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Rickets in the Middle East: role of environment and genetic predisposition

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Abstract

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

Obiective: 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 (age 6 mo – 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 gene polymorphisms.

Results: Epidemiological factors had a key role in pursue of rickets; Egyptian and Turkish patients had lower ($P < 0.01$) dietary calcium intake than controls and the recommended dietary intakes (RDI), and serum 25-hydroxyvitamin D [25(OH)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(OH)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 RDI for calcium and vitamin D.

Introduction

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 women, spending most time indoors, and exclusive or prolonged breastfeeding 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$(OH)_2$D] 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 is a prospective multicenter collaboration, designed and executed by the European Society for Paediatric 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, age 11.7 ± 8.2 months) and 50 controls (30 males and 20 females, age 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 – December 2004. Both patients and controls were recruited from university and community outpatient hospitals in Cairo, Egypt (latitude 30.01N, corresponding to New Orleans, LA, USA), and Erzurum and Van, Turkey (latitude 39.57N, corresponding to Philadelphia, PA, USA, and 38.30N, 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 non rachitic patients coming to the outpatient, 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, and Marmara, Istanbul, and of 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 semi-quantitative 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 Welcome Trust classification (21) was evaluated. Epidemiological questionnaire included living conditions, family data, socio-economic status by parental education and family income, and sunlight exposure by clothing type and time spent outdoor. 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-day nutrition diary was estimated and compared with the recommended dietary intakes (RDI) by the National Institute of Health (NIH), USA (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/L) 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.

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, CA, USA, 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, USA) as previously described (27). 1α-hydroxylase and 24-hydroxylase activity were estimated from the ratio of the product/substrate [$1,25(OH)_2D$/25(OH)D and $24,25(OH)_2D$/25(OH)D, respectively] and expressed as per cent (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 < 9% and intra-assay variability < 7%. All blood samples were measured in duplicates.

VDR gene polymorphisms
In all patients and controls, and in 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 leucocytes by direct polymerase chain reaction 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 non-parametric 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 (chi-square) 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 program. 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 socio-economic status were worse, and the time spent outdoor 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 not differ between patients and controls other than maternal education which was unexpectedly higher in patients than in 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 in controls. The proportion of Turkish patients who received prophylactic vitamin D supplements (400 IU/day) 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 (Tab. 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 $1,25(OH)_2D/25(OH)D$ and $24,25(OH)_2D/25(OH)D$ ratio, in comparison with controls. Serum levels of calcium, phosphate, and PTH were lower in Egyptian than in 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/L). 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 in 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 have been 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. 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 only in 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 Epypt, 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 $1,25(OH)_2D/25(OH)D$ ratio (Fig. 2B). In Egypt, but not in Turkey, patients with the *FF* genotype had lower serum $1,25(OH)_2D$ levels and $1,25(OH)_2D/25(OH)D$ 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.

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 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 in controls, suggesting that it is not a social disease. Only maternal nutrition during nursing and the estimated time spent outdoor 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 two hours of sunlight per week if they are fully clothed with no hat to reach vitamin D sufficiency (29), and

the mean time spent outdoor was above this cut-off 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, as the adequate intake for calcium varies with age we estimated it as per cent 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 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/L) 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 suggests 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 to 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 in spite of the lower osteocalcin levels were 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

$1,25(OH)_2D/25(OH)D$ 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 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 in patients, lower serum 25(OH)D levels were associated with an increased $1,25(OH)_2D/25OHD$ 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 hemodyalised *BB* patients compared to 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 non negligible 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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Figure legends

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

Figure 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 $1,25(OH)_2D/25(OH)D$ ratio in all controls.
(C) VDR *Bsm*I genotype associations with dietary calcium intake, expressed as per cent 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 $1,25(OH)_2D/25(OH)D$ ratio in Egyptian patients.
To convert values for serum 25(OH)D to nmol/L, multiply by 2.5. To convert values for serum $1,25(OH)_2D$ to pmol/L, multiply by 2.4.
*, $P < 0.05$; °, $P < 0.02$; and $^{\#}$, $P < 0.01$ *vs. bb*.
^, $P < 0.05$; and ‡, $P < 0.001$ *vs. Bb*.
†, $P < 0.05$ *vs. BB*.
**, $P < 0.02$ *vs. Ff*.

Figure 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 per cent of NIH-RDI (22). Similar proportions were observed for calcium intake expressed as per cent 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.

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

Inclusion criteria	Exclusion criteria
Patients	
Age range 6 mo – 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/day)	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/day
Written informed consent	
Controls	
Age range 6 mo – 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/day)	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/day
Written informed consent	

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
number 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 age (yr)	35.2 ± 8.5	37.2 ± 7.9	NS	31.5 ± 6.2	32.6 ± 6.2	NS
mother age (yr)	28.6 ± 6.4	29.6 ± 6.6	NS	28.6 ± 6.7	28.9 ± 6.6	NS
Socio-economic status						
father education*	2.4 ± 1.1	2.4 ± 1.2	NS	2.2 ± 0.8	2.6 ± 1.1	<0.05
mother education*	2.3 ± 1.1	1.7 ± 0.9	<0.05	1.3 ± 0.5	2.2 ± 1.1	<0.0001
family income°	2.3 ± 0.6	2.2 ± 0.5	NS	2.1 ± 0.9	2.6 ± 0.9	<0.02
Sunlight exposure						
child clothing#	3.6 ± 1.3	3.6 ± 1.0	NS	2.2 ± 1.1	2.7 ± 1.5	NS
time spent outdoor (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 (mo)	29.1 ± 14.5	35.4±25.2	NS	30.3 ± 17.4	64.6 ± 65.5	<0.001
prenatal care (% adequate)	47	45	NS	25	67	<0.001
regular visit (%)	29	40	NS	15	57	<0.0001
nutrition during nursing (% adequate)	57	90	<0.05	46	83	<0.01
clothing during pregnancy#	2.1 ± 1.1	2.2 ± 1.0	NS	2.4 ± 0.8	2.8 ± 0.9	<0.05
time spent outdoor during pregnancy (h/wk)	10.7 ± 17.4	8.6 ± 5.3	NS	17.4 ± 15.8	19.5 ± 13.7	NS

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Continue TABLE 2

Nutrition						
nutritional status[§]	1.3 ± 0.5	1.1 ± 0.2	NS	1.3 ± 0.5	1.2 ± 0.4	NS
breastfeeding (%)	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 % as specified.
[*]Scored as follows: 1 = no education; 2 = primary school; 3 = secondary school; 4 = high school; 5 = university.
[°]Scored (standardized for each country separately) as follows: 1 = very low; 2 = low; 3 = average; 4 = more than average.
[#]Five body sites were considered (face, arms, hands, limbs, and feet) and one point for each exposed site was assigned.
[§]Scored as follows: 1 = well nourished; 2 = undernourished.

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[b]	9.4 ± 0.6	<0.001	7.3 ± 1.5	9.9 ± 0.8	<0.001
Phosphate (mg/dL)	3.0 ± 0.6[a]	5.2 ± 0.6	<0.001	3.6 ± 1.5	5.1 ± 0.8	<0.001
Intact PTH (pg/m/L)	188.4 ± 88.2[a]	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[a]	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
$1,25(OH)_2D$/25(OH)D ratio (%)	0.7 ± 0.3	0.4 ± 0.2	<0.01	0.8 ± 0.6	0.4 ± 0.3	<0.01
$24,25(OH)_2D$/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/L)	6546.8 ± 13368.1[b]	29.6 ± 15.0	<0.05	2035.9 ± 5100.2	63.4 ± 24.6	<0.05
Osteocalcin (µg/L)	13.3 ± 3.7[b]	19.3 ± 3.9	<0.001	15.4 ± 3.9	15.6 ± 2.8	NS
PINP (µg/L)	499.4 ± 61.1[c]	322.6 ± 114.5	<0.001	412.9 ± 74.1	277.4 ± 81.9	<0.001
ICTP (µg/L)	54.6 ± 30.9	12.4 ± 5.1	<0.001	47.7 ± 29.7	22.2 ± 13.2	<0.001

Data are mean ± SD.
[a] $P < 0.05$ *vs.* Turkish patients.
[b] $P < 0.02$ *vs.* Turkish patients.
[c] $P < 0.0001$ *vs.* Turkish patients.
To convert values for serum calcium and phosphate to mmol/L, multiply by 0.25 and 0.323, respectively. To convert values for serum PTH to ng/L, multiply by 1.0. To convert values for serum 25(OH)D and $24,25(OH)_2D$ to nmol/L, multiply by 2.5. To convert values for serum osteocalcin to nmol/L, multiply by 0.171. To convert values for serum $1,25(OH)_2D$ to pmol/L, multiply by 2.4.

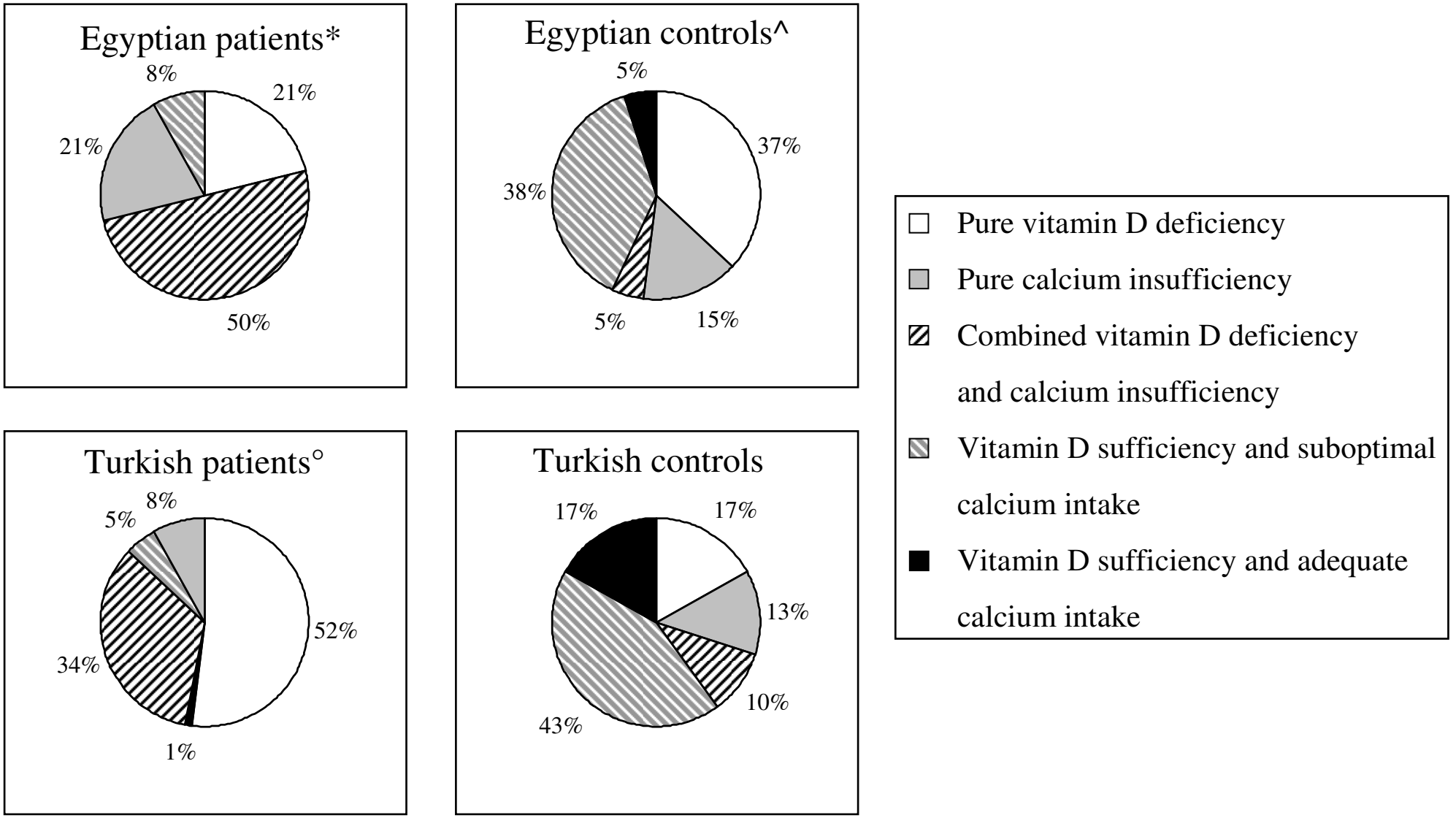

Figure 1. Baroncelli et al. ↑ TOP

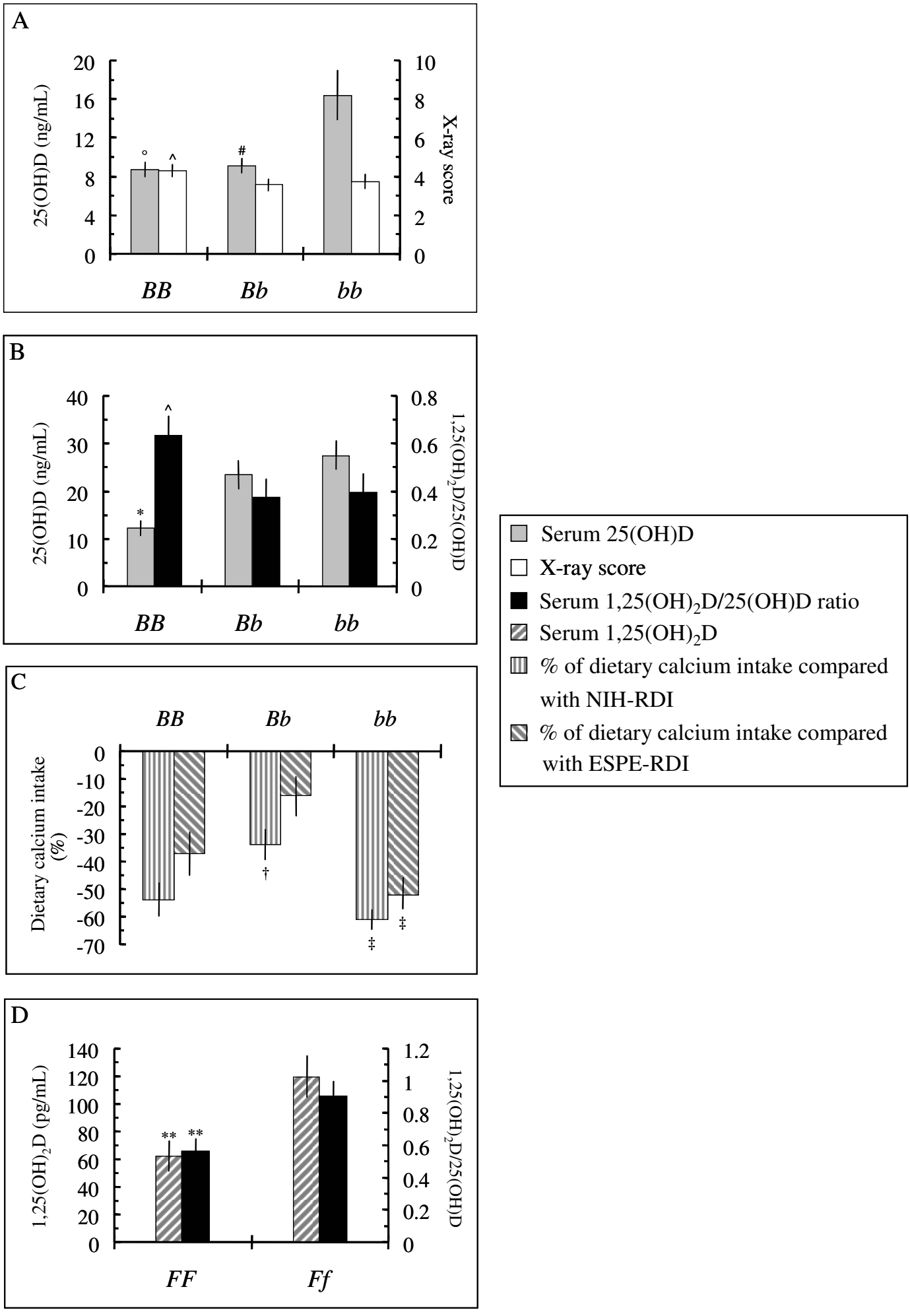

Figure 2. Baroncelli et al. ↑ TOP

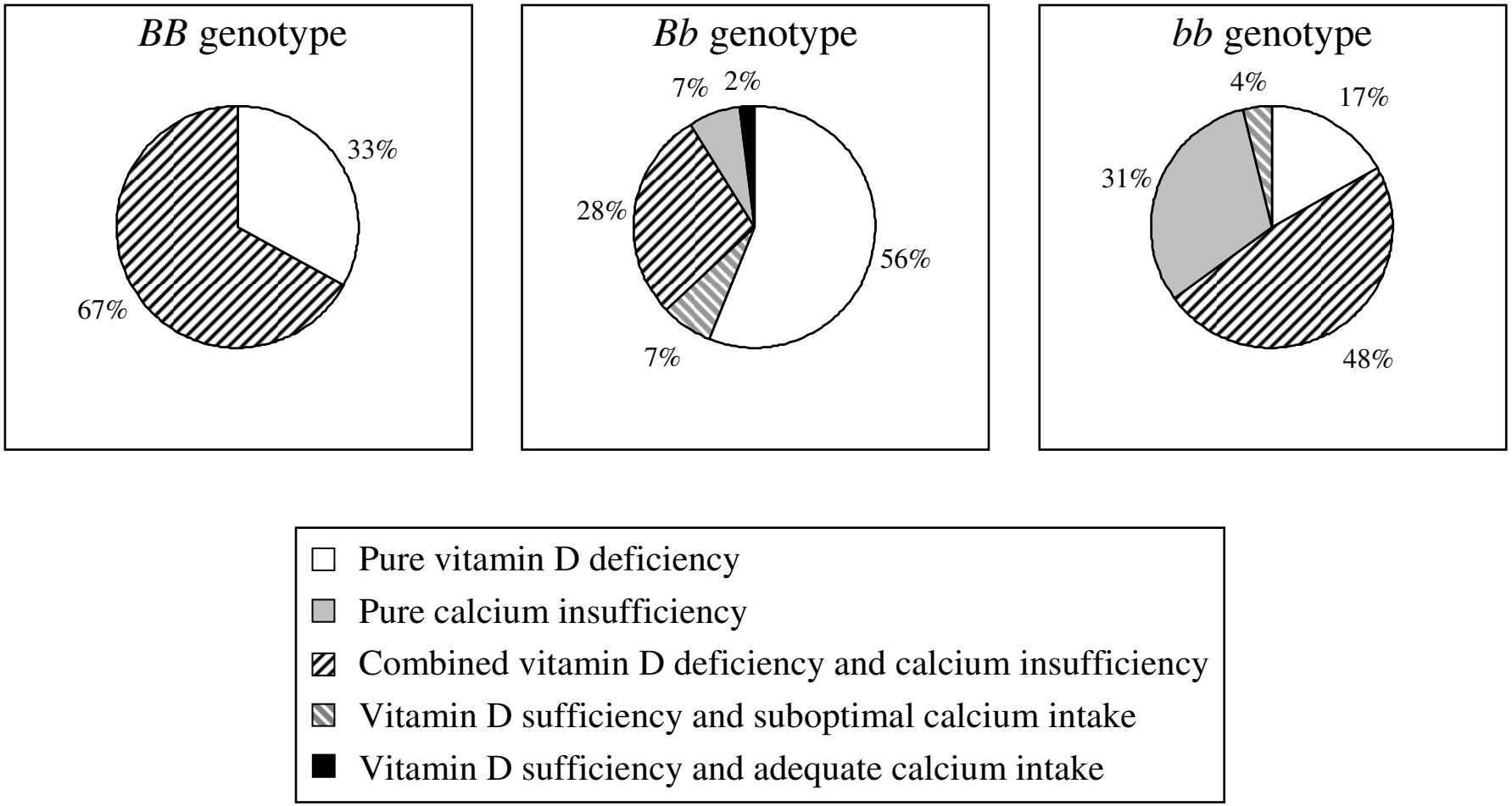

Figure 3. Baroncelli et al. ↑ TOP