Vitamin D receptor gene polymorphisms in Turkish children with vitamin D deficient rickets

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Vitamin D deficient rickets is prevalent in Turkey and a considerable number of children are at risk of growth retardation, impaired bone formation and fracture. In order to check whether vitamin D receptor (VDR) gene polymorphism relates to the vitamin D deficient rickets, we analyzed VDR gene FokI, TaqI and ApaI polymorphisms in 24 Turkish vitamin D deficient rickets patients and 100 healthy controls. We found that "A" (ApaI) allele is more abundant in patients than controls (83 vs 57%, p=0.002) but there were no significant differences for FokI (p=0.693) and TaqI (p=0.804) allele frequencies between patients and controls. We also showed that the frequency of Tt and Aa genotypes was significantly decreased in patients. Our results indicated that VDR gene polymorphisms might be an important factor for genetic susceptibility to vitamin D deficient rickets in the Turkish population.

Key words: vitamin D receptor gene polymorphisms, vitamin D deficient rickets.

Vitamin D deficiency rickets is prevalent today in many developing countries while it is on the rise in the developed ones. Hence, Turkey is of no exception, with a frequency of rickets of 6% among children under three years according to one recent study. Not only infants but also a considerable number of adolescent girls, pregnants and nursing mothers are at risk of vitamin D deficiency in Turkey[1].

Among the factors responsible for the high prevalence of vitamin D deficiency in developing countries and its resurgence in developed countries are: limited sunshine exposure due to individuals' spending more time indoors to watch television and work on computer or their avoiding sunshine intentionally for fear of air pollution and skin cancer development. Traditional clothing (covered dress) further limits the exposure time to sunshine and thus decreases the endogenous synthesis of vitamin D[1,2].

Vitamin D regulates calcium and phosphate homeostasis in the body and has a positive impact on bone mineralization[3]. The most active form of vitamin D (a steroid hormone), 1,25 $(OH)_2$ D, exerts its effect on target tissues through the vitamin D receptor (VDR). VDR is a ligand-dependent transcription factor and belongs to the steroid hormone receptor superfamily. The liganded VDR results in dimerization of the receptor and it forms homodimers or heterodimers with one of the retinoid X receptors (RXRa, RXRb, and RXRg). VDR homodimers or VDR-RXR heterodimers bind to specific enhancer elements, referred to as vitamin D response element, and activate target gene transcription[4,5]. Many tissues contain VDR and thus 1,25 $(OH)_2$D is expected to affect these tissues and cells like epidermis, macrophages, prostate, breasts, pancreas, and parathyroid glands[6,7].

The emerging field in nutrition science, so-called nutritional genomics (nutrigenomics), draws attention to the fact that development of certain conditions of diseases may be linked to the polymorphisms that individuals carry. Presence of certain polymorphisms renders the host susceptible for certain diseases even in the presence of recommended intake of

the offending nutrient. Whether this is so for vitamin D and calcium is not yet clear. There have been some studies conducted in Africa indicating a possible link between certain VDR polymorphisms and calciopenic rickets[8].

The VDR protein is encoded by the VDR gene, which is linked to 12q13.1. VDR gene is about 100kb, consists of 9 exons and has highly polymorphic sites. Several polymorphisms in the VDR gene have been reported so far, including FokI, TaqI and ApaI. FokI, which is a translation start codon polymorphism, is located in exon 2, and due to the T to C transition, translation initiates 3 amino acid downstream of the first ATG. The other polymorphism, which is localized in exon 9, is TaqI and ATT codon is converted to ATC, but either of them encodes isoleucine amino acid. ApaI is an intronic polymorphism, which is a G/T transition, localized in intron 8[9,10].

In this study, we therefore determined VDR genotypes (FokI, TaqI and ApaI) of 24 Turkish children diagnosed with vitamin D deficiency rickets and compared the allelic frequencies of these polymorphisms with those of normal children.

Material and Methods

The present study included 24 children with vitamin D deficient rickets ranging in age from 3 to 32 months and 100 children with no history of rickets as controls. Patients were recruited from the outpatient pediatric clinics of Atatürk University Faculty of Medicine, Erzurum, a province located in the East of Turkey with a high frequency of rickets.

The diagnosis of rickets was confirmed both biochemically and radiologically in children who presented to the clinics with a different combination of signs and symptoms like tetany, craniotabes, rachitic rosary, Harrison's groove, delayed closure of anterior fontanelle, delayed dentition, enlarged wrists and bowed legs.

Individual's peripheral blood was obtained and genomic DNA was extracted from leukocytes by salting out method. Exon 2 and intron8/exon9 of the VDR gene were amplified by polymerase chain reaction (PCR). PCR products were digested with FokI (37°C, 1.5h), TaqI (65°C, 1h) and ApaI (37°C, 2h) and subjected to electrophoresis in 3% agarose gel. Digested fragments were visualized after staining with ethidium bromide. VDR genotypes of each subject were identified according to the digestion patterns.

Distribution of VDR genotypes and allelic frequencies of patients were calculated and compared with healthy individuals (controls) using chi-square (χ^2) test. Conformance to Hardy-Weinberg equilibrium was assessed using χ^2 test as well. P-values less than 0.05 were considered to indicate statistical significance.

Results

The present study was performed among 24 rickets patients and 100 healthy individuals. The allelic frequencies of FokI, TaqI and ApaI are shown in Table I. "F", "T", "A" indicate the absence and "f", "t", "a" the presence of digestion sites for FokI, TaqI and ApaI, respectively. Our findings indicate that "A" (ApaI) allele is more abundant in patients than controls (83 vs 57%, p=0.002). However, there were no significant differences for FokI (p=0.693) and TaqI (p=0.804) allele frequencies between patients and controls.

Table I. VDR Allelic Frequencies of Patients and Controls

Polymorphism	Alleles	Patients (n:24)	Controls (n:100)	P values
FokI	F	0.77	0.73	0.693
	f	0.23	0.27	
TaqI	T	0.56	0.59	0.804
	t	0.44	0.41	
ApaI	A	0.83	0.57	0.002
	a	0.17	0.43	

Table II shows the distribution of VDR genotypes between patients and controls. We observed that the most common genotypes were FF, TT, AA in patients and FF, Tt, Aa in controls. The frequency of TT, tt and AA genotypes was higher in patients than controls, while the frequency of Tt and Aa was significantly decreased in patients (p<0.001). In contrast, the genotype distribution of FokI was not significantly different between patients and controls (p=0.793).

The genotype distributions of all polymorphisms in controls and FokI in patients were in Hardy-Weinberg equilibrium. There was a departure from the equilibrium for TaqI and ApaI polymorphisms.

Table II. Distribution of VDR Genotypes

Group	N	Genotypes					
		n	%	n	%	n	%
		FF		Ff		ff	
Patients	24	15	63	7	29	2	8
Controls	100	55	55	36	36	9	9
		TT		Tt		tt	
Patients	24	13	54	1	4	10	42
Controls	100	35	35	49	49	16	16
		AA		Aa		aa	
Patients	24	19	79	2	8	3	13
Controls	100	30	30	55	55	15	15

Discussion

Recently, there have been many efforts to investigate an association between VDR polymorphisms and several diseases in different populations[11-13]. In our study, we report VDR gene polymorphisms (FokI, TaqI and ApaI) in vitamin D deficient rickets for the first time in Turkey.

Multiple polymorphic variations exist in the VDR gene, each of which could have different types of consequences. VDR polymorphisms can affect VDR mRNA/protein level, stability, translation efficiency and protein-protein interactions[14]. More than 25 different polymorphisms are currently known to be present at the VDR gene and most are in/near the regulatory areas rather than coding sequences. The most studied polymorphisms include FokI, TaqI and ApaI.

FokI is an exonic polymorphism, which leads to T/C transition, and variant alleles generate two VDR gene products that differ in length by three amino acids. Some studies have shown that "F" allele and FF genotype may be more advantageous for bone mineralization[15]. However, we did not observe differences in the frequencies of "F" allele and FF genotypes between patients and controls. In contrast to our results, Lu et al.[16] reported that "F" alleles and FF genotypes were more common in patients suffering from vitamin D deficient rickets. Likewise, Fischer et al.[8] indicated that "F" allele was more abundant in rickets.

TaqI and ApaI polymorphisms are localized in 3' regulatory region and are in linkage disequilibrium with 3'UTR. TaqI is another exonic polymorphism that does not affect the amino acid sequence of encoded protein. We demonstrated a significant increase in TT and tt and decrease in Tt genotypes in patients. However, Fischer[8] and Kaneko et al.[17] reported that neither allele nor genotype frequencies of TaqI were significantly different among rickets and controls in Nigeria and Mongolia populations, respectively. ApaI, which is an intronic polymorphism, affects neither splicing site nor transcription factor binding site. We showed that the frequencies of "A" allele and AA genotype were increased and Aa genotype was decreased in rickets compared to controls; however, Wei-Ping et al.[18] indicated that the distribution of ApaI polymorphism between rickets and controls was balanced. Although these polymorphisms seem to be non-functional, they can be used as a marker to detect a functional allele due to the linkage disequilibrium. The 3'UTR region of the VDR gene is involved in the regulation of gene expression, so these polymorphisms may play an important role in mRNA stability. It is possible that different allelic frequencies and VDR genotypes among populations can occur due to the gene-gene and gene-environment interactions[10,19].

We demonstrated that VDR gene polymorphisms might be an important factor for genetic susceptibility to vitamin D deficient rickets in the Turkish population. Further studies will be needed to determine the functional consequences of different VDR alleles. Considering the ethnic background of the patients in different countries, it may be normal to find different allelic frequencies among patients. If a causal relationship could be established between certain polymorphisms and vitamin D deficient rickets, carriers of these particular polymorphisms might be supplemented with more vitamin D

(more than the recommended daily dose of 200-400 IU) and calcium in order to prevent the development of poor bone mineralization and rickets (personalized dietetic approach of nutrigenomics).

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