Rickets in the Middle East: Role of Environment and Genetic Predisposition

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Context: The Middle East has a high incidence of rickets, and it is also common in Europe-dwelling children of Middle Eastern origin.

Objective: The objective of the study was to explore the mechanisms leading to rickets in children of the Middle East.

Design and Setting: We conducted a prospective study in 98 rachitic and 50 controls (aged 6 months to 4 yr) from university and community outpatient hospitals in Egypt and Turkey.

Main Outcome Measures: We collected epidemiological, maternal, nutritional, radiographic, and biochemical parameters; markers of bone turnover; and vitamin D receptor (VDR) gene polymorphisms.

Results: Epidemiological factors had a key role in pursuit of rickets; Egyptian and Turkish patients had lower ($P < 0.01$) dietary calcium intake than controls and the recommended dietary intakes, and serum 25-hydroxyvitamin D levels were reduced in patients, the difference with controls being significant ($P < 0.001$) only in Turkey, although rickets was more severe in Egypt as determined by the x-ray score ($P < 0.05$). In Turkey, the *F* VDR allele frequency was significantly ($P < 0.05$) increased in patients. The *BB* VDR genotype was associated with lower serum 25-hydroxyvitamin D levels in both patients and controls and with severity of rickets.

Conclusions: In Turkey most patients had vitamin D deficiency, whereas in Egypt they had mostly calcium insufficiency combined with vitamin D deficiency. In this environ, VDR genotypes may predispose to rickets by increased frequency of the *F* allele. The unique environs and genetic predisposition have to be accounted for in the design of preventive measures, rather than using European or American recommended dietary intake for calcium and vitamin D. **(*J Clin Endocrinol Metab* 93: 1743–1750, 2008)**

Despite ample sunlight, the Middle East has a high incidence of rickets (1–3). Moreover, rickets is common in children of Middle Eastern origin living in European countries (4–9) and in Australia (10). The cause of rickets in Middle Eastern children remains an enigma. Limited sunlight exposure has been blamed on cultural practices, such as clothing and veiling in Muslim

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Abbreviations: BAP, Bone alkaline phosphatase isoenzyme; ESPE, European Society for Pediatric Endocrinology; ICTP, carboxyterminal telopeptide of type I collagen; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; $24,25(OH)_2D$, 24,25-dihydroxyvitamin D; 25(OH)D, serum 25-hydroxyvitamin D; PINP, propeptide of type I procollagen; RDI, recommended dietary intake; VDR, vitamin D receptor.

women, spending most time indoors, and exclusive or prolonged breast-feeding without vitamin D supplements (1–3, 11). However, a significant number of children in these studies had normal serum 25-hydroxyvitamin D [25(OH)D] and high 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] levels, suggesting mechanisms beyond vitamin D deficiency (1, 2, 12–14). A relative insensitivity to vitamin D was suggested to occur in children of the Middle East, as indicated by the evidence that high doses of vitamin D were needed to cure rickets (13–15). Studies in South African (16) and Nigerian children (12, 14, 17) demonstrated that, rather than vitamin D deficiency, a dietary calcium deficiency may be a factor in the etiology of rickets in these countries.

The present study was a prospective multicenter collaboration, designed and executed by the European Society for Pediatric Endocrinology (ESPE) Bone Club. The aim of study was to explore the mechanisms leading to rickets in children of the Middle East, with an intention to provide a scientific basis for large-scale prevention. The study was designed to compare affected against unaffected children in Egypt and Turkey, in which rickets incidence is as high as 13 and 6%, respectively (18, 19). Characterization included epidemiological and nutritional aspects, clinical and radiographic findings, calcium-phosphate metabolism, biochemical markers of bone turnover, and genetic polymorphism of the vitamin D receptor (VDR).

Subjects and Methods

Subjects

A total of 98 rachitic (63 males and 35 females, aged 11.7 ± 8.2 months) and 50 controls (30 males and 20 females, aged 14.2 ± 8.3 months) from Egypt and Turkey were enrolled in the study, examined, and blood drawn simultaneously throughout the year to exclude seasonal variation from January to December 2004. Both patients and controls were recruited from university and community outpatient hospitals in Cairo, Egypt (latitude 30.01 N, corresponding to New Orleans, LA), and Erzurum and Van, Turkey (latitude 39.57 N, corresponding to Philadelphia, PA, and 38.30 N, corresponding to Palermo, Sicily, Italy, respectively). Selection criteria for patients and controls participating to the study are given in Table 1. Exclusion criteria were based on the history, physical examination, and laboratory testing. Controls were brothers or sisters of nonrachitic patients coming to the outpatient clinic or children with minor illnesses as upper respiratory infections.

The study protocol was approved by the ethical committees of the Universities of Ain Shams (Cairo, Egypt) and Marmara (Istanbul, Turkey) and all the participating centers and was conducted according to the Declaration of Helsinki II. Written informed consents were obtained from the parents of both patients and controls.

Study design

Children were examined, a semiquantitative food frequency questionnaire was administered (20), and a parent or guardians were interviewed. In all children, nutritional status based on weight and presence or absence of edema according to the Wellcome Trust classification (21) was evaluated. Epidemiological questionnaire included living conditions, family data, socioeconomic status by parental education and family income, and sunlight exposure by clothing type and time spent outdoors. A maternal questionnaire included interlude to next pregnancy, prenatal and nursing period care and nutrition, clothing type and time spent outdoor during pregnancy, and calcium and vitamin D supplementation. Nutritional data included type of milk fed during infancy, (breast, cow, goat, or formula), dietary calcium intake, and calcium and vitamin D supplementation.

In both patients and controls, dietary calcium intake by a 3-d nutrition diary was estimated and compared with the recommended dietary intakes (RDI) by the National Institute of Health (NIH; Bethesda, MD) (22) and the ESPE Bone Club (23). Calcium insufficiency was arbitrarily defined as less than 50% of the RDI, and suboptimal intake was defined as between 51 and 85% of the RDI. A dietary calcium intake higher than 86% was considered adequate. A value of 25(OH)D under or above 15 ng/ml (37.5 nmol/liter) was defined as vitamin D deficiency or sufficiency, respectively (24, 25). Serum 25(OH)D levels were also assessed in the mothers of Turkish patients.

Radiographic analysis

In all patients, radiographic assessment of the severity of rickets was examined by standard x-rays of both wrist and knee and scored from 0 (normal) to 10 points (severe) by three observers independently according to Thacher *et al.* (26), and the mean values were used for analyses. Wrist was scored for both radius and ulna and knee for both distal femur and proximal tibia separately.

TABLE 1. Selection criteria in patients and controls for participation in the study

Inclusion criteria	Exclusion criteria
Patients	
Age range 6 months to 4 yr	History of prematurity
Clinical, biochemical, and radiograghic signs of rickets	Renal, liver, intestinal, cardiac, or central nervous system disease
No treatment with vitamin D (except prophylactic supplementation dose, 400 IU/d)	Chronic disease
	Bone disease (with the exclusion of rickets)
No treatment with calcium	Tuberculosis
No medications interfering with calcium-phosphate metabolism	Family history of hereditary forms of rickets
Only one child per family	Treatment with vitamin D or vitamin D supplements above 400 IU/d
Written informed consent	
Controls	
Age range 6 months to 4 yr	History of prematurity
Normal and healthy with no symptoms or signs of rickets	Chronic disease
No treatment with vitamin D (except prophylactic supplementation dose, 400 IU/d)	Bone disease
	Severe acute disease
No treatment with calcium	Tuberculosis
No medications interfering with calcium-phosphate metabolism	Family history of hereditary forms of rickets
Only one child per family	Treatment with vitamin D or vitamin D supplements above 400 IU/d
Written informed consent	

Assays

Serum calcium and phosphate levels were measured by standard methods. Serum intact PTH, osteocalcin, and bone alkaline phosphatase isoenzyme (BAP) levels were measured in a single laboratory by a two-site immunoradiometric assay (Allegro and human osteocalcin, Nichols Institute, San Juan Capistrano, CA; and Tandem-R Ostase; Hybritech Europe, Liege, Belgium, respectively). Serum 25(OH)D, $1,25(OH)_2D$, and 24,25-dihydroxyvitamin D [$24,25(OH)_2D$] levels were measured in a single laboratory by a competitive binding RIA (DiaSorin, Stillwater, MN) as previously described (27). 1α-Hydroxylase and 24-hydroxylase activity were estimated from the ratio of the product/substrate [$1,25(OH)_2D$ to 25(OH)D and $24,25(OH)_2D$ to 25(OH)D, respectively] and expressed as percent (27). Serum aminoterminal propeptide of type I procollagen (PINP) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) levels were measured by RIA (Orion Diagnostic, Espoo, Finland). For all measurements, interassay variability was less than 9% and intraassay variability less than 7%. All blood samples were measured in duplicates.

VDR gene polymorphisms

In all patients and controls and the mothers of patients, VDR gene polymorphisms at intron 8 (*Bsm*I) and exon 2 (*Fok*I) were determined for 296 alleles in DNA extracted from leukocytes by direct PCR fragment sequencing (28). Alleles were designated *b* or *B* for intron 8 polymorphism when the restriction enzyme site for *Bsm*I was present or absent, and *f* or *F* for exon 2 polymorphism when the restriction enzyme site for *Fok*I was present or absent, respectively.

Statistical analysis

Comparison of clinical and biochemical data between patients and controls was determined by a nonparametric Mann-Whitney rank-sum test and the comparison of proportions by z test with Yates correction. Differences for VDR gene alleles, genotypes, and combined genotype distribution between patients and controls and mothers of patients within each population, as well as between the two populations, were analyzed by the χ^2 test for three-by-three and two-by-two tables. Simple regression analyses were carried out among the biochemical parameters, dietary calcium intake, or radiographic score of rickets. All statistical analyses were performed by Statview 4.5 program (Abacus Concepts, Inc., Berkley, CA). Data are expressed as mean ± SD unless otherwise stated. A value of $P < 0.05$ was considered significant.

Results

Epidemiology, maternal care, and nutrition

Epidemiological, maternal, and nutritional data are summarized in Table 2. In Turkey, family size, overcrowding, income, and socioeconomic status were worse, and the time spent outdoors was lower in patients, compared with controls. Among the maternal factors, interlude to the next pregnancy, prenatal care, physician visits, nutrition during nursing, and exposed body surface were lower in the mothers of patients. In Egypt, epidemiological data did

TABLE 2. Epidemiological, maternal, and nutritional findings in patients and controls

	Egypt			Turkey		
	Patients (n = 30)	Controls (n = 20)	*P*	Patients (n = 68)	Controls (n = 30)	*P*
Epidemiology						
Living conditions						
Household size (m^2)	79.3 ± 23.0	72.5 ± 11.2	NS	96.3 ± 29.0	103.2 ± 24.8	NS
Household size (m^2/people)	15.5 ± 7.5	15.1 ± 6.3	NS	12.9 ± 5.4	23.7 ± 8.8	<0.0001
No. of children	3.2 ± 1.4	3.1 ± 1.5	NS	4.1 ± 2.6	2.3 ± 1.1	<0.001
People/rooms	2.4 ± 0.8	2.6 ± 0.5	NS	3.1 ± 1.3	1.7 ± 0.8	<0.0001
Father's age (yr)	35.2 ± 8.5	37.2 ± 7.9	NS	31.5 ± 6.2	32.6 ± 6.2	NS
Mother's age (yr)	28.6 ± 6.4	29.6 ± 6.6	NS	28.6 ± 6.7	28.9 ± 6.6	NS
Socioeconomic status						
Father's education[a]	2.4 ± 1.1	2.4 ± 1.2	NS	2.2 ± 0.8	2.6 ± 1.1	<0.05
Mother's education[a]	2.3 ± 1.1	1.7 ± 0.9	<0.05	1.3 ± 0.5	2.2 ± 1.1	<0.0001
Family income[b]	2.3 ± 0.6	2.2 ± 0.5	NS	2.1 ± 0.9	2.6 ± 0.9	<0.02
Sunlight exposure						
Child's clothing[c]	3.6 ± 1.3	3.6 ± 1.0	NS	2.2 ± 1.1	2.7 ± 1.5	NS
Time spent outdoors (h/wk)	7.0 ± 10.6	18.4 ± 12.7	<0.01	2.2 ± 4.2	5.7 ± 6.3	<0.01
Maternal care						
Interlude to next pregnancy (months)	29.1 ± 14.5	35.4 ± 25.2	NS	30.3 ± 17.4	64.6 ± 65.5	<0.001
Prenatal care (percent adequate)	47	45	NS	25	67	<0.001
Regular visit (%)	29	40	NS	15	57	<0.0001
Nutrition during nursing (percent adequate)	57	90	<0.05	46	83	<0.01
Clothing during pregnancy[c]	2.1 ± 1.1	2.2 ± 1.0	NS	2.4 ± 0.8	2.8 ± 0.9	<0.05
Time spent outdoors during pregnancy (h/wk)	10.7 ± 17.4	8.6 ± 5.3	NS	17.4 ± 15.8	19.5 ± 13.7	NS
Nutrition						
Nutritional status[d]	1.3 ± 0.5	1.1 ± 0.2	NS	1.3 ± 0.5	1.2 ± 0.4	NS
Breast-feeding (%)	57	70	NS	33	10	<0.05
Prophylactic vitamin D supplements (%)	0	20		9	83	<0.0001
Dietary calcium intake (mg/d)	316 ± 177	494 ± 175	<0.01	307 ± 160	418 ± 166	<0.01

Data are mean ± SD or percent as specified.

[a] Scored as follows: 1, no education; 2, primary school; 3, secondary school; 4, high school; 5, university.

[b] Scored (standardized for each country separately) as follows: 1, very low; 2, low; 3, average; 4, more than average.

[c] Five body sites were considered (face, arms, hands, limbs, and feet), and one point for each exposed site was assigned.

[d] Scored as follows: 1, well nourished; 2, undernourished.

not differ between patients and controls other than maternal education, which was unexpectedly higher in patients than controls. However, the time spent by the child outdoors and proper maternal nutrition during nursing were lower in patients, compared with controls. Nearly all mothers of patients and controls did not receive calcium or vitamin D supplements during pregnancy or lactation. The type of milk fed during infancy did not differ between patients and controls (data not shown) as well as nutritional status, and only a third of Egyptian and Turkish patients were classified as underweight (60–80% of the expected weight for age but no edema). The proportion of breast-fed infants did not differ among Egyptians, whereas it was higher in Turkish patients than controls. The proportion of Turkish patients who received prophylactic vitamin D supplements (400 IU/d) during the first year of life was lower than that of controls, whereas none of the Egyptian patients and only a small proportion of Egyptian controls received vitamin D supplements. No patient or control received calcium supplements. Both Egyptian and Turkish patients had reduced mean dietary calcium intake in comparison with their respective controls (Table 2); however, mean calcium intake was markedly below the NIH and ESPE RDI in Egyptian (46 and 60%, respectively) and Turkish (60 and 74%, respectively) patients as well as in Egyptian controls (60 and 67%, respectively), whereas it was somewhat below the NIH RDI (75%) but normal against ESPE RDI in Turkish controls (96%).

Biochemical and radiographic data and their correlation

Biochemical data are reported in Table 3. Both Egyptian and Turkish patients had marked hypocalcemia and hypophosphatemia and higher serum levels of PTH, BAP, PINP, and ICTP as well as $1,25(OH)_2D$ to 25(OH)D and $24,25(OH)_2D$ to 25(OH)D ratio, in comparison with controls. Serum levels of calcium, phosphate, and PTH were lower in Egyptian than Turkish patients. Serum 25(OH)D and $24,25(OH)_2D$ levels were lower in Turkish patients, compared with controls, whereas serum 25(OH)D levels did not differ ($P = 0.074$) between Egyptian patients and controls possibly due to the smaller number of subjects and to a wider variation of the levels in patients. Serum $1,25(OH)_2D$ levels did not differ between patients and controls in both countries. Serum osteocalcin levels were lower in Egyptian patients, compared with their own controls and Turkish patients. Serum 25(OH)D levels were very low in the mothers of Turkish patients (4.7 ± 2.6 ng/ml, 11.8 ± 6.5 nmol/liter).

Positive correlations were found between serum calcium and serum 25(OH)D ($r = 0.23$, $P < 0.05$), $1,25(OH)_2D$ ($r = 0.37$, $P < 0.01$), or osteocalcin ($r = 0.22$, $P < 0.05$) levels. Serum 25(OH)D levels correlated negatively with serum PTH levels ($r = -0.29$, $P < 0.01$) and positively with serum $1,25(OH)_2D$ ($r = 0.44$, $P < 0.001$).

Rickets was severe in all patients, with scores being greater in Egyptian patients than Turkish patients (8.1 ± 2.2 and 7.0 ± 2.6, $P < 0.05$, respectively). The severity of rickets, as manifested by the x-ray score, was predicted by serum levels of phosphate ($r = -0.30$, $P < 0.01$), BAP ($r = 0.31$, $P < 0.01$), and ICTP ($r = 0.22$, $P < 0.05$).

Biochemical parameters and severity of rickets did not differ between malnourished and well-nourished patients (data not shown).

Stratification of patients and controls according to vitamin D status and calcium intake

With regard to serum 25(OH)D levels and dietary calcium intake, children were classified into five groups, defined as pure vitamin D deficiency, pure calcium insufficiency, combined vitamin D deficiency and calcium insufficiency, vitamin D sufficiency with suboptimal calcium intake, and vitamin D sufficiency with adequate calcium intake (Fig. 1). The proportion of these groups was significantly different between patients and controls as well as between Egyptian and Turkish patients but not between Egyptian and Turkish controls. Most Egyptian patients (71%) had calcium insufficiency or vitamin D deficiency, with a 50% of the total having a combination of both situations. As many as 29% of Egyptian patients had normal serum 25(OH)D levels.

TABLE 3. Biochemical results in patients and controls

	Egypt			Turkey		
	Patients (n = 30)	Controls (n = 20)	*P*	Patients (n = 68)	Controls (n = 30)	*P*
Calcium (mg/dl)	6.6 ± 0.9[a]	9.4 ± 0.6	<0.001	7.3 ± 1.5	9.9 ± 0.8	<0.001
Phosphate (mg/dl)	3.0 ± 0.6[b]	5.2 ± 0.6	<0.001	3.6 ± 1.5	5.1 ± 0.8	<0.001
Intact PTH (pg/ml)	188.4 ± 88.2[b]	28.9 ± 10.9	<0.001	257.9 ± 153.0	28.6 ± 8.1	<0.001
25(OH)D (ng/ml)	14.3 ± 11.3[b]	21.2 ± 15.4	NS	10.1 ± 7.9	25.5 ± 13.1	<0.001
$1,25(OH)_2D$ (pg/ml)	79.9 ± 44.1	67.9 ± 31.3	NS	64.3 ± 47.1	81.7 ± 25.6	NS
$24,25(OH)_2D$ (ng/ml)	1.2 ± 0.6	1.1 ± 0.6	NS	1.1 ± 0.9	1.7 ± 1.0	<0.01
$1,25(OH)_2D$ to 25(OH)D ratio (%)	0.7 ± 0.3	0.4 ± 0.2	<0.01	0.8 ± 0.6	0.4 ± 0.3	<0.01
$24,25(OH)_2D$ to 25(OH)D ratio (%)	9.9 ± 3.5	7.0 ± 3.8	<0.02	12.7 ± 8.0	8.5 ± 7.1	<0.01
BAP (μg/liter)	6546.8 ± 13368.1[a]	29.6 ± 15.0	<0.05	2035.9 ± 5100.2	63.4 ± 24.6	<0.05
Osteocalcin (μg/liter)	13.3 ± 3.7[a]	19.3 ± 3.9	<0.001	15.4 ± 3.9	15.6 ± 2.8	NS
PINP (μg/liter)	499.4 ± 61.1[c]	322.6 ± 114.5	<0.001	412.9 ± 74.1	277.4 ± 81.9	<0.001
ICTP (μg/liter)	54.6 ± 30.9	12.4 ± 5.1	<0.001	47.7 ± 29.7	22.2 ± 13.2	<0.001

Data are mean ± SD. To convert values for serum calcium and phosphate to millimoles per liter, multiply by 0.25 and 0.323, respectively. To convert values for serum PTH to nanograms per liter, multiply by 1.0. To convert values for serum 25(OH)D and $24,25(OH)_2D$ to nanomoles per liter, multiply by 2.5. To convert values for serum osteocalcin to nanomoles per liter, multiply by 0.171. To convert values for serum $1,25(OH)_2D$ to picomoles per liter, multiply by 2.4.

[a] $P < 0.02$ *vs.* Turkish patients.

[b] $P < 0.05$ *vs.* Turkish patients.

[c] $P < 0.0001$ *vs.* Turkish patients.

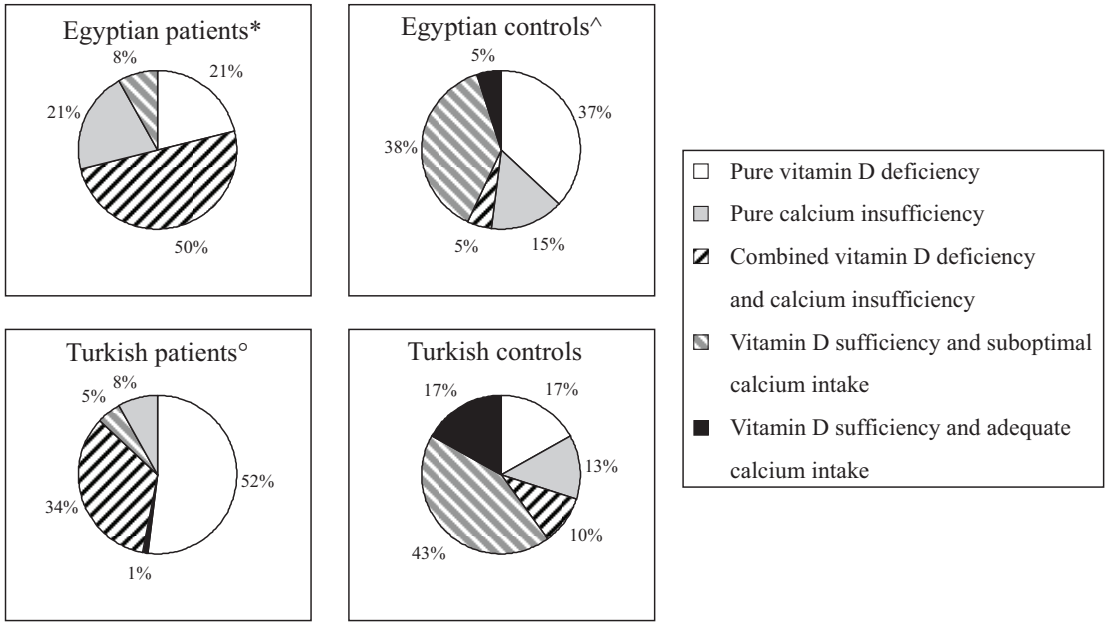

FIG. 1. Comparison among the groups of patients and controls classified according to serum 25(OH)D levels and dietary calcium intake, expressed as percent of the NIH RDI (22). Similar proportions were observed for calcium intake expressed as percent of ESPE RDI (23) (data not shown). *, $P < 0.01$ *vs.* Egyptian controls and $P < 0.05$ *vs.* Turkish patients; °, $P < 0.0001$; *, P = NS *vs.* Turkish controls.

Most Turkish patients had vitamin D deficiency, pure or combined with calcium insufficiency (52 and 34%, respectively). Vitamin D sufficiency with suboptimal calcium intake was evident in only a small percentage of patients (8 and 5% among Egyptian and Turkish patients, respectively). The severity of rickets was not different among the five groups (data not shown). Moreover, in Egyptian and Turkish controls, 37 and 17% had pure vitamin D deficiency, and 15 and 13% had pure calcium insufficiency, respectively. One patient and three controls in Egypt and nine patients and seven controls in Turkey had an adequate calcium intake; among these, all but one patient and four controls had vitamin D deficiency. Therefore, only one Turkish patient (1%) and six controls (Egypt, n = 1, 5%; Turkey, n = 5, 17%) showed an adequate calcium intake and vitamin D sufficiency.

VDR allele frequencies, and association of VDR polymorphism with biochemical parameters, dietary calcium intake, and severity of rickets

No difference was observed between patients and controls for *Bsm*I genotype or allele frequencies (*B* allele frequencies: Egypt, 0.37 and 0.42; Turkey, 0.43 and 0.42; *b* allele frequencies: Egypt, 0.63 and 0.58, Turkey, 0.57 and 0.58, respectively). In both countries a tendency to increased *FF* genotype frequency in patients against controls was observed (Egypt, 63 *vs.* 55%; Turkey, 53 *vs.* 47%), although it did not reach significance; the *ff* genotype was absent in Egyptians. In Turkey, the frequency of the *F* allele was increased and that of the *f* allele was decreased in patients against controls (0.75 *vs.* 0.65, and 0.25 *vs.* 0.35, respectively, $P = 0.024$); similar results were observed for the entire rickets group, compared with the entire group of controls (0.77 *vs.* 0.70, and 0.23 *vs.* 0.30, respectively, $P = 0.04$). There was no difference in allele frequencies between patients and their mothers for both *Bsm*I and *Fok*I genotypes (data not shown).

Figure 2 shows the associations of VDR *Bsm*I and *Fok*I genotypes with biochemical parameters, dietary calcium intake, and severity of rickets in patients and/or controls. The *BB* genotype in both patients (Fig. 2A) and controls (Fig. 2B), as well as the *Bb* genotype in patients only (Fig. 2A), were associated with lower serum 25(OH)D levels; in patients, the *BB* genotype was also associated with higher x-ray score (Fig. 2A), and the *bb* genotype was associated with the lowest calcium intake (Fig. 2C). In controls, the *BB* genotype was associated with the highest $1,25(OH)_2D$ to 25(OH)D ratio (Fig. 2B). In Egypt, but not in Turkey, patients with the *FF* genotype had lower serum $1,25(OH)_2D$ levels and $1,25(OH)_2D$ to 25(OH)D ratio, compared with patients with the *Ff* genotype (Fig. 2D), but otherwise there was no difference in biochemistry or severity of rickets (data not shown).

Stratification of patients and controls according to VDR polymorphisms, vitamin D status, and calcium intake

Patients' stratification according to serum 25(OH)D levels and dietary calcium intake was different among the VDR *Bsm*I genotypes (Fig. 3) but not among the *Fok*I genotypes (data not shown). A combined vitamin D deficiency with calcium insufficiency and a pure vitamin D deficiency were the main risk factors in homozygous *BB* and heterozygous *Bb* patients, respectively. No patient with the *BB* genotype had vitamin D sufficiency with suboptimal or adequate calcium intake or a pure calcium insufficiency; the proportion of patients with pure calcium insufficiency was the highest in the *bb* genotype. Controls' stratification did not show any significant difference among the VDR *Bsm*I genotypes (data not shown); only one control with vitamin D deficiency and suboptimal calcium intake carried the *BB* genotype.

Discussion

This study shows that rickets in the Middle East is multifactorial and that rachitic children living in Egypt and Turkey have some distinct characteristics.

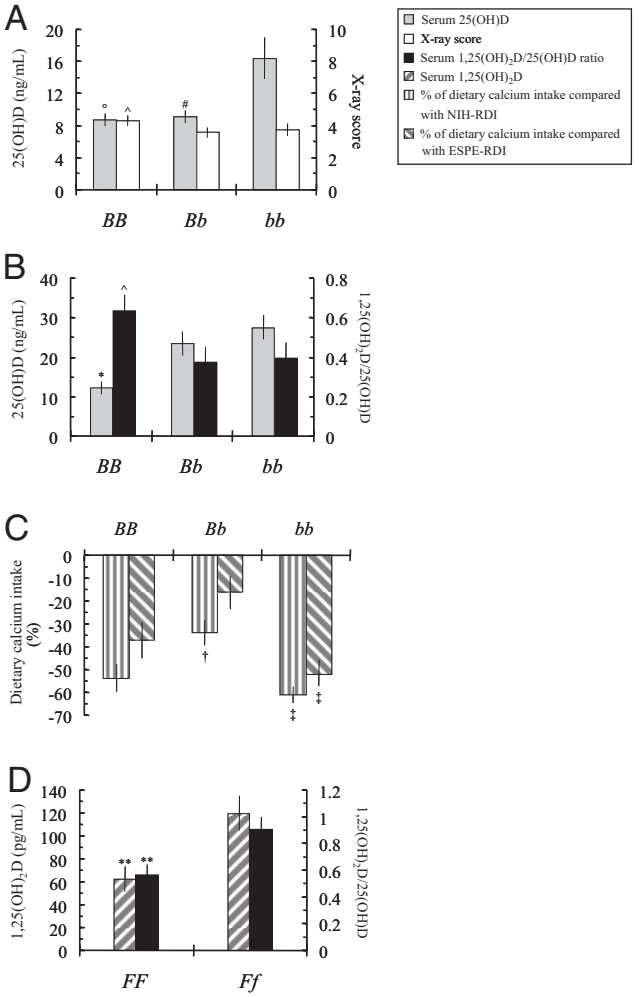

FIG. 2. Data are means ± SEM. A, VDR *Bsm*I genotype associations with serum 25(OH)D levels and x-ray score in all patients. B, VDR *Bsm*I genotype associations with serum 25(OH)D levels and serum $1,25(OH)_2D$ to 25(OH)D ratio in all controls. C, VDR *Bsm*I genotype associations with dietary calcium intake, expressed as percent of the NIH RDI (22) and ESPE RDI (23) in all patients. D, VDR *Fok*I genotype associations with serum $1,25(OH)_2D$ levels and serum $1,25(OH)_2D$ to 25(OH)D ratio in Egyptian patients. To convert values for serum 25(OH)D to nanomoles per liter, multiply by 2.5. To convert values for serum $1,25(OH)_2D$ to picomoles per liter, multiply by 2.4. *, $P < 0.05$; °, $P < 0.02$; #, $P < 0.01$ *vs. bb*; ^, $P < 0.05$; ‡, $P < 0.001$ *vs. Bb*; †, $P < 0.05$ *vs. BB*; **, $P < 0.02$ *vs. Ff*.

In Turkey, rickets is a disease of the underprivileged, strongly correlated with negative social background and insufficient exposure to sunlight; a lack of vitamin D supplementation appears to be decisive for the development of the disease. During the first year of life only 9% of patients received vitamin D supplements against 83% of controls, and the estimated time spent outdoor was less in patients than in controls. Exposed body surface by the mothers of patients during pregnancy was smaller and the number of children was higher than that of the mothers of controls. Moreover, serum 25(OH)D levels were severely reduced in the mothers of patients, as was also found by other studies (1, 3, 9, 23), suggesting that reduced vitamin D stores likely contributed to the development of rickets in their children and strengthening the need for vitamin D supplement's recommendation for mother and baby.

In Egypt, rickets was not related to living conditions or maternal clothing during pregnancy, and paradoxically, maternal education was higher in patients than controls, suggesting that it is not a social disease. Only maternal nutrition during nursing and the estimated time spent outdoors were less in patients than in controls, but in the sun-flooded Egypt, it is expected to be sufficient to maintain a normal vitamin D status. In fact, infants require approximately 2 h of sunlight per week if they are fully clothed with no hat to reach vitamin D sufficiency (29), and the mean time spent outdoors was above this cutoff in Egyptian patients.

Malnutrition was not a primary cause of rickets in both countries. The mean dietary calcium intake was lower in patients than in controls, and in Egypt it was well below the NIH (22) and ESPE RDI (23) but not low enough to be labeled calcium deficiency (daily intake < 200 mg) (2, 3, 14, 16, 17). Yet no reliable data on the lowest calcium intake that would cause rickets are evident (30). In the absence of reliable indicators of nutritional adequacy for calcium, estimates of calcium insufficiency are based largely on adequacy of dietary intake related to the estimated requirements, but this approach may be complicated by the fact that RDIs for calcium vary with expert authorities, and they could differ between Egypt and Turkey populations. Moreover, because the adequate intake for calcium varies with age, we estimated it as percent of some RDIs (22, 23), and a threshold value of −50% was used to separate the children with a condition of possible calcium insufficiency from those with a possible condition of suboptimal calcium intake. Only a few Egyptian and Turkish children had an adequate calcium intake. Likely, a pure calcium insufficiency was not the sole cause of rickets in some of our patients (up to 21% in Egyptian patients and up to 8% in Turkish patients). By contrast to patients with calcium deficiency rickets in whom serum $1,25(OH)_2D$ levels are elevated (14, 17, 24, 31), our patients had normal serum levels, and calcium deficiency rickets has been usually observed at an older age than that of our patients (12, 14, 16).

Definition of vitamin D deficiency has varied, depending on the study and the age of patients, but it has been shown that serum 25(OH)D levels less than 15 ng/ml (37.5 nmol/liter) are usually associated with rickets (25). Vitamin D deficiency may be exacerbated further by increased catabolism of 25(OH)D as a consequence of secondary hyperparathyroidism due to calcium deficiency/insufficiency (3, 24). The negative correlation of serum 25(OH)D levels with serum PTH levels and the positive correlation with serum calcium levels support this mechanism in our patients.

Although the definition of children by a different calcium intake standard and/or by a different serum 25(OH)D levels threshold could affect their stratification, our results suggest that Egyptian and Turkish patients have, at least in part, different mechanisms causing rickets (3, 24). In fact, the stratification of patients showed that vitamin D deficiency, pure or associated with calcium insufficiency, was found to be frequent in Turkish patients (up to 86%), whereas in Egyptians the most frequent situation (50%) was defined as calcium insufficiency associated with vitamin D deficiency. The cause of rickets in 8% of Egyptian and 5% of Turkish patients showing a vitamin D sufficiency with a suboptimal calcium intake and in only one Turkish patient with vitamin D sufficiency and adequate calcium intake was not clearly defined. This may suggest that additional factors, including genetic factors, may have a key role in the pathogenesis of the disease in some patients.

The higher BAP levels, despite the lower osteocalcin levels, were

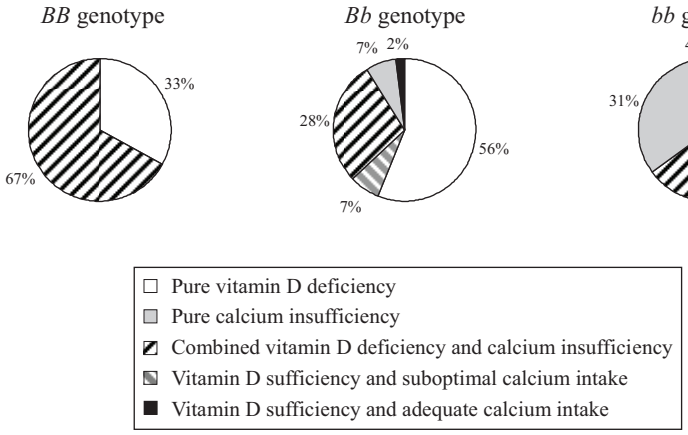

FIG. 3. VDR *Bsm*I genotype associations among the groups of patients classified according to serum 25(OH)D levels and dietary calcium intake, expressed as percent of NIH RDI (22). Similar proportions were observed for calcium intake expressed as percent of the ESPE RDI (23) (data not shown). $P < 0.01$ among the VDR *Bsm*I genotypes; $P < 0.05$, *Bb vs. bb* for pure calcium insufficiency.

compatible with an arrest of the osteoblast phenotype in the developmental phase immediately preceding matrix mineralization induced by vitamin D deficiency (32) and/or calcium insufficiency according to the positive correlation between serum calcium and osteocalcin levels. Severity of rickets was predicted by serum BAP, phosphate, and ICTP levels, reflecting the development of secondary hyperparathyroidism (32).

Although social and nutritional factors may explain the high prevalence of rickets in the Middle East, additional genetic factors, interacting with various degrees of vitamin D deficiency, and/or calcium insufficiency may increase predisposition in some children. An Egyptian study reported some differences in palmar dermatoglyphics between rachitic infants and controls (33). Whereas the evolutionary context of VDR polymorphism has not been investigated so far, haplotypes in the VDR locus have been shown to have significant phenotypic association (34). The prevalence of the *F* allele was increased and that of the *f* allele was decreased in rickets against controls, in agreement with findings in Nigerian children with calcium deficiency rickets (35). The *F* allele confers a transcriptionally somewhat more efficient VDR (36), and its increased prevalence suggests an evolutionary adaptation to a vitamin D and calcium insufficient environment or lifestyle (37). In Egyptian patients, serum $1,25(OH)_2D$ levels and $1,25(OH)_2D$ to 25(OH)D ratio were lower in *FF* homozygotes, as expected to occur in subjects with a more effective VDR. In healthy adolescents, a greater calcium absorption was found in *FF* homozygotes, compared with those of *ff* homozygotes and *Ff* heterozygotes (38); however, the positive effect of the *FF* genotype is limited whether dietary calcium is severely restricted (39). Egyptian controls also had a poor dietary calcium intake, but it was higher, compared with that of patients, suggesting that it is the interaction of VDR polymorphism with reduced calcium intake and vitamin D status that could determine the individual susceptibility to developing rickets.

In addition to the VDR *F* allele effect, we showed that the VDR *B* allele may predispose an individual to vitamin D deficiency, whereas the *b* allele was more frequent in patients with reduced calcium intake; moreover, rickets with the homozygous *BB* genotype was more severe. Homozygous *BB* genotype association with lower serum 25(OH)D levels was evident in both patients and controls; in controls, but not patients, lower serum 25(OH)D levels were associated with an increased $1,25(OH)_2D$ to 25OHD ratio, suggesting that vitamin D status may be regulated by VDR polymorphism. Associations between VDR genotypes and bone and mineral metabolism have been described, in agreement with results found in control and rachitic children; in *BB* individuals, lower bone mineral density (40, 41) and lower $1,25(OH)_2D$ and higher PTH and BAP in hemodyalized *BB* patients, compared with the *bb* group (42), had been reported. Moreover, dietary calcium intake was positively associated with bone mineral density only among persons with the *bb* genotype (40). In our study, homozygous *BB* and heterozygous *Bb* patients were associated mainly with pure vitamin D deficiency and combined vitamin D deficiency with calcium insufficiency, in agreement with reports of apparently greater than usual doses of vitamin D, associated with calcium supplementation, to cure rickets in children of the Middle East (13, 15).

In Mongolian children with rickets, frequencies of VDR genotypes for polymorphisms in intron 8 (*Bsm*I) and exon 9 (*Taq*I), which are in linkage disequilibrium, were similar in patients and controls, as in our study for *Bsm*I (43).

In conclusion, our study demonstrates that rickets in the Middle East is determined by nutrition but also by the environment, lifestyle, and genetic predisposition. As such, it should be not labeled pure nutritional rickets. The rachitic children suffer mostly from calcium insufficiency combined with vitamin D deficiency in Egypt and from vitamin D deficiency associated with insufficient mother and child care in Turkey.

A nonnegligible percentage of controls of both countries had pure vitamin D deficiency and/or calcium insufficiency, which may suggest that other factors are involved in the pathogenesis of rickets.

Although little is known about the evolutionary adaptive changes of the VDR genotypes, its polymorphisms may predispose to rickets in the given environs and nutrition. The unique environs and genetic predisposition have to be accounted for in the design of preventive measures rather than using European or American RDI for calcium and vitamin D.

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References

1. **Bereket A** 2003 Rickets in developing countries. In: Hochberg Z, ed. Vitamin D and rickets. Basel, Switzerland: Karger; 220–232
2. **Pettifor JM** 2004 Nutritional rickets: deficiency of vitamin D, calcium, or both? Am J Clin Nutr 80(Suppl 6):1725S–1729S
3. **Thacher TD, Fischer PR, Strand MA, Pettifor JM** 2006 Nutritional rickets around the world: causes and future directions. Ann Trop Paediatr 26:1–16
4. **Meulmeester JF, van den Berg H, Wedel M, Boshuis PG, Hulshof KF, Luyken R** 1990 Vitamin D status, parathyroid hormone and sunlight in Turkish, Moroccan and Caucasian children in The Netherlands. Eur J Clin Nutr 44:461–470
5. **Yeste D, Carrascosa A** 2003 Nutritional rickets in childhood: analysis of 62 cases. Med Clin 121:23–27
6. **Mallet E, Gaudelus J, Reinert P, Le Luyer B, Lecointre C, Leger J, Loirat C, Quinet B, Benichou JJ, Furioli J, Loeuille GA, Roussel B, Larchet M, Freycon F, Vidailhet M, Varet I** 2004 Symptomatic rickets in adolescents. Arch Pediatr 11:871–878
7. **Glerup H, Rytter L, Mortensen L, Nathan E** 2004 Vitamin D deficiency among immigrant children in Denmark. Eur J Pediatr 163:272–273
8. **Holvik K, Meyer HE, Haug E, Brunvand L** 2005 Prevalence and predictors of vitamin D deficiency in five immigrant groups living in Oslo, Norway: the Oslo Immigrant Health Study. Eur J Clin Nutr 59:57–63
9. **Erkal MZ, Wilde J, Bilgin Y, Akinci A, Demir E, Bödeker RH, Mann M, Bretzel RG, Stracke H, Holick MF** 2006 High prevalence of vitamin D deficiency, secondary hyperparathyroidism and generalized bone pain in Turkish immigrants in Germany: identification of risk factors. Osteoporos Int 17:1133–1140
10. **Robinson PD, Högler W, Craig ME, Verge CF, Walker JL, Piper AC, Woodhead HJ, Cowell CT, Ambler GR** 2006 The re-emerging burden of rickets: a decade of experience from Sydney. Arch Dis Child 91:564–568
11. **Hochberg Z** 2003 Rickets. Past and present. In: Hochberg Z, ed. Vitamin D and rickets. Basel, Switzerland: Karger; 1–13
12. **Okonofua F, Gill DS, Alabi ZO, Thomas M, Bell JL, Dandona P** 1991 Rickets in Nigerian children: a consequence of calcium malnutrition. Metabolism 40: 209–213
13. **Essawy M, El Kholy M, El Husseiny S, Awad Z** 1992 Plasma concentration of 1,25-dihydroxyvitamin D before and during treatment of vitamin D deficiency rickets. Pediatric Rev Commun 6:145–152
14. **Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Isichei CO, Reading JC, Chan GM** 1999 A comparison of calcium, vitamin D, or both for nutritional rickets in Nigerian children. N Engl J Med 341:563–568
15. **Abdullah MA, Salhi HS, Bakry LA, Okamoto E, Abomelha AM, Stevens B, Mousa FM** 2002 Adolescent rickets in Saudi Arabia: a rich and sunny country. J Pediatr Endocrinol Metab 15:1017–1025
16. **Pettifor JM, Ross P, Wang J, Moodley G, Couper-Smith J** 1978 Rickets in children of rural origin in South Africa: is low dietary calcium a factor? J Pediatr 92:320–324
17. **Thacher TD, Fisher PR, Pettifor JM, Lawson JO, Isichei CO, Chan GM** 2000 Case-control study of factors associated with nutritional rickets in Nigerian children. J Pediatr 137:367–373
18. **Awwaad S, Khalifa AS, Naga MA, Tolba KA, Fares R, Gaballa AS, Hayeg OE, Wahhab SA** 1975 A field survey on child health in a rural community in Egypt. J Trop Med Hyg 78:20–25
19. **Ozkan B, Buyukavcı M, Aksoy H, Tan H, Akdağ R** 1999 Incidence of rickets among 0-to-3 years-old children in Erzurum. Çocuk Sağlığı ve Hastalıkları Dergisi 42:389–396
20. **Rockett HR, Breitenbach M, Frazier AL, Witschi J, Wolf AM, Field AE, Colditz GA** 1997 Validation of a youth/adolescent food frequency questionnaire. Prev Med 26:808–816
21. **Wellcome Trust Working Party** 1970 Classification of infantile malnutrition. Lancet 2:302–303
22. **National Institutes of Health Consensus Conference** 1994 NIH consensus development panel on optimal calcium intake. JAMA 272:1942–1948
23. **Hochberg Z, Bereket A, Davenport M, Delemarre-Van de Waal H, De Schepper J, Levine MA, Shaw N, Shoenau E, Weisman Y, Zadik Z** 2002 Consensus development for the supplementation of vitamin D in childhood and adolescence. Horm Res 58:39–51
24. **DeLucia MC, Mitnick ME, Carpenter TO** 2003 Nutritional rickets with normal circulating 25-hydroxyvitamin D: a call for reexamining the role of dietary calcium intake in North American infants. J Clin Endocrinol Metab 88:3539–3545
25. **Holick MF** 2006 Resurrection of vitamin D deficiency and rickets. J Clin Invest 116:2062–2072
26. **Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Manaster BJ, Reading JC** 2000 Radiographic scoring method for the assessment of the severity of nutritional rickets. J Trop Pediatr 46:132–139
27. **Tiosano D, Weisman Y, Hochberg Z** 2001 The role of vitamin D receptor in regulating vitamin D metabolism: a study of vitamin D-dependent rickets, type II. J Clin Endocrinol Metab 86:1908–1912
28. **Marti G, Audí L, Esteban C, Oyarzabal M, Chueca M, Gussinye M, Yeste D, Fernández-Cancio M, Andaluz P, Carrascosa A** 2004 Association of vitamin D receptor gene polymorphism with type 1 diabetes mellitus in two Spanish populations. Med Clin 123:286–290
29. **Specker BL, Valanis B, Hertzberg V, Edwards N, Tsang RC** 1985 Sunshine exposure and serum 25-hydroxyvitamin D concentrations in exclusively breast-fed infants. J Pediatr 107:372–376
30. **American Academy of Pediatrics Committee on Nutrition** 1999 Calcium requirements of infants, children, and adolescents. Pediatrics 104:1152–1157
31. **Oginni LM, Sharp CA, Badru OS, Risteli J, Davie MW, Worsfold M** 2003 Radiological and biochemical resolution of nutritional rickets with calcium. Arch Dis Child 88:812–817
32. **Baroncelli GI, Bertelloni S, Ceccarelli C, Amato V, Saggese G** 2000 Bone turnover in children with vitamin D deficiency rickets before and during treatment. Acta Paediatr 89:513–518
33. **Shawky RM, El Kholy M, Mostafa N** 1991 A genetic study of vitamin D deficiency rickets: dermatoglyphics. Egypt J Pediatr 8:377–391
34. **Grundberg E, Lau EM, Pastinen T, Kindmark A, Nilsson O, Ljunggren O, Mellström D, Orwoll E, Redlund-Johnell I, Holmberg A, Gurd S, Leung PC, Kwok T, Ohlsson C, Mallmin H, Brändström H** 2007 Vitamin D receptor 3′ haplotypes are unequally expressed in primary human bone cells and associated with increased fracture risk: the MrOS Study in Sweden and Hong Kong. J Bone Miner Res 22:832–840
35. **Fischer PR, Thacher TD, Pettifor JM, Jorde LB, Eccleshall TR, Feldman D** 2000 Vitamin D receptor polymorphisms and nutritional rickets in Nigerian children. J Bone Miner Res 15:2206–2210
36. **Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, Tonai T, Nishisho T, Mori S, Takeda E** 1997 A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res 12:915–921
37. **Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP** 2004 Genetics and biology of vitamin D receptor polymorphisms. Gene 338:143–156
38. **Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA** 1999 Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. J Bone Miner Res 14:740–746
39. **Abrams SA, Griffin IJ, Hawthorne KM, Chen Z, Gunn SK, Wilde M, Darlington G, Shypailo RJ, Ellis KJ** 2005 Vitamin D receptor Fok1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty. J Bone Miner Res 20:945–953
40. **Kiel DP, Myers RH, Cupples LA, Kong XF, Zhu XH, Ordovas J, Schaefer EJ, Felson DT, Rush D, Wilson PW, Eisman JA, Holick MF** 1997 The *Bsm*I vitamin D receptor restriction fragment length polymorphism (bb) influences the effect of calcium intake on bone mineral density. J Bone Miner Res 12: 1049–1057
41. **Ferrari SL, Rizzoli R, Slosman DO, Bonjour JP** 1998 Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? J Bone Miner Res 13:363–370
42. **Chudek J, Karkoszka H, Schmidt-Gayk H, Ritz E, Kokot F** 2000 Plasma parathyroid hormone, phosphatemia and vitamin D receptor genotype: are they interrelated? J Nephrol 13:54–58
43. **Kaneko A, Urnaa V, Nakamura K, Kizuki M, Seino K, Inose T, Takano T** 2007 Vitamin D receptor polymorphism among rickets children in Mongolia. J Epidemiol 17:25–29