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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; Schauber 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 halotypes. 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), *ApaI* 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).

Table 1: Primary sequences.

	Forward Primer	Reverse Primer
BsmI	*5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3'*	*5'-AACCAGCGGAAGAGGTCAAGGG-3'*
FokI	*5'GATGCCAGCTGGCCCTGGCACTG3'*	*5'ATGGAAACACCTTGCTTCTTCTCCCTC-3'.*
ApaI and *TaqI*	*5'- AGAGCATGGACAGGGAGCAAG-3'*	*5'-GCAACTCCTCATGGCTGAGGTCTCA-3'*

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.

Gene	Polymorphism	Genotype	Patient Group			Allele Frequency FQ		Control Group		Allele Frequency FQ		Compherison of Mutant Allele Carriers versus Homozygot Wild Individuals			Compherison of Heterozygous Individuals versus Homozygot Wild Individuals		
			Genotype	Number of Individuals	%	Allele		Number of Individuals	%	Allele	FQ	Odds Ratio	95% CI	P value	Odds Ratio	95% CI	P value
VDR	FokI	Wild Heterozygous Mutant	FF Ff Ff	19 23 4	41.3 50 8.7	F f	0.67 0.33	53 30 13	55.2 31.3 13.5	F F	0.70 0.30	1.75	0.8598 to 3.5681	0.122	**2.13**	**1.0053 to 4.5493**	**0.048**
	BsmI	Wild Heterozygous Mutant	bb Bb BB	0 36 10	0 78.3 21.7	b B	0.39 0.61	0 70 26	0 72.9 27.1	b B	0.36 0.64	0.74	0.3252 to 1.7198	0.494	0.51	0.0101 to 26.6296	0.743
	ApaI	Wild Heterozygous Mutant	GG GT TT	5 28 13	10.9 60.9 28.2	G T	0.41 0.59	25 42 29	26 43.8 30.2	G T	0.48 0.52	**2.88**	**1.0264 to 8.1225**	**0.044**	**3.33**	**1.1403 to 9.7440**	**0.027**
	TaqI	Wild Heterozygous Mutant	TT TC CC	16 23 7	34.8 50 15.2	T C	0.60 0.40	45 39 12	46.9 40.6 12.5	T C	0.67 0.33	1.65	0.7995 to 3.4235	0.174	1.6587	0.7689 to 3.5779	0.197

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 *BmsI* 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 *BmsI* 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, el., 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 *TagI 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.

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