Genetic, Environmental, and Disease-Associated Correlates of Vitamin D Status in Children with CKD

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Abstract

Background and objectives Vitamin D deficiency is endemic in children with CKD. We sought to investigate the association of genetic disposition, environmental factors, vitamin D supplementation, and renal function on vitamin D status in children with CKD.

Design, setting, participants, & measurements Serum 25-hydroxy-vitamin D, 1,25-dihydroxy-vitamin D, and 24,25-dihydroxy-vitamin D concentrations were measured cross-sectionally in 500 children from 12 European countries with CKD stages 3–5. All patients were participants of the Cardiovascular Comorbidity in Children with Chronic Kidney Disease Study, had CKD stage 3–5, and were age 6–18 years old. Patients were genotyped for single-nucleotide polymorphisms in the genes encoding 25-hydroxylase, vitamin D binding protein, 7-dehydrocholesterol reductase, and 24-hydroxylase. Associations of genetic status, season, local solar radiation, oral vitamin D supplementation, and disease-associated factors with vitamin D status were assessed.

Results Two thirds of patients were vitamin D deficient (25-hydroxy-vitamin D <16 ng/ml). 25-Hydroxy-vitamin D concentrations varied with season and were twofold higher in vitamin D–supplemented patients (21.6 [14.1] versus 10.4 [10.1] ng/ml; $P<0.001$). Glomerulopathy, albuminuria, and girls were associated with lower 25-hydroxy-vitamin D levels. 24,25-dihydroxy-vitamin D levels were closely correlated with 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D ($r=0.87$ and $r=0.55$; both $P<0.001$). 24,25-dihydroxy-vitamin D concentrations were higher with higher c-terminal fibroblast growth factor 23 and inversely correlated with intact parathyroid hormone. Whereas 25-hydroxy-vitamin D levels were independent of renal function, 24,25-dihydroxy-vitamin D levels were lower with lower eGFR. Vitamin D deficiency was more prevalent in Turkey than in other European regions independent of supplementation status and disease-related factors. Single-nucleotide polymorphisms in the vitamin D binding protein gene were independently associated with lower 25-hydroxy-vitamin D and higher 24,25-dihydroxy-vitamin D.

Conclusions Disease-related factors and vitamin D supplementation are the main correlates of vitamin D status in children with CKD. Variants in the vitamin D binding protein showed weak associations with the vitamin D status.

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Introduction

Vitamin D status depends on environmental and endogenous factors and affects a multitude of physiologic processes, such as bone mineral metabolism, the immune system, muscle metabolism, and the cardiovascular phenotype (1–3). Although insufficient vitamin D levels are found in a significant proportion of the general pediatric population (4–6), vitamin D deficiency seems even more common in children with CKD (7–11) and has not declined over the past few decades (8). Vitamin D from nutritional intake and dermal synthesis is converted to 25-hydroxy-vitamin D [25(OH)D] in the liver. Although 1,25-dihydroxy-vitamin D [1,25$(OH)_2$D] can be produced by a variety of tissues, the processing to the active form by 1α-hydroxylase mainly takes place in the kidneys (12). The latter step is controlled by endocrine pathways; in particular, 1α-hydroxylase is activated by parathyroid hormone and inhibited by c-terminal fibroblast growth factor 23, calcium, and phosphorus (13). 25(OH)D is degraded mainly to the inactive metabolite 24,25-dihydroxy-vitamin D [24,25$(OH)_2$D] and 1,25$(OH)_2$D is degraded to 1,24,25-trihydroxy-vitamin D (12,14).

Recent genome–wide association studies have uncovered associations of vitamin D levels with proteins involved in the synthesis, transport, and metabolism of 25(OH)D: 7-dehydrocholesterol reductase (DCHR7), an enzyme reducing the vitamin D_3 precursor 7-dehydroxycholesterol to cholesterol; 25-hydroxylase (CYP2R1), the enzyme converting vitamin D_3 to 25(OH)D; and vitamin D binding protein (DBP or GC) (15). Single-nucleotide polymorphisms

(SNPs) in the genes encoding for DHCR7, CYP2R1, and DBP have been linked to vitamin D deficiency in the general population (15,16) and the risk of developing vitamin D deficiency–associated diseases, including bone fractures (17), malignancy (18), and autoimmune disease (19).

In this study, we comprehensively analyzed the status of vitamin D metabolites in the largest pediatric CKD cohort studied to date, encompassing a wide variation of geographic distribution, nutritional and cultural habits, and vitamin D supplementation practices. Our study focused on the interaction of genetic and environmental factors in the setting of CKD.

Materials and Methods

Patients and Study Design

Vitamin D metabolite levels [25(OH)D, 1,25$(OH)_2$D, and 24,25$(OH)_2$D] were measured in children and adolescents between 6 and 18 years old with CKD stage 3–5 before dialysis or transplantation. All patients were participants of the observational Cardiovascular Comorbidity in Children with Chronic Kidney Disease Study and enrolled at 55 pediatric nephrology centers in 12 European countries (20). Only patients with a complete vitamin D status and genetic information were included in this cross-sectional analysis. By these criteria, 500 of 704 active patients at the baseline visit were eligible for analysis. The study was approved by all local ethical committees and has been performed in accordance with the principles of the Helsinki Declaration. Written informed consent was given by the parents and adolescents, and oral assent was given by younger children.

For this study, clinical information and biospecimens collected at study entry (between September of 2009 and July of 2011) were analyzed.

Documentation and Definitions

The primary renal diagnoses were categorized as congenital anomalies of the kidneys and urinary tract, glomerulopathies, CKD after AKI, inflammatory disorders, metabolic disorders, and other or unknown The GFR was estimated according to the Schwartz equation on the basis of serum creatinine, cystatin C, and urea (21), and patients were categorized to CKD stages 3a, 3b, 4, and 5 by eGFR ranges of 45–60, 30–44, 15–29, and <15 ml/min per 1.73 m^2, respectively.

Genome–wide SNP genotyping was performed by hybridization on Illumina 2.5M Omni chips, with subsequent imputation to 10 million SNPs. Imputation was performed using the public 1000 Genomes data as a reference panel (22,23). For this study, SNPs associated with vitamin D insufficiency in the general population were used as candidate SNPs (15,16,24) (*i.e.*, in the genes encoding for CYP2R1 [rs10741657], DBP [rs7041 and rs2282679], DHCR7 [rs12785878], and 24-hydroxylase [CYP24A1; rs6013897]). Results were obtained from imputed data for rs10741657 and rs12785878.

A detailed medication history was recorded, including the dosage and application modality of vitamin D. Cholecalciferol or ergocalciferol was used for nutritional vitamin D supplementation, and calcitriol, 1-α-calcidiol, or paricalcitol was used as the active vitamin D analog. Patients were considered as vitamin D supplemented if treatment had started at least 1 week before blood sampling.

Regional solar irradiation was estimated from sunlight radiation maps (www.solargis.info) according to geographic location. Season was classified as winter for November through April and summer for May through October according to the month of blood and urine collection. Patient history was recorded, and a physical examination at the time of blood sampling was performed for anthropometry measurements and pubertal status. Vitamin D status was classified as replete with serum 25(OH)D level >30 ng/ml, insufficient at 16–30 ng/ml, deficient at 5–15.9 ng/ml, and severely deficient at <5 ng/ml in accordance with the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines (25).

Laboratory Techniques

All biochemical analyses were performed centrally. Standard laboratory techniques were used to measure the serum and urinary concentrations of albumin, creatinine, phosphate, and calcium. Intact parathyroid hormone (iPTH) was measured with the Elecsys ECLIA (Roche Diagnostics, Indianapolis, IN), and C–terminal c-terminal fibroblast growth factor was measured with the Immutopics ELISA (San Clemente). Vitamin D metabolites were measured *via* liquid chromatography-mass spectrometry/mass spectrometry using an Acquity UPLC coupled to a Quattro Premier Mass Spectrometer (Waters, Milford, MA) as described by Aronov *et al.* (26). The lower limit of quantification was 25 pg/ml for all vitamin D metabolites. The interassay and intra–assay coefficients of variation were ≤25%.

Statistical Analyses

Serum levels of 25(OH)D and 24,25$(OH)_2$D were below the detection limit (DL) in very few samples, for which concentration was assumed to be $DL/\sqrt{2}$. For 1,25$(OH)_2$D, approximately 30% of values were below the DL and handled using the distribution–based multiple imputation method (27). Imputation yielded five complete datasets; these were analyzed separately, and the results were pooled. The influence of potential environmental and disease–associated covariates on vitamin D status was analyzed using multivariable linear regression modeling of log–transformed vitamin D levels. Backward variable selection was performed with a threshold of P=0.15.The influence of genetic polymorphisms on vitamin D levels was tested on the basis of the reduced models. The interaction between albuminuria and glomerulopathy on vitamin D levels was tested in multivariate analysis.

Group comparisons were carried out using chi-squared, t, or Mann–Whitney tests in the case of two groups and chi-squared test, ANOVA, or Kruskal–Wallis test in the case of more than two groups as appropriate. For seasonal and geographic differences, nonparametric Hodges–Lehmann estimators with corresponding 95% confidence intervals were calculated. Descriptive P values <0.05 were considered statistically significant. Data were analyzed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Patient Characteristics

Patient characteristics are given in Table 1. Mean age was 12 years old. Ethnicity was white in 94.6% of the

Table 1. Patient characteristics

Parameter	All	CKD Stage				*P* Value
		3a	3b	4	5	
N	500	37	162	265	36	
Age, yr	12.0±3.3	12.9±2.8	11.9±3.3	12.0±3.4	12.2±3.1	0.36
Boys, %	64.2	70.3	67.3	62.6	55.6	0.44
Biochemistry						
eGFR, ml/min per 1.73 m^2	28.2±10.36	51.0±3.96	35.9±4.19	22.4±4.19	13.4±1.32	
Albuminuria, g/g creatinine	0.37 (1.17)	0.08 (0.21)	0.22 (0.78)	0.46 (1.56)	1.10 (2.39)	<0.001
Serum albumin, g/L	38.8±6.10	38.8±4.86	39.3±6.26	39.0±6.00	35.9±8.58	0.20
Serum calcium,[a] mg/dl	8.79±0.99	8.72±1.14	8.82±0.78	8.81±1.02	8.60±1.39	0.73
Serum phosphorus, mg/dl	4.76±1.11	4.57±1.30	4.47±1.04	4.83±0.96	5.75±1.60	<0.001
Mineral hormones						
Plasma iPTH, ng/ml	116 (127)	63 (62)	88 (83)	148 (143)	221 (214)	<0.001
Serum cFGF23, kRU/L	184 (202)	134 (115)	129 (138)	221 (196)	599 (901)	<0.001
Serum 25(OH)D, ng/ml	11.1 (11.3)	10.2 (9.33)	13.0 (10.2)	11.0 (11.6)	8.86 (11.9)	0.14
Serum 1,25$(OH)_2$D, pg/ml	70.0 (92.3)	60.0 (92.3)	80.0 (100)	60 (82.3)	55.0 (72.3)	0.07
Serum 24,25$(OH)_2$D, ng/ml	0.39 (0.53)	0.36 (0.41)	0.45 (0.55)	0.38 (0.47)	0.38 (0.47)	0.06
Medications						
Vitamin D supplements	56 (11.2%)	2 (5.4%)	17 (10.3%)	29 (10.9%)	8 (22.2%)	0.12
Active vitamin D analog	254 (50.8%)	14 (37.8%)	71 (43.8%)	149 (56.2%)	20 (55.6%)	0.03

Data are given as means±SDs, medians (interquartile ranges), or *n* (%) as appropriate. *P* values resulted from chi-squared tests, ANOVA, or the nonparametric Kruskal–Wallis test. iPTH, intact parathyroid hormone; cFGF23, c-terminal fibroblast growth factor 23; 25(OH)D, 25-hydroxy-vitamin D; 1,25(OH)2D, 1,25-dihydroxy-vitamin D; 24,25(OH)2D, 24,25-dihydroxy-vitamin D.
[a]Corrected for serum albumin concentration.

patients. Renal hypodysplasia was the underlying diagnosis in 69.4% of patients, glomerulopathies were the underlying diagnosis in 7.6%, tubulointerstitial and metabolic disorders were the underlying diagnosis in 12%, CKD after AKI was the underlying diagnosis in 4%, and other diagnoses were the underlying diagnosis in 7%. The most common glomerulopathy was FSGS (*n*=16) followed by rapid progressive GN (*n*=4) and IgA nephropathy (*n*=4). Blood samples were obtained in the winter season in 57% of the patients. Annual local solar irradiation at the place of residence was <1200 kW/m^2 in 25.0%, 1200–1600 kW/m^2 in 40.4%, and >1600 kW/m^2 in 34.6% of patients. Age, radiation, and the season of blood sampling were distributed similarly across CKD stages. The genotype frequency distribution of the selected SNPs in *DHCR7*, *GC*, *CYP2R1*, and *CYP24A1* is given in Table 2.

Vitamin D Metabolite Concentrations

Descriptive statistics of the vitamin D metabolite concentrations are given in Tables 1 and 2 and by region of origin in Supplemental Table 1. The vast majority of patients (94.2%) showed 25(OH)D levels below the repletion cutoff (30 ng/ml), and 68.2% were considered 25(OH)D deficient [25(OH)D<16 ng/ml]. Serum 1,25$(OH)_2$D levels were highly correlated with 25(OH)D (r=0.56; P<0.001). In multivariate analysis, 25(OH)D levels showed the strongest association with 1,25$(OH)_2$D [Table 3, correlates of serum 1,25$(OH)_2$D concentration]. Serum 24,25$(OH)_2$D levels correlated with both 25(OH)D (r=0.87; P<0.001) and 1,25$(OH)_2$D concentrations (r=0.55; P<0.001) (Figure 1).

Vitamin D Supplementation

Oral vitamin D supplements were administered in 11.2% of all patients at a median daily dose of 1000 IU/m^2 body surface area. Vitamin D supplementation prevalence increased with declining eGFR from 5.4% in CKD stage 3a to 22.2% in CKD stage 5. In the vitamin D–replete group, 41.4% of patients were vitamin D supplemented compared with 21.5% in the insufficient group and 4.7% in the deficient group. Vitamin D supplementation was associated with twofold higher 25(OH)D (P<0.001) and almost threefold higher 24,25$(OH)_2$D levels (P<0.001) (Table 2). Treatment with active vitamin D analogs was associated with significantly higher 1,25$(OH)_2$D (70 [102] ng/ml versus 60 [82] ng/ml; P=0.05) and 24,25$(OH)_2$D (0.41 [0.58] ng/ml versus 0.37 [0.47] ng/ml; P=0.03) levels in univariate analysis. In multivariate analysis, 25(OH)D levels showed a stronger association than treatment with an active vitamin D analog. Patients with a higher dosage of active vitamin D had relatively lower 1,25$(OH)_2$ levels.

Seasonal and Geographic Factors

25(OH)D levels from blood samples collected in the winter were a median of 4.8 ng/ml lower (95% confidence interval, 3.5 to 6.1) compared with those in the summer season. Among nonsupplemented patients, children in regions with solar radiation >1600 kW/m^2 per year showed in median 3.8 ng/ml lower 25(OH)D levels compared with those in regions with lower sunlight intensity (95% confidence interval, −5.2 to −2.5; P<0.001) in univariate analysis. In this regard, analysis of 25(OH)D levels by treatment center revealed a highly significant inverse

Table 2. Genetic and environmental correlates of vitamin D levels

	Patients, %	25(OH)D, ng/ml	*P* Value	Vitamin D Deficient, %	$1,25(OH)_2D$, pg/ml	*P* Value	$24,25(OH)_2D$, ng/ml	*P* Value
Environmental correlates								
Region								
Western Europe	27.4	18.1 (14.3)	<0.001	41.6	70 (85)	0.004	0.72 (0.70)	<0.001
Central Europe	9.4	14.2 (9.0)		59.6	80 (130)		0.60 (0.59)	
Southern Europe	7.6	13.1 (8.1)		68.4	70 (70)		0.38 (0.51	
Turkey	55.6	9.1 (8.4)		82.7	50 (80)		0.31 (0.32)	
Local solar radiation								
Low, <1200 kW/m²	25.0	16.0 (15.0)	<0.001	51.2	76 (40)	<0.001	0.67 (0.75)	<0.001
Medium, 1200–1600 kW/m²	40.4	11.4 (11.0)		67.3	75 (107)		0.40 (0.57)	
High, >1600 kW/m²	34.6	9.0 (8.5)		81.5	50 (80)		0.29 (0.32)	
Glomerulopathy								
Yes	7.6	6.8 (9.4)	<0.001	86.8	50 (87)	0.21	0.67 (0.71)	0.17
No	92.4	11.6 (11.3)		66.7	70 (88)		0.31 (0.32)	
Season								
Winter	57	8.9 (9.3)	<0.001	77.9	50 (62)	<0.001	0.31 (0.39)	<0.001
Summer	43	14.6 (10.9)		55.4	90 (90)		0.55 (0.53)	
Vitamin D supplementation								
Yes	11.2	21.6 (14.1)	<0.001	28.1	100 (85)	<0.001	0.90 (0.60)	<0.001
No	88.8	10.4 (10.1)		73.2	60 (90)		0.35 (0.44)	
Genetic correlates								
GC (rs7041)								
Major homozygotes	36.4	12.4 (12.9)	0.43	63.7	70 (99)	0.27	0.38 (0.57)	0.59
Heterozygotes	45.0	11.0 (10.3)		70.7	70 (79)		0.39 (0.50)	
Minor homozygotes	18.6	10.7 (10.3)		71.0	60 (79)		0.43 (0.52)	
GC (rs4588)								
Major homozygotes	54.0	11.8 (12.8)	0.74	66.3	70 (100)	0.18	0.38 (0.52)	0.05
Heterozygotes	37.8	10.6 (9.65)		71.4	60 (78)		0.37 (0.50)	
Minor homozygotes	8.2	12.5 (8.58)		65.9	70 (96)		0.61 (0.73)	
GC (rs2282679)								
Major homozygotes	53.8	11.5 (12.8)	0.74	66.2	70 (100)	0.21	0.38 (0.52)	0.06
Heterozygotes	38.0	10.7 (10.2)		71.6	60 (78)		0.38 (0.48)	
Minor homozygotes	8.2	12.5 (10.8)		65.8	70 (96)		0.61 (0.74)	
CYP2R1 (rs10741657)								
Major homozygotes	44.6	10.6 (9.8)	0.27	72.7	70 (87)	0.70	0.37 (0.52)	0.25
Heterozygotes	44.0	12.8 (11.9)		65.0	60 (89)		0.4 (0.52)	
Minor homozygotes	11.4	12.8 (14.3)		63.2	50 (82)		0.45 (0.67)	
DHCR7 (rs12785878)								
Major homozygotes	45.6	12.6 (12.2)	0.30	65.4	60 (90)	0.99	0.44 (0.62)	0.08
Heterozygotes	40.4	11.1 (10.4)		69.8	65 (89)		0.38 (0.45)	
Minor homozygotes	14.0	9.7 (10.7)		72.9	70 (75)		0.35 (0.36)	

Table 2. (Continued)

	Patients, %	25(OH)D, ng/ml	*P* Value	Vitamin D Deficient, %	$1,25(OH)_2D$, pg/ml	*P* Value	$24,25(OH)_2D$, ng/ml	*P* Value
CYP24A1 (rs6013897)								
Major homozygotes	63.0	11.8 (12.8)	0.17	34.3	60 (89)	0.09	0.4 (0.56)	0.13
Heterozygotes	29.4	10.6 (9.65)		25.2	60 (90)		0.35 (0.47)	
Minor homozygotes	7.6	12.5 (8.58)		36.8	80 (80)		0.47 (0.42)	

Concentrations are given as medians and interquartile ranges. *P* values resulted from the nonparametric Wilcoxon rank sum test (two groups) or the Kruskal–Wallis test (more than two groups). Genetic correlates: *GC*, vitaminD binding protein; *CYP2R1*, 25-hydroxylase; *DCHR7*, 7-dehydrocholesterol reductase; *CYP24A1*, 24-hydroxylase. 25(OH)D, 25-hydroxy-vitamin D; 1,25(OH)2D, 1,25-dihydroxy-vitamin D; 24,25(OH)2D, 24,25-dihydroxy-vitamin D.

U–shaped relationship: 25(OH)D levels are positively associated with local solar radiation in regions with <1300 kW/m^2 per year but sharply declined with higher radiation above this cutoff level (Figure 2). Patients from Turkey exhibited significantly lower 25(OH)D and $24,25(OH)_2D$ levels than those from other European regions, including patients from Italy and Portugal living at comparable geographic latitude (Table 2) ($P<0.001$). During summer time, nonsupplemented Turkish boys had significantly higher 25(OH)D levels than nonsupplemented Turkish girls, whereas no sex difference was observed in children from the other European regions. During wintertime, no sex differences were evident in any region. In the multivariate analysis of the total cohort, the lower 25(OH)D and $24,25(OH)_2D$ levels of Turkish patients seemed independent of vitamin D supplementation, solar radiation, season, and underlying renal disease.

Renal Diagnosis and Function

25(OH)D levels were significantly lower in patients with glomerulopathies compared with in patients with other diagnoses ($P<0.001$) (Table 2). This difference was confirmed in the multivariate analysis [Table 3, correlates of serum 25(OH)D concentration, Supplemental Table 2, full model]. There was a significant interaction between albuminuria and glomerulopathies, indicating an even stronger association of albuminuria with 25(OH)D levels in patients with glomerulopathies.

In patients with glomerulopathies, $24,25(OH)_2D$ was relatively higher than in other patients, and albuminuria was positively associated with $24,25(OH)_2D$ levels. In this regard, there was no significant interaction between the presence of a glomerulopathy and the extent of albuminuria ($P=0.07$) [Table 3, correlates of serum $24,25(OH)_2D$ concentration].

Whereas 25(OH)D and $24,25(OH)_2D$ levels did not differ across CKD stages in the population as a whole (Table 1), significantly lower levels were observed in nonvitamin D–supplemented patients with CKD stage 5 compared with those with stage 3 or 4 for both 25(OH)D (6.99 [5.25] versus 10.7 [10.7]; $P<0.01$) and $24,25(OH)_2D$ (0.2 [0.19] versus 0.37 [0.46] ng/ml; $P<0.003$). In the multivariate analysis, eGFR was confirmed as being significantly associated with $24,25(OH)_2D$ but not with 25(OH)D levels.

Endocrine Factors

By univariate analysis, 25(OH)D and $24,25(OH)_2D$ were negatively associated with iPTH ($r=-0.24$; $P<0.001$ and $r=-0.3$; $P<0.001$, respectively) but not with c-terminal fibroblast growth factor (cFGF23). In the multivariable regression, iPTH was associated with lower $24,25(OH)_2D$ levels, and cFGF23 was associated with higher $24,25(OH)_2D$ levels [Table 3, correlates of serum $24,25(OH)_2D$ concentration, Supplemental Table 3, full model].

Genetic Variants

Vitamin D levels by genetic polymorphisms of the whole cohort are listed descriptively in Table 2, genetic correlates, and another subdivision by region of origin is shown in Supplemental Table 1. Among the variants selected for multivariable analysis in *CYP2R1*, *CYP24A1*, *GC*, and *DHCR7*, only polymorphisms in the *GC* gene exhibited a

Table 3. Environmental and genetic correlates of (log–transformed) serum 25-hydroxy-vitamin D, 1,25-dihydroxy-vitamin D, and 24,25-dihydroxy-vitamin D concentrations

Parameter	Estimate	95% CI	*P* Value
Correlates of serum 25(OH)D concentration			
Intercept	2.48	2.25 to 2.72	<0.001
Age, 10 yr	−0.16	−0.31 to −0.00	0.05
Sex, girl	−0.13	−0.24 to −0.03	0.02
Season, summer	0.37	0.26 to 0.48	<0.001
Albuminuria, g/g	−0.07	−0.10 to −0.04	<0.001
Vitamin D supplementation	0.61	0.44 to 0.79	<0.001
Region of origin: Turkey	−0.32	−0.43 to −0.21	<0.001
Glomerulopathy	−0.45	−0.65 to −0.25	<0.001
Glomerulopathy × albuminuria, g/g	−0.02	−0.03 to −0.00	0.02
GC1s-1s[a]	0.13	0.02 to 0.24	0.02
Correlates of serum 1,25(OH)$_2$D concentration			
Intercept	2.27	1.63 to 2.90	<0.001
25(OH)D, log	0.73	0.62 to 0.83	<0.001
Sex, girl	−0.21	−0.37 to −0.09	<0.01
Active vitamin D analog	0.20	0.02 to 0.37	0.03
Active vitamin D analog × dosage	−0.31	−0.54 to 0.08	<0.01
cFGF23, kRU/L log	−0.05	−0.14 to 0.03	0.21
Metabolic	0.29	−0.10 to 0.68	0.14
Correlates of serum 24,25(OH)$_2$D concentration			
Intercept	−4.11	−4.55 to −3.66	<0.001
1,25(OH)$_2$D, log	0.06	0.03 to 0.10	<0.001
25(OH)D, log	1.04	0.99 to 1.10	<0.001
Serum albumin, 10 g/L	−0.05	−0.12 to 0.02	0.16
Sex, girl	0.05	−0.01 to 0.11	0.11
eGFR, 10 ml/min per 1.73 m^2	0.08	0.05 to 0.11	<0.001
cFGF23, kRU/L, log	0.11	0.07 to 0.15	<0.001
iPTH, ng/ml log	−0.04	−0.06 to −0.02	<0.001
Glomerulopathy	0.78	0.23 to 1.33	<0.01
Albuminuria, g/g	0.02	0.00 to 0.05	0.04
Glomerulopathy × albuminuria, g/g	−0.01	−0.03 to 0.00	0.07
Region of origin: Turkey	−0.17	−0.23 to −0.11	<0.001
GC rs4588, major allele no.	−0.05	−0.10 to −0.01	0.04

95% CI, 95% confidence interval; 25(OH)D, 25-hydroxy-vitamin D; *GC*, vitamin D binding protein gene; 1,25(OH)2D, 1,25-dihydroxy-vitamin D; cFGF23, c-terminal fibroblast growth factor 23; 24,25(OH)2D, 24,25-dihydroxy-vitamin D; iPTH, intact parathyroid hormone.
[a]Major homozygous for both rs7041 and rs4588.

weak association with vitamin D status. Patients carrying one or two minor alleles of rs7041 showed approximately 10% lower 25(OH)D concentrations than major allele homozygotes. In multivariate analysis, 25(OH)D levels were significantly higher for patients who were homozygous for the major allele of both rs7041 and rs4588 of the *GC* gene when correcting for age, glomerular disease, albuminuria, season, region, and vitamin D supplementation [Table 3, correlates of serum 25(OH)D concentration]. Conversely, 24,25(OH)$_2$D levels were significantly lower for patients with at least one major allele of rs4588 [Table 3, correlates of serum 1,25(OH)$_2$D concentration].

Discussion

This study provides a comprehensive analysis of modifiable and nonmodifiable correlates of the vitamin D status of children with CKD. The study cohort covered a large geographic area with substantial variation of climatic conditions, ethnic composition, nutritional and cultural attitudes, and health care policies. To our knowledge, our study is the first to address the role of common variants in vitamin D–regulating genes on vitamin D status in patients with CKD.

Our study across 55 pediatric nephrology centers impressively shows the high current prevalence of vitamin D deficiency in the European pediatric CKD population. Only one in 17 children had a 25(OH)D level considered optimal according to the KDOQI definition (25), and two thirds of the cohort were classified as deficient. Our findings confirm previous reports of the prevalence of vitamin D insufficiency and deficiency in children with CKD ranging between 16% and 82% (6,7,9,10).

CKD

Conflicting evidence has been reported regarding a potentially increasing prevalence of vitamin D deficiency with advancing CKD (6,9,28,29). When adjusting for genetic, environmental, and supplementation variables, we did not observe a major association of renal function

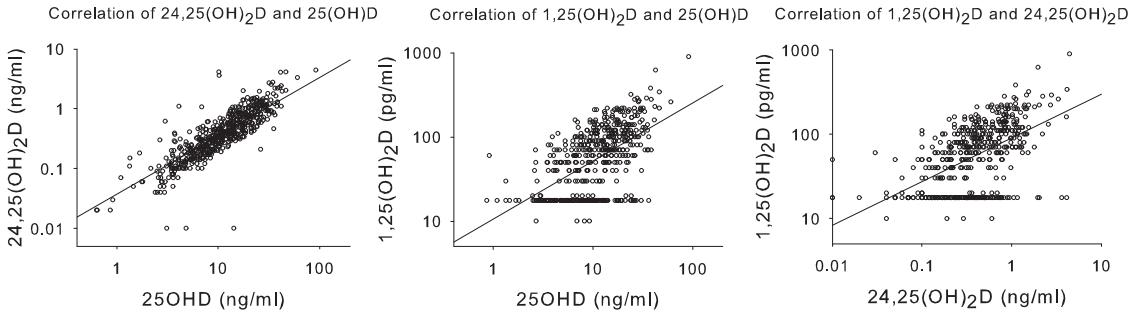

Figure 1. | **Correlation of vitamin D metabolites.** 1,25$(OH)_2$D, 1,25-dihydroxy-vitamin D; 24,25$(OH)_2$D, 24,25-dihydroxy-vitamin D; 25(OH)D, 25-hydroxy-vitamin D.

on 25(OH)D levels, suggesting that disease-associated factors other than declining eGFR *per se* contribute to the high prevalence of vitamin D deficiency in CKD.

Vitamin D Supplementation

Patients with vitamin D supplementation had twice as high serum 25(OH)D levels and a 70% lower prevalence of vitamin D deficiency. The positive association of supplementation was independent of age and the severity of renal failure. This observation is in keeping with the results of controlled pediatric trials in children with mild to moderate CKD (7,30), whereas previous observational studies, probably because of their smaller sample size, had been unable to link supplementation to vitamin D levels (6,9,29). However, on the background of vitamin D deficiency in 73% of nonsupplemented patients, the overall supplementation rate of 11% seems inappropriately low.

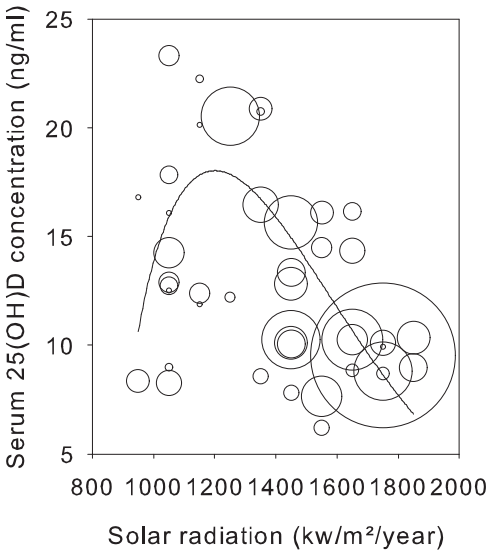

Figure 2. | **25-Hydroxy-vitamin D [25(OH)D] levels for each center by solar radiation.** Each circle represents one center and is sized according to patient number per center; 25(OH)D levels are given as median per center.

Season and Ethnic Factors

Although lower vitamin D levels during the winter season were expected, the relationship of vitamin D concentrations with the region of origin was surprising. This result prevailed in the multivariate analysis and when only patients without vitamin D supplementation were analyzed. We speculate that cultural factors, such as clothing habits with higher skin cover in girls, and genetic diversity contribute to this finding. Previous studies in Turkish children found sex differences in 25(OH)D levels and associations to concealing clothing in healthy children (31–33). An additional factor may be the slightly darker skin among Turkish patients compared with central European populations, because skin pigmentation has been shown to negatively influence ultraviolet B–induced vitamin D synthesis (34). In Europe and Turkey, dairy milk products usually are not fortified with vitamin D. Therefore, the main dietary sources of vitamin D are fish products. Surveys of the general population actually show high disparities in the daily fish consumption in Europe and Turkey, with a significantly lower consumption of fish in Turkey (about one third of the consumption in Europe). Southern European countries (*e.g.*, Italy and Greece) are reported to have higher rates of fish consumption compared with northern regions (Germany and The Netherlands) (35,36). Although this could further explain the very low vitamin D levels in Turkey, it may play a pivotal role in the explanation of the U-shaped curve of vitamin D levels throughout Europe and Turkey.

Albuminuria and Glomerulopathies

Our observation of the association between glomerulopathies and low 25(OH)D levels confirms the findings of previous studies (6,29). Loss of DBP in patients who are proteinuric has been assumed as the likely underlying mechanism (29). Notably, 24,25$(OH)_2$D levels were relatively higher in patients with glomerulopathies and positively correlated with albuminuria. Lower 25(OH)D levels in patients with glomerulopathies may, therefore, partially be explained by more rapid degradation of 25(OH)D secondary to DBP loss and thus, reduced vitamin D binding capacity in patients with gross proteinuria (29).

24,25$(OH)_2$D

We observed strong independent associations of both 25(OH)D and 1,25$(OH)_2$D with 24,25$(OH)_2$D levels,

confirming the role of $1,25(OH)_2D$ as the main inducer of CYP24A1. In addition, iPTH and cFGF23 showed independent opposite associations with $24,25(OH)_2D$, pointing to their important regulatory roles in vitamin D metabolism. The positive, highly significant relationship of cFGF23 with $24,25(OH)_2D$ levels supports its role as an antagonist to $1,25(OH)_2D$ (29,37,38) and agonist of CYP24A1, serving to protect the organism from calcium overload (39). However, in the literature, evidence is not consistent in this regard (40).

Finally, although 25(OH)D levels were largely independent of eGFR, we noted an independent association of borderline significance of $24,25(OH)_2D$ with eGFR, similar to observations in both the pediatric and adult CKD population (29,41).

Genetic Influence

We systematically studied the role of common genetic variants on vitamin D status in our pediatric CKD cohort. Common variants in *GC* are associated with lower DBP serum levels in the general population (16,42,43). In this cohort, only a weak association of lower 25(OH)D levels and higher $24,25(OH)_2D$ with a polymorphism in the *GC* gene was found.

Additional polymorphisms (*e.g.*, in genes involved in cholesterol synthesis or hydroxylation of vitamin D metabolites) have been associated with vitamin D deficiency (15,24). However, contrary to these findings in healthy cohorts, we could not verify the influence of the described polymorphisms in our cohort. This may partly be attributed to the fact that our cohort was an ethnically rather homogenous group, and differences were not sufficiently great to detect genetic variabilities. We hypothesize that, in this cohort, environmental and disease-associated factors are more relevant in a cohort of CKD and may render negligible the influence of small genetic variations.

Although the large cohort assessed in a wide geographic area and the first exploration of the role of common genetic variants on vitamin D metabolism in pediatric CKD are the most important strengths of this study, our work is limited by its cross–sectional, descriptive character, which precludes demonstration of cause and effect relationships. Also, in the absence of DBP measurements, we can only speculate about genotype–dependent quantitative and/or qualitative differences in DBP and their effects on vitamin D status in children with different types of kidney disease and stages of CKD. Because the participants of this study were mainly white, the results should not be generalized to other ethnic groups, although our data warrant further investigation in other ethnic populations.

In conclusion, we present a unique comprehensive study describing the vitamin D status and its correlates in children with CKD. Vitamin D deficiency is widespread in European children with CKD, and current supplementation practices are only partially efficient in repleting vitamin D stores. Vitamin D levels are influenced by a complex interplay of environmental, disease- and treatment-associated, and ethnic and genetic factors. Vitamin D levels should be monitored, and supplementation practices should be reviewed and adapted accordingly.

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A list of the investigators contributing to the 4C Study is in the Supplemental Appendix.

Disclosures

None.

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