

Research article

## Vitamin D receptor gene polymorphisms in nephrotic syndrome children

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### Abstract

**Background:** Vitamin D mechanism of action is organized by vitamin D receptors (VDRs), which are affected by diverse genetic polymorphisms, comprising FokI, ApaI, TaqI and BsmI restriction fragment length polymorphisms. It has been declared to be associated with several diseases. The aim of our study was to define the frequency and the association of VDR gene polymorphic variants in Egyptian children with nephrotic syndrome (NS). **Subjects and methods:** This study included 93 children classified as 50 children with nephrotic syndrome and 43 healthy children as control subjects. The four VDR polymorphic sites (FokI, ApaI, TaqI and BsmI) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (RFLP) in EDTA blood. Serum vitamin D (25(OH)D<sub>3</sub>), parathyroid hormone (PTH), intact parathyroid hormone (iPTH) and fibroblast growth factor-23 (FGF-23) concentrations were assayed by ELISA (enzyme linked immunosorbent assay). **Results:** Genotype and allele frequencies were compared among nephrotic syndrome patients and controls. There was no significant difference ( $p > 0.05$ ) at three polymorphic sites FokI, ApaI and TaqI, except at BsmI a significant correlation was observed ( $P = 0.013$ ). There was significant correlation between ApaI and albumin /creatinine ( $p = 0.016$ ) in NS. Vitamin D and PTH levels were significantly lower in NS than controls ( $p = 0.001$ ) and ( $p = 0.006$ ) respectively, where iPTH and FGF-23 levels were significantly higher in NS ( $p = 0.002$ ) and ( $p = 0.047$ ) respectively. There were significant variances in the frequency of four-marker haplotype alleles in NS cases and controls. **Conclusion:** Our results revealed that BsmI VDR gene polymorphism may have a significant role in children having nephrotic syndrome.

### Introduction

Nephrotic syndrome (NS) is the most common glomerular disorder in children; it is characterized by massive proteinuria [1], hypoalbuminemia resulting into edema, and hypercholesterolemia, usually responding to steroids [2]. Nephrotic children frequently exhibit disturbances of mineral metabolism, such as deficiency of circulating vitamin D (1,25(OH)<sub>2</sub>D<sub>3</sub>) and hypocalcemia, leading to secondary hyperparathyroidism and decreased bone mineral density. It is usually associated with hyperphosphatemia, and elevated fibroblast growth factor-23 (FGF-23) formed by osteoblastic cells and ends up to renal phosphate wasting [3].

Parathyroid hormone (PTH) and FGF-23 can conquer the expression of type II sodium-phosphate cotransporters (are secondary active transporters, which means they use an electrochemical gradient as a means of energy) in the proximal tubule of the kidney and reduce serum 1,25-

dihydroxy D<sub>3</sub> (active form of vitamin D). In addition, FGF-23 inhibits PTH and 1 $\alpha$ -hydroxylase expression, thus reduces vitamin D levels. Vitamin D and its metabolites are conveyed in the circulation through binding to a plasma protein, vitamin D binding protein (DBP), which shares many structural and evolutionary similarities with albumin [4].

Vitamin D is an essential fat-soluble vitamin that has a major role in skeletal mineral deposition and regulation of hormones like insulin, renin, FGF, and PTH production [5-7]. Intracellular, vitamin D acts through the vitamin D receptor (VDR), a nuclear transcription factor to which vitamin D binds through the carboxyl-terminal ligand-binding domain. Genetic sequence encoding VDR may differ producing polymorphic forms similar to the variants formed on digestion with restriction enzymes like ApaI and TaqI. A subset of VDR polymorphic variants are linked with decreased vitamin D activity and may result in increasing risk and tendency to several bone and

endocrinal disorders, like osteoporosis, rickets, urolithiasis, and also diabetes mellitus type 1 [8-12].

Vitamin D receptor (VDR) is an intracellular hormone receptor, which binds precisely to vitamin D active formula. It intermingles with target-cell nuclei and produces multiple biological effects for example immunity, growth, bone mineralization, cells differentiation, and plays different functions in different body organs. Some studies reported the significance of BsmI restriction enzyme polymorphism of the vitamin D receptor (VDR) gene and the polymorphisms of the gene in diabetes [13, 14]. Nephrotic syndrome pathogenesis up till now is indecisive and debatable and is thought to be arbitrated via the immune system. Frequent polymorphisms found in the VDR gene were reported to be associated with many skeletal as well as non-skeletal diseases [15]. As VDR acts as a potent immunomodulator and NS pathogenesis has been associated with biochemical derangements resulting into metabolic disease.

BsmI and ApaI are proved to be silent single nucleotide polymorphisms (SNPs) which have no irregularities in the coding amino acids sequence like in FokI, however they may disturb gene expression by regulating mRNA stability [16]. The Cdx2 polymorphism which is a changing of guanine (G) to adenine (A) in the promoter section of the VDR gene, exactly at the binding site for a specific transcription factor in the intestine known as Cdx2. The A allele interacts to the Cdx2 transcription factor with an increased affinity and produces high transcriptional activity. As a result, the A allele may exaggerate VDR expression in the intestine, and consequently in an increased bone mineral density through an improved intestinal absorption of calcium [16].

The aim of this study was to define the frequency and the association of Vitamin D receptor (VDR) gene polymorphic variants in children suffering from nephrotic syndrome.

## Subjects and methods

### Subjects

The present study included 93 children, 50 children with primary nephrotic syndrome recruited from Pediatric Nephrology Clinic at Children's Hospital, Cairo University, Egypt and 43 healthy children from the same population with no history of renal diseases as control subjects. They were age, sex, and ethnically matched.

Inclusion criteria of NS cases were the presence of nephrotic-range proteinuria (protein excretion of more than 40 mg/m<sup>2</sup>/h), generalized edema, hypoalbuminemia (serum albumin <3 gm/dl) and hypercholesterolemia (serum cholesterol>200 mg/dl). Urine albumin /creatinine ratio>2. Patients with impaired kidney function, those

with end stage renal disease (ESRD) on dialysis and the secondary NS were excluded from the current study.

Each NS patient was subjected to full medical history taking focusing on frequency of relapses, response to steroid therapy, and need of other immunosuppressive or cytotoxic drugs. Physical examination of all included cases and control children focusing on body mass index (BMI) calculated by the formula [BMI= weight (kg) / height (m<sup>2</sup>)], and blood pressure measurement. Renal biopsies of included patients were reviewed and NS cases were categorized into two subgroups based on their pathological findings [minimal-change nephrotic syndrome (MCNS & focal segmental glomerulosclerosis (FSGS)].

### Ethical considerations

Informed written consent was obtained from all the children's guardians. The study had been approved by the Ethical committee of National Research Centre (NRC) in Egypt (Approval No.10010008).

### Collection and processing of samples

Approximately 5–8 ml of blood was collected from all the study subjects in appropriate tubes for subsequent laboratory analyses. Serum was isolated by centrifugation and was used for the biochemical analyses. For genotyping, the blood was collected in tubes containing the anticoagulant EDTA and were stored at -20°C till DNA extraction for genotyping.

### Biochemical and laboratory parameters

Biochemical assessments were done for all cases and controls including serum creatinine, albumin, cholesterol, calcium, phosphorus and alkaline phosphatase using the automated clinical chemistry analyzer (Olympus AU 400 analyzer-Germany) as well as complete blood picture using CoulterT890 (Coulter Counter, Harpenden, UK). Urine albumin /creatinine ratio was determined for all study subjects.

### Extraction of genomic DNA

DNA was extracted using QIAamp DNA Blood Mini Kits -50- Catalog no. 51104 supplied by (Qiagen GmbH, Germany). DNA concentration was determined by NanoDrop 2000c Spectrophotometer (Thermo Fisher).

### Genotyping of VDR gene polymorphisms

VDR genotyping of 50 NS patients was performed based on polymerase chain reaction-restriction fragment length polymorphism (RFLP). Following amplification, products were digested with restriction enzymes BsmI, ApaI, TaqI and FokI.

Amplification of DNA by real-time polymerase chain reaction using the Quantistudio 12 Flex (Applied Biosystems, CA 94404, USA). Amplified PCR products

were genotyped for TaqI A/G (rs731236): assay C\_2404008\_10; FokI A/G (rs2228570): assay C\_12060045\_20; BsmI C/T(rs15444410) assay C\_8716062\_10 and ApaI A/C (rs7975232) using the TaqMan genotyping RFLP (Applied Biosystems, Foster City, CA, USA). Polymorphisms were detected according to the digestion pattern generated for the amplified DNA fragment using the restriction enzymes [37].

The PCR reaction was set up in a 20 µl reaction volume including 20-30ng DNA, primers and 10 µl TaqMan Universal PCR Master Mix in 96-well PCR plates. The PCR assay was carried out according to manufacturer's instructions including one step of 10 min at 95°C followed by 40 cycles of DNA denaturation at 95°C for 15s and annealing/extension at 60°C for 1 min. Final products were analyzed by TaqMan Genotype software.

#### **Assay of FGF-23, PTH, iPTH and 25-hydroxyvitamin D in serum**

Enzyme linked immunosorbent assay (ELISA) was used to measure the concentration of serum FGF-23 by UsCan Life kit Cat.no. #ED746h (Ray Biotech, inc., USA);PTH and iPTH intact by DRG International, Inc.,(USA). (EIA-3645); and 25(OH) D<sub>3</sub> (total) by ELISA kit Cat # EIA-5396, DRG Diagnostics GmbH, (Germany).

#### **Statistical analysis**

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests [17].Correlations between quantitative variables were done using Spearman correlation coefficient [18]. Logistic regression was done to detect odds ratio (OR) and 95% confidence interval (CI) of genotypes and alleles [19], we made a new variable by multiplying the genes polymorphisms. Two-sided p-values less than 0.05 were considered as statistically significant.

#### **Results**

This study included 50 children with nephrotic syndrome (NS), 32 of them were males (64.7%) and 18 were females (35.3%); mean age was (8.21 ± 3.54 years). All NS cases were on steroid therapy at assessment. 18 children (36%) of NS cases were steroid resistant NS (failed of response after 4 weeks of daily oral steroid

therapy of 60 mg/ m<sup>2</sup> /day followed by three pulses of methylprednisolone). Twenty children (40%) of the studied NS cases were on cytotoxic drugs in addition to steroid therapy. Seventeen patients had frequent relapsing NS (had two consecutive relapses or two of four relapses in any 6 months period). Eighteen patients (36%) were on calcium channel blocker as antihypertensive therapy (not tabulated). Clinical characteristics and biochemical markers of the nephrotic patients and healthy controls were evaluated. Serum calcium, phosphorus, alkaline phosphatase, albumin, and urine albumin/creatinine ratio showed highly significant difference between the healthy and nephrotic children groups (Table1).

#### **VDR's ApaI restriction site polymorphism**

The allele frequency for the A and C alleles (rs7975232) of ApaI were 67(68.8%) and 31(31.2%) respectively in the patient group and 58(67.4%) and 28(32.6%) in the control group. There was no significant difference between allele frequency (p>0.05) and genotype frequency (p>0.05) between NS and control groups.

#### **VDR's TaqI restriction site polymorphism**

The allele frequency for the G and A (rs731236) of TaqI were 70(71.9%) and 28(28.1%) in the patient group and 45(52.3%) and 41(47.7%) in the control group. There was no significant difference in genotype frequency (p>0.050); as well as in the allele frequency (p>0.05) between NS and control groups.

#### **VDR's FokI restriction site polymorphism**

In children with nephrotic syndrome, incidence of G and A alleles (rs2228570) of FokI were 58(59.4%) and 40(40.6%) respectively, while 50(58.1%) and 36(41.9%) in the control group. There was no significant difference in genotype frequency (p>0.05) and allele frequency (p>0.05) between NS and control groups.

#### **VDR's BsmI restriction site polymorphism**

Frequencies of the alleles T and C (rs15444410) of BsmI were 43(43.8%) and 55(56.2%) in NS patient and 29 (33.7%) and 57 (66.3%) in the control group. Our results revealed that the risk of the disease was increased in CT (mutant) genotype of BsmI and there was significant difference (p=0.013) between NS and control groups (Table 2).

To see the cumulative effect, we analyzed our data of the VDR gene among patients and controls in different combinations of genes. There was a strong significance between BsmI and ApaI or BsmI and TaqI polymorphisms (Table 3).

**Table1. Clinical characteristics and biochemical markers of patients and controls.**

	NS Patients (N=50) Mean $\pm$ SD	Healthy controls (N=43) Mean $\pm$ SD	P value
Age (years)	8.21 $\pm$ 3.54	10.0 $\pm$ 03.81	0.211
Gender: Male n (%)	32(64.7%)	25.3 (58.8%)	0.451
Female n (%)	18(35.3%)	17.7 (41.2%)	
BMI (kg/m <sup>2</sup> )	22.06 $\pm$ 5.66	20.60 $\pm$ 1.44	0.1034
SBP (mmHg)	109.41 $\pm$ 12.36	95.54 $\pm$ 9.70	< 0.0001*
DBP (mmHg)	71.47 $\pm$ 11.15	61.55 $\pm$ 10.10	< 0.0001*
Albumin (g/dL)	3.04 $\pm$ 1.14	4.92 $\pm$ 0.39	< 0.0001*
Creatinine (mg/dL)	0.45 $\pm$ 0.09	0.53 $\pm$ 0.33	0.321
Calcium (mg/dL)	8.89 $\pm$ 0.98	10.91 $\pm$ 0.40	< 0.0001*
Phosphorus (mg/dL)	4.45 $\pm$ 0.62	3.90 $\pm$ 0.58	< 0.0001*
Cholesterol (mg/dL)	215.7 $\pm$ 45.9	172.7 $\pm$ 14	< 0.0001*
ALP (U/L)	296.7 $\pm$ 84.7	138 $\pm$ 59.3	< 0.0001*
White blood cells [ $\times 10^3$ /mm <sup>3</sup> ]	8.79 $\pm$ 2.56	3.57 $\pm$ 1.42	< 0.0001*
Hemoglobin (g/dL)	12.45 $\pm$ 1.52	14.20 $\pm$ 1.50	< 0.0001*
Platelets [ $\times 10^3$ /mm <sup>3</sup> ]	378 $\pm$ 110.46	325 $\pm$ 109.25	0.236
Urine alb. /creat. (mg/dL)	3.30 $\pm$ 5.60	1.90 $\pm$ 0.2	< 0.0001*

BMI (body mass index), SBP (systolic blood pressure), DBP (diastolic blood pressure), ALP (alkaline phosphatase), alb/creat. (albumin/ creatinine ratio).

All data are expressed as mean $\pm$ SD

\*P value <0.05 was considered significant.

**Table 2. The count and the percentage (%) of allele frequency BsmI in cases and control groups.**

		Patients	Control	P value	OR	95% CI	
		Count (%)	Count (%)			lower	Upper
Bsm I	TT	15 (31.2)	3(7.0)		Wild		
	CT	12 (25.0)	23 (53.5)	<b>0.013*</b>	0.104	0.018	0.620
	CC	22 (43.8)	17 (39.5)	0.103	0.247	0.046	1.326
	Allele T	43 (43.8)	29 (33.7)		Wild		
	Allele C	55 (56.2)	57 (66.3)	0.316	0.654	0.285	1.499

\* Significant correlation at P<0.05.

**Table3. VDR gene among patients and controls.**

		Patients		Control		P value
		Count	%	Count	%	
TaqI=T1 BsmI=B1	TaqI & BsmI	9	18.8%	3	7.0%	0.330
TaqI=T1 BsmI=B0		19	37.5%	13	30.2%	0.595
TaqI=T0 BsmI=B1		6	12.5%	0	0.0%	0.070
TaqI=T0 BsmI=B0		15	31.3%	27	62.8%	<b>0.031*</b>
BsmI=B1 ApaI=A0	BsmI & ApaI	15	31.3%	3	7.0%	<b>0.028*</b>
BsmI=B0 ApaI=A1		3	6.3%	6	14.0%	0.661
BsmI=B0 ApaI=A0		31	62.5%	34	79.1%	0.312

\*Significant value (p<0.05).

0=mutant + heterozygous, 1=wild type genotype

As shown in table (4), serum vitamin D, PTH levels were significantly lower in NS than controls ( $11.55 \pm 5.4$  vs  $27.85 \pm 17.1$ ) ( $p=0.001$ ), ( $25.3 \pm 12.2$  vs  $52 \pm 32.1$ ) ( $p=0.006$ ) and iPTH, FGF-23 levels was significantly higher in NS ( $88.69 \pm 39.03$  vs  $22.34 \pm 9.72$ ) ( $p=0.002$ ), ( $32.47 \pm 36.84$  vs  $19.32 \pm 14.28$ ) ( $p=0.047$ ) than controls.

The results of the relation between genotypes and other parameters of patients revealed a significant correlation between FokI gene and alb/creat. ( $p=0.01$ ) and cholesterol ( $p=0.032$ ). There was also significant correlation between ApaI gene and alb./creat. ( $p=0.016$ ) and there was significant correlation between albumin and cholesterol ( $p=0.007$ ), iPTH and albumin ( $p=0.027$ ) in NS. The distribution of the frequency of four-marker haplotype alleles (VDR-FokI, VDR-BsmI, VDR-ApaI, and VDR-

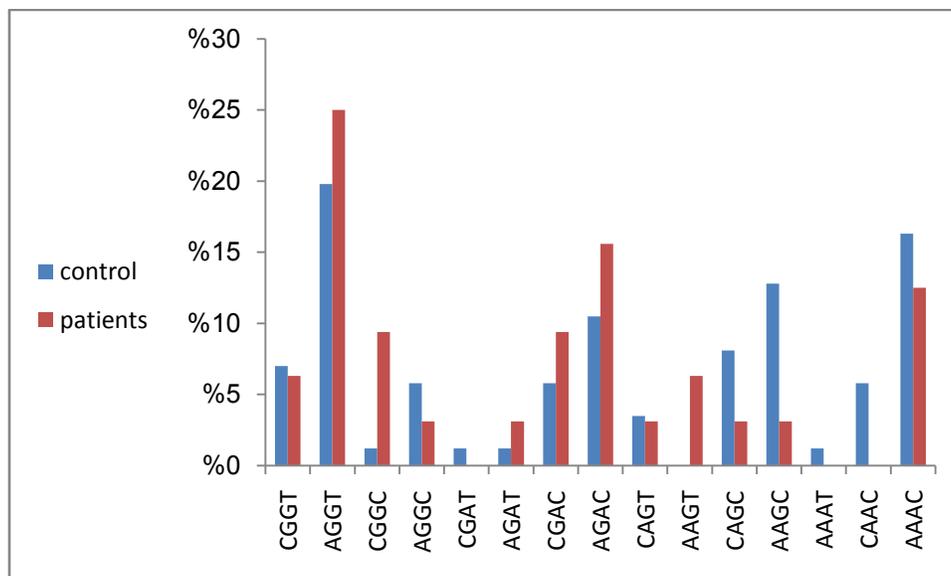
TaqI) in NS cases and controls were shown in (figure1), there were significant variances in the distribution.

**Table 4. Vitamin D, parathyroid hormone, intact parathyroid hormone and fibroblast growth factor-23 in patient and control groups.**

Parameters	Patients (N=50)	Healthy Controls (N=43)
Vit. D (ng/mL)	$11.55 \pm 5.43^*$	$27.85 \pm 6.67$
PTH (pg/mL)	$25.31 \pm 12.12^{**}$	$52.10 \pm 2.18$
iPTH (pg/mL)	$88.69 \pm 39.03^{**}$	$22.34 \pm 9.72$
FGF-23 (pg/mL)	$32.47 \pm 56.84^*$	$19.32 \pm 14.28$

\* Significant difference than control group at P-value < 0.05.

\*\* Highly significant difference than control group at P-value < 0.01.



**Figure 1. Four-marker (FokI(A/G); BsmI(C/T); ApaI(A/C); and TaqI(A/G) polymorphisms) haplotype estimated prevalence (%) in Nephrotic syndrome patients and healthy individuals. There are significant variances in the distribution.**

## Discussion

Vitamin D and its nuclear vitamin D receptor (VDR) are widely involved in biological processes including bone mineralization, modulation of the immune response and regulation of cell proliferation and differentiation. Variations in the Vitamin D or VDR have thus been linked to several diseases, including osteoarthritis, diabetes, cancer, cardiovascular disease and tuberculosis. Nephrotic syndrome pathogenesis up till now is indecisive and debatable and is thought to be arbitrated via the immune system [5-6].

In this work we study the restriction fragment length polymorphisms (RFLP) for BsmI, ApaI, FokI and TaqI to define the frequency and the association of VDR gene polymorphic variants in Egyptian children suffering from nephrotic syndrome (NS). Studies investigated the

correlation between vitamin D levels and biochemical abnormalities in nephrotic syndrome have not specified steady association between serum vitamin D and low serum PTH and Ca levels. These findings may be explained by the free hormone hypothesis. Vitamin D is a hormone, mainly bound to vitamin D binding protein (DBP) and <1% of vitamin D in the serum exists in free form. The major formula (85–90%) of vitamin D is firmly bound to DBP and a minor formula (10–15%) is loosely bound to albumin. Bioavailable vitamin D (alb.-bound hormone combined with the free fraction) levels have been shown to have a better association with serum Ca and PTH than total vitamin D in NS patients [3].

Tubular dysfunction, in patients with NS and renal Fanconi syndrome is associated with high rate of DBP urinary excretion. It has been suggested that augmented urinary loss of DBP may contribute to the decrease in total vit. D levels in proteinuria patients [20]. Though,

Doorenbos *et al.* [21] reported that antiproteinuric treatment protocols decrease urinary loss of DBP, but had no effect on vitamin D levels in chronic kidney diseases. Thus, urinary DBP may be considered as a marker of renal interstitial inflammation and fibrosis [22]. DBP and vitamin D in the serum are found to be low in type 1 diabetes mellitus (T1DM) [23], chronic liver [24] and kidney diseases [20-25]. Carpenter *et al.* [26] found that the common single nucleotide polymorphisms rs4588 in the *GC* gene encoding DBP affects DBP serum level, thus in turn affects serum 25(OH)D levels in infants and toddlers. Similarly, Jassil *et al.* [28] reported that factors affecting DBP alter the interpretation of 25(OH)D levels. They analyzed the effects of other endocrine hormones and diseases on DBP levels [27].

Beside augmented fibroblast growth factor-23 (FGF-23) secretion by osteocytes [29], FGF-23 concentrations may also increase due to accumulation in the serum as a result of reduced glomerular filtration. FGF-23 have a small molecular weight, like that of Cystatin C, which also increase in serum in patients with reduced renal clearance [30]. Studies showed that elevated FGF-23 levels suppress 1-alpha hydroxylase, exaggerate vitamin D deficiency, and cause secondary hyperparathyroidism [31], PTH acts on bone cells to surge expression of FGF-23 [32]. Our results revealed significant correlation between FokI gene and alb./creat. ( $p=0.001$ ) and cholesterol ( $p=0.032$ ) respectively. There was also significant correlation between ApaI gene and alb./creat. ( $p=0.016$ ). Moreover, we found serum levels of 25(OH) vitamin D were significantly lower in nephrotic syndrome patients than controls ( $p=0.001$ ). Our results are supported by study by Yang *et al.* [35].

The results show increased cholesterol this may be due to increased production of lipoproteins which go with increased hepatic albumin formation as a result of hypoalbuminemia. Nevertheless, serum cholesterol levels have been found to be independent of albumin formation rates. Reduced plasma oncotic pressure may have a role in increased hepatic lipoprotein synthesis. Also causative to the dyslipidemia of NS are idiosyncrasies in controlling enzymes, like lecithin-cholesterol acyltransferase lipoprotein [2, 32].

Dissimilarity in DNA sequences take place frequently in the population and has a considerable biological effect on the development of certain diseases. The gene product (protein) is determined by exons 2–9. Exons 7–9 have a crucial role in attaching to vitamin D. Allelic differences in VDR are tangled in their function, signifying in turn that allelic differences in VDR lead to functional modifications in the effectiveness of 1,25-dihydroxy D3 as an immunosuppressive hormone. VDR gene polymorphism (including TaqI, FokI, ApaI and BsmI genotypes) is identified to be genetically determined and affected by ethnicity [33].

In our work we studied the frequency and association of ApaI, TaqI, FokI and BsmI polymorphisms that may be considered as one of the genetic risk factors for NS. We observed the allele frequency distribution of TaqI, FokI and ApaI and there was no significant difference could be found among the patient group when compared to the control group. In case of an allele of BsmI (CT) restriction site, there was a highly significant difference between NS patient and control groups ( $p=0.013$ ). We demonstrated that the haplotypes frequencies exhibited significant differences between the patient and the control groups when the four-marker haplotype incidence was evaluated, as shown in Figure (1).

Similar to our results, Al-Eisa AA and Haider MZ found no significant difference of TaqI, FokI and ApaI between idiopathic nephrotic syndrome patients and control group. They do not support the use of VDR TaqI or ApaI gene polymorphisms as genetic markers of idiopathic nephrotic syndrome nor do they predict steroid responsiveness in children with the disease [34]. While research on systemic lupus erythematosus nephritis, plus secondary nephrotic syndrome with or without renal impairment, described a certain relationship of renal association with the BsmI genotype polymorphism, which is a mutual single-nucleotide polymorphism of the VDR gene. This is in accordance with our results [35].

NS has been commonly thought to be a primary immune disease characterized by an immune controlling disproportion between Th1 and Th2 cytokines, that are supposed to play key roles in both the pathogenesis and prognosis of the disease [36]. Meanwhile, the significant part of VDR gene polymorphism in NS might be facilitated via its direct consequence on the role and effectiveness of 1,25-dihydroxy D3, that is thought to be a hormone suppressing immunity, that adversely controls the manufacture of dissimilar cytokines and down regulation of the immune system [37].

Studies on VDR gene polymorphism in children with NS are infrequent. In contrast to our study, Jafar *et al.* found a significant variance in the rate of ApaI polymorphic genotype among NS Indian patients of East Asian origin. While there are no significant variance in the TaqI genotype frequency among the same group of patients when compared to normal healthy controls [38]. In our group of patients, we did not find any relationship of the two polymorphic genotypes ApaI and TaqI with NS. The difference in both the ethnic and genetic circumstantial of the Arab population from that of the East Asian population might easily elucidate the variance in the outcomes of the two researches.

In our study we observed that CT of BsmI, strongly associated with NS ( $p=0.013$ ). VDR gene BsmI polymorphism CT genotype was found to be positively associated with the development of diabetic nephrotic syndrome in a study on the online Chinese database (CNKI and Wanfang) and English database [39].

Differentiation of VDR genotypes may be concomitant with the pathogenesis of the NS, or it may lead to modify the VDR construction eventually leading to transformed receptor function, and to conclude it may also increase or decrease the expression of VDR protein thus generating disease. Most of tissues in the body like heart, kidney, stimulated T and B subtypes of lymphocytes etc. have the intranuclear VDR receptors for 1, 25 dihydroxyvitamin D<sub>3</sub>. Consequently, it is not unexpected that 1,25 dihydroxyvitamin D<sub>3</sub> has multiple biological effects which are non-calcemic in nature [40], and comprise effects on immunity, muscle, vasculature, growth and variation of numerous cell types [41]. VDR gene polymorphisms appear to be an important genetic determinant in cause and progression of NS, it is considering to be an important predisposition risk factor for NS. In our study we observed that CT of BsmI, strongly associated with NS (p=0.013). Further studies in this area will open a number of options and will allow an advance anticipation of the clinical outcome.

### Conclusion

Our study suggests that the BsmI polymorphism may be a risk factor for susceptibility to nephrotic syndrome among Egyptian children population. Further studies of VDR gene polymorphic variations (FokI, ApaI, TaqI and BsmI) as being genetic contributing factor of vulnerability to nephrotic syndrome in children is recommended. Conferring to our findings, the genotype dissimilarities of the VDR gene polymorphisms may be one of the causes in the development of nephrotic syndrome, which is a polygenic and multifactorial autoimmune illness. Finally, these findings advocate that the BsmI VDR polymorphism may be considered as a risk factor of NS and could be targeted in the protocols to prevent NS later on.

### Conflict of Interest

All authors declare no conflict of interest and no any financial support or relationships that may pose conflict of interest relevant to this work. All authors have contributed significantly and are in agreement with the content of the manuscript. Authors have full control of all primary data and that they agree to allow the journal to review their data if requested.

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