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*Original Article*

# Role of *VDR* gene polymorphisms with community acquired pneumonia in North Indian children: a case-control study

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**Abstract:** Community-acquired pneumonia (CAP) is a leading cause of death in children under five years of age globally. Currently, the vitamin D receptor (*VDR)* gene is an emerging factor that regulates inflammatory pathways that may alter the response to infections and possibly modify the outcome of CAP. The objective of this study was to investigate the association *of VDR* gene polymorphisms *ApaI, FokI, TaqI, BsmI* with CAP in children aged 2-59 months. Hospitalized children aged (2-59 months) with WHO-defined CAP were included as cases after parental consent. Age-matched healthy controls were recruited from the immunization clinic of the hospital within one week of the recruitment of the case. Children with a clinical diagnosis of cystic fibrosis and congenital heart disease were excluded. Four *VDR* gene polymorphisms, *ApaI*, *FokI, TaqI, BsmI* were genotyped by using PCR-RFLP. From Oct-2016 to Oct-2019, 160 cases (34.37% females) and 160 controls (47.5% females) were recruited. Mean age of the cases was 26.30±23.10 months and controls 25.93±15.99 months. In *FokI* (rs2228570 polymorphism, heterozygous genotype (CT) [OR=2.06, 95% CI=1.25-3.39, *P*=0.00] and mutant allele (T) [OR=1.45, 95% CI=1.06-2.00, *P*=0.02] were found to be associated with the risk of CAP. In *VDR* gene, *FokI* polymorphism predisposes to CAP in Indian children.

**Keywords:** Community-acquired pneumonia, polymorphism, *VDR* gene, inflammation, infection, polymorphism, *ApaI, FokI, TaqI, BsmI*

## Introduction

Community-acquired pneumonia (CAP) is responsible for 5.9 million deaths in children under five years of age, of which 1.2 million deaths occur in India. India has the highest under-five mortality rate of 48 per 1000 live births and about 16% deaths are due to CAP [1]. CAP can be caused both by bacteria and viruses [2]. According to the World Health Organization, CAP in a child aged 2-59 months is defined as presence of cough and/or difficult breathing, where the respiratory rate is above age specific cut off, with or without chest indrawing, this is classified as pneumonia. However, in presence of general danger signs, namely not able to drink, persistent vomiting, convulsions, lethargic or unconscious, stridor in a calm child or severe malnutrition, this is classified as severe pneumonia or very severe disease [3]. Pneumonia can progress to severe pneumonia either due to delayed treatment or due to environmental or a host related factors [4].

Despite timely and effective treatment also, there could be adverse outcomes in cases of CAP. One possible reason may be host physiological and genetic factors, vitamin D being one of them. In a recent meta-analysis, Vitamin D supplementation was found to have protective effect against acute lower respiratory tract infections [5]. Active form of vitamin D has been considered as a growth factor for alveolar cells type-II, and conversion of 25D to active form of vitamin D, 1,25(OH)2D3 was considered as a key regulator of epithelial proliferation in terms of lung development and repair [6].

In humans, vitamin D receptor (VDR) is a nuclear transcription factor. VDRs are expressed on various immune cells including activated CD4+

and CD8+ T cells, B cells, neutrophils, macrophages, and dendritic cells. In complex with 1,25di-hydroxy (OH) vitamin D3, VDR regulates the expression of more than 900 genes [7]. VDR in turn is encoded by the *VDR* gene, located on the 12$^{th}$ chromosome at the position of 12q13-14 [8]. *VDR* gene polymorphisms are associated with many pulmonary diseases namely bronchiolitis caused largely by the Respiratory syncytial virus (RSV) infections, asthma, atopy, pneumonia and tuberculosis [9-13].

Various polymorphisms in the *VDR* gene have been identified, but the four polymorphisms rs7975232 or *ApaI*, rs2228570 or *FokI*, and rs731236 or *TaqI*, rs1544410 or *BsmI* have been most researched [14]. *VDR* gene polymorphisms, specifically *FokI,* have been associated with and acute respiratory tract infections (ALTRI) in Canadian as well as Egyptian children [15, 16]. However, data on the association of *VDR* gene polymorphisms with WHO defined CAP is still scanty and possibly there is no data from India. Therefore, in this study, our primary objective was to assess the association of *VDR* gene polymorphisms, namely rs7975232 or *ApaI*, rs2228570 or *FokI*, and rs731236 or *TaqI*, rs1544410 or *BsmI* with susceptibility to WHO defined CAP among North Indian children. Our secondary objective was to assess the association of above polymorphisms with mortality among hospitalized cases.

## Material and methods

### *Study setting*

This was a hospital-based case-control study conducted at the Department of Pediatrics, King George's Medical University (KGMU), Lucknow, India, from Oct 2016-Oct 2019. Ethical approval for the study was obtained from the Institutional Ethics Committee (letter no. 575/Ethics/R-Cell-16). Written informed consent was obtained from the parent/legal guardian of the child before enrollment in the study.

### *Inclusion/exclusion criteria*

All children (2-59 months) hospitalized in the pediatric ward of KGMU were screened for eligibility as cases. Included were children aged 2-59 months, hospitalized with symptoms of WHO-defined CAP and whose parents consented for participation [3]. Those with the clinical diagnosis of cystic fibrosis and congenital heart disease were excluded.

Age-matched healthy controls were recruited from the immunization clinic of the hospital within one week of the recruitment of the case. Excluded were those who had clinical diagnosis of acute respiratory illnesses or chronic respiratory illnesses or had been ever hospitalized for CAP.

Children were classified as having no malnutrition, moderate malnutrition, (weight for height (WH), Z score between <-1SD to <-2SD), severe acute malnutrition (SAM) (WH, Z score <-3SD) on the basis of anthropometric measurements [17]. Thrombocytopenia and thrombocytosis are defined as platelet counts <150,000/L or >400,000/L, respectively [18]. On the basis of optical scattering principle, routine hematological variables were analyzed by automated cell counter analyzer (Swelab cell counter).

### *Data collection*

Data were collected on a pre-designed and pretested questionnaire. Face-to-face interviews were conducted with parents/caregivers to note socio-demographic details like age, consanguinity. All the clinical details were collected from the patients' medical records including duration of medical stay.

Environmental characteristics such as place of residence, number of rooms and windows, separate cooking space and the fuel used for cooking, a distance of residence from heavy traffic, garden/farm field around the house, type of road nearby residence were also taken into consideration (Data not given). Immunization history was noted from the immunization record of the child. In case an immunization record was unavailable, parents were asked about the immunization status of the child. Anthropometric measurements of the recruited subjects were recorded by the researcher. Weight (in kg) was recorded by an electronic weighing machine. Stadiometer was used to measure the height (in cm) for children who were ≥2 years, while an infantometer was used for younger children. All the digits were corrected to one decimal unit.

**Table 1.** Demographic characteristics among cases and controls

| Variables | Cases (N=160) n (%) | Controls (N=160) n (%) | *p* value |
|---|---|---|---|
| Age (months) (m ± SD) | 26.30±23.10 | 25.93±15.99 | 0.87 |
| Sex | | | Ref |
| Male | 105 (65.63) | 84 (52.5) | 0.02 |
| Female | 55 (34.37) | 76 (47.5) | |
| Consanguinity | | | |
| Yes | 21 (13.12) | 30 (18.75) | 0.17 |
| Weight (kg) (M ± SD) | 10.17±4.14 | 10.33±2.13 | 0.66 |
| Height (cm) (m ± SD) | 77.55±20.64 | 80.91±10.06 | 0.06 |
| Duration of hospital stay | 9.20±2.46 | | |

*Molecular work*

Two milliliters of venous blood was drawn from each participant under aseptic conditions and collected in ethylenediaminetetraacetic acid (EDTA) vial for the genotyping. Phenol chloroform method was used for DNA extraction. Qualitative estimation of DNA samples was done on 0.8% agarose gel electrophoresis. Purity and quantitative assessment were done by using a spectrophotometer. We selected four polymorphisms rs7975232 or *ApaI*, rs2228570 or *FokI*, and rs731236 or *TaqI*, rs1544410 or *BsmI* with at least 10% minor allele frequency in the Indian population. Genotyping was performed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Amplified products were obtained by using reported primers [14]. PCR (Applied Biosystems, Foster City, USA) was done by using the following conditions: initial denaturation at 95°C for 2 min followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for *FokI* and *BsmI* and 57.5°C for *ApaI* and 54.5 °C for *TaqI*, for 15 seconds, and extension at 72°C for 30 seconds. The final extension of the reaction was ended at 72°C. PCR products of rs7975232 or *ApaI*, rs2228570 or *FokI*, and rs731236 or *TaqI*, rs1544410 or *BsmI* were verified by 2% agarose gel electrophoresis.

*Sample size*

The sample size was calculated by using online OSSE (Online sample size estimator) software based on the previous study [14]. For cases, minor allele frequency was used 5% and for controls, it was taken 15%. The power of the study was taken 80% and the significance level was considered 5%. Case to control ratio was 1:1.

*Statistical analysis*

All data, entered in MS excel was double-checked and analyzed using statistical software SPSS (v15, Chicago, Illinois, USA) and INSTAT (version 3.0). All the demographic variables were recorded as frequencies and percentages. Chi-square test without Yates correction was used for all the categorical variables. In case of any frequency was less than five then Fisher exact test was used. All continuous variables were represented as mean ± Standard Deviation (S.D.) and analyzed using an independent t-test. Hardy Weinberg Equilibrium principle was applied for all the cases and controls separately. To better define the association between genotypes and pneumonia, three genetic models (dominant, overdominant, recessive) were used. Two-sided *p*-value <0.05 was taken as significant.

## Results

*Demographic characteristics*

A total of 320 subjects (160 cases and 160 controls) were recruited in this study from Oct 2016-Oct 2019. Most of the recruited children were males (65.6%, 105/160 cases and 52.5%, 84/160 were controls). **Table 1** depicts the demographic characteristics of cases and controls. The mean ages of the case and control group were 26.30±23.10 months and 25.93±15.99 months respectively. Regarding immunization status, 63 (39.4%) children were completely immunized in cases and 77 (48.13%) in controls (*P*=0.12). Among recruited children, 54 (33.75%) were delivered by cesarean section in cases and 36 (22.5%) in controls; those of the cases were also found to be associated (*P*=0.02). Characteristics of cooking space were studied and exclusive use of biomass fuel for cooking (*P*=0.01) was found to be positively associated among cases.

**Table 2** depicts the laboratory parameters among expired and survived cases. Out of 160 CAP cases, 14 (8.8%) expired, 146 (91.2%) survived and 32 (20%) had SAM. Among survived

**Table 2.** Laboratory parameters among expired and survived patients

| Parameters | Survived (N=146) | Expired (N=14) | *p* value |
|---|---|---|---|
| Hemoglobin (gm/dl) | 9.91±1.39 | 8.94±1.04 | 0.01 |
| Total Leukocyte Count (cells/mm$^3$) | 15037.05±7315.1 | 17928.57±7075.1 | 0.16 |
| Polymorphs (%) | 62.76±10.88 | 64.0±8.48 | 0.68 |
| Lymphocytes (%) | 28.02±6.79 | 26.0±5.96 | 0.28 |
| Platelets (lac/mm$^3$) | 2.07±0.72 | 1.11±0.30 | <0.00 |

cases, 25 (17.12%) had SAM while in expired cases 7 (50.0%) had SAM and found to be associated with mortality (*P*=0.00). Routine laboratory findings have been compared between survived and expired. Mean hemoglobin (gm/dl) was 8.94±1.04 and mean platelet count 1.11±0.30 were significantly lower in expired patients. No significant association was observed in total leukocyte counts, polymorphs, and lymphocyte counts of patients.

*Association of VDR gene polymorphisms with community-acquired pneumonia*

Genotypic and allelic distribution of *VDR* gene polymorphisms (rs7975232 or *ApaI*, rs2228570 or *FokI*, and rs731236 or *TaqI*, rs1544410 or *BsmI*) among cases and controls are given in **Table 3**. The frequency distribution of CC, CT and TT genotypes of rs2228570 (*FokI*) polymorphism in cases were 29.3%, 50.62% and 20% as compared to controls 45%, 37.5% and 17.5% respectively. Increased risk of CAP was found in children carrying CT genotype of rs2228570 (*FokI*) polymorphism [OR=2.06, 95% CI=1.25-3.39, *P*=0.00] whereas, those carrying T allele of this variant were 1.45 times susceptible to CAP [OR=1.45, 95% CI=1.06-2.00, *P*=0.02]. No significant association was observed among *ApaI* (rs7975232) *TaqI* (rs731236), *BsmI* (rs1544410) with CAP. Comparison of genotypic frequency distribution of cases and controls with different genetic models showed increased risk of CAP in dominant [CC Vs CT+TT, OR=1.96, 95% CI=1.24-3.12, *P*=0.00] and over-dominant models [CC+TT Vs CT, OR=1.70, 95% CI=1.09-2.66, *P*=0.02] of rs2228570 (*FokI*) polymorphism.

*Association of genotypic and allelic frequencies of VDR gene polymorphisms with hospital mortality*

**Table 4** depicts the association of genotypic and allelic frequencies of *VDR* gene polymorphisms among expired and survived as compared to controls. Mutant homozygous (CC) [OR=9.22, 95% CI=2.17-39.19, *P*=0.00] and mutant allele (C) [OR=4.07, 95% CI=1.78-9.33, *p*=0.00] of *TaqI* polymorphism and mutant allele (A) [OR=2.70, 95% CI=1.06-6.87, *P*=0.03] of *BsmI* polymorphism were found to be associated with mortality in CAP children. In genetic models, dominant [OR=3.42, 95% CI=1.12-10.46, *p*=0.02] and recessive model [OR=7.94, 95% CI=1.98-31.77, *P*=0.00] of *TaqI* polymorphism were also found to be associated with mortality in the children with CAP.

*Haplotype analysis*

To find a better independent role of the mutation occurring in the allele, we conducted haplotype analysis (SHesis software available online). Among eight haplotypes: ACTA*, ACTG*, ATTG*, CCCG*, CCTA*, CCTG*, CTCG*, CTTG* increased risk of CAP was found with CCTA* haplotype [OR=2.80, 95% CI=1.19-6.57, *P*=0.01].

## Discussion

The current case control study was done in northern India to find the association of *VDR* gene polymorphisms with susceptibility to WHO defined CAP in children aged 2-59 months. We found an increased risk of CAP with CT genotype and T allele of rs2228570 or *FokI* polymorphism. Similarly, among all genetic models, *FokI* (rs2228570) was found to be associated with increased risk of CAP in dominant and over-dominant models. Mutant homozygous (CC) genotype and mutant allele (C), dominant and recessive models of *TaqI* polymorphism and mutant allele (A) of *BsmI* polymorphism were found to be associated with hospital mortality in children with CAP.

VDR induces the regulatory regions of target genes after binding with 1,25(OH)2D3. VDR assists the formation of large protein complexes, whose dynamic activities are crucial for modifications in transcription. These activities

**Table 3.** Genotypic and allelic frequencies of the *VDR* gene polymorphisms among cases and controls

| Polymorphisms | Controls (N=160) (n%) | CAP (N=160) (n%) | Crude OR ,95% CI, *p* value |
|---|---|---|---|
| *rs7975232 (ApaI)* | | | |
| CC | 65 (40.62) | 69 (43.12) | Ref |
| AC | 57 (35.62) | 60 (37.5) | 0.99 (0.60-1.62) 0.97 |
| AA | 38 (23.75) | 31 (19.37) | 0.76 (0.42-1.37) 0.37 |
| C | 187 (58.4) | 198 (62.0) | Ref |
| A | 133 (41.6) | 122 (38.0) | 0.86 (0.63-1.18) 0.37 |
| CC | 65 (40.62) | 69 (43.12) | Ref |
| AC+AA (Dominant) | 95 (59.38) | 91 (56.88) | 0.90 (0.57-1.40) 0.73 |
| CC+AA | 103 (64.37) | 100 (62.5) | Ref |
| AC (Over dominant) | 57 (35.63) | 60 (37.5) | 1.08 (0.68-1.70) 0.82 |
| CC+AC | 122 (76.25) | 129 (80.6) | Ref |
| AA (Recessive) | 38 (23.75) | 31 (19.4) | 0.77 (0.45-1.31) 0.34 |
| *rs2228570 (FokI)* | | | |
| CC | 72 (45.0) | 47 (29.3) | Ref |
| CT | 60 (37.5) | 81 (50.62) | 2.06 (1.25-3.39) 0.00 |
| TT | 28 (17.5) | 32 (20.0) | 1.75 (0.93-3.27) 0.07 |
| C | 204 (63.75) | 175 (54.68) | Ref |
| T | 116 (36.25) | 145 (45.32) | 1.45(1.06-2.00) 0.02 |
| CC (Dominant) | 72 (45.0) | 47 (29.4) | Ref |
| CT+TT | 88 (55.0) | 113 (70.6) | 1.96 (1.24-3.12) 0.00 |
| CC+TT | 100 (62.5) | 79 (49.4) | Ref |
| CT (Over dominant) | 60 (37.5) | 81 (50.6) | 1.70 (1.09-2.66) 0.02 |
| CC+CT | 132 (82.5) | 128 (80.0) | Ref |
| TT (Recessive) | 28 (17.5) | 32 (20.0) | 1.17 (0.67-2.06) 0.56 |
| *rs731236 (TaqI)* | | | |
| TT | 128 (80.0) | 120 (75.0) | Ref |
| TC | 22 (13.75) | 29 (18.1) | 1.40 (0.76-2.58) 0.27 |
| CC | 10 (6.25) | 11 (6.9) | 1.17 (0.48-2.86) 0.72 |
| T | 278 (87) | 269 (84.0) | Ref |
| C | 42 (13) | 51 (16.0) | 1.25 (0.80-1.95) 0.31 |
| TT (Dominant) | 128 (80.0) | 120 (75.0) | Ref |
| TC+CC | 32 (20.0) | 40 (25.0) | 1.33 (0.78-2.26) 0.28 |
| TT+CC | 138 (86.2) | 131 (81.9) | Ref |
| TC (Over dominant) | 22 (13.8) | 29 (18.1) | 1.38 (0.75-1.14) 0.28 |
| TT+TC | 150 (93.7) | 149 (93.1) | Ref |
| CC (Recessive) | 10 (6.3) | 11 (6.9) | 1.10 (0.45-2.68) 0.82 |
| *rs154410 (BsmI)* | | | |
| GG | 119 (74.4) | 131 (81.87) | Ref |
| GA | 30 (18.7) | 19 (11.87) | 0.57 (0.30-1.07) 0.08 |
| AA | 11 (6.9) | 10 (6.25) | 0.82 (0.33-2.01) 0.67 |
| G | 268 (84.0) | 281 (88.0) | Ref |
| A | 52 (16.0) | 39 (12.0) | 0.71 (0.45-1.11) 0.14 |
| GG (Dominant) | 119 (74.4) | 131 (81.9) | Ref |
| GA+AA | 41 (25.6) | 29 (18.1) | 0.64 (0.37-1.09) 0.10 |
| GG+AA | 130 (81.3) | 141 (88.1) | Ref |
| GA (Over dominant) | 30 (18.7) | 19 (11.9) | 0.58 (0.31-1.08) 0.09 |
| GG+GA | 149 (93.1) | 150 (93.75) | Ref |
| AA (Recessive) | 11 (6.9) | 10 (6.25) | 0.90 (0.37-2.19) 0.82 |

**Table 4.** Genotype and allelic frequencies of *VDR* gene polymorphisms among survived and expired patients

| Polymorphisms | Survived (N=146) | Expired (N=14) | Crude-OR, 95% CI, *p* value |
|---|---|---|---|
| *rs7975232 (ApaI)* | | | |
| CC | 63 (43.1) | 6 (42.8) | Ref |
| AC | 55 (37.7) | 5 (35.7) | 0.95 (0.27-3.30) 0.94 |
| AA | 28 (19.2) | 3 (21.5) | 1.12 (0.26-4.82) 0.99 |
| C | 181 (61.9) | 17 (60.7) | Ref |
| A | 111 (38.1) | 11 (39.3) | 1.05 (0.47-2.33) 0.89 |
| CC (Dominant) | 63 (43.1) | 6 (42.8) | Ref |
| AC+AA | 83 (56.9) | 8 (57.2) | 1.01 (0.33-3.06) 0.98 |
| CC+AA | 91 (62.3) | 9 (64.3) | Ref |
| AC (Over-dominant) | 55 (37.7) | 5 (35.7) | 0.91 (0.29-2.88) 0.88 |
| CC+AC | 118 (80.8) | 11 (78.5) | Ref |
| AA (Recessive) | 28 (19.2) | 3 (21.5) | 1.14 (0.30-4.39) 0.73 |
| *rs2228570 (FokI)* | | | |
| CC | 43 (29.4) | 4 (28.6) | Ref |
| CT | 74 (50.7) | 7 (50.0) | 1.01 (0.28-3.67) 0.99 |
| TT | 29 (19.9) | 3 (21.4) | 1.11 (0.23-5.34) 0.99 |
| C | 160 (54.8) | 15 (53.6) | Ref |
| T | 132 (45.2) | 13 (46.4) | 1.05 (0.48-2.28) 0.90 |
| CC (Dominant) | 43 (29.4) | 4 (28.6) | Ref |
| CT+TT | 103 (70.6) | 10 (71.4) | 1.04 (0.31-3.51) 0.99 |
| CC+TT | 72 (49.3) | 7 (50.0) | Ref |
| CT (Over-dominant) | 74 (50.7) | 7 (50.0) | 0.97 (0.32-2.91) 0.96 |
| CC+CT | 117 (80.1) | 11 (78.6) | Ref |
| TT (Recessive) | 29 (19.9) | 3 (21.4) | 1.10 (0.28-4.20) 0.99 |
| *rs731236 (TaqI)* | | | |
| TT | 113 (77.4) | 7 (50.0) | Ref |
| TC | 26 (17.8) | 3 (21.4) | 1.86 (0.45-7.69) 0.40 |
| CC | 7 (4.8) | 4 (28.6) | 9.22 (2.17-39.19) 0.00 |
| T | 252 (86.3) | 17 (60.7) | Ref |
| C | 40 (13.7) | 11 (39.3) | 4.07 (1.78-9.33) 0.00 |
| TT (Dominant) | 113 (77.4) | 7 (50.0) | Ref |
| TC+CC | 33 (22.6) | 7 (50.0) | 3.42 (1.12-10.46) 0.02 |
| TT+CC | 120 (82.2) | 11 (78.6) | Ref |
| TC (Over dominant) | 26 (17.8) | 3 (21.4) | 1.25 (0.32-4.83) 0.72 |
| TT+TC | 139 (95.2) | 10 (71.4) | Ref |
| CC (Recessive) | 7 (4.8) | 4 (28.6) | 7.94 (1.98-31.77) 0.00 |
| *rs154410 (BsmI)* | | | |
| GG | 122 (83.6) | 9 (64.3) | Ref |
| GA | 16 (10.9) | 3 (21.4) | 2.54 (0.62-10.38) 0.18 |
| AA | 8 (5.5) | 2 (14.3) | 3.38 (0.62-18.39) 0.17 |
| G | 260 (89.1) | 21 (75.0) | Ref |
| A | 32 (10.9) | 7 (25.0) | 2.70 (1.06-6.87) 0.03 |
| GG (Dominant) | 122 (83.6) | 9 (64.3) | Ref |
| GA+AA | 24 (16.4) | 5 (35.7) | 2.82 (0.86-9.17) 0.07 |
| GG+AA | 130 (89.1) | 11 (78.6) | Ref |
| GA (Over-dominant) | 16 (10.9) | 3 (21.4) | 2.21 (0.55-8.79) 0.22 |
| GG+GA | 138 (94.5) | 12 (85.7) | Ref |
| AA (Recessive) | 8 (5.5) | 2 (14.3) | 2.87 (0.54-15.09) 0.21 |

prompt the expression of networks of target genes which unite to adapt definite biological responses [19]. In humans, *VDR* gene is positioned at the long at the long arm of the 12th chromosome, at the location 12q13-14 and contains six promoter regions [8]. *FokI* (rs2228570), polymorphism is found on the 2nd exonic position of the *VDR* gene and causes the replacement of the thymine (T) by the cytosine (C) at the first ATG site (ATG to ACG). This replacement results in the synthesis of three amino acids shorter protein (M4,423 amino acids long VDR). *ApaI* and *BsmI* are intronic SNPs and are found on intron-8 and affect gene expression by changing the mRNA stability. *TaqI* is located in the 9th exon of the *VDR* gene. This silent codon change is characterized by the replacement of C with T, resulting in ATC to ATT codon change [20].

We found *FokI* polymorphism to be associated with increased risk of CAP. Similar findings were reported in pediatric population from Egypt and Canada [15, 16]. Another polymorphism (rs2239185) of *VDR* gene, not analyzed by us, has been reported to be associated with increased risk of CAP in Chinese Han population [12]. We did not find *ApaI* and *TaqI* polymorphism associated with CAP which is similar to findings reported in Saudi population [21]. However, we have found *TaqI* and *BsmI* polymorphisms to be associated with increased risk of mortality in our study. To the best of our knowledge, this has not been reported by anyone till date.

This is perhaps the first case-control study to assess the association of *VDR* gene polymorphisms (rs2228570 or *FokI*, rs1544410 or *BsmI*, rs7975232 or *ApaI*, and rs731236 or *TaqI*) with CAP in the north Indian population. We have used WHO classification of CAP, and have clearly defined inclusion and exclusion criteria. These give internal and external validity to the work. We did not assess serum vitamin D levels in cases and controls that was one of the limitations of our study.

We conclude that *FokI* (rs2228570) polymorphism of the *VDR* gene is a risk factor for CAP. Further genome-wide association studies and their functional implications with *VDR* gene polymorphisms are required with different study designs in various populations.

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## Disclosure of conflict of interest

None.

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