Abstract

Background. The IL-4 receptor alpha-chain locus is a candidate gene for atopy and allergic disease. Several polymorphisms have been investigated within the gene; however, the results are ambiguous. Polymorphism in the promoter region of the gene was suggested to be crucial for asthma and atopy susceptibility.

Objectives. The aim of this study was to evaluate the association of the C−3223T polymorphism in the promoter region and the I50V polymorphism in the coding region of the IL−4Rα gene with susceptibility to atopy and atopic diseases.

Material and Methods. Children diagnosed with atopic disease and having a positive IgE−dependent response (SPT, specific IgE, total IgE) and children with no features of atopy serving as a control group were recruited. The patients and controls were genotyped for both polymorphisms, C−3223T and I50V.

Results. The presence of the C−3223T mutant allele corresponded with atopy status, whereas such a relationship was not found for I50V.

Conclusions. These results imply that C−3223T may play an important role in the pathogenesis of atopy and atopic diseases (Adv Clin Exp Med 2007, 16, 6, 735–741).

Key words: atopy, genetic polymorphism, IL−4 receptor.

Streszczenie

Wprowadzenie. Gen dla łańcucha α receptora IL-4 jest idealnym kandydatem na gen podatności na astmę i atopowe choroby alergiczne. Polimorfizm regionu promotorowego genu jest sugerowany jako kluczowy czynnik determinujący podatność na astmę i atopię.

Cel pracy. Ocena asocjacji polimorfizmów C-3223T i I50V z podatnością na atopię i atopowe choroby alergiczne w grupie dzieci.

Material i metody. Do badania włączono dzieci atopowe z rozpoznaniem atopowej choroby alergicznej i pozytywnym miernikiem odpowiedzi IgE−zależnej (PTS, IgE specyficzne, IgE całkowite) oraz dzieci bez cech atopii, które stanowiły grupę kontrolną. Porównano dystrybucję genotypów i alleli dla obu polimorfizmów C-3223T i I50V.

 Wyniki. Wykazano statystycznie istotną różnicę w rozkładzie genotypów i alleli dla C-3223T, podczas gdy zależności tego rodzaju nie udało się wykazać dla I50V.


Słowa kluczowe: atopia, polimorfizm genetyczny, receptor IL-4.
During the last 30 years, the incidence of atopic diseases, including asthma, has increased considerably. This is considered to be caused mainly by changes in environmental factors, although atopy is undoubtedly a genetically determined feature, as shown by several research projects based on genome scanning, positional cloning, and association analysis. Despite enormous efforts by researchers, the question of which genes determine susceptibility to atopy and atopic diseases remains open. The IL-4 receptor \( \alpha \)-chain gene, located on chromosome 16 (16p12.1-p11.2) \[1\] represents an excellent candidate gene for such research. The alpha chain of the IL-4 receptor is an important element in the transmission pathway of a signal induced both by IL-4 and IL-13, two key immunological factors with regard to allergic processes. Therefore, the search for polymorphisms in the gene’s functional regions has been of great interest to researchers since at least 1990, when the first polymorphism, characterized by an isoleucine shift to valine at 50 position (I50V) was described \[2\]. A substantial number of reports have been published to date, but the results thereof are, unfortunately, inconsistent. The majority of the studies were limited to the search for possible mutations related to atopy and atopic diseases in the gene’s coding region. However, in more recent studies it was suggested to extend the search to the intron regions and the gene promoter \[3\]. In 2001, a new polymorphism (C-3223T) was described in the promoter region of the IL-4 receptor alpha-chain gene. A relationship between C-3223T and decreased level of the soluble receptor form of the IL-4 receptor (sIL-4R), a regulatory particle of ambivalent character, was found as well. The promoter polymorphism represents a potentially important factor in regulating gene function since it disrupts the sequence for transcription factors, such as ATF, TF11, CREB, and AP-1. Moreover, it was shown that C-3223T is in linkage disequilibrium with another SNP, I50V, in the gene’s coding region, for which some studies showed an association with atopic asthma \[4\]. The aim of this study was to evaluate the association of the C-3223T and I50V polymorphisms with susceptibility to atopic diseases in children.

**Material and Methods**

**Study Population**

The patient group consisted of 40 children (42.5% girls and 57.5% boys) with an average age of 9.25 years (range: 3–16 years). Their detailed medical histories were recorded. The qualification criteria for the patients were the occurrence of allergic disease symptoms such as asthma, allergic rhinitis, or atopic dermatitis within at least 12 months prior to the study. Most of the children had symptoms of more than one allergic disease. The patients also fulfilled at least one of the following inclusion criteria: positive results of skin prick tests to at least one common environmental allergen (Allergopharma) and/or an increased level of specific IgE of at least 0.7 kU/l induced by common allergens and/or an increased level of total IgE (≥ 100 IU/ml). The patient group was regarded as an atopic group due to the inclusion criteria. For the control group, 36 children (47.22% girls and 52.77% boys) with an average age of 11.14 years (range: 2–17 years) were considered. The qualification criterion for the control group was an absence of allergic symptoms in the patient’s and his/her family’s past medical history. All the children were recruited from the Department of Pediatrics, Allergy, and Cardiology at Wroclaw Medical University. The clinical profiles of the two groups are shown in Table 1. The study was approved by the local ethics committee.

**Genotyping**

DNA was extracted from peripheral blood leukocytes following standard protocols (DNAmini-kit, Qiagen). The determination of the C-3223T and I50V polymorphisms of the IL-4 receptor alpha-chain gene was performed by SSP-PCR according to the protocol described by Hackstein \[5\].

**Statistical Analysis**

Genotype and alleles frequencies were compared using the chi-squared (\( \chi^2 \)) test or Fisher’s exact test. Logistic regression models were used to analyze associations with age, sex, and genotype as co-variables. Because of the low prevalence of homozygous variant genotypes, these genotypes were grouped together with heterozygous genotypes for logistic regression. The homozygous genotype with the higher percentage was used as the reference. Linkage disequilibrium (D’) between SNP loci was measured according to Lewtin \[6\]. A \( p \) value of 0.05 or less was regarded as significant.

**Results**

**Genotype and Allele distribution of IL-4R\( \alpha \) Polymorphisms**

The distribution of the genotypes for the C-3223T and I50V polymorphisms was compliant with Hardy-Weinberg equilibrium. To assess the associa-
tion with susceptibility to atopy, the frequencies of the genotypes and alleles for the two IL-4Rα polymorphisms C-3223T and I50V in atopic children and non-atopic controls were compared. Based on statistical analysis, considerable differences in the distributions of the C-3223T alleles ($\chi^2, p < 0.01$) and genotypes (CT + TT vs. CC, $\chi^2, p < 0.001$) were demonstrated. Logistic regression showed that subjects with a mutated allele (CT + TT) presented an almost sevenfold greater risk of atopy than subjects in whom the CC genotype was found (Tab. 2).

There were no statistically significant differences in the distributions of the genotypes and alleles between the atopic and the control groups for the I50V polymorphism (Tab. 3). No linkage disequilibrium was found between I50V and C-3223T ($p = 0.2$).

**Association Between Atopy-Related Phenotypes and the IL-4Rα Polymorphisms**

In the next stage, associations between the genotypes and alleles for both the tested polymorphisms and the occurrence of a specific disease, the type of allergen sensitization, and the place of residence (urban or rural) were analyzed. The greatest association was found for C-3223T genotypes and allergic rhinitis and asthma (Fisher’s test, $p < 0.01$). The highest frequency of TT homozygotes was observed in the group of asthmatic patients compared with other diagnoses (22.73%). The greatest risk related to the CT+TT vs. CC genotype was noted for allergic rhinitis. No statistically significant results were obtained for atopic dermatitis.

Considering the type of allergen sensitization, the greatest association was shown for the CT+TT genotype and allergy to pollen and house dust mites (Fisher’s test, $p < 0.001$). In logistic regression, the greatest risk was obtained for the CT + TT vs. CC genotype and allergy to animal fur (OR 19.55), for house dust mites (OR 8.75) and pollen (OR 7.11) (Tab. 4).

Considering the place of residence, a greater association of C−3223T genotype and atopy was found in urban children (Fisher’s test, $p < 0.01$) than in rural inhabitants (Fisher’s test, $p < 0.05$). Conversely, a higher risk for atopy was obtained in the rural group in logistic regression, but the model was not statistically significant.

For I50V, no statistically significant differences were noted in the distributions of genotypes and alleles between the study groups with regard to the character of allergic disease diagnosis, allergen sensitization type, and place of residence.

**Discussion**

Several studies related to the chromosome 16 region (16p12.1-p11.2) have broadened the knowledge of IL-4Rα participation in atopic disease pathogenesis, but the results obtained do not present an unambiguous answer as to the genetic determination of atopy. None of the three studies based on genome scanning demonstrated an asso-
The present study, based on a case-control analysis, demonstrated an association between C-3223T IL-4Rα gene variations and atopy status \((p < 0.001)\). Children carrying the T-3223 mutation (homozygotes and heterozygotes) revealed an almost sevenfold greater risk of occurrence of any atopic disease. In the next stage, the analysis was continued by a more precise definition of the phenotype and narrowing down the calculations to the diagnosis of a specific atopic disease or sensitization to specific allergens. Comparing the frequencies of the genotypes and alleles in the patient groups defined in this way and in the control group, similar results were obtained \((p < 0.01)\). The highest risk was noted for allergic rhinitis, whereas no statistically significant differences in genotype distribution were found for atopic dermatitis. Considering sensitization to a specific allergen group, the highest risk, associated with the C-3223T genotype, was demonstrated for allergy to animal fur, then for house dust mites and pollen, but in the first case the small size of the group induces some caution. No statistically significant differences in the frequencies of the genotypes and alleles for C-3223T were observed between controls and children sensitized for food allergens and fungi.

It was also decided to include the environmental effect, defined as the place of residence, rural or urban, within the least five years, in this analysis. However, the influence of this factor was hard to interpret due to the small size of the group of children living in a rural area.

Few studies have been carried out to date regarding C-3223T polymorphism in relation to susceptibility to atopic diseases. In 2004, a Swedish study related to haplotypes consisting of 3 SNPs (C-3223T, Q551R, I50V) confirmed a relationship between TVR haplotype \((T-3223, V50, R551)\) and atopic asthma. The patients who were
homo- and heterozygous for T, V, and R alleles also showed a milder form of asthma and a decreased level of sIL-4R. For the T-3223 allele itself, a statistically significant relationship with decreased sIL-4R serum level and a more active form of the disease was observed. Asthmatic patients revealed higher serum levels of sIL-4 than the control group [8]. In the next study considering C-3223T, 61 SNPs were analyzed within 35 genes potentially related to an immunological phenotype corresponding to allergic diseases. The evaluation included the association between selected polymorphisms and IL-5, IL-10, IL-13, and IFN-γ production, infant wheezing, occurrence of atopic eczema in the first year of life, concentration of total IgE, eosinophils, and sensitization in the first year of life. However, a significant role of C-3223T was not demonstrated [9]. In two studies related to atopic dermatitis, an association between susceptibility to this disease and the occurrence of C-3223T alleles was confirmed. Special attention should be paid to a report by Japanese authors who undertook functional research related to the IL-4Rα promoter. In their project they identified six polymorphisms by sequencing, for which five, including C-3223T, an association with atopic dermatitis was demonstrated. In the subsequent stage they analyzed haplotypes, also confirming a relationship with this disease entity. Lastly, evaluating the influence of polymorphisms on promoter transcription activity in several independent experiments, they did not find such dependence. Interesting is the result of computer analysis for the promoter, the aim of which was to define binding sites for transcription factors. The result of this study indicated the T-3223 allele as the most probable binding region for GATA-3, a factor that is responsible for the differentiation of Th2 lymphocytes [10]. In another study related to atopic dermatitis it was shown that the C-3223T polymorphism is characteristic only for eczema in atopic patients. No association was confirmed in the patients with non-atopic eczema [11].

The coupling disequilibrium between C-3223T and I50V described by Hackstein [4] and presented in numerous studies as a polymorphism revealing a correlation with susceptibility to asthma and atopy helped to decide on a further analysis considering this correlation. However, after comparing the distributions of the genotypes and alleles of the I50V polymorphism between the atopic and control groups, statistically significant associations with atopy status, diagnosis of a specific allergic disease, or a particular type of sensitization was still not found. The results were also not influenced by consideration of sex, family history of allergic diseases, and place of residence.

I50V is one of the most “popular” polymorphisms, especially with regard to functional importance. In two extensive studies, Mitsuyasu et al. [12, 13] confirmed an association between the polymorphism and atopic asthma, increased level of total IgE, and allergy to house dust mites. They also recognized the functional significance of SNP. In 1999, Tan et al. [14] made an attempt to replicate the results of the Japanese scientists in a study based on association analysis, locating a study group within the three main ethnic groups.

### Table 4. Association between C-3223T genotypes and atopy-related phenotypes (asthma, allergic rhinitis, atopic dermatitis, sensitization to particular allergens); odds ratios (OR) were calculated by logistic regression

<table>
<thead>
<tr>
<th>Diagnosis of allergic disease (Rozpoznanie choroby alergicznej)</th>
<th>OR (95% CI)#</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma (Ahma) n = 22</td>
<td>6.99 (1.87–26.08)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Allergic rhinitis (Alergiczny nieżyt nosa) n = 24</td>
<td>7.58 (2.07–27.79)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Atopic dermatitis (Atopowe zapalenie skóry) n = 8</td>
<td>4.88 (0.77–30.98)</td>
<td>0.18</td>
</tr>
<tr>
<td>Type of allergen sensitization (Rodzaj alergenu) House dust mites (Kurz) n = 26</td>
<td>8.75 (2.37–32.75)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pollen (Pyłki) n = 29</td>
<td>7.11 (2.12–23.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fur (Sierść zwierząt) n = 12</td>
<td>19.55 (2.03–188.24)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fungi (Grzyby) n = 8</td>
<td>5.25 (0.8–33.93)</td>
<td>0.11</td>
</tr>
<tr>
<td>Food (Pokarm) n = 5</td>
<td>6.07 (0.5–74.38)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* logistic regression model CT + TT vs. CC, statistically significant values are bolded, p < 0.05.
* χ² test or Fisher’s exact test, CT + TT vs. CC model.
* regresja logistyczna model CT + TT vs CC, pogrubioną czcionką zaznaczono wyniki statystycznie istotne p < 0.05.
* test χ² lub dokładny test Fishera model CT + TT vs CC.
of Singapore. Unlike their predecessors, they showed that the I50 allele occurred with a lower frequency in the atopic group than in the control group, while V50 was quite common in the first group. Similarly, Ober [3] demonstrated that I50 is transmitted with considerably less frequency to ill subjects than the mutated V50 allele. In the year 2000, Japanese authors carried out a functional study of transfectants and peripheral blood mononuclear cells loaded with the first or the second variant, evaluating CD23 expression and IgE production after stimulation with IL-4. In the I50 transfectants the level of STAT6 activation and e-chain transcription was higher, similarly to I50 in PBMCs, where the expression level of CD23 and IgE was increased. The authors demonstrated a statistically significant difference between the allele distributions in healthy subjects and atopic asthmatics in the tested population. Such a relationship was not found for non-atopic asthma. The morphisms of the interleukin-4 receptor gene: the dichotomy of extrinsic and intrinsic atopic dermatitis reflected by combined analysis of monocyte immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin-13 receptor gene: the dichotomy of extrinsic and intrinsic atopic dermatitis. J Invest Dermatol 2004, 122, 843–845.

References


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