Association of Vitamin D Receptor Gene Polymorphisms in Children With Atopic Diseases
Research Article

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Summary
Interaction of environmental factors and the gene polymorphisms has been shown to increase the risk of resulting allergic diseases. In addition to vitamin D forming the cellular immune response, polymorphisms of vitamin D receptors (VDR) has been shown to contribute to asthma and atopy. Our aim is to show the VDR gene polymorphisms in children in our region with atopic constitutions. A total of 46 children between 2-16 years with diagnosis of atopic dermatitis were included in the study. Blood samples were used to investigate polymorphisms of VDR genotypes FokI C>T (rs2228570), ApaI A>C (rs7975232), BsmI G>A (rs1544410) and TaqI C>T (rs731236). Comparing heterozygous mutation of FokI and ApaI in patients with those with no mutation (wild), the atopy risk was increased 2.13 and 3.33 times, respectively (p<0.05). In addition comparing patients with ApaI heterozygous and homozygote mutation with wild patients, the atopy risk was increased 2.88 times (p<0.05). The role of VDR gene polymorphisms in development of atopy is not fully understood. We believe that different approaches to the timing and amount of vitamin D added to the diet, especially of children and individuals at risk of atopy, should be developed and this may help reduce the incidence of asthma and atopy in the population.
I. Introduction:
Genetic polymorphisms are important markers allowing us to determine individual differences that influence susceptibility to the certain diseases. While some gene polymorphisms (alleles) increase the risk of disease, some reduce it. Some polymorphic alleles are known to only affect the risk of disease under the influence of certain environmental factors. It has been shown that the mutual interaction of environmental factors and mixed gene groups plays a role in the development of allergic diseases such as asthma, allergic rhinitis and atopic dermatitis. Widespread genome analysis of the long arm of the 12th chromosome has identified a region that is linked to phenotypes related to asthma and allergies (Raby BA, et al., 2003).

Vitamin D, a basic actor in the metabolism of bone and calcium, also forms the cellular immune response and has an important role in cell proliferation (D’Ambrosio D, et al., 1998; Lemire JM, et al., 1995; O’Kelly J, et al., 2002; Lee J, et al., 2011; Dombrowski Y, et al., 2010; Schaubler J and Gallo RL, 2008; Frieri M and Valluri A, 2011). Vitamin D receptor (VDR) is a receptor connecting mainly 1α,25-dihydroxyvitamin D3, and is an important molecule in calcium metabolism and bone turnover. Structurally it shows similar properties to steroid and thyroid hormone receptors (Baker AR, et al., 1988).

Poon A, et al. (2004) in a study of people living in the eastern Canadian region of Quebec reported a relationship between sensitivity to asthma and atopy and VDR gene polymorphisms and haplotypes. Their findings show that VDR gene polymorphisms contribute to asthma and atopy. Early exposure to dietary or additional vitamin D supplements was estimated to be a risk factor for later allergies and asthma (Wjst M, 2004; Milner JD, et al., 2004; Wjit M and Dold S, 1999). Vitamin D receptor polymorphisms have been widely researched for relationships to a variety of diseases (Gyorffy B, et al., 2002; Park BS, et al., 1999; Simmons JD, et al., 2000). However in Turkey, where atopy is widespread, there was no study found on VDR gene polymorphisms and allergic diseases. The aim of our study is to show the VDR gene polymorphisms in children in our region with atopic diseases.

II. Materials and Methods:
The study began after permission was granted by Canakkale 18 Mart University (COMU) School of Medicine Clinical Research Ethics Committee. A total of 46 children, 2-16 years old, monitored by the Pediatric Health and Disease, and Dermatology clinics of COMU School of Medicine Research and Application Hospital with diagnosis of atopic dermatitis, asthma, allergic rhinitis and allergic conjunctivitis who agreed to participate were included in the study. The patient group all had atopic dermatitis diagnosis according to the criteria of Hanifin JM and Rajka G (1980). A control group of 96 individuals was chosen from cases without personal or family history of atopy.

Blood samples were collected in tubes containing EDTA. Genomic DNA was extracted from whole blood using the DNA extraction kit (Fermentas). Vitamin D receptor gene (VDR) genotyping for four different polymorphic sites (FokI C>T (rs2228570), Apal A>C (rs7975232), BsmI G>A (rs1544410) and TaqI C>T (rs731236)) was completed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Primer’s sequences are shown in Table 1.

The PCR reaction volume was 50µl. 25 µl Dream Taq Green PCR master mix (2X), was mixed with 1 µl Forward primer (0.2 µM) 1 µl reverse primer (0.2 µM), 5 µl DNA sample, and 18 µl water. PCR condition was performed at 95 °C for 3 min (initial denaturation), 30 cycles at 95 °C for 30 s, Tm-5 for 30 s, 72 °C for 1 min and 72 °C for 5 min (final extension).
PCR products were digested with BsmI, FokI, ApaI and TaqI restriction enzymes. Enzyme digestion reaction was completed with 17 µl water, 2 µl 10X Fast digest Green buffer, 10 µl PCR product and 1 µl enzyme.

The BsmI, FokI, and ApaI enzymes were incubated at 37°C for 5 min; TaqI enzyme was incubated at 65 °C for 5 min.

The digested PCR products were identified by ethidium bromide staining of separated fragments in a 2% agarose gel electrophoresis.

The BsmI product “b” allele fragments had size of 648bp and 175bp (wild type) and “B” allele fragments had size of 823bp (mutant), FokI “F” allele fragments were 273bp (wild type) and “f” allele fragment size was 198bp and 75bp (mutant), ApaI “G” allele fragments size was 531bp, 214bp (wild type), “T” allele fragments size was 745bp (mutant) and TaqI “T” allele fragments size of 496 bp, 249 bp (wild type), while “C” allele fragment size was 293, 251, and 201bp (mutant).

The results determined the homozygous wild, and heterozygous and homozygous mutant genotypes and allele frequencies were calculated. To compare the genotypes and allele frequencies of the patient group with the control group, the Odd’s ratios and p values were calculated. Results with p values less than 0.05 were accepted as statistically significant.

### III. Results

A total of 46 pediatric patients, 22 male and 24 female, were included in the study. The average age was 9.45±8.53 (2-16). All of the patients had a diagnosis of atopic dermatitis and 13 had allergic rhinitis, 4 had allergic conjunctivitis, 10 had allergic asthma, 4 had allergic rhinitis+allergic conjunctivitis, 10 had allergic rhinitis+allergic asthma, 1 had allergic conjunctivitis+allergic asthma and 4 had allergic-rhinitis + allergic - conjunctivitis + alle-rgic asthma respectively.

For all children the BsmI, FokI, ApaI and TaqI mutations were examined. The evaluation results of homozygote, heterozygote and normal (wild) genotypes of these polymorphisms are given in Table 2.

In terms of FokI comparing patients with heterozygous mutation with those without mutation (wild), those with heterozygous mutation had a risk of atopy increased 2.13 times (odds ratio: 2.1386).

As the p value of the result was less than 0.05, it was statistically significant. In terms of ApaI patients with heterozygous mutation had a 3.33 greater risk of atopy than wild genotyped patients (odds ratio: 3.3333) (p<0.05).

In addition for patients with ApaI heterozygous and homozygous mutations the atopy risk was increased 2.88 times compared to wild genotyped patients (odds ratio: 2.88) (p<0.05).
Table 2: Allele frequencies of patient and control group for VDR gene polymorphism genotype.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Patient Group</th>
<th>Allele Frequency</th>
<th>Control Group</th>
<th>Allele Frequency</th>
<th>Comparison of Mutant Allele Carries versus Homozygous Wild Individuals</th>
<th>Comparison of Heterozygous Individuals versus Homozygous Wild Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild Heterozygous Mutant</td>
<td>FF</td>
<td>19</td>
<td>41.3</td>
<td>F</td>
<td>0.67</td>
<td>0.33</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>FF</td>
<td>23</td>
<td>50</td>
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<td>0.33</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>FF</td>
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<td>0.39</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>BB</td>
<td>10</td>
<td>21.7</td>
<td>B</td>
<td>0.39</td>
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<td></td>
<td></td>
<td></td>
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<td>TT</td>
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<td></td>
<td></td>
<td>TC</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
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<td></td>
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<td>12</td>
<td>46.9</td>
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</table>

IV. Discussion:

VDR gene polymorphisms are known to be responsible for a variety of immune and inflammatory diseases as diabetes, psoriasis and Crone’s disease. (Gyorffy B, et al., 2002; Park BS, et al., 1999; Simmons JD, et al., 2000). One of the factors blamed for development of allergic diseases is VDR gene polymorphisms. In our study of children with atopy, the ApaI and FokI polymorphisms, especially, were significantly high.

Comparing the VDR gene polymorphisms of atopic pediatric patients in the population of Canakkale with healthy controls, the most significant result was for ApaI polymorphism with both heterozygote and homozygote mutant genotypes having risk increased by 2.8-3.3 times and this risk being identified as statistically significant.

In this situation the FokI and TaqI polymorphisms (odds ratio clearly high but p value greater than 0.05) the mutant allele frequency is clearly high compared with healthy controls which may indicate that the allele frequency is insufficient in the population due to the number in the patient group.

For BsmI polymorphism comparing in homozygous, heterozygous and wild patients the odds ratio is less than 1: 0.3962, 0.5177 and 0.7479, respectively. In other words these values show that the polymorphism in BsmI is protective against atopy. However it is not statistically significant due to our insufficient patient number.

Poon A, et al. (2004) in a study of VDR gene polymorphisms BsmI, ApaI and TaqI from asthma and atopic patients in Canada found results parallel to our own. The difference in their study was that all three polymorphisms were statistically significant, due to their larger case number. Raby BA, et al. (2004) in a study of asthmatic children and their families in Germany reported that the ApaI polymorphism was significant in the Caucasian group.
In our study the ApaI polymorphism was statistically significant leading us to believe that among the 4 VDR gene polymorphisms (BsmI, FokI, ApaI and TaqI) for atopy and asthma the most important is ApaI.

In the Chinese han population a study of VDR gene only analyzed BsmI and FokI and no polymorphic genotype was found to be statistically significant with odds ratio of 1.44 and 1.15 for BsmI and FokI respectively (Fang WL, et al., 2009).

In our results the allele frequencies for VDR gene polymorphisms in our population were similar to the Caucasian population and differed from the Chinese population. Just like other genetic studies in our study, Turkish population was found similar to Caucasian population than Chinese population.

Heine G, et al. (2013) in a study of adult patients with atopic dermatitis found the BsmI G, ApaI C and TaqI T alleles were higher than healthy controls. The results are parallel to our study, while the FokI polymorphism was not found to be significant for adult atopic dermatitis it was significant in our study. Studies in the literature mainly focus on VDR gene polymorphism and asthma showing that more studies are required especially for atopy in the pediatric age group.

Makishima M, et al. (2002) showed that vitamin D receptor is also a receptor for the secondary bile acid, lithocholic acid. This may be related to the itching complaint of polymorphic patients. In addition the critical role of VDR in immunoregulation may be an important factor in susceptibility of patients with inherited polymorphism to asthma and atopy.

In conclusion the inherited polymorphic variations in vitamin D receptors may be added to the factors that affect atopy with roles played by polymorphic genetic factors and environmental factors. The role of VDR gene polymorphism in development of atopy is not fully understood.

We believe that different approaches to the timing and amount of vitamin D added to the diet, especially of children and individuals at risk of atopy, should be developed and this may help reduce the incidence of asthma and atopy in the population. Suspicious environmental factors must also be evaluated in further studies.

Understanding etiology of asthma and atopic diseases is very important for treatment and prevention. Illumination of genetic basis is not only important for etiology but also very critic for determining risky population and personalized medicine.

Further and detailed studies are needed to explain the role of VDR gene in atopic diseases with large amount of case and control numbers.

References:


