyperimmunoglobulinemia E than those who have IL-1 alpha -889/C allele. Regarding the genotypes, persons who are heterozygotes for IL-1 alpha -889 have eight times higher possibility to develop hyperimmunoglobulinemia E unlike the heterozygotes for IL-1 beta -511 and IL-1 beta +3962 where the susceptibility is 3.8 or 2.3 times. Our investigation didn’t show any association between hyperimmunoglobulinemia E and IL-1R and IL-1RA polymorphisms (alleles or genotypes).

IL-12, as part of the IL-12/IFN gamma axis, plays an important role in the host defence from the intracellular pathogens [33, 34]. The results from the polymorphisms of IL-12 and their role is still unclear. It was shown that additionally to the polymorphisms located in the promoter region also the polymorphism in the 3'UTR is in correlation with the intensity of the secretion of the protein [35, 36].

We examined the possible role of polymorphisms in the 3'UTR of the IL-12B gene in the pathogenesis of hyperimmunoglobulinemia E. The results showed that there is no significant difference in allele frequencies or in the genotypes, suggesting that 3'UTR polymorphisms have none or negligible effect in the pathogenesis of hyperimmunoglobulinemia E in the Macedonian population. A possibility remains that there is some role of 3'UTR polymorphisms in the pathogenesis of hyperimmunoglobulinemia E through its association with another functional polymorphism in the IL-12/IFN gamma axis [37, 38].

IFN gamma is also a participant in the IL-12/IFN gamma axis, in addition to IL-12, IL-12R and the receptor for IFN gamma. He is one of the most important Th1 cytokines that participate in the host defense established through the activation of macrophages [39]. IFN gamma plays a key role in defense against viruses and intracellular organisms. The analysis of the data found significant differences only for IFN gamma +874 / A: T genotype. Individuals who are heterozygous for this polymorphism have almost six times higher chances to develop hyperimmunoglobulinemia E. The possible significance of this polymorphism arises from the fact that this polymorphism is in significantly unbalanced relationship with the other two polymorphisms of the IFN gamma gene, and it is known that they are associated with certain diseases [40].

It is believed that the role of TGF-beta1 is associated with its anti-inflammatory and profibrotic activities, such as regulation of growth and differentiation of cells, release of cytokines and angiogenesis, production of extra-cellular matrix and restoration of tissues [41, 42] and the creation of elastin [43, 44]. The results showed an association with hyperimmunoglobulinemia E only for TGF-beta1 cdn 25 polymorphism. We found protective association only for TGF-beta1 cdn 25/G allele, while the /C, /C:G genotype and /CC haplotype showed negative association with hyperimmunoglobulinemia E.

Tumor - necrosis factor alpha (TNF-alpha) is a proinflammatory cytokine that acts synergistically with IFN gamma in the activation of the macrophages [45]. TNF-alpha has a role in airway remodeling and alters the function of smooth muscle cells [46], and because of that it is considered as a major factor in the genesis and the maintenance of the lung damage in patients with certain respiratory system diseases [47, 48]. Our results showed association between TNF-alpha -238 A/G polymorphism and hyperimmunoglobulinemia E. TNF-alpha -238/A allele,
/A:G genotype and /GA haplotype showed negative association with hyperimmunoglobulinemia E, while TNF-alpha -238/G showed protective association. The question remains whether this association represents an independent effect of TNF-alpha or it is a result of his association with the HLA-A1, B17 and DR7 [49].

Interleukin 2 is a cytokine produced by T cells during an immune response. It can be also produced by eosinophils and epithelial cells from the respiratory system. It stimulates the growth, differentiation and survival of number of immune cells [50]. In our study we did not find any significant association between allele frequencies, genotypes and haplotypes in the IL-2 -330 and IL-2 +160 polymorphisms and hyperimmunoglobulinemia E.

Interleukin - 4 (IL-4) is a multifunctional Th2 cytokine, which reduces Th1 cell response. IL-4 may also stimulate the production of mucus, as well as hyperplasia of the goblet cells in the bronchial submucosa [51]. In this study we examined alleles frequency, genotypes and haplotypes in three polymorphisms in the IL-4 gene at positions -1098 , -590 and -33. Results showed positive association between IL-4 -33/C allele, /C:C genotype and /GCC and hyperimmunoglobulinemia E. Analysis of the frequencies showed negative association between IL-4 -33/T allele and hyperimmunoglobulinemia E, which increases from 6 times to 17.8 times in homozygous T genotype, to 47.3 times in TCT haplotype and 52 times in GTT haplotype. Only /G:G genotype from IL-4 -1098 polymorphism showed significant association. People who have this genotype have 27.2 times greater chance to develop hyperimmunoglobulinemia E in comparison with the individuals who are carriers of the other genotypes. For the alleles, genotypes and haplotypes in IL-4 -590 and IL-4R alpha +1902 polymorphisms with didn’t find any associations with hyperimmunoglobulinemia E.

It is known that IL-6 plays an important role in the initial phase of the innate immune response [52, 53]. We investigated the association of two polymorphisms in the IL-6, -174C/G and nt565 A/G. The results showed that IL-6 -174/G:G genotype and IL-4/GA haplotype are associated with hyperimmunoglobulinemia E (positive).

IL-10 is a possible anti-inflammatory Th2 cytokine which inhibits the replication of on macrophages/ monocytes and T lymphocytes, reduces the production of the proinflammatory cytokines in the inflammatory responses [54-57] and inhibits the expression of the co-stimulatory molecules and the molecules of class 2 of MHC on the macrophages surfaces [58]. We examined the association between 3 single nucleotide polymorphisms in the region of the gene promoter for IL-10 (-1082 A/G, -819 C/T and -592 A/C) and hyperimmunoglobulinemia E. We analyzed the polymorphisms and we did not find any significant association between the investigated alleles and hyperimmunoglobulinemia E. But the analysis of the genotypes showed positive association between IL-10 -1082/A:A genotype (homozygous for the A allele) and the heterozygous genotype of the same polymorphism with hyperimmunoglobulinemia E.

Although the mechanisms which are associated with polymorphisms which makes changes in the gene expression are still not well known, today we known that hyperimmunoglobulinemia E is partly under polygenic control. The role inn the pathogenesis of hyperimmunoglobulinemia E is played not only by the large number of alleles located on different genes and even on the chromosomes, but also by the interaction between the genes and surrounding. We still don’t know whether the polymorphisms alone by themself contribute for protection/susceptibility of hyperimmunoglobulinaemia E. Because of this it is possible that various ethnic groups will show different associations with cytokine polymorphisms.

The number of patients in our study is very small. In the association studies, there are possibilities that some positive results might be spurious and some negative findings might be a consequence of low statistical power. It could be due to their small sample size or methodological shortcomings, such as the selection of an appropriate control group. It is necessary to investigate cytokine gene polymorphisms in our population in well defined subgroups of phenotypes with bigger number of participants in order to have more precise conclusions for genetic background of development of hyperimmunoglobulinemia E in Macedonians. However, multicentre studies and/or meta-analysis of the patients with hyperimmunoglobulinaemia E and association with cytokine polymorphisms should be very useful, as it was shown before with our published data about cytokine polymorphism associations [59-61].

The results of this study for the association of the cytokine polymorphisms with hyperimmunoglobulinaemia E in the Macedonians showed positive association with four alleles, 10 genotypes and 5 haplotypes and negative associations with four alleles, 3 genotypes and 1 haplotype. The results can be used for future meta analyses of the association of cytokine polymorphisms with patients with hyperimmunoglobulinemia E.

References
3. Hill, H. R., Quie, P. G. Raised serum-IgE levels and defective neutrophil chemotaxis in three children with eczema and...


