Potential role of vitamin D receptor-related polymorphisms in bronchopulmonary dysplasia

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Abstract

Background: The potential contribution of vitamin D and its receptor (VDR) to bronchopulmonary dysplasia (BPD) in preterm neonates is still unknown. The objective of the study was to test the relationship between VDR Taq 1 and Fok 1 gene polymorphisms and BPD in preterm neonates. VDR Fok 1 and Taq 1 gene polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Result: No statistically significant differences of genotypic distributions and allele frequencies of Fok 1 and Taq 1 VDR polymorphisms were detected between cases and controls. Moreover, no risk association was detected between both polymorphisms and BPD development in preterm neonates. Homozygous mutant (ff) genotype was the least frequent genotype among BPD and non-BPD groups (2.6%, 13.0% respectively) (p = 0.1). The same was detected for the mutant (CC) genotype frequency in both groups (10.5% and 15.2%, respectively). However, Taq 1 VDR polymorphism was significantly associated with the severity of BPD, as the genotypes with mutant allele C (CC +CT) were more frequent among severe cases (52.2%).

Conclusion: Fok 1 and Taq 1 VDR polymorphisms have no role in BPD development in preterm neonates. However, the presence of a mutant allele of Taq 1 VDR polymorphism may be associated with a more severe form of the disease.

Keywords: Bronchopulmonary dysplasia, Polymorphism, Premature neonates, Vitamin D receptor

Background

Bronchopulmonary dysplasia (BPD) is a worldwide major challenging consequence of prematurity and has a significant heritability [1]. In the USA alone, yearly reported new cases were about 10,000–15,000 [2]. Moreover, another study reported that BPD incidence ranged from 20% in preterm infants up to 60% in extremely preterm infants who were born before 26 weeks of gestation [3]. Although the major inflammatory role in the pathogenesis of BPD due to prematurity and perinatal triggering factors has been established, the genetic predisposition mechanisms remain unknown [4, 5]. According to twin studies, molecular factors represent about 53–82% of the variance in predisposition to BPD [6, 7]. Bronchopulmonary dysplasia is associated with future risk of reactive airway disease [3], infant mortality, and conflicting neurodevelopmental outcomes [8]. There are limited therapies available to prevent BPD despite recent advances, hence comes the role of genetic variants [2].

The effect of vitamin D on bone and mineral metabolism has been well-known through the role of the vitamin D receptor (VDR) that acts as a ligand-activated transcription factor [9].

The VDR is expressed in numerous systems other than skeletal, such as immune and respiratory systems [10].
The effect of vitamin D on several morbidities, such as multiple sclerosis, diabetes, and malignancies, has been established through affecting immunity and cell proliferation [11]. Several researchers reported the important role of vitamin D and its receptor in the pathogenesis of chronic lung diseases through interactions between genes related to cellular proliferation, differentiation, inflammation, and immunity [12]. Furthermore, some studies reported its important role as a regulator for intrauterine lung development [13–15]. In animal studies, a low level of vitamin D with pregnancy tends to modify alveolar epithelial-mesenchymal signaling and reduce tracheal width, thus increasing airway resistance and reducing lung compliance, which results in lung hypofunction in fetal mice [16]. Additionally, in human studies, the impact of vitamin D on the production of pulmonary surfactant has been confirmed [17]. As it has an important role as a growth factor for type-II alveolar pneumocytes, local elaboration of VDR was considered an innovative mechanism that may affect the epithelial growth of lung development and modulation [18]. So, further studies are still needed to assess the relationship between VDR polymorphisms and BPD.

The aim of this study was to determine the possible association between VDR Fok 1 and Taq 1 gene polymorphisms and BPD susceptibility in Egyptian preterm neonates.

Methods
Subjects
This case-control study included 84 newborns and was carried out at the neonatal intensive care unit (NICU) of Kasr AlAiny Hospitals, Cairo University, over a period of 1 year, starting from December 2018 to November 2019.

Infants were recruited into the study if they had been admitted to NICU and born prematurely with a gestational age of ≤ 32 weeks and a birth weight of ≤ 1500 g. Newborns who fulfilled the criteria for the BPD diagnosis joined the case group (38 cases), while the matched control group (46 cases) was selected among premature cases that had been admitted to NICU for different purposes but did not fulfill the criteria for the diagnosis of BPD. A BPD diagnosis and severity were carried out depending on the National Institute of Child Health and Human Development (NICHD) severity-based definition of BPD [19]. For those born at less than 32 weeks of gestation, BPD was defined as the need for oxygen support of more than 21% for at least 28 days and a subsequent assessment at 56 days postnatal age or discharge. At the time of assessment, infants with no oxygen need were considered as mild BPD. Moderate BPD was considered in cases needing less than 30% oxygen, while severe BPD was considered in cases with a need for positive pressure and/or oxygen support of ≥ 30% [19]. BPD risk factor-related data were collected from medical records of both groups: gestational age, birth weight, gender, mode of delivery, duration of mechanical ventilation, duration of oxygen therapy, administration of surfactant, presence of patent ductus arteriosus requiring treatment, duration of hospitalization, and mortality. Additionally, some prematurity-related complications were taken into account. They included respiratory distress syndrome (RDS) that was considered in premature infants when they presented shortly after birth with clinical signs of respiratory distress with the need for supplemental oxygen (FiO2 >0.21) to achieve oxygen saturation >90% and evidence of respiratory acidemia in blood gasses (pH < 7.25 and PCO2 > 60 mmHg) [20].

The typical radiological findings and grading of RDS were considered in the chest x-ray of all cases: hypoxexpansion and diffuse fine granular appearance (grade I), air bronchogram caused by atelectasis of the alveoli (grade II), ground-glass opacities (grade III), or white lungs caused by diffuse bilateral atelectasis (grade IV) [21]. Sepsis was diagnosed by a positive blood culture or a positive C-reactive protein and the immature-to-total-neutrophil ratio of more than 0.2 with concomitant clinical signs of sepsis. Sepsis in the first three postnatal days was defined as early-onset sepsis (EOS), while later sepsis was defined as late-onset sepsis (LOS) [22]. Necrotizing enterocolitis (NEC) was detected based on modified Bell staging criteria [23], and cases with grade Ib or more were considered. Intraventricular hemorrhage (IVH) was diagnosed by cranial ultrasound, and we only considered cases with grade II or more.

All newborns suspected of having genetic diseases or congenital anomalies were excluded from both groups. The study protocol was approved by the Ethics Committee of Faculty of Medicine, and it conformed to the provisions of the Declaration of Helsinki of 1964 and its later amendments or comparable ethical standards. An informed written consent was obtained from parents/ surrogates of each child before enrollment in this study.

Methods
Detection of vitamin D receptor polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)
Genomic DNA of included subjects was isolated from ethylenediaminetetraacetic acid (EDTA)-treated whole blood, using a G-spin TM total DNA extraction kit
Genotyping of vitamin D receptor polymorphism (Fok1 and Taq1 sites) was performed using the PCR-RFLP technique. The primer sequences used were as follows: for Fok1 polymorphism, forward primer: 5′-AGCTGGCCCTGGCAGCTTCTGCTCT-3′, reverse primer: 5′-ATGGAAAACACCTTGCTTTCTCTCCCT-3′ [24], while for the Taq1 polymorphism, forward primer: 5′-CAG AGC ATG GAC AGG GAG CAAG-3′, reverse primer: 5′-GCAACTCCTCATGGCTGAGGTCTCA-3′ [25]. Amplification was done using 2X PCR Master mix Solution (iTaqTM) (iNtRON biotechnology, Korea) in a total volume of 20μl.

For Fok1 polymorphism amplification, an initial denaturation at 95 °C for 3 min was followed by 35 cycles consisting of 30 s of denaturation at 94 °C, 30 s of annealing at 60 °C, and an extension for 30 s at 72 °C, and then, a final extension at 72 °C for 5 min. For Taq1 polymorphism amplification, an initial denaturation at 95 °C for 3 min was followed by 35 cycles consisting of 45 s of denaturation at 93 °C, 30 s of annealing at 66 °C, and an extension for 45 s at 72 °C and then a final extension at 72 °C for 5 min.

Amplification products were subjected to restriction digestion by the enzyme Fok1 (Enzymomics, Korea) and Taq1 (Enzymomics, Korea), respectively. Fragments were separated on 2% agarose gel, and bands were visualized by ethidium bromide staining under ultraviolet (UV) light. Fok1 reaction yielded one fragment of 265 bp indicating homozygous wild genotype (FF) (Fig. 1); Taq1 reaction yielded two fragments of 495 bp and 245 bp indicating a homozygous wild genotype (FF) (Fig. 2).

Sample size calculation was performed using the Power and Sample Size Calculation program version 3.0.43. It was based on the following inputs: the power of 90%, type 1 error 0.05, an equal number of candidates in both cases and controls, true difference in mean between groups 0.9, and a standard deviation 3.8. Thirty-eight subjects were found in each group.

The data was analyzed using Microsoft Excel 2010 and a statistical package for social science (SPSS version 24.0) for Windows (SPSS IBM, Chicago, IL). Continuous normally distributed variables were presented as mean ± SD with a 95% confidence interval using the frequencies and percentage for categorical variables; a p value < 0.05 was considered statistically significant. To compare the means of normally distributed variables between groups, Student’s t-test was performed. \( \chi^2 \) test or Fisher’s exact test was used to determine the distribution of categorical variables between groups. Haplotype analysis was done using the haplotype analysis software v1.05. The status of Hardy–Weinberg equilibrium (HWE) was checked through the analysis of genotype distribution. Effect modifications were evaluated by stratification; statistical interaction was assessed by including main effect variables and their product
terms in the multiple stepwise backward logistic regression model.

Results
Clinical and demographic data
The study involved 84 preterm neonates, in which 38 patients were diagnosed as BPD and 46 without BPD. The BPD group comprised 16 males (42.1%) and 22 females (57.9%); their mean age ± SD was 30 ± 1.5 weeks. While the non-BPD group comprised 24 males (52.2%) and 22 females (47.8%), their mean age ± SD was 31 ± 1.3 weeks. Among the control group, 32 (69.6%) cases were less than 30 weeks, while, among the cases, 15 (39.5%) were less than 30 weeks.

Antenatal variables were comparable between BPD and non-BPD groups with no significant differences, except for gestational age, as the BPD group has significantly lower age. On the other hand, BPD cases required more frequent O2 supply, mechanical ventilation (MV), and inotropic support (p < 0.001), and they were admitted for longer periods in comparison to the non-BPD group.

The BPD cases were classified according to the severity of the disease into three groups: mild cases 4 (10.6%), moderate cases 17 (44.7%), and severe cases 17 (44.7%). No significant statistical difference in disease severity between BPD cases was found with an age of fewer than 30 weeks versus those aged 30 weeks or more. Among those with 30 or more weeks, three mild, eleven moderate, and nine severe cases were observed versus one mild, six moderate, and eight severe cases in patients less than 30 weeks (p value 0.642).

Hemodynamically, the BPD group showed significant patent ductus arteriosus (PDA) in 23 cases (60.5%) in comparison to the 11 cases in the non-BPD group (23.9%) (p = 0.01). Regarding the occurrence of neonatal sepsis, late-onset neonatal sepsis was more frequent in the BPD group than the non-BPD group. All demographic and clinical data are shown in Table 1.

Fok 1 and Taq 1 VDR polymorphism results
The controls and BPD cases fit in the Hardy-Weinberg equilibrium for both Fok1 and Taq1 genotypes and have a p value > 0.05.

Regarding Fok 1 VDR polymorphism genotypic distribution (FF, Ff, ff), no significant statistical difference was detected between the studied groups (p value >0.05); homozygous mutant (ff) genotype was the least frequent genotype among BPD and non-BPD groups (2.6%, 13.0% respectively) (p value 0.1). Moreover, the allelic distributions of F and f alleles did not differ significantly between the cases and the controls (p value >0.05).

Regarding Taq 1 genotype distribution, the difference in genotypic distribution (TT, TC, CC) was not statistically significant between the cases and the controls (p value >0.05); the homozygous mutant (CC) genotype frequency was the least frequent genotype in BPD and non-BPD groups (10.5% and 15.2% respectively). Furthermore, there was no statistical difference between the allelic distributions (T allele, C allele) in the studied groups where the mutant (C) allele frequencies were 27% and 35% in patients and control groups, respectively, (p = 0.7).

Wild genotypes and wild alleles of both Fok 1 and Taq 1 VDR polymorphisms were taken as references for the risk assessment, and analysis revealed no significant association between both polymorphisms and the risk of BPD in our preterm neonates (Table 2). Moreover, no risk association between both polymorphisms and BPD development when cases are more stratified according to gestational age (≥30 weeks and <30 weeks) (Table 3).

Haplotype association analysis revealed no significant differences between the cases and the controls regarding the frequency of different haplotypes (Table 4).

Taq 1 VDR polymorphism was significantly associated with the severity of BPD as the genotypes with mutant allele C (CC +CT) were more frequent among severe cases (52.2%). But such association was not detected for Fok 1 VDR polymorphism (Table 5).

No associations were detected between Taq 1 and Fok 1 VDR polymorphism genotypes and the disease outcome among preterm newborns (Table 5).

Risk assessment for the development of BPD
In studying the univariate analysis of potential clinical risk factors for the development of BPD, gestational age, duration of hospitalization, administration of O2, and mechanical ventilation together with inotropes and surfactant had a potential effect on the development of BPD. Moreover, late-onset sepsis, PDA, pneumothorax, IVH, and NEC were associated with an increased risk of BPD (Table 6).

Multiple stepwise backward logistic regression was conducted to find the significant predictors for grouping (control/case). The independent variables entered on step 1 are Taq1, Fok1 VDR gene polymorphisms, gestational age, late-onset neonatal sepsis, PDA, Apgar at 5 min, and RDS grade depending on chest x-ray findings. The model was significant X2 (49.27) and p value < 0.001. It can independently explain the change in the grouping by 44.4% (r2 0.444). The significant predictors in the model were RDS severity and Apgar at 5 min, which means that an increase in the severity of RDS is more significantly associated with BPD cases than with the controls (p<0.001, OR 9.98, 95% CI 3.2–31.12). Every unit increase in Apgar score at 5 min increases the probability of the patient not having a disease (control) (p 0.002, OR 0.49, 95% CI 0.32–0.77), * P value <0.05 is significant, while **P value < 0.01 is highly significant.
| Table 1 Demographic and clinical data                                             | Non-BPD, N= 46 | BPD, N= 38 | p value |
|---------------------------------------------------------------------------------|----------------|------------|---------|
| **Gestational age**                                                             | 31±1.3         | 30±1.5     | 0.003   |
| **Gender**                                                                      |                |            |         |
| Male                                                                            | 24 (52.2%)     | 16 (42.1%) | 0.5     |
| **Mode of delivery**                                                            |                |            |         |
| CS                                                                               | 36 (78.3%)     | 30 (78.9%) | 0.9     |
| VD                                                                              | 10 (21.7%)     | 8 (21.1%)  | 0.9     |
| **Birth weight (g)**                                                            | 1350.43±24.66  | 1298.79±38.97 | 0.2     |
| **History of PROM**                                                             |                |            |         |
| Yes                                                                             | 11 (23.9%)     | 11 (28.9%) | 0.7     |
| **Antenatal steroid**                                                           |                |            |         |
| Yes                                                                             | 17 (37.0%)     | 13 (34.2%) | 0.8     |
| **Multiple gestation**                                                          |                |            |         |
| Yes                                                                             | 10 (21.7%)     | 6 (15.8%)  | 0.5     |
| **Preeclampsia**                                                                |                |            |         |
| Yes                                                                             | 6 (13%)        | 7 (18.4%)  | 0.16    |
| **Duration of admission (days)**                                                 |                |            |         |
| Mean ± SD                                                                       | 32.67±1.68     | 55.21±2.95 | 0.001   |
| **Duration of O₂ (days)**                                                       | 12.91±0.93     | 44.11±3.0  | 0.001   |
| **Mechanical ventilation**                                                      |                |            |         |
| Yes                                                                             | 7 (15.2%)      | 33 (86.8%) | 0.001   |
| **MV duration**                                                                 | 0.46±0.17      | 25.63±2.03 | 0.001   |
| **Apgar at 1 min**                                                              |                |            |         |
| Mean±SD                                                                         | 4.9±1.9        | 2.6±1.2    | 0.01    |
| **Apgar at 5 min**                                                              |                |            |         |
| Mean±SD                                                                         | 6.7±1.47       | 5.2±1.1    | 0.01    |
| **Inhaled steroid duration (days)**                                             |                |            |         |
| Yes                                                                             | 6.57±0.89      | 31.87±3.35 | 0.001   |
| **Intake of inotropes**                                                         |                |            |         |
| Yes                                                                             | 18 (39.1%)     | 38 (100.0%)| 0.001   |
| **Inotropes duration (days)**                                                   |                |            |         |
| Yes                                                                             | 2.87±0.61      | 16.47±1.51 | 0.001   |
| **Onset of trophic feeding (days)**                                             |                |            |         |
| Yes                                                                             | 2.35±0.08      | 3.29±0.36  | 0.01    |
| **TPN**                                                                         |                |            |         |
| Yes                                                                             | 43 (93.5%)     | 38 (100.0%)| 0.7     |
| **Duration of TPN (days)**                                                      | 13.85±1.09     | 31.74±1.68 | 0.001   |
| **Patent ductus arteriosus**                                                    | 11 (23.9%)     | 23 (60.5%) | 0.001   |
| **Early onset sepsis**                                                          |                |            |         |
| Yes                                                                             | 19 (51.4%)     | 18 (48.6%) | 0.3     |
| **Late onset sepsis**                                                           |                |            |         |
| Yes                                                                             | 33 (47.1%)     | 37 (52.9%) | 0.01    |
| **Admission chest X-ray**                                                       |                |            |         |
| Normal                                                                          | 2 (4.3%)       | 0 (0.0%)   | 0.2     |
| Mild RDS                                                                        | 34 (73.9%)     | 8 (21.1%)  | 0.001   |
| Moderate RDS                                                                    | 5 (10.9%)      | 7 (18.4%)  | 0.3     |
| Severe RDS                                                                      | 5 (10.9%)      | 23 (60.5%) | 0.001   |
| **Pneumothorax**                                                                |                |            |         |
| Yes                                                                             | 1 (2.2%)       | 25 (65.8%) | 0.001   |
| **IVH**                                                                         |                |            |         |
Discussion

BPD is a relevant chronic lung disease attributed to prematurity. Various studies considered the genetic aberrations involved in the development of BPD and were not conclusive. However, they highlighted several genes, variants, and pathways involved in the susceptibility to BPD [26].

The gene encoding the VDR is placed on chromosome 12; it contains 11 exons and 75 kb spans of genomic DNA [27]. There are more than two hundred polymorphisms of the VDR gene. The most described were Fok I, Bsm I, Apa I, and Taq I polymorphisms. The Fok I is in exon 2 and results in the construction of a longer and less active protein, while Bsm1, Apa1, and Taq1 polymorphisms are located between exons 8 and 9 [24].

The current study revealed that neither Fok 1 nor Taq 1 was associated with susceptibility to BPD in Egyptian neonates. Moreover, the allelic distribution of F and f alleles, on the one hand, and T and C alleles, on the other hand, respectively, was similar in both groups. To the best of our knowledge, Koroglu et al. [29] was the only study that evaluated possible associations of both polymorphisms and BPD. It reported a significant association of mutant Fok1 genotype as a risk factor for the development of BPD (detected in 53.1% of the cases versus 33.9% in the control group); furthermore, the mutant Taq 1 polymorphism is located at the untranslated region (UTR) of the VDR gene, which has an important role in the regulation of mRNA stability and protein translation, and thus the altered VDR levels may affect vitamin D signaling [28].

Table 1 Demographic and clinical data (Continued)

|                          | Non-BPD, N= 46 | BPD, N= 38 | p value |
|--------------------------|----------------|------------|---------|
| Yes                      | 9 (19.6%)      | 23 (60.5%) | 0.001   |
| Necrotizing enterocolitis|                |            |         |
| Yes                      | 6 (13.0%)      | 15 (39.5%) | 0.02    |
| Jaundice                 |                |            |         |
| Yes                      | 32 (69.6%)     | 32 (84.2%) | 0.4     |
| Intake of surfactant     |                |            |         |
| Yes                      | 7 (15.2%)      | 18 (47.4%) | 0.01    |
| Outcome                  |                |            |         |
| Died                     | 3 (6.5%)       | 12 (31.6%) | 0.01    |
| Living                   | 43 (93.5%)     | 26 (68.4%) | 0.001   |

CS cesarean section, VD vaginal delivery, PROM premature rapture of membrane, MV mechanical ventilation, TPN total parenteral nutrition, RDS respiratory distress syndrome, IVH intraventricular hemorrhage

Table 2 Genotypic distribution and allelic frequencies of Taq 1 and Fok 1 VDR polymorphisms

|                                | Non-BPD (N=46), No. (%) | BPD (N=38), No. (%) | 1p value | Odds ratio | 95% CI |
|--------------------------------|-------------------------|---------------------|----------|------------|--------|
| **Fok1 VDR gene polymorphism** |                         |                     |          |            |        |
| FF                             | 26 (56.5%)              | 20 (52.6%)          | 0.8      | 1          |        |
| Ff                             | 14 (30.4%)              | 17 (44.7%)          | 0.3      | 1.579      | 0.631–3.948 |
| ff                             | 6 (13.0%)               | 1 (2.6%)            | 0.1      | 2.500      | 0.024–1.947 |
| Ff+ff                          | 20 (43.5%)              | 18 (47.4%)          | 0.7      | 1.170      | 0.493–2.774 |
| F allele                       | 66 (71.7%)              | 57 (75%)            | 0.8      | 1          |        |
| f allele                       | 26 (28.3%)              | 19 (25%)            | 0.6      | 0.846      | 0.425–1.686 |
| **Taq1 VDR gene polymorphism** |                         |                     |          |            |        |
| TT                             | 18 (39.1%)              | 15 (39.5%)          | -        | 1          |        |
| TC                             | 21 (45.7%)              | 19 (50.0%)          | 0.8      | 1.086      | 0.431–2.737 |
| CC                             | 7 (15.2%)               | 4 (10.5%)           | 0.6      | 0.686      | 0.168–2.799 |
| TC+CC                          | 28 (60.9%)              | 23 (60.5%)          | 0.9      | 0.986      | 0.409–2.376 |
| T allele                       | 57 (62%)                | 49 (64.5%)          | 0        | 1          |        |
| C allele                       | 35 (38%)                | 27 (35%)            | 0.7      | 0.897      | 0.478–1.686 |

1p value less than 0.05 is statistically significant
CI confidence interval
Genotype of Taq1 VDR polymorphism was considered to have a protective effect against BPD (detected in 12.8% of the cases versus 25.8% in the control group). However, in their study, after controlling the gestational age and birth weight variables, Fok1 polymorphism did not have a significant effect on BPD susceptibility. Contrary to their results, our study of the Egyptian preterm neonates revealed a low frequency of the mutant genotype of Fok1 (2.6% in BPD cases, 13% in non-BPD cases) and mutant genotype of Taq1 polymorphism (10.5% in BPD cases, 15.2% in non-BPD cases). These frequencies of different expressions could be attributed to different ethnicity, as was suggested by previous studies [30, 31]. The inconsistencies between studies expressing genetic risks may be due to genetic heterogeneity, gene-to-environment and/or gene-to-gene interactions, as well as population admixture [32].

The present study revealed that mutant genotypes (CC+CT) of Taq1 VDR polymorphism were significantly more frequent among severe cases of BPD; this finding was not detected for Fok1 polymorphism. Although Taq1 polymorphism is nonfunctional, it shares other functional polymorphisms and a complex gene network affecting the expression of the VDR gene, and this could explain its relation to the severity of the disease [28]. Vitamin D and VDR may also play a role in the pathogenesis of chronic lung diseases through epigenetic control of the inflammatory process, immune regulation, and cellular proliferation [12]. Moreover, VDR receptor polymorphism may contribute to a higher incidence of respiratory tract infections through their effects on innate immunity [33]. Alongside all these factors that may explain the link between VDR

| Table 3 | Genotypic distribution of Taq 1 and Fok 1 VDR polymorphisms among different age groups |
|---------|--------------------------------------------------------------------------------------|
| Age >30 | Fok1 VDR gene polymorphism                                                          |
|         | BPD (N=23), No. (%)                                                                  |
|         | 1p value Odds ratio 95% CI                                                          |
|         | FT                                     | FF (70%) | 13 (56.5%) | 0.6997 | 0.769 | 0.202–2.917 |
|         | Ff+ff                                  | 7 (50%) | 10 (43.5%) |          |       |            |
|         | Taq1 VDR gene polymorphism                                                         |
|         | BPD (N=15), No. (%)                                                                 |
|         | 1p value Odds ratio 95% CI                                                          |
|         | TT                                     | 7 (50%) | 9 (39.1%)  | 1       |       |            |
|         | TC+CC                                  | 7 (50%) | 14 (60.2%) | 0.518   | 1.555 | 0.406–5.947 |
| Age <30 | Fok1 VDR gene polymorphism                                                         |
|         | BPD (N=15), No. (%)                                                                 |
|         | 1p value Odds ratio 95% CI                                                          |
|         | FT                                     | 19 (59.4%) | 7 (46.7%) |          | 1       |       |
|         | Ff+ff                                  | 13 (40.6%) | 8 (53.3%) | 0.415   | 1.670 | 0.485–5.746 |
|         | Taq1 VDR gene polymorphism                                                         |
|         | BPD (N=15), No. (%)                                                                 |
|         | 1p value Odds ratio 95% CI                                                          |
|         | TT                                     | 11 (34.4%) | 6 (40%) |          | 1       |       |
|         | TC+CC                                  | 21 (65.5%) | 9 (60%) | 0.708   | 0.785 | 0.222–2.782 |

*p value less than 0.05 is statistically significant
CI confidence interval

The inconsistencies between studies expressing genetic risks may be due to genetic heterogeneity, gene-to-environment and/or gene-to-gene interactions, as well as population admixture [32].

The present study revealed that mutant genotypes (CC+CT) of Taq1 VDR polymorphism were significantly more frequent among severe cases of BPD; this finding was not detected for Fok1 polymorphism. Although Taq1 polymorphism is nonfunctional, it shares other functional polymorphisms and a complex gene network affecting the expression of the VDR gene, and this could explain its relation to the severity of the disease [28]. Vitamin D and VDR may also play a role in the pathogenesis of chronic lung diseases through epigenetic control of the inflammatory process, immune regulation, and cellular proliferation [12]. Moreover, VDR receptor polymorphism may contribute to a higher incidence of respiratory tract infections through their effects on innate immunity [33]. Alongside all these factors that may explain the link between VDR

| Table 4 | Combined Taq1 and Fok1 haplotype frequencies among cases and controls |
|---------|---------------------------------------------------------------------|
| Haplotype | Non-BPD (N=42), % | BPD (N=43), % | p value |
| FT       | 42 45.7% | 43 56.6% | 0.3     |
| FC       | 15 16.3% | 6 7.9% | 0.1     |
| FF       | 24 26.1% | 14 18.4% | 0.3     |
| FC       | 11 12.0% | 13 17.1% | 0.4     |

BPD bronchopulmonary dysplasia

| Table 5 | The association between Taq1 and Fok1 VDR polymorphism genotypes and both the outcome and disease severity among BPD cases |
|---------|---------------------------------------------------------------------|
| Variable | Taq1 VDR genotyping | p value |
|         | TT | TC+CC |
| Outcome | Died | 4 (26.7%) | 8 (34.8%) | 0.6 |
|         | Living | 11 (73.3%) | 15 (65.2%) | 0.6 |
| Severity of BPD | Mild | 5 (33.3%) | 5 (33.3%) | 0.02 |
|         | Moderate | 7 (46.7%) | 10 (43.5%) | 0.02 |
|         | Severe | 11 (55.0%) | 13 (52.2%) | 0.02 |
| Fok1 VDR genotypes | FF | Ff+ff |
| Outcome | Died | 8 (40.0%) | 4 (22.2%) | 0.1 |
|         | Living | 12 (60.0%) | 14 (77.8%) | 0.1 |
| Severity of BPD | Mild | 1 (5.0%) | 3 (16.7%) | 0.5 |
|         | Moderate | 8 (40.0%) | 9 (50.0%) | 0.5 |
|         | Severe | 11 (55.0%) | 6 (33.3%) | 0.5 |

BPD bronchopulmonary dysplasia
polymorphism, vitamin D axis, and severity of BPD, there are several studies that have recognized a relationship between low serum vitamin D level [34], vitamin D binding protein (Gc globulin) [35], and subsequent risk of BPD development.

The current study revealed significant associations between some clinical factors and the risk of BPD, such as lower gestational age, long duration of oxygen therapy, mechanical ventilation, significant PDA, late-onset sepsis, severe RDS, surfactant therapy, and long admission duration. These findings were in agreement with several other studies [29, 36, 37]. Our study also showed that BPD cases were significantly associated with NEC, IVH similarly to Landry et al. [38]. And Mailaparambil et al. [39] concluded that BPD was more associated with some clinical factors rather than genetic polymorphisms.

Moreover, some researchers explored the relationships between Fok 1 and Taq polymorphisms and the development of these BPD-related risk factors in premature babies, as Ustun et al. [40] suggested that Taq1 polymorphism may be considered as a risk factor of RDS, while Mokhtar et al. [41] revealed a significant association of Fok 1 polymorphism and neonatal sepsis whereas Barchitta et al. [42] revealed a significant role of Fok 1 polymorphism with gestational duration and birth weight.

The study limitations here could be attributed to the relatively small sample size enrolled in the study, as well as a single tertiary center experience; thus, further studies with larger cohorts are recommended to confirm the role of these SNPs as molecular incriminators to the addressed pathology. Likewise, 25 (OH) D serum levels should have been examined in order to study the link between the studied genetic polymorphisms and its serum levels.

### Conclusion

Our findings revealed that neither the Fok 1 nor the Taq 1 were associated with an increased risk of BPD. Moreover, the variant mutants of both polymorphisms are detected at a low frequency among Egyptian preterm neonates. The mutant genotypes (CC+CT) of Taq 1 VDR polymorphism were significantly more frequent among severe cases of BPD. Further studies on a larger group of preterm neonates and more genome-wide association studies are required to eradicate the inconsistencies found so far and to elucidate the role of VDR polymorphisms in relation to susceptibility to BPD and its severity.

### Abbreviations

- BPD: Bronchopulmonary dysplasia
- VDR: Vitamin D receptor
- PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism
- EOS: Early-onset sepsis
- LOS: Late-onset sepsis
- NEC: Necrotizing enterocolitis
- PDA: Patent ductus arteriosus
- IVH: Intraventricular hemorrhage
- RDS: Respiratory distress syndrome

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### Reprints

Nil.

### Authors’ contributions

Dr. WA is an assistant professor of Pediatrics at Faculty of Medicine, Cairo University; she is the principal investigator (PI) of the study. Dr. N. AS is a lecturer of Pediatrics at the Faculty of Medicine, Cairo University; she was responsible for writing the manuscript. Dr. AG is an assistant professor of Hematology department at the Faculty of Medicine, Cairo University; she participated in this study by performing the practical part of the study and is the corresponding author. Finally, Dr. R. AW is a lecturer of clinical pathology, Faculty of Medicine, Cairo University; she participated in the statistical analysis. All authors were responsible for selection of the cases, collection of the patient's samples as well as obtaining the informed consent from the chosen cases. They also participated in doing DNA extraction for gene polymorphism, statistical analysis, and result interpretation. Finally, all authors have read and approved the manuscript.

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### Availability of data and materials

Nil.

### Ethics approval and consent to participate

An informed written consent was obtained from parents/surrogates of each child before enrollment, and the study was approved by the faculty of medicine (Kasr Alainy Hospitals) ethical committee. All procedures performed in the study involving human participants were in accordance with the ethical standards of the faculty's ethical research committee and with the

### Table 6 Univariate analysis of clinical variables influencing the risk of BPD

| Variable                        | OR   | 95% CI       | p value          |
|---------------------------------|------|--------------|------------------|
| Gestational age                 | 0.624| 0.45–0.867   | 0.005**          |
| Sex (male gender)               | 0.667| 0.281–1.584  | 0.3              |
| Birth weight (g)                | 0.999| 0.997–1.001  | 0.2              |
| Duration of hospitalization     | 1.12 | 1.064–1.18   | 0.001**          |
| Mode of delivery                | 0.96 | 0.336–2.739  | 0.9              |
| Duration O2                     | 1.255| 1.139–1.382  | 0.001**          |
| Mechanical ventilation          | 36.771| 10.665–126.78 | 0.001**         |
| Duration of MV                  | 1.503| 1.125–2.009  | 0.006**          |
| Surfactant                      | 5.014| 1.797–13.99  | 0.002**          |
| Inhaled steroid duration        | 1.277| 1.143–1.427  | 0.001**          |
| Early onset sepsis              | 1.3  | 0.54–3.0     | 0.6              |
| Late onset sepsis               | 14.6 | 1.81–117.6   | 0.01*            |
| Patent ductus arteriosus        | 4.879| 1.907–12.48  | 0.001**          |
| Pneumothorax                    | 86.538| 10.684–700.97 | 0.001**         |
| Intraventricular hemorrhage     | 6.304| 2.374–16.739 | 0.001**          |
| Necrotizing enterocolitis       | 4.348| 1.481–12.761 | 0.007**          |

OR: odds ratio, CI: confidence interval
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