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POPULATION STUDY ARTICLE

Association of vitamin D receptor gene FokI polymorphism and susceptibility to CAP in Egyptian children: a multicenter study

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BACKGROUND: Community-acquired pneumonia (CAP) is the leading cause of child deaths around the world. Recently, the vitamin D receptor (VDR) gene has emerged as a susceptibility gene for CAP.
OBJECTIVES: To evaluate the association of the VDR gene Fok I polymorphism with susceptibility to CAP in Egyptian children.
METHODS: This was a multicenter case-control study of 300 patients diagnosed with CAP, and 300 well-matched healthy control children. The VDR Fok I (rs2228570) polymorphism was genotyped by *PCR*-restriction fragment length polymorphism (*RFLP*), meanwhile serum 25-hydroxy vitamin D (25D) level was assessed using *ELISA* method.
RESULTS: The frequencies of the VDR *FF* genotype and *F* allele were more common in patients with CAP than in our control group (OR = 3.6; (95% CI: 1.9–6.7) for the *FF* genotype; $P = 0.001$) and (OR: 1.8; (95% CI: 1.4–2.3) for the *F* allele; $P = 0.01$). Patients carrying the VDR *FF* genotype had lower serum (25D) level (mean; 14.8 ± 3.6 ng/ml) than *Ff* genotype (20.6 ± 4.5 ng/ml) and the *ff* genotype (24.5 ± 3.7 ng/ml); $P < 0.01$.
CONCLUSION: The VDR gene Fok I (rs2228570) polymorphism confers susceptibility to CAP in Egyptian children.

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INTRODUCTION

Community-acquired pneumonia (CAP) is one of the most serious pediatric infections, with an estimated annual incidence to be 33 cases per 1000 children in developed countries. CAP is more common, more severe and is the number one killer of children in the developing world.[1] A significant number of CAP patients develop systemic inflammatory response syndrome (SIRS) and sepsis.[2] Lung injury resulting in acute respiratory failure (ARF) is the primary complication of pneumonia. Environmental and genetic interplay may affect molecular and cellular processes that cause lung injury in CAP.[3] One such environmental factor is vitamin D, as sun exposure is its major source, but it is also provided by some foods. Vitamin D is a group of fat-soluble seco-steroids, plays a crucial role for bone and mineral homeostasis. 1, 25-dihydroxyvitamin D (1, 25D) serves as the active form of vitamin D and binds to the vitamin D receptor (VDR) expressed at the nuclei of target tissues.[4] Other than bone, VDR is found on the majority of immune cells; suggesting that vitamin D could modulate both innate and adaptive immunity and also regulate the inflammatory cascade.[5] Recent studies have shown that vitamin D and its receptor cast as a pleiotropic regulator of human physiology involved in modification of immune competence, cancer chemoprevention, cardio-protection, propensity to auto-immune diseases and infectious disease risk.[6]

Vitamin D receptor signaling induces the expression of antimicrobial peptides (AMPs) such as cathelicidin (LL-37), β-defensin, and toll-like receptors (TLRs), molecules important for innate defense against both bacterial and viral infections owing to their chemotactic action and endotoxin neutralization.[5,7] At the same time, (1, 25D) can interfere with the instruction of an adaptive immune response pathway, by down-regulating the signals of transcription factors as NF-κB and exerting an anti-inflammatory effect.[8] An earlier study by Muhe et al.[9] reported that clinical vitamin D deficiency was associated with 13-fold increased risk of CAP in Ethiopian children. Furthermore, inadequate vitamin D stores in children have also been linked to severity and outcome of the disease particularly in the Middle East.[10] A randomized controlled trial performed in Kabul concluded that children with pneumonia who received a single high-dose of oral vitamin D_3 were less likely to have a repeat episode in the ninety days after supplementation.[11]

Despite these reports, final proof of a causal role of vitamin D status in CAP is still lacking. A genetic approach could be the only way to clarify this issue. The human *VDR* gene maps to chromosome 12q13. Although >470 single nucleotide polymorphisms (SNP) have been identified, only a few of them modulate vitamin D uptake. Four polymorphisms of this gene (FokI, BsmI, ApaI, and TaqI) have been the most studied.[12]

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The FokI polymorphism creates an alternative start codon in exon 2 which leads to transcription of two VDR proteins with different structure i.e., a long variant (*f*-VDR) or a short one (*F*-VDR).[12] The FokI polymorphism has a unique role as it is not in linkage-disequilibrium (LD) with any of the VDR polymorphisms.[13] The FokI *F* allele was found to influence immune cells behavior and may be associated with altered immune function, resulting in a more active immune system.[14]

Given the sparse data on VDR and CAP, we conducted this study to evaluate the association of the VDR gene Fok I polymorphism with susceptibility to CAP in young Egyptian children, and we also estimated serum 25D concentrations to assess its relation to Fok I polymorphism.

METHODS

This was a prospective multicenter case-control study performed in Zagazig University, Ain-Shams and Cairo University hospitals from March 2016 through April 2018. The study protocol was approved by the ethical committees of Zagazig, Ain-Shams and Cairo Universities, Egypt and written informed consent from parents of each participant was provided in accordance with the Declaration of Helsinki.

All participants belonged to the same ethnic group: African Caucasian. A total of 300 children; who had CAP as diagnosed in the Pediatric Departments of the study hospitals, were recruited in this study. The study involved children aged from 6 months to 6 years. For the purpose of this study pneumonia was defined according to the previously published guidelines.[15,16] Pneumonia was defined as community-acquired if children had no history of hospitalization during the two weeks prior to admission.[17]

Exclusion criteria

Children were excluded if they were recently hospitalized (4 weeks before admission), or had acute bronchiolitis or any alternative respiratory diagnosis, congenital heart disease (CHD), immunodeficiency, rickets, chronic hepatic or renal disease; and genetic or neurological disorders. Postoperative children and patients who received vitamin D or calcium supplementations during the past three months were also excluded.

Severity criteria

patients were further classified into three groups 'mild, moderate or severe disease' according to the British Thoracic Society guidelines.[17] Severe sepsis (SS) was defined as systemic inflammatory response syndrome (SIRS) in association with multi-organ dysfunction.[18] Acute Respiratory failure was defined as partial pressure of oxygen in arterial blood [PaO_2] ≤ 50 mmHg in room air or [PaO_2/FiO_2] ratio ≤ 250 under oxygen administration and in the absence of cyanotic CHD.

The control population comprised three hundred healthy children aged 6 months to 6 years who attended the Pediatric Departments for preoperative evaluation for elective surgery (all without respiratory symptoms). To limit the effect of seasonal variation of vitamin D photosynthesis controls were also matched with cases by season at enrollment (winter or non-winter). All participants were subjected to detailed history taking and thorough clinical examination.

Blood sampling. Upon enrollment, a blood sample was obtained from each subject and divided into 2 ml blood collected into EDTA-containing tubes for genomic DNA isolation. Then, sera were separated and stored at −20 °C until processing.

Estimation of serum 25-hydroxy vitamin D concentration. Serum 25D was estimated using a commercial ELISA kit according to the manufacturer's instructions (K2110, immune-diagnostic, Dutch Company, Holland). Vitamin D deficiency was considered as levels of 25D <20 ng/ml and severe deficiency for levels <10 ng/mL.[19]

Genomic DNA isolation. Genomic DNA from venous samples of all subjects was isolated using the Invisorb Spin Micro DNA Kit (Stratec Molecular GmbH, Berlin, Germany) according to the manufacturer's protocol. DNA was stored at −20 °C till the time of use.

Genotyping of VDR Fok *I gene polymorphisms*. All subjects were genotyped for the *Fok* I (rs2228570) polymorphism located in exon 2 of the VDR gene by polymerase chain reaction– restriction fragment length polymorphism (PCR-RFLP) methodology. A 265 base-pair region was amplified by polymerase chain reaction (PCR), using the sense primer 5′- AGCTGGCCCTGGCACTGACTC TGCTCT-3′ and the antisense primer, 5′-ATGGAAACACCTTGCTT GCTTCTCCCTC-3′, as previously described.[20]

Statistical analysis

The VDR *Fok* I genotype and allele frequencies in patients with CAP and controls were tested for Hardy–Weinberg equilibrium. The *Chi-square* test was used to determine differences in the VDR *Fok* I allele frequencies and genotype distribution between patients and controls. *Fisher's exact* test was used when the frequency was <5.The odds ratio (OR) and 95% Confidence Intervals (95% (CI)) were calculated for disease susceptibility in relation to the studied VDR *Fok* I gene variants. Multiple logistic regression analysis was performed to evaluate the independent effect of VDR *Fok* I genotypes on the clinical outcome of CAP. The Student's *t*-test and analysis of variance (ANOVA) test were used to compare numeric variables within groups. P value < 0.05 was considered to be statistically significant. All analyses were performed using SPSS for Windows, version 22.0 (IBM Corp., Armonk, NY).

RESULTS

Of the 300 children with CAP enrolled in the study, 162 (54%) were males. The mean patient age was 2.3 years ranging from 6 to 72 months. The control group was well-matched for age, gender, ethnicity, and season at enrollment (all $P > 0.05$; Table 1).

The mean serum 25D level was lower in our patients compared to the control group (17.5 ± 4.4 ng/ml vs 36.7 ± 6.5 ng/ml; $P < 0.01$); Table 1.

Regarding severity, there were 102 (34%) patients identified as mild pneumonia, 117 (39%) as moderate and 81 (27%) as severe cases. Eighty-seven patients (29%) required admission to the ICU during their inpatient stay and 27 (9%) patients died. Eighty-one patients (27%) developed severe sepsis. Thirty-three cases (11%) were complicated with acute respiratory failure (Table 1).

Distribution of the VDR genotypes and alleles among studied subjects are shown in Table 2. The genotype frequencies of the VDR *Fok* I in patient and control groups were compatible with Hardy–Weinberg expectations.

The *Fok* I distributions were different between patients and control children. Homozygous *FF* was more common (21%) in patients than in healthy controls (6%). Children carrying the *FF* genotype had a 3.6 fold higher susceptibility to CAP (OR = 3.6; (95% CI: 1.9–6.7); $P = 0.001$). The occurrence of *F* allele was significantly more frequent in patients (44% vs. 30%; OR: 1.8; (95% CI: 1.4–2.3); $P = 0.01$); by contrast, a significant decrease in the frequency of the *f* allele was found in comparison to control children (56% vs. 70%; OR: 0.55; (95% CI: 0.4–0.7); $P = 0.01$); Table 2.

Thirty-two patients (51%) with the VDR *FF* genotype had severe pneumonia compared to (16 and 24%) in those carrying the *ff* or *Ff* genotypes, $P < 0.01$; Table 3). Severe sepsis was more frequent in the VDR *FF* homozygous subjects (52%) compared to (26%) in *Ff*

Table 1. Demographic, laboratory and clinical characteristics of patients with community-acquired pneumonia (CAP) and healthy controls

Characteristics	Patients ($n = 300$)	Controls ($n = 300$)	*P*
Age, median (Range)	2.3 (6mon–6years)	2.6 (6mon–5.8 years)	>0.05
Gender, *n* (%)			
M/F	162 (54)/138 (46)	165 (55)/135 (45)	>0.05
Season at enrollment (winter/non-winter)	58 (19)/242 (81)	60 (20)/240 (80)	>0.05
Weight for age *z*-score	−0.7 (−1.2 to 0.4)	−0.6 (−1.1 to 0.6)	>0.05
C-reactive protein, mg/l	38.5 (11.6–86)	4.6 (2.8–5.9)	<0.01
WBC count, $\times 10^9$/L	14.5 (12.3–19.4)	6.3 (4.5–7.6)	<0.05
Neutrophil cells, Percentage	67.2 (58.3–86.8)	43.4 (41–52.6)	<0.05
Neutrophil cells, $\times 10^9$/L	9.7 (7.1–16.7)	2.8 (1.8–4.1)	<0.05
serum 25(OH) vitamin D (ng/ml)	17.5 ± 4.4	36.7 ± 6.5	<0.01
Pneumonia severity, *n* (%)			
Mild	102 (34)	—	
Moderate	117 (39)	—	
Severe	81 (27)	—	
ICU admission, *n* (%)			
NO	213 (71)	—	
YES	87 (29)	—	
Severe sepsis, *n* (%)			
NO	219 (73)	—	
YES	81 (27)	—	
Acute respiratory Failure *n* (%)			
NO	267 (89)	—	
YES	33 (11)	—	
Hospital mortality, *n* (%)			
NO	273 (91)	—	
YES	27 (9)	—	

CAP community-acquired pneumonia, *BMI* body mass index, *WBC* white blood cells, *ICU* intensive care unit
Values in parentheses are percentages or data are presented as median (range)
P value < 0.05 indicates a significant difference

heterozygous individuals and (12%) in *ff* genotypes. The VDR *ff* gene variant confers protection against sepsis ($P = 0.001$). The VDR *Fok* I genotypes were not associated with the risk of acute respiratory failure in CAP children ($P = 0.375$). Patients who required admission to the ICU during their inpatient stay had Fok I distributions as follow: the *ff* genotype (19%), *Ff* genotype (28%), and *FF* genotype (48%). The *FF* genotype carried the risk for ICU admission ($P = 0.004$). The ICU mortality was in significant association with the *FF* genotype (21%), meanwhile both *Ff* and *ff* gene variants were not (8 and 3%; respectively); ($P = 0.001$; Table 3).

Our logistic regression model showed a significant positive association between the VDR *FF* gene variant and susceptibility to sepsis, ICU admission, and hospital mortality as did the Fok I *F* allele (OR: 3.7; (95% CI: 1.4–10.2) for the *FF* genotype; $P = 0.001$) and (OR: 1.5; (95% CI: 1.3-5.8) for the *F* allele; $P = 0.03$.

About half of our CAP cases 159 (53%) were vitamin D deficient with 15% being severely deficient; meanwhile 141 (47%) cases had normal vitamin D levels. By contrast, 66 (22%) of the control children had vitamin D deficiency and 234 (78%) had normal vitamin D levels ($P < 0.01$; Fig. 1). Vitamin D deficient subjects had 4 folds higher susceptibility to CAP than did children with normal vitamin D level (OR = 4; (95% CI: 2.5–6.33), $P < 0.01$; Fig. 1).

Patients carrying the VDR *FF* gene variant had lower serum 25D level (mean; 14.8 ± 3.6 ng/ml) than *Ff* genotype (20.6 ± 4.5 ng/ml) and the *ff* genotype (24.5 ± 3.7 ng/ml); $P < 0.01$.The mean serum 25D concentration was 15 ± 0.8 ng/ml for the *Fok* I *F* allele and 24.7 ± 1.4 ng/ml for the *f* allele; $P < 0.01$, Table 3.

Table 2. Distribution of the VDR *Fok* I genotypes, alleles and serum 25-hydroxyvitamin D in patients with community-acquired pneumonia (CAP) and controls

Genotype	Patient group		Control group		OR (95% CI)	*P*
	n (300)	%	*n* (300)	%		
VDR *Fok* I						
f/f	99	(33)	138	(46)	0.75 (0.52–1.08)	0.1
F/f	138	(46)	144	(48)	Referent	
F/F	63	(21)	18	(6)	3.6 (1.9–6.7)	0.001
Alleles						
f	336	(56)	420	(70)	0.55(0.4–0.7)	
F	264	(44)	180	(30)	1.8 (1.4–2.3)	0.01
Serum 25(OH) vitamin D (ng/ml)	17.5 ± 4.4		36.7 ± 6.5			<0.01[a]

CAP community-acquired pneumonia; *VDR* vitamin D receptor, *OR* odds ratio, *CI* 95% confidence interval
Values in parentheses are percentages or data are presented as mean ± SD.
P value < 0.05 indicates a significant difference. Chi-square test
[a]Student *t*-test

Table 3. Association of the VDR *Fok* I genotypes with disease severity, clinical outcome and serum 25-hydroxyvitamin D in patients with community-acquired pneumonia (CAP)

VDR *Fok* I Genotype	***ff*** ($n = 99$) *n* (%)	***Ff*** ($n = 138$) *n* (%)	***FF*** ($n = 63$) *n* (%)	***P***
CAP severity				
Mild	52 (52)	38 (25)	12 (27)	
Moderate	31 (32)	67 (49)	19 (30)	
Severe	16 (16)	33 (24)	32 (51)[b]	<0.01
Severe sepsis	12 (12)	36 (26)	33 (52)[b]	0.001
Acute respiratory Failure	10 (10)	13 (9)	10 (16)	0.375
Clinical outcome				
ICU admission	19 (19)	38 (28)	30 (48)[b]	0.004
Hospital mortality	3 (3)	11 (8)	13 (21)[b]	0.001
Serum 25(OH) vitamin D (ng/ml)	24.5 ± 3.7	20.6 ± 4.5	14.8 ± 3.6	<0.01 [a]

CAP community-acquired pneumonia, *VDR* vitamin D receptor, *ICU* Pediatric intensive care unit
P value < 0.05 indicates a significant difference. *Chi-square* test
[a]*ANOVA* test
[b]Significant difference between each three genotypes group

DISCUSSION

Childhood CAP is a common cause of mortality around the world.[21] As early as 1975, a link was hypothesized between vitamin D and CAP.[22] Experimental work confirmed that administration of vitamin D ameliorates pulmonary inflammatory responses while enhancing innate defense mechanisms against respiratory pathogens.[23] In particular, the activation of the VDR by 1, 25D stimulate the endogenous synthesis of Cathelicidin which is cleaved to generate the active cationic peptide, LL-37. Cathelicidin is highly expressed at barrier sites including respiratory epithelium, thus provides a pivotal first line defense for the innate immune system.[24] In addition, vitamin D regulates phagocytosis dependent and antibody dependent macrophages which protect

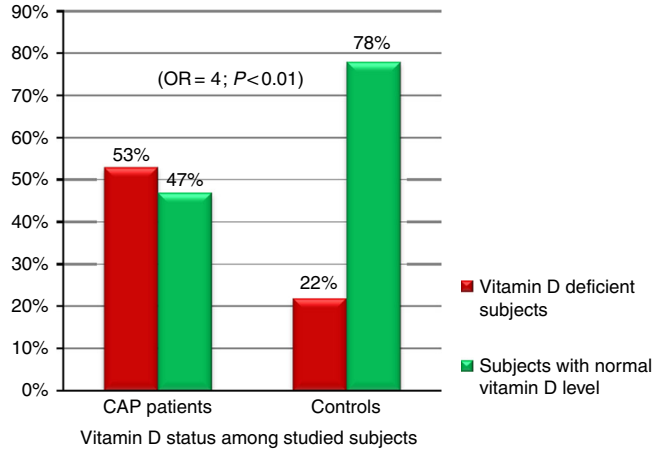

Fig. 1 Vitamin D status among studied subjects

from respiratory infections.[25] Vitamin D was also reported as a growth factor for alveolar cells type-II, and local conversion of 25D to 1, 25D was once considered as a key modulator of epithelial proliferation in the context of lung development and repair.[26]

Because of potential immune-modulatory activity of vitamin D and its effects on pulmonary cell biology, the VDR gene polymorphisms may affect individuals' susceptibility, illness severity, and the clinical outcome of CAP.

In this study, the VDR *Fok* I genotypic distribution was different between studied groups. The *FF* genotype and *F* allele were more frequent in patients than in control group. A child with the *FF* genotype had 3.6-increased odds for CAP risk, thus revealing that our cases were more susceptible to pneumonia. By contrast, the *Fok* I *f* allele showed a significant negative association with CAP risk suggesting that *f* allele confers protection against CAP.

SNPs in the VDR gene have been reported to be associated with a variety of physiological and pathological phenotypes. Recent studies discovered an association between some VDR gene variants with host susceptibility to pulmonary tuberculosis,[27] symptomatic pertussis[28] and RSV bronchiolitis in children.[29] To date, few reports concerned the association of the VDR gene SNPs with susceptibility to CAP.[30,31] Li et al. studied the VDR *Fok* I gene SNP (rs2239185) on genomic DNAs of 91 Chinese Han population, compared to 94 ethnicity-matched controls. The authors demonstrated that the TT genotype of rs2239185 in the VDR gene may be a genetic risk factor for CAP, and the T allele at the same position may be associated with CAP susceptibility and severity.[30] Roth et al.[31] investigated the role of VDR gene *SNPs* in acute lower respiratory tract infections among Canadian children. By contrast, they reported that the odds of LRTI for children with the *Fok* I *ff* gene variant were increased relative to those with the *Fok* I *FF* genotype. They concluded that a function altering SNP in the VDR gene was associated with more risk for LRTI.

The inconsistent findings between our study and previously reported data are likely related to different ethnicity or to genetic and environmental interplay.

A high prevalence of vitamin D deficiency was demonstrated in pediatric and adolescent populations across the globe. It has been postulated that vitamin D deficiency may contribute to the epidemic of LRTI, including CAP, among Egyptian, Ethiopian, and Japanese cohorts.[9,32,33]

Of note, vitamin D deficiency was detected in about half (53%) of CAP patients with (15%) being severely deficient; although the study population reside in Delta Egypt with plenty of sunny weather. In line with our expectations, the mean serum 25D level was significantly decreased in children with CAP compared to the control group. Moreover, children with the *Fok* I *FF* genotype had significantly lower serum 25D concentrations relative to the *Ff* and *ff* gene variants. This finding confirms the results of recently published reports.[34]

1, 25D regulates its own serum concentration and its precursor 25D by a negative feedback loop via the VDRs. Unlike BsmI, ApaI and TaqI polymorphisms, FokI polymorphism gives rise to VDR proteins of different lengths. The long allelic variant, i.e., *f*-allele has less biological activity than the shorter *F*-VDR,[14] therefore, allow more synthesis of 25D, which could explain the observed higher concentrations of vitamin D in children with the FokI *ff* gene variant.

Our findings support the possibility that the VDR gene SNPs may contribute to inter-individual variations in the course of illness and clinical outcome of CAP.

Among studied children with CAP, the presence of the VDR allele *F* or the *FF* gene variant; being associated with lower serum 25D levels; constitute risk factor for developing severe sepsis, ICU admission and mortality. Our results confirm and extend the previous findings of Das et al.[35] who suggested that the VDR polymorphisms can be potentially used as genetic markers for assessing sepsis risk in Indian population.

In experimental models of sepsis, 1, 25D administration has been shown to modulate systemic inflammatory cytokine response and was associated with improved coagulation parameters in sepsis related disseminated intravascular coagulation.[36] In vitro study by Liu et al. confirmed that 1, 25D treatment of cultured macrophages infected with *M. tuberculosis* resulted in enhanced expression of cathelicidin, and improved killing of the microorganisms.[37] Cathelicidin has a broad antimicrobial spectrum against gram negative and positive bacteria, fungi and mycobacteria.[24]

Based on a review of the epidemiology of sepsis, it was hypothesized that vitamin D insufficiency is a risk factor for sepsis worldwide. That hypothesis was quickly supported by Jeng et al.,[38] who found that those admitted to ICU with or without sepsis had much lower serum vitamin D levels than others in the community. Inamo et al.[33] suggested a correlation between severe 25D deficiencies and the need of supplementary oxygen and ventilator support in children with acute LRTI.

Moreover, Lee et al.[39] reported increased hospital mortality in the critically ill with vitamin D deficiency that may be explained by immune and endothelial cell dysfunction. In particular, endothelial cell dysfunction has been supposed to be a potential cause of multiple organ dysfunction syndrome (MODS).[40] It is possible that vitamin D deficiency amplifies the impaired immune regulation and metabolic derangement observed in sepsis, which may lead to worse clinical outcome than would be experienced in children with normal vitamin D status.

At the molecular level, vitamin D suppresses the amassing of mRNA for interleukin (IL)-12, GM-CSF, and interferon –γ while IL-4, IL-10, and transforming growth factor β (TGF-β) production is enhanced, resulting in inhibition of the overall helper T cell type 1 response (Th1) and further shifts the cytokine profile toward Th2 dominance. The VDR and its ligand, 1, 25D, has been shown to enhance the differentiation of naive CD4 + T lymphocytes toward a helper T cell type 2.[41] Hence, Vitamin D exerts both antimicrobial and anti-inflammatory effects against infectious pathogens.[8] In Vitamin D deficiency or even insufficiency, there is decreased expression of the VDRs resulting in impaired clearance of microbes and uncontrolled pulmonary inflammation that leads to progressive lung damage with impaired oxygenation.[42]

To our knowledge, ours is the first such study to evaluate the association between the VDR *Fok* I polymorphism and the susceptibility to CAP in Egyptian children.

Since the small sample size was one of our limitations; it may be necessary to adopt a genome-wide association studies; particularly in our developing countries.

A lack of detailed dietary intake or sun exposure data among children with CAP was another limitation in our study. We have studied only one SNP in the VDR gene which might represent LD with yet-to-be-identified other VDR gene markers. As vitamin D measurements and the CAP diagnosis were concurrent, cause and effect assumptions are difficult. Other VDR polymorphisms (i.e., BsmI, ApaI, and TaqI) should be genotyped concurrently with evaluation of vitamin D status in children with CAP to validate these findings on different ethnic populations.

CONCLUSION

The VDR gene Fok I (rs2228570) polymorphism confers susceptibility to CAP in Egyptian children.

Finally, Vitamin D deficiency may be a modifiable risk factor for morbidity in CAP and represents a promising target for intervention taking into account the VDR polymorphisms.

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AUTHOR CONTRIBUTIONS

H.A.Z. submitted the manuscript. M.A.A. designed the study. A.M.K. collected clinical data and coordinated the sample collection (Zagazig University). N.M.A. collected clinical data and coordinated the sample collection (Ain-Shams University). M.M.S. collected clinical data and coordinated the sample collection (Cairo University). M.S.H. and H.A.A.E. performed the statistical analysis. M.A.N. and A.A.S. helped to draft the manuscript. A.M.S., A.A.M., and M.E.H. wrote the manuscript. A.A.A., M.T.Z., and S.S.A.E. critically revised the final version. A.M.A., R.M.N., and G.M.A. performed laboratory analysis and genotyping. All authors read and approved all the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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