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Vitamin D receptor gene polymorphisms in chronic kidney disease Egyptian children: effect on biochemical markers of bone mineral disorders

Polimorfizmy genu receptora witaminy D w przewlekłej chorobie nerek egipskich dzieci: wpływ na biochemiczne markery zaburzeń mineralnych kości

[1]Manal F. Elshamaa, [2]Eman Fathy Eryan, [2]Hanan M. Hamed, [2]Iman A.S. Khalifa, [3]Solaf Kamel, [3]Mona H. Ibrahim, [3]Dina Kandil, [3]Hebatallah Farouk, [3]Mona Raafat, [3]Dalia A. Abd-El Haleem, [3]Eman Mahmoud, [4]Gamila S.M. El-Saeed, [1]Maha M.A. Abo Hashish, [1]Enas M. Abd Elhamid, [2]Shaimaa Sayed

[1]Pediatrics Department, National Research Centre, Cairo, Egypt
[2]Pediatrics Department, Cairo University, Cairo, Egypt
[3]Clinical Pathology Department, National Research Centre, Cairo, Egypt
[4]Department of Medical Biochemistry, National Research Centre, Cairo, Egypt

Abstract

Aim of the study: The aim of this study was to assess the association between four vitamin D receptor (VDR) single nucleotide polymorphisms BsmI (rs1544410), ApaI (rs7975232), FokI (rs2228570) and TaqI (rs731236) and the susceptibility to chronic kidney disease (CKD) in Egyptian children and to evaluate their association with mineral status in these patients.

Material and methods: The current study included 305 patients with CKD and 100 apparently healthy children. We measured the serum vitamin D (VD), parathyroid hormone (PTH) level and fibroblast growth factor 23 (FGF-23) levels by ELISA method. The genotyping of the four *VDR* gene variants was carried out by PCR-RFLP technique.

Results: The TaqI AG & the BsmI TT genotypes were associated with a significantly higher risk of CKD. The expression of 25-OH D serum level was decreased in patients with TaqI GG & AG genotypes groups and in patients with BsmI TT genotype group The expression of PTH serum level was increased in patients with BsmI CT genotype group. The expression of FGF-23 serum level was increased in patients with Taq1 AG genotype group. We found 3 specific haplotypes; AGCA, AGCC and GGCA for healthy controls.

Conclusions: Our study showed an association between VDR TaqI, BsmI polymorphisms and the susceptibility to CKD. The existence of VDR variants affected the protein expression of VD, FGF-23 and PTH. The AGCA, AGCC and GGCA haplotypes were considered as protective factors against the development of renal nephropathy in our population.

Key words:
chronic kidney disease, secondary hyperparathyroidism, *VDR* polymorphisms, children.

Introduction

Chronic kidney disease (CKD) has emerged as a major public health issue in recent years [1]. End-stage renal disease (ESRD) is a major health and economic concern for people all over the world [2]. Changes in metabolic processes can occur as a result of renal dysfunction. One of the most well-known metabolic aggravations of CKD is an unbalance in mineral status [3]. Recent studies revealed that the development of ESRD is influenced by both environmental and genetic variables [4, 5]. More significantly, some single-gene polymorphisms (e.g., as VD receptor [VDR]) were related to the risk of chronic renal failure (CRF).

Chronic kidney disease has been linked to impaired renal production of the biologically active form of vitamin D (VD); 1,25 dihydroxy VD [1,25$(OH)_2D_3$] [6]. The effects of VD are exerted by the VDR. Vitamin D is a well-known central regulator of bone and calcium metabolism. Numerous studies have revealed that VD may play a role in a variety of renal disorders [7, 8]. Recent studies revealed that CKD patients with and without dialysis were shown to have a lower level of VD, which was related with vascular calcification, anemia, and cardiovascular mortality in ESRD patients [9].

The proximal renal tubule regulates VD through its receptor. The VDR has been demonstrated to regulate 1-hydroxylase and 24-hydroxylase activity in proximal convoluted tubule cells by

Manal Fouad Elshamaa
Department of Pediatrics
National Research Centre
33 EL BWHOUS
12311 Cairo, Egypt
e-mail: manal_elshmaa@hotmail.com

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"sensing" the level of circulating VD [10]. The *VDR* gene length is 100kb and located on the long arm of chromosome 12 at 13.11 position [11]. The DNA binding domain is encoded by exons 2–3, while the ligand binding domain is encoded by exons 6–9 [12]. Several genetic variants have been identified in the *VDR* gene that have been linked to a variety of disorders, including osteoporosis, cancer, short stature, and diabetes [13, 14].

Single nucleotide polymorphisms (SNPs) are commonly found in the *VDR* gene, and these variations may have an impact on transcriptional regulation, which may then affect VDR protein expression [15]. Unfortunately, any effect of these polymorphisms has not been clear as the results are conflicting. The ApaI (rs7975232), BsmI (rs1544410), FokI (rs2228570), and TaqI (rs7975232) SNPs are the most prevalent kinds of *VDR* gene SNPs (rs731236). There have been earlier researches on the relation between *VDR* gene variation and the risk of CKD [16–18]; on the other hand, the conclusions of these assessments are always debatable.

Fibroblast growth factor 23 (FGF-23) is a protein that plays a critical role in the phosphate regulation process [19]. Osteocytes are the primary source of FGF-23, this protein regulates phosphate, $1,25(OH)_2D_3$ metabolism, and PTH [20, 21]. The increased FGF-23 levels, block one of the key steps in the conversion of 25(OH)D3 to active $1,25(OH)_2D_3$, a process that damages calcium absorption, raises PTH and causes abnormal bone turnover [22].

This study aimed to assess the association between four VDR SNPs ApaI (rs7975232), BsmI (rs1544410), FokI (rs2228570) and TaqI (rs731236) and the susceptibility to CKD in Egyptian pediatric population. We sought to determine whether VDR polymorphisms ApaI, BsmI, FokI and TaqI. are involved with the serum levels of $25(OH)D_3$, FGF-23, PTH, phosphorus and calcium and in CKD children.

Material and methods

Population study

This was a case-control study that included 305 children with CKD stages 2–5, as measured by the National Kidney Foundation's estimated glomerular filtration rate (e-GFR) [23], (recruited from Pediatric Nephrology Unit at Children's Hospital, and 100 healthy controls. The CKD group comprised 100 CKD stage 5 on hemodialysis (HD) and 205 CKD stages 2–4 patients (100 children with systemic lupus erythematosus (SLE), and 105 children with nephrotic syndrome recruited from the immunology and nephrology outpatients and were not hospitalized). The inclusion criteria for all CKD children were age $<$ 18 years with CKD stages from 2–5 determined by eGFR [23]. The inclusion criteria for HD children included a constantly elevated serum creatinine level above the normal range (ranging from 3.4 to 15.8 mg/dl) and were dialyzed for not less than 6 months. Patients' age, gender, duration of chronic dialysis and medical conditions, were recorded during enrollment. Patients with active cancers, primary hyperparathyroidism, genetic calcium disorders like autosomal dominant hypercalciuric hypocalcemia, and cirrhosis were excluded from the study. Hundred apparent healthy children were enrolled as controls, matched by age and sex, and with no clinical signs or family history of renal disease and no medications taken at the time of the study. They were chosen from the pediatric clinic of the Medical Research Centre throughout the same period.

The ethics committee of our Centre approved the study's protocol (Registration number = 13,169). Each participants or their parents gave a written informed consent prior to the enrolment.

Collection and processing of samples

A round 5 to 8 ml blood samples from the subjects in this study and were subjected to laboratory analysis. Centrifugation was employed to separate the serum, which was then used for the biochemical tests. Blood was collected and stored at –20°C in tubes containing the anticoagulant EDTA until DNA extraction for molecular analysis.

Biochemical and laboratory parameters

Biochemical analysis (i.e., albumin, serum creatinine, cholesterol, phosphorus, calcium and alkaline phosphatase [ALP]) for each patient and control were performed using an automated clinical chemistry analyzer (Olympus AU 400 analyzer-Germany) as well as a complete blood picture utilising the CoulterT890 blood analyzer (Coulter Counter, Harpenden, UK).

PTH, FGF-23, and 25(OH)D3 assay

Uscan Life kit (Cat.no. #ED746h) (Ray Biotech, inc., USA) was used to test serum FGF-23 concentrations by Enzyme linked immunosorbent assay (ELISA). PTH and 25(OH) D3 (total) concentrations were measured by DRG International, Inc. kit (EIA-3645) (USA) & Cat # EIA-5396, DRG Diagnostics GmbH, kit (Germany) respectively, using ELISA.

DNA extraction

QIAamp DNA Blood Mini Kits (Qiagen GmbH, Germany) was used to extract the DNA. The concentration and purity of the extracted DNAs was determined by NanoDrop 2000c Spectrophotometer (Thermo Fisher).

Genotyping of VDR gene polymorphisms

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to genotype the examined groups (RFLP). Restriction enzymes BsmI, ApaI, TaqI, and FokI were used to digest the products after they had been amplified. The DNA was amplified using the Quantistudio 12 Flex (Applied Biosystems, CA 94404, USA) by real-time polymerase chain reaction (rt-PCR). The TaqMan genotyping RFLP (Applied Biosystems, Foster City, CA, USA) was used to the amplified PCR products 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) The restriction enzymes were used to detect according to the digestion pattern generated for the amplified DNA fragment. The PCR reaction was carried out in 96-well PCR plates with the final volume 20 μl for a reaction volume. The PCR reaction mixture included 10 μl TaqMan Universal PCR Master Mix, 20–30 ng DNA, and

primers. The PCR assay contained a pre-denaturation step of 10 min at 95°C followed by followed by 40 cycles of DNA denaturation at 95°C for 15 s and annealing-extension at 60°C for 1 min. The TaqMan Genotype software was used to analyze the final products.

Statistical analysis

The data analysis was done by the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). The mean, median, standard deviation, minimum, and maximum values were used to summarize quantitative data, while frequency (count) and relative frequency (percentage) were used to summarize categorical data. The Kruskal-Wallis and Mann-Whitney tests were used to do non-parametric comparisons between quantitative variables. The χ^2 test was used to compare categorical data. When the expected frequency is less than 5, the exact test was used. The Spearman correlation coefficient was used to determine the correlations between quantitative variables. For the purpose of comparing reported and expected SNP genotypes, the Hardy-Weinberg (H-W) model was used. The frequencies of SNP genotypes were calculated under the H-W equilibrium assumption (HWE). The χ^2 test was used to compare the observed genotype frequency distributions of the SNP antigens to those predicted by the HWE. Statistical significance was defined as *p*-values less than 0.05.

Results

Description of the study population

This study enrolled 405 individuals in total, including 305 people with CKD and 100 healthy volunteers. Polysulphone membranes and bicarbonate dialysate were used for patients on HD with blood flow rates ranging from 80–150 ml/min and dialysate flow rates of 500 ml/min at three times per week. In sealed containers, dialysate fluids were made from concentrated salt solutions and bicarbonate powder.

Table I showed the study participants demographic characteristics and biochemical parameters. The mean age of patients and controls were 11.54 ±6.00 and 12.84 ±3.73 years ($p = 0.11$) respectively.

Biochemical parameters

Considerable changes in biochemical concentrations were noticed between CKD patients and healthy controls serum with

Table I. Demographic characteristics and biochemical parameters of CKD children and healthy controls

Parameter	Controls ($n = 100$)	CKD patients ($n = 305$)	*p*-value
Age (years)	11.54 ±6.00	12.84 ±3.73	0.11
Age of onset (years)		6.89 ±3.34	–
Gender (M/F)	51 (51%)/49 (49%)	129 (42.3%)/176 (57.7%)	0.128
Hb (g/dl)	12.41 ±0.82	11.08 ±2.09	< 0.001
HCT	34.54 ±1.36	32.51 ±4.75	< 0.001
WBCs ($\times 10^3/mm^3$)	6.96 ±2.60	8.52 ±3.63	< 0.001
PLTs ($\times 10^3/mm^3$)	238.10 ±78.29	291.40 ±124.69	< 0.001
Albumin (g/dl)	4.43 ±0.43	3.57 ±0.83	< 0.001
Ca (mg/dl)	9.64 ±0.81	8.96 ±0.97	< 0.001
P (mg/dl)	5.09 ±0.62	4.52 ±1.18	< 0.001
ALP (U/l)	210.48 ±58.81	425.64 ±327.81	< 0.001
$25(OH)D_3$ (ng/ml)	23.93 ±6.41	13.34 ±5.76	< 0.001
PTH (pg/ml)	41.79 ±20.11	213.57 ±355.17	0.003
FGF-23 (pg/ml)	15.38 ±7.63	62.94 ±172.84	0.061

Variables are presented as means ± standard deviations, numbers or percentage as applicable.
M/F male/female; Hb – hemoglobin; HCT – hematocrit, WBCs – white blood cells; PLTs – platelets; Ca – calcium; P – phosphorous; ALP – total alkaline phosphate; PTH – parathyroid hormone, 25(OH)D – 25 hydroxy vitamin D_3, FGF-23 – fibroblast growth factor 23, $p < 0.05$ was considered significant

regard to calcium (8.96 ±0.97 mg/dl vs. 9.64 ±0.81 mg/dl, $p < 0.001$), phosphorous (4.52 ±1.18 mg/dl vs. 5.09 ±0.62 mg/dl, $p < 0.001$), ALP (425.64 ±327.81 U/l vs. 210.48 ±58.81, $p < 0.001$), 25(OH)D3 (13.34 ±5.76 pg/ml vs. 23.93 ±6.41, $p < 0.001$), PTH (213.57 ±355.17 pg/ml vs. 41.79 ±20.11 pg/ml, $p = 0.003$) and FGF-23 (62.94 ±172.84 pg/ml vs. 15.38 ±7.63, $p = 0.061$), respectively (Table I).

SNP selection and genotyping

The distribution of the four VDR polymorphisms (BsmI, FokI, ApaI, and TaqI) in CKD patients and healthy controls is shown in Table II. In the controls, the genotype frequencies of the four polymorphisms investigated coincided with HWE ($p > 0.05$).

The rs 731236 TaqI A/G SNP

The genotype distribution of the rs 731236 TaqI A/G SNP differed significantly ($p < 0.05$) between the patients and the controls. The AA, AG, and GG geno-types were found in 55 (18.0%), 138 (45.2%), and 112 (36.7%) and 35%, and 31 (31%), 34 (34%), and 35 (35%) in CKD children and controls, respectively. Using the rs 731236 TaqI A/G AA genotype as a reference, logistic regression analysis revealed that the AG ($p = 0.005$, adjusted

Table II. Distribution of VDR polymorphisms among CKD children and healthy controls

		Controls ($n = 100$)		CKD patients ($n = 305$)		*p*-value	OR	95% CI	
		Count	%	Count	%			Lower	Upper
rs 731236 Taq I A/G	AA	31	31.0	55	18.0	Reference			
	AG	34	34.0	138	45.2	0.005	2.288	1.283	4.080
	GG	35	35.0	112	36.7	0.047	1.804	1.009	3.225
	A allele	96	48.0	248	40.7	Reference			
	G allele	104	52.0	362	59.3	0.069	1.347	0.977	1.857
rs 2228570 Fok I A/G	AA	19	19.0	79	25.9	Reference			
	AG	39	39.0	124	40.7	0.394	0.765	0.413	1.417
	GG	42	42.0	102	33.4	0.087	0.584	0.315	1.082
	A allele	77	38.5	282	46.2	Reference			
	G allele	123	61.5	328	53.8	0.057	0.728	0.525	1.009
rs1544410 Bsm I C/T	CC	37	37.0	104	34.1	Reference			
	CT	57	57.0	149	48.9	0.769	0.930	0.573	1.508
	TT	6	6.0	52	17.0	0.017	3.083	1.223	7.773
	C allele	131	65.5	357	58.5	Reference			
	T allele	69	34.5	253	41.5	0.081	1.345	0.964	1.877
rs7975232 Apa I A/C	AA	50	50.0	136	44.6	Reference			
	AC	37	37.0	135	44.3	0.237	1.341	0.824	2.183
	CC	13	13.0	34	11.1	0.915	0.962	0.470	1.969
	A allele	137	68.5	407	66.7	Reference			
	C allele	63	31.5	203	33.3	0.642	1.085	0.770	1.528

OR – odds ratio; CI – confidence interval; CKD – chronic kidney disease; *p*-value for comparison of genotype frequencies between control and CKD groups; VDR – vitamin D receptor

OR=2.288, 95% confidence interval [CI]: 1.283–4.080) genotype was linked with a significantly higher risk of CKD than the AA genotype. The rs 731236 TaqI A/G; G allele frequency was 362 (0.593) among the patients and 104 (0.520) among the controls ($p = 0.069$, adjusted OR = 1.347, 95% CI: 0.977–1.857).

The rs1544410 BsmI C/T SNP

We found significant differences in the genotype distribution of the rs1544410 BsmI C/T SNP between the patients and the controls ($p < 0.05$). The frequencies of the CC, CT, and TT genotypes were 104 (34.1%), 149 (48.9%), and 52 (17.0%) among CKD children and 37 (37%), 57 (57%), and 6 (6%) among controls, respectively ($p = 0.017$). By logistic regression analysis, when utilizing the rs rs1544410 BsmI C/T CC genotype as the reference, the TT ($p = 0.017$, adjusted OR = 3.083, 95% CI: 1.223–7.773) genotype was associated with a significantly higher risk of CKD than the CC genotype. The rs1544410 BsmI C/T T allele frequency was 253 (0.415) among the patients and 69 (0.345) among the controls ($p = 0.081$, adjusted OR = 1.345, 95% CI: 0.964–1.877).

The rs 2228570 FokI A/G and rs7975232 ApaI A/C

There were no significant differences of these genotypes between the CKD and control groups.

The effect of the association of the four investigated SNPs on the risk of CKD was subsequently evaluated using haplotype association analysis, as shown in Table III. We found 3 specific haplotypes for healthy controls. The AGCA haplotype with frequency 28 (14.0%) vs. 40 (6.6%) in CKD children, $p = 0.004$, adjusted OR = 0.400, 95% CI: 0.215–0.741. The AGCC haplotype with frequency 15 (7.5%) vs. 25 (4.1%) in CKD children, $p = 0.045$, adjusted OR = 0.466, 95% CI: 0.221–0.984. The GGCA with frequency 15 (7.5%) vs. 24 (3.9%) in CKD children, $p = 0.036$, adjusted OR = 0.447, 95% CI: 0.211–0.949. In our population, we found that these three haplotypes were linked to reduced risk of renal nephropathy development. These three haplotypes may operate as preventative measures against the onset of renal nephropathy. VDR haplotypes were not linked to an increased risk of CKD in children ($p > 0.05$).

The biochemical markers of CKD mineral and bone disorders (CKD- MBD) in the TaqI and BsmI genotypes are shown in Table IV.

Table III. Summary of haplotype association analysis of various *VDR* gene polymorphisms in patients with CKD and healthy controls

Haplotypes	Controls		CKD patients		*p*-value	OR	95% CI	
	n	%	*n*	%			Lower	Upper
AACA	33	16.5	118	19.3	Reference			
AACC	11	5.5	17	2.8	0.053	0.432	0.185	1.012
AATA	2	1.0	14	2.3	0.390	1.958	0.423	9.050
AGCA	28	14.0	40	6.6	0.004	0.400	0.215	0.741
AGCC	15	7.5	25	4.1	0.045	0.466	0.221	0.984
AGTA	0	0.0	19	3.1	0.998		–	–
AGTC	7	3.5	15	2.5	0.304	0.599	0.226	1.591
GACA	17	8.5	60	9.8	0.969	0.987	0.509	1.915
GACC	10	5.0	54	8.9	0.299	1.510	0.694	3.286
GATA	2	1.0	12	2.0	0.512	1.678	0.358	7.873
GATC	2	1.0	7	1.1	0.979	0.979	0.194	4.937
GGCA	15	7.5	24	3.9	0.036	0.447	0.211	0.949
GGCC	2	1.0	19	3.1	0.204	2.657	0.589	11.994
GGTA	40	20.0	120	19.7	0.513	0.839	0.496	1.420
GGTC	16	8.0	66	10.8	0.675	1.154	0.591	2.252

OR – odds ratio; CI – confidence interval; CKD – chronic kidney disease; *p*-value for comparison of genotype frequencies between control and CKD groups; VDR – vitamin D receptor

Table IV. Levels of markers of CKD-MBD across significant rs 731236 Taq 1 A/G and rs1544410 Bsm 1 C/T VDR genotypes in CKD children

CKD	rs 731236 Taq 1 A/G				rs1544410 Bsm 1 C/T			
	AA (n = 55)	AG (n = 138)	GG (n = 112)	p-value	CC (n = 104)	CT (n = 149)	TT (n = 52)	p-value
25(OH)D_3 (ng/ml)	16.31 ±6.88	13.05 ±4.96	12.25 ±5.63	< 0.001	13.72 ±6.63	14.17 ±5.42	10.24 ±3.39	< 0.001
PTH (pg/dl)	476.57 ±567.09	226.96 ±309.85	68.05 ±120.06	< 0.001	215.96 ±454.78	272.29 ±317.61	41.69 ±29.29	< 0.001
FGF-23 (pg/ml)	20.65 ±24.33	101.34 ±248.40	37.07 ±50.74	0.005	51.23 ±89.44	86.91 ±232.13	15.86 ±15.58	0.003
Age (years)	11.55 ±3.90	11.53 ±4.10	11.55 ±8.50	0.188	10.85 ±4.11	12.69 ±7.40	9.71 ±3.96	0.004
Age of onset (years)	5.84 ±2.66	6.74 ±3.65	7.61 ±3.08	0.002	6.38 ±3.46	7.08 ±2.99	7.38 ±3.89	0.154
Hb (g/dl)	10.63 ±2.26	10.57 ±2.13	11.96 ±1.63	< 0.001	11.34 ±1.64	10.68 ±2.40	11.66 ±1.82	0.012
WBCs ($\times 10^3$/mm^3)	10.74 ±5.67	8.21 ±2.59	7.83 ±3.10	0.011	8.91 ±4.63	8.54 ±2.96	7.55 ±2.53	0.215
PLTs ($\times 10^3$/mm^3)	272.94 ±119.61	268.90 ±106.36	327.02 ±139.42	0.006	289.72 ±109.97	286.31 ±141.57	309.88 ±103.52	0.228
Albumin (g/dl)	3.76 ±0.62	3.39 ±0.76	3.76 ±1.08	0.006	3.44 ±0.85	3.68 ±0.82	3.23 ±0.76	0.126
Ca (mg/dl)	9.06 ±0.76	8.73 ±0.81	9.17 ±1.19	0.056	8.98 ±0.93	8.89 ±0.96	9.21 ±1.08	0.046
P (mg/dl)	5.20 ±1.83	4.33 ±0.64	4.30 ±0.97	0.067	4.73 ±0.92	4.38 ±1.42	4.48 ±0.51	0.006
ALP (U/l)	667.86 ±525.50	403.83 ±238.20	323.29 ±188.70	0.001	555.90 ±428.79	333.72 ±225.95	393.03 ±175.40	0.001

Variables are presented as means ± standard deviations.
Hb – hemoglobin; HCT – hematocrit; WBCs – white blood cells; PLTs – platelets; Ca – calcium; P – phosphorous; ALP – total alkaline phosphate; PTH – parathyroid hormone; 25(OH)D_3 – 25 hydroxyvitamin D; FGF-23 – fibroblast growth factor 23; $p < 0.05$ was considered significant

TaqI genotypes

The expression of 25(OH)D_3 serum level was decreased in patients with TaqI GG and AG genotypes groups (12.25 ±5.63 pg/ml, and 13.05 ±4.96 ng/ml) than in TaqI AA genotype group (16.31 ±6.88 ng/ml; $p < 0.001$ and $p < 0.05$, respectively). There was a statistically significant difference ($p = 0.003$) in the expression of FGF-23 serum levels between patients with TaqI AG genotype group (101.34 ±248.40 pg/ml) and TaqI AA genotype group (20.65 ±24.33 pg/ml).

BsmI genotypes

Patients in the BsmI TT genotype group had significant lower levels ($p = 0.006$) of 25(OH)D_3 serum expression (10.24 ±3.39 ng/ml) than those in the Bsm1 CC genotype group (13.72 ±6.63 ng/ml). Patients with BsmI CT genotype group (272.29 ±317.61 pg/ml) had significant higher expression of PTH serum level than in BsmI CC genotype group (215.96 ±454.78 pg/ml) ($p = 0.009$) and in BsmI TT genotype group (41.69 ±29.29 pg/ml; $p = 0.006$).

Positive correlation was found between 25-OH D and FGF-23 serum levels ($r = 0.193$, $p = 0.001$). Both 25-OH D and FGF-23 serum levels were correlated with patients age and age of onset of CKD ($r = 0.171$, $p = 0.003$, $r = 0.246$, $p < 0.001$, $r = 0.193$, $p = 0.001$, $r = 0.232$, $p < 0.001$, respectively).

Discussion

Chronic kidney disease is a major concern for patients and health care systems all around the world. Both activation of the endocrine VDR and the synthesis of calcitriol [1,25(OH)D_3] depend on the kidneys as it is the primary location for the conversion of 25-OH D to calcitriol [24]. Four *VDR* gene poly-

morphisms TaqI, BsmI, Foki and ApI were examined in this our study for their association with CKD risk as well as their effect on VD, PTH and FGF-23 serum concentrations. In Egyptian children, we found that the intronic SNPs of the genes Taq1 and Bsm1 to be significantly linked to CKD risk. This result was in agreement with previous Egyptian study [25–27]. Also, we had detected significant differences in VD levels in patients with the Taq1 GG genotype and the BsmI TT genotype of *VDR* gene. No significant differences were observed between CKD children and controls at Fok1 or Apa1 restriction sites.

Some polymorphisms may be more prevalent in a particular race, which may affect their chance of developing the disease. According to earlier investigations [11, 28], ethnic differences in the distribution of VDR polymorphisms have also been found [11, 28]. The Asian population had a significantly lower frequency of the Bsm1 B allele (7%) than other populations studied (Africans 36%, and 42% for Caucasians). Variations in VDR polymorphism frequency may help explain the racial variance in the markers of CKD-MBD. Differentiation of VDR genotypes may directly or indirectly disrupt the cytokine relationship. It could be a contributing factor in the development of CKD, or it could alter the structure of the VDR. Ultimately this leads to altered receptor function, which may increase or decrease the expression of VDR protein thereby causing the disease.

Despite the fact that the Bsm1 polymorphism is located in the intronic region, it has the potential to affect VDR expression by disrupting a splice site for VDR mRNA transcription, resulting in a shortened or alternatively spliced protein product [29] or they could change the mRNA product [30]. We found that BsmI genotypes TT were substantially linked to CKD, demonstrating that shortened proteins are produced, resulting in reduced binding with the VDR, which may lead to CKD.

Several researchers have investigated the link between VDR polymorphisms and the calcium/PTH/calcitriol axis in an attempt to understand the intricacy behind the pathophysiologic mechanisms of CKD-MBD, and inconsistent results were obtained [31].

In the ESRD, the synthesis of calcitriol has been potently disturbed [32]. There was a significant difference in the amount of this hormone between the CKD children and the control groups in the current study. Indeed, the reduced VD expression found in patients with CKD may be due to a variety of factors, including genetics and environmental factors. Reduced 1-hydroxylase (CYP27B1) activity is seen in patients with kidney disease because their renal mass decreases. As a result, the active form of VD is less likely to be produced when CYP27B1 activity is reduced [33]. In the CDK patients, the existence of *VDR* gene polymorphisms can influence the VD expression. We reported that the TaqI GG and BsmI TT genotypes in CKD children were associated with significant differences in VD levels. In the presence of VD, defective activation of VDR affects the expression of a number of genes. These genes control the appearance of hypertension, renal damage, immune disorder, hyperphosphoremia, and secondary hyperparathyroidism, because they are associated with skeletal integrity and mineral homeostasis [24]In the current study, PTH levels differed significantly between BsmI genotypes, with patients with the CT genotype having increased PTH levels than those with the CC and TT genotypes. In predialysis CKD and transplant patients, the effect of BsmI on parathyroid function was noticed. Predialysis CKD patients with the BsmI genotype had a slower progression of secondary hyperparathyroidism, according to Marco *et al*. [34], while it was reported that transplant patients with BsmI genotypes had lower PTH levels [35]. In contrast to our findings, other research has found no significant changes in PTH levels between BsmI genotypes. BsmI genotype distribution varies widely among ethnic groups, making it difficult to compare the results of individual investigations.

The presence of T alleles has been associated to the molecular mechanisms through which BsmI VDR polymorphisms affect hyperparathyroidism. T alleles have been linked strongly to lower *VDR* gene transcription and/or m RNA stability as previously reported, as a result, the regulation of calcitriol on parathyroid glands had been affected [35]. For instance, patients with CC genotypes are less likely than those with TT genotypes to have decreased 1-hydroxylase. Consequently, those with the T allele are less likely to have the calcitriol levels essential to suppress parathyroid cell proliferation and PTH release.

Furthermore, in our study, FGF-23 levels differed significantly between CKD children and healthy controls. In CKD patients, having the TaqI AG genotype was related with considerably greater levels of FGF-23 expression, according to our findings. Many studies approve the elevation of FGF-23 during ESRD [33, 36]. Indeed, FGF-23 is critical in maintaining normal serum phosphate or phosphorus levels and in regulating VD metabolism. It decreases calcitriol levels, promotes phosphaturia, and inhibits parathyroid hormone release [37]. Calcitriol, FGF-23, and PTH may have antagonistic effects on one another [38]. According to several experts, this pathology in patients with CKD is predominantly caused by an elevation in FGF-23 levels, which results in a reduction in $1,25(OH)_2D_3$ levels and likely stimulation of the VDR. As a result, hypocalcemia develops, resulting in persistent PTH secretion [39].

In children with CKD, we found a positive correlation between FGF-23 and VD. We can assume that the physiological suppressive action of higher FGF-23 levels is disrupted, as mediated by FGF receptors and the co-receptor Klotho. Indeed, many changes in these receptors have previously been described in ESRD [40]. Higher VD levels and defective FGF-23 action may confer greater protection against the development of renal nephropathy.

In our study, the presence of the AGCA, AGCC and GGCA haplotypes were associated with lower risk of CKD development. In our population, these three haplotypes were thought to be protective against the development of renal nephropathy. In terms of VDR function, the Bsm-Apa-Taq haplotype is the most commonly studied [41, 42]. It was found that the BAt haplotype had a stronger response to various therapies than the baT haplotype when it came to improving bone mineral density [40, 41]. This was described in terms of good mRNA stability and half-life, which would theoretically lead to more VDR protein being

made in the target cells with the best response to the VD effect. According to Thakkinstian *et al*. [42], the most frequent haplotype for the *VDR* gene, apart from ethnicity was baT, followed by BAt and bAT in Caucasians, and bAT and BaT in Asians. In another study, there was no correlation between BsmI and TaqI haplotype and colorectal cancer risk in the Korean population, however the haplotype "fbaT" was shown to be related with a lower risk of colorectal cancer [43].

Limitations

VDR is regulated by genetic and environmental factors. The main environmental factors associated with this regulation are diet, sunlight exposure, and pollution. The mechanism of this regulation is not completely understood but it could be through epigenetic mechanisms. it is reported that the response to VD supplementation could be modified by the variants of the *VDR* gene [44] So, our limitations in this single centered study included the lacking information related to the dietary history of VD and the ultraviolet B-rays exposure. Also, we could not determine the seasonal variation in $25(OH)D_3$ levels. Further study could be done to understand of the role of *VDR* gene polymorphisms in the modification of the VD supplementation response.

Conclusions

The current study showed that VDR TaqI and BsmI polymorphisms were associated with an increased risk of CKD in children. We have demonstrated that secondary hyperparathyroidism was predicted by BsmI CT genotype in CKD pediatric population. In addition, Taq1 GG and BsmI TT genotypes might be an important determinant of an individual's susceptibility to $25(OH)D_3$ deficiency. There were three haplotypes (i.e., AGCA, AGCC, and GGCA) that were thought to be protective against renal nephropathy in our population. VD, FGF-23, and PTH factor expression in association with *VDR* gene variations should give critical information regarding the molecular mechanisms implicated in CKD. For a more thorough evaluation of the impact of these common factors on ESRD risk, the interactions between genes and the environment are also essential. Such relationships can also aid in the progress of new managements, that would lead to improve public health.

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