Polymorphisms of fibronectin-1 (rs3796123; rs1968510; rs10202709; rs6725958; and rs35343655) are not associated with bronchopulmonary dysplasia in preterm infants

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Abstract Bronchopulmonary dysplasia (BPD) is a chronic lung disease that mainly affects premature newborns. Many different factors, increasingly genetic, are involved in the pathogenesis of BPD. The aim of the study is to investigate the possible influence of fibronectin SNP on the occurrence of BPD. The study included 108 infants born between 24 and 32 weeks of gestation. BPD was diagnosed based on the National Institutes of Health Consensus definition. The 5 FN1 gene polymorphisms assessed in the study were the following: rs3796123; rs1968510; rs10202709; rs6725958; and rs35343655. BPD developed in 30 (27.8%) out of the 108 preterm infants. Incidence of BPD was higher in infants with lower APGAR scores and low birthweight. Investigation did not confirm any significant prevalence for BPD development in any genotypes and alleles of FN1. Further studies should be performed to confirm the role of genetic factors in etiology and pathogenesis of BPD.

Keyword Fibronectin-1 · Polymorphism genes · Bronchopulmonary dysplasia

Introduction Bronchopulmonary dysplasia (BPD) is a severe lung disease of premature neonates born from 22 to 28 weeks of gestational age [1, 2]. As gestational age and birth weight decline, the occurrence of this disease increases and, as such, BPD persists to be the most prevalent complication associated with prematurity [3, 4]. Despite the fact that this condition is usually seen in premature neonates, BPD has been observed in full-term infants who require an aggressive form of ventilator therapy for 28 days or longer [5, 6]. Signs and symptoms such as nasal flaring, grunting, tachypnoea, and increased respiratory effort can be associated with BPD. Although many causative factors are associated with BPD, preterm delivery is the leading cause [7]. Other causes also contribute to the pathogenesis of BPD at a lower propensity, which include prenatal infections, patent ductus arteriosus, mechanical breathing, and postnatal infection [8]. Although BPD stems from combined exposures to both prenatal and postnatal influences such as those noted above, there is rising concern in the genetic contribution to the development of BPD. The findings of studies in both adults and neonates indicate that after acute lung injury, fibronectin may play a major role in the development of pulmonary fibrosis [9]. Fibronectin is a multi-domain glycoprotein present in nearly all vertebrate tissues and organs [10]. Glycoprotein fibronectin, of high molecular weight, is present in association with basement membranes, as well as in insoluble form in interstitial connective tissue and in soluble form in extracellular fluids such as amniotic, plasma, and cerebrospinal fluid [11]. In a variety of biological processes, fibronectin plays a crucial role in regard to extracellular matrix adhesion, cell migration, blood coagulation, and wound healing. Fibronectin and other pulmonary cytokines are important players in the successful and orderly repair of wounds; however, the
excessive development of these mediators can contribute to an amplification of the normal process of healing with the overall outcome of pulmonary fibrosis. There are studies proving FN mRNA and protein level increased in early acute BPD with their levels appearing greatest during the chronic reparative stage of BPD [12]. Results suggest a significant role for pulmonary cell-derived FN in the early inflammatory and later proliferative stages of BPD. Therefore, it was decided to investigate the possible influence of fibronectin polymorphisms on the occurrence of BPD [13]. In summarizing the current literature on fibronectin in bronchopulmonary dysplasia, we intend to lay the groundwork for further explanation of the process by which single-nucleotide fibronectin polymorphisms may lead to the development of fibrotic disease in BPD.

Materials and methods

Study population

The study includes a population of 108 infants born from 22 + 6 to 33 + 6 weeks of gestation, hospitalized at the Department of Neonatology (III level hospital) in the Clinical Hospital of Gynecology and Obstetrics of Poznan University of Medical Sciences between the years 2014 and 2018. The study did not include neonates born from multiple pregnancies, from pregnancies complicated by death of one of the fetuses, chromosomal abnormalities, TORCH (toxoplasmosis, other, rubella, cytomegalovirus, herpes) complex inflammation, or inherited errors of metabolism and infants without antenatal steroid therapy.

Clinical features

The following factors that may be associated with the development of BPD were studied: gender, gestational age (GA; weeks), birth weight (BW, grams), mode of delivery (vaginal birth vs cesarean section); APGAR score in 1st and 5th minute, pH and blood base excess (BE) in cord blood, intrauterine infection, surfactant administration, ventilation mode, and its duration. Infants delivered outside the Clinical Hospital of Gynecology and Obstetrics of Poznan University were also analyzed for developing bronchopulmonary dysplasia.

BPD diagnosis

Bronchopulmonary dysplasia was diagnosed based on the National Institutes of Health Consensus definition. BPD was defined as the need for at least one form of oxygen supplementation either at 28 postnatal days or 36 weeks of post-menstrual age (PMA) [14, 15].

Studied polymorphisms

We studied 5 single-nucleotide polymorphisms of fibronectin gene which are the following: rs3796123; rs1968510; rs10202709; rs6725958; and rs35343655 [16, 17]. A sample of blood was taken directly after the delivery and banked. Genomic DNA was extracted from blood leukocytes using QIAamp DNA Blood Mini Kit (QIAGEN Inc; Germany). Genotyping was performed using polymerase chain reaction (PCR) procedures. For the detection of FN1 (rs3796123) mutation PCR was amplified with starters: 5'-ACC AAT gCC AgG ATT CAg AgG-3', 5'-CCC AAC TTA ggC ATg AgA gC-3' (PCR product 234 bp long), and hydrolyzed with Alul restriction enzyme (Thermo Scientific). The following genotypes were obtained: AA 152, 82 bp; AT 234, 152, 82 bp; TT 234 bp. For detection of the FN1 (rs1968510) mutation, PCR was amplified with starters: 5'-gTT TgT TgT gTC AgT gTA gTA-3', 5'-TgC ATT AgC gTT ATg gCC ATg-3' (PCR product 784 bp long), and hydrolyzed with TaqI restriction enzyme (Thermo Scientific). The following genotypes were obtained: GG 594, 190 bp; GA784, 594,190 bp, and AA 784 bp. The FN1 (rs10202709) polymorphism was detected using starters: 5'-CAg gACT Tgg ATg gTg gTA A-3', 5'-CTC Agg ACT Tgg ATg gTg gTA A-3', 5'-CAG gAA CgA gAA CgA gAA gTT ggg ATg AT-3', 5'-CAC gAA CgA gAA CgA gAA gTT ggg ATg AT-3', 5'-gTA CCA TgT TAC TTgTgg AAT AgA g-3'. PCR product (206 bp long) was hydrolyzed with HindIII restriction enzyme (Thermo Scientific) and found the following genotypes: CC 138, 68 bp; CT 206, 138,68 bp; TT 206 bp. For detection of the FN1 (rs6725958) mutation, PCR was amplified with starters: 5'-CTC Agg ACT Tgg ATg gTg gTA A-3', 5'-TCA TTT CCC AAT AAA AgT ACA CTg-3' (PCR product 256 bp long), and hydrolyzed with HaeIII restriction enzyme (Thermo Scientific). The following genotypes were obtained: CC 171, 85 bp; AC 256, 171, 85 bp, and AA 256 bp. The FN1 (rs35343655) polymorphism was detected using starters: 5'-ACT gAA gTg CTC ggg ATg AT-3', 5'-CAC gAA CgA gAA AAT gTT ggg ATg AT-3', 5'-CTC Agg ACT Tgg ATg gTg gTA A-3', 5'-TCA TTT CCC AAT AAA AgT ACA CTg-3' (PCR product 236 bp long) was hydrolyzed with MspI restriction enzyme (Thermo Scientific) and found the following genotypes: GG 139,97 bp; GA 236,139,97 bp; AA 236 bp. Informed consent was obtained from all parents. The study was approved by the Bioethics Committee of Poznan University of Medical Sciences (no. 66/14 and 799/16) (Table 1).

Statistical analysis

Statistical analysis was performed using CytelStudio version 10.0 (CytelStudio Software Corporation, Cambridge, Massachusetts, United States) and Statistica version 10 (Stat Soft, Inc., Tulsa, Oklahoma, United States). The
results are presented as a percentage for categorical variables, or median and range for non-normally distributed continuous variables as tested by the Shapiro–Wilk test. A p value of less than 0.05 indicates statistical significance. The Fisher exact probability test, the chi-square test, Fisher Freeman Halton, and Chi-squared test with Yates’s correction were all used to evaluate the association between BPD and categorical variables such as gender, GA, BW, type of delivery, birth asphyxia, intrauterine infection, Apgar score, and delivery outside tertiary referral hospitals. Differences in non-normally distributed continuous variables were compared by the U Mann–Whitney test.

### Results

Table 2 shows the demographic and clinical characteristics of enrolled infants. In our study population, 30 (27.8%) infants developed BPD. The incidence of BPD was inversely proportional to multiple criteria, such as birth weight [incidence was significantly higher in newborns with birth weight less than 1000 g (76.7% vs 23.3%); p = 0.000052] and lower Apgar score in first (6(1–10) vs 4.5(1–7); p = 0.006) and fifth minute of life [7(4–10) vs 7(1–9); p = 0.001] had higher incidence of BPD. BPD developed more often in children diagnosed with intrauterine infection (76.7% vs 23.3%; p = 0.0165). Analysis showed higher prevalence of BPD in children ventilated conventionally than in non-invasive mode (80.0% vs 20.0%, p = 0.0001). Average duration of ventilation of children with BPD was 53.5 days (8–106, p < 0.0001). Our investigation did not confirm any significant prevalence for BPD development in any genotypes/alleles of FN1. Genotype and allele distribution of the polymorphisms in infants with or without BPD is presented in Table 3.

### Discussion

Preterm birth has been associated with an increased risk of both early and late severe clinical difficulties. An increased risk in complications is observed in extremely preterm (< 28 weeks of GA) and very preterm infants (28–31 weeks of GA) [3, 4]. With the addition of chronic neurological problems, chronic respiratory problems due to bronchopulmonary dysplasia (BPD) are the most common long-term complications of prematurity [4, 18].

We used binomial exact test to examine the association between the single-nucleotide polymorphism of fibronectin-1 and a risk factor of occurrence of bronchopulmonary dysplasia in newborns, born before the 34th week of gestation. Our investigation did not confirm any significant prevalence for BPD development in any of the assessed genotypes or alleles of FN1. Besides these finding, we observe the role of recognized risk factors in developing bronchopulmonary dysplasia, including birth weight, lower Apgar score in first and fifth minute of life, children diagnosed with intrauterine infection, and in children ventilated conventionally.

In our study population, 30/108 infants developed BPD, with an average duration of required ventilation of around 55 days.

BPD contributes significantly to morbidity and mortality in neonatal intensive care units [19]. Bronchopulmonary dysplasia is the result of a multimodal process leading to a severe, lifelong disease, caused by an imbalance between lung damage and repair [20, 21]. The definition includes the need for mechanical ventilation and oxygen supplementation at 28 days of age and 36 weeks of pregnancy. Despite significant advances in treatment over the past two decades, the incidence of BPD is still over 30% in premature babies less than 30 weeks of pregnancy in most European countries.
Diagnosis has been associated with an increased risk of abnormal somatic and psychomotor development [23]. There has been animal experiments that indicate that premature infants with BPD may be at a higher risk of developing chronic obstructive pulmonary disease later in life [24, 25].

The factors contributing to the development of this disease can be divided into prenatal, perinatal, and postnatal causes. Among the prenatal and perinatal factors are genetic factors, immaturity of surfactant homeostasis, intrauterine and perinatal infections, and weak lung growth caused by placental insufficiency. Postnatal risk factors include mechanical ventilation, fluid overload, and nutritional deficiencies [26].

The results from a plethora of studies in neonatal care suggest that fibronectin may play a key role in the development of pulmonary fibrosis following acute lung injury [9]. FN1 has been observed to change modalities based on its surrounding environment. FN1 has been reported to hold a soluble form in plasma and an insoluble form when associated with the extracellular matrix, where FN1 binds to tissues such as collagen. Fibronectin-1 is important for alveolarization and the structural homeostasis of the alveoli [27]. Sinkin and colleagues examined the distribution of pulmonary FN by in situ hybridization (for mRNA) and immunohistochemistry (for protein) in neonatal autopsy lung specimens, comparing lungs with BPD to those without.

### Table 2: Demographic and clinical characteristics of enrolled infants

|                                | BPD     | p       |
|--------------------------------|---------|---------|
|                                | Yes     | No      |
| Gender                         |         |         |
| Male                           | 18 (60%)| 44 (56.4%)| 0.788<sup>a</sup> |
| Female                         | 12 (40%)| 34 (43.6%)|         |
| Gestational age (week)         |         |         |
| <29                            | 8 (26.7%)| 29 (37.2%)| 0.303<sup>a</sup> |
| ≥29                            | 22 (73.3%)| 49 (62.8%)|         |
| Birth weight (g)               |         |         |
| <750                           | 7 (23.3%)| 7 (9.0%)| 0.000052<sup>a</sup> |
| 750–1000                       | 16 (53.3%)| 16 (20.5%)|         |
| >1000                          | 7 (23.3%)| 55 (70.5%)|         |
| Apgar score                    |         |         |
| 1st minute                     | 4.5 (1–7)| 6 (1–10)| 0.006<sup>b</sup>| 0.001<sup>b</sup> |
| 5th minute                     | 7 (1–9)| 7 (4–10)|         |
| Mode of delivery               |         |         |
| Vaginal                        | 16 (53.3%)| 31 (39.7%)| 0.202<sup>a</sup> |
| Cesarean section               | 14 (46.7%)| 47 (60.3%)|         |
| pH 1                           | 7.35 (7.00–7.51)| 7.31 (6.98–7.53)| 0.123<sup>b</sup>| 0.132<sup>b</sup> |
| BE 1                           | −1.4 (−11.0–+2.3)| −3.0 (−16.9–+10.6)|         |
| Intrauterine infection         |         |         |
| Yes                            | 23 (76.6%)| 40 (51.2%)| 0.0165<sup>a</sup> |
| No                             | 7 (23.3%)| 38 (48.8%)|         |
| Inborn                         | 27 (90%)| 69 (88.5%)| 0.909<sup>c</sup> |
| Outborn                        | 3 (10%)| 9 (11.5%)|         |
| Deaths                         |         |         |
| Yes                            | 0 (0%)| 13 (16.7%)| 0.0178<sup>d</sup> |
| No                             | 30 (100%)| 65 (83.3%)|         |
| Surfactant administration      |         |         |
| Yes                            | 25 (83.3%)| 30 (38.5%)| <0.0001<sup>a</sup> |
| No                             | 5 (16.7%)| 48 (61.5%)|         |
| Ventilation mode               |         |         |
| Non-invasive                   | 6 (20%)| 48 (61.5%)| 0.0001<sup>a</sup> |
| Conventional                   | 24 (80%)| 30 (38.5%)|         |
| Duration of ventilation [days] |         |         |
| ≤100                           | 53.5 (8–106)| 9 (1–60)| <0.0001<sup>b</sup> |

Dell Statistica (data analysis software system), version 10. software.dell.com. BPD bronchopulmonary dysplasia. aChi-square test; bMann–Whitney test; cChi-square test with Yate’s correction; dFisher’s exact test.

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[22]. [23]. [24]. [25].
Table 3 Genotype and allele distribution of infants with or without BPD

| Polymorphism          | Genotype  | Yes | No | p value | OR      |
|-----------------------|-----------|-----|----|---------|---------|
| FN1 rs3796123 (AluI)  | Genotype  | 17  | 42 | 0.756   |         |
| AA                    |           | 1   |    |         | References |
| TT                    |           | 1   | 6  | 0.412 (0.008–3.85) |
| AT                    |           | 12  | 30 | 0.988 (0.371–2.576) |
| Allele                |           |     |    | 0.723   |         |
| A                     |           | 46  | 114|         | References |
| T                     |           | 14  | 42 | 0.826 (0.379–1.724) |
| FN1 rs1968510 (TaqI)  | Genotype  | 25  | 70 | 0.542   |         |
| GG                    |           |     |    | 0.283   | References |
| AA                    |           | 1   | 0  |         | –       |
| GA                    |           | 4   | 8  |         | 1.4 (0.283–1.724) |
| Allele                |           |     |    | 0.319   |         |
| A                     |           | 54  | 148|         | References |
| T                     |           | 6   | 8  |         | 2.056 (0.559–7.087) |
| FN1 rs10202709 (HindIII) | Genotype | 22  | 52 | 0.708   |         |
| CC                    |           |     |    | 0.914   | References |
| TT                    |           | 1   | 6  | 0.394 (0.008–3.585) |
| CT                    |           | 7   | 20 | 0.827 (0.258–2.425) |
| Allele                |           |     |    | 0.47    |         |
| C                     |           | 51  | 124|         | References |
| T                     |           | 9   | 32 | 0.684 (0.268–1.602) |
| FN1 rs6725958 (HaeIII) | Genotype | 8   | 26 |         | References |
| CC                    |           | 7   | 1  | 1.034 (0.271–3.865) |
| AA                    |           | 15  | 22 | 0.486   | 1.625 (0.538–5.156) |
| Allele                |           |     |    | 0.486   |         |
| C                     |           | 31  | 82 | 0.415   | References |
| A                     |           | 37  | 74 | 1.323 (0.718–2.442) |
| FN1 rs35343655 (MspI) | Genotype | 6   | 2  | 1       | References |
| AA                    |           | 46  | 20 | 1       | References |
| GG                    |           | 26  | 8  | 0.767 (0.07–4.803) |
| GA                    |           | 2   |    |         | 1.083 (0.089–7.826) |
| Allele                |           |     |    | 0.625   |         |
| A                     |           | 38  | 48 |         | References |
| G                     |           | 118 |    | 0.776 (0.339–1.680) |

Results are expressed as absolute number of patients (percentage). The odds ratio (OR) and 95% confidence intervals (95% CI) are expressed as the following. AA denotes homozygosity for the A-encoded FN1 rs3796123 (AluI) polymorphism variant; TT homozygosity for the T-encoded FN1 rs3796123 (AluI)T polymorphism variant; AT heterozygosity for FN1 rs3796123 (AluI) polymorphism. GG denotes homozygosity for the G-encoded FN1 rs1968510 (TaqI) polymorphism variant; AA homozygosity for the A-encoded FN1 rs1968510 (TaqI) A polymorphism variant; GA heterozygosity for FN1 rs1968510 (TaqI) polymorphism. CC denotes homozygosity for the C-encoded FN1 rs10202709 (HindIII) polymorphism variant; TT homozygosity for the T-encoded FN1 rs10202709 (HindIII) T polymorphism variant; CT heterozygosity for FN1 rs10202709 (HindIII) polymorphism. CC denotes homozygosity for the C-encoded FN1 rs6725958 (HaeIII) polymorphism variant; AA homozygosity for the A-encoded FN1 rs6725958 (HaeIII) A polymorphism variant; CA heterozygosity for FN1 rs6725958 (HaeIII). AA denotes homozygosity for the A-encoded FN1 rs35343655 (MspI) polymorphism variant; GG homozygosity for the G-encoded FN1 rs35343655 (MspI) polymorphism variant; AG heterozygosity for FN1 rs35343655 (MspI) Cytel Studio version 11.1.0 (January 05, 2016). FN1 fibronectin-1, BPD bronchopulmonary dysplasia. Test used for calculation: binomial exact test.
FN mRNAs were detected in vascular endothelium, macrophages, fibroblasts, vascular, airway smooth muscle, chondrocytes, and pulmonary parenchyma in children with and without bronchopulmonary dysplasia, but not in epithelial cells. Fibronectin levels were the most increased in early acute BPD, while in stable BPD the observed levels were significantly lower [28].

Several studies have documented an increased expression of FN1 in clinical BPD, including plasma, endotracheal aspirates, BAL fluid, and lung tissue [29]. The excessive production of FN1 may result in excess and increased propensity of the healing process. The potential role of fibronectin as a possible marker was described by Watts and colleagues [9]. Further investigation by Jeffrey and others indicated that tracheal lavage fibronectin/albumin ratio from patients with BPD was elevated from the 3rd week of life [19]. Additional examination of BPD and FN1 by Zhang and others suggested that decreased miR-206 expression in BPD may be responsible for the underlying factor of increasing levels of FN1 noted in the lungs of BPD patients; since FN1 has been associated to be a target of miR-206 [30, 31].

FN1 shows angiogenic properties and a potential to interact with other key regulators of the extracellular matrix proteins, such as TGF-β1 and VEGF. FN1 activation is believed to be induced by inflammation. Kallapur and McAdams provided evidence for the potential of different levels of inflammation affecting FN1 severity [32].

The fibronectin gene has been assessed in relation to various disorders [27]. Polymorphisms and mutations in the FN1 gene have a multimodal association and have been shown to play a role in renal glomerulopathy, spondylometaphyseal dysplasia, lung fibrosis in systemic sclerosis, knee osteoarthritis, lung cancers, gastric cancers, breast cancers, and schizophrenia [16, 33–40].

In our study, we assessed the significance of fibronectin gene polymorphism in the incidence of BPD in 108 infants born in 22 + 6 and 33 + 6 weeks of gestation. Within this group, 30 (27.8%) infants developed BPD and none of the five gene polymorphisms assessed (rs3796123; rs1968510; rs10202709; rs6725958; and rs35343655) showed a significantly higher prevalence of BPD.

The relationship of single-nucleotide polymorphisms of fibronectin in neonatal diseases has been highlighted in the previous studies [41]. One genotype (TT-FN1 rs10202709 gene) and one allele (T-FN1 rs10202709 gene) of the FN1 gene showed a significantly higher prevalence in IVH. Infants with SNP TT in the rs10202709 gene were 7 times more likely to develop IVH stage II to IV. Infants with T-FN1 rs10202709 allele were also more than twice as likely to develop IVH stage II to IV. Although basal lamina is crucial also in proper lung development, this study did not confirm any significant prevalence for BPD development in any genotypes or alleles of FN1.

However, there has been studies that show the significant role that genetics plays in the pathogenesis of BPD [41]. In Caucasian populations, an interconnection between FGFR-4 SNP rs1966265 polymorphism and both—BPD and RDS frequency has been described [42]. The A/A genotype, encoding for isoleucine instead of valine, was noted to have a protective influence in this study. FGFR-4 appears to be fundamental in alveolar formation. Weinstein et al. demonstrated hyperoxia-exposed mice presented a BPD-like lung pattern with a reduced expression of FGFR-3 and FGFR-4 [43], further highlighting the protective role of the FGFR gene.

In African-American newborns, other genetic variations—IL-18RAP rs3771150 and IL-18R1 rs3771171—have been associated with susceptibility for the development of BPD [44]. These genes may contribute to AA BPD pathogenesis via inflammatory-mediated processes.

Furthermore, genetic rearrangements in Toll-like receptors indicate different odds of BPD—TLR6 (rs5743827) in African Americans, TLR 2 SNPs in non-Hispanic Caucasians, and TLR4 (rs11536898) in both study groups [45]. Single SNPs in TLR2, TLR4, and TLR6 were associated with BPD. Similar to FGFR-3 and FGFR-4, TLR6 SNP rs5743827 was associated with decreased risk for BPD. On the other hand, rs11536898 in TLR4 was associated with BPD infants, with the A-allele having twice the higher risk for BPD compared with those with the C-allele in both the total sample and in the African-American population. Among non-Hispanic Caucasians, additional tagging SNPs in TLR2 and TLR6 were associated with BPD. It is speculated that TLR6 mutations impact susceptibility to Urea plasma respiratory tract colonization, duration, and the severity of inflammatory response that contributes to bronchopulmonary dysplasia. Since TLR6 interacts with TLR2 and attenuates NF-κB activation—this suggests that mutations in these TLRs weaken the immune response, increasing susceptibility to infection.

To date, only a few studies have been published in regard to FN1 rs10202709 polymorphism and rs6725958 polymorphism. Yand and colleagues studied the influence of, inter alia, FN1 rs6725958 and rs10202709 SNPs on a possible risk factor in developing knee osteoarthritis. Only rs940739A/T genotype appeared to be associated with a higher risk for knee osteoarthritis [46]. Moreover, Murat and colleagues assessed the FN1 rs10202709 as a risk factor of calcium oxalate stones in the Uighur population but with no significant correlation [47].

Our investigation did not confirm any significant prevalence for BPD development in any of the studied genotypes or alleles of FN1. Certainly, this study has a few limitations. Firstly, a relatively small-sized control group. A larger research group is pivotal to ultimately rule out any role of fibronectin single-nucleotide polymorphisms in the
Conclusion

Exploring genetic variations in the fibronectin-1 physiological pathway of functioning may broaden the current understanding of genetic susceptibility to bronchopulmonary dysplasia. Our study did not confirm any significant prevalence for BPD development in any of the studied genotypes or alleles of FN1. Further studies with larger samples are required.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11001-022-04397-1.

Author contributions KK and DS designed research; KK, KG, AS, DS, JAA, SRA, and LMK collected and analyzed the data; KK, DS, JAA, AS, SRA, LMK, MS-B, KD, AS-M, and GK performed research; GK was responsible for PCR procedure. All authors commented on the manuscript at all grades.

Data availability The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Poznan University of Medical Sciences (66/14 and 799/16).

Informed consent Informed consent was obtained from all subjects involved in the study.

References

1. Jobe AH (2015) Animal models, learning lessons to prevent and treat neonatal chronic lung disease. Front Med 7(2):49. https://doi.org/10.3389/fmed.2015.00049
2. Xu YP (2016) Bronchopulmonary dysplasia in preterm infants born at less than 32 weeks gestation. Glob Pediatr Health. https://doi.org/10.1177/2333794X16668773
3. Wadhawan R, Vohr BR, Fanaroff AA, Perritt RL, Duara S, Stoll BJ, Goldberg R, Laptook A, Poole K, Wright LL et al (2003) Does labor influence neonatal and neurodevelopmental outcomes of extremely-low-birth-weight infants who are born by cesarean delivery? Am J Obstet Gynecol 189:501–506. https://doi.org/10.1067/s0002-9378(03)00360-0
4. Stoll BJ, Hansen NI, Bell EF et al (2010) Neonatal outcomes of extremely preterm infants from the NICHD neonatal research network. Pediatrics 126(3):443–456
5. Charafeddine L et al (1999) Atypical chronic lung disease patterns in neonates. Pediatrics 103:759–765
6. Lavoie PM, Pham C, Jang KL (2008) Heritability of bronchopulmonary dysplasia, defined according to the consensus statement of the national institutes of health. Pediatrics 122:479–485. https://doi.org/10.1542/peds.2007-213
7. D’Angio CT, Maniscalco WM (2004) Bronchopulmonary dysplasia in preterm infants: pathophysiology and management strategies. Paediatr Drugs 6(5):303–330. https://doi.org/10.2165/00148581-200406050-00004
8. Pasha AB, Chen XQ, Zhou GP (2018) Bronchopulmonary dysplasia: pathogenesis and treatment. Exp Ther Med 16(6):4315–4321. https://doi.org/10.3892/ethm.2018.6780
9. Colunga Biancatelli RML, Solopov P, Dimitropoulou C, Catravas JD (2021) Age-dependent chronic lung injury and pulmonary fibrosis following single exposure to hydrochloric acid. Int J Mol Sci 22(16):8833. https://doi.org/10.3390/ijms22168833
10. Henderson B, Nair S, Pallas J, Williams MA (2011) Fibronectin: a multidomain host adhesin targeted by bacterial fibronectin-binding proteins. FEMS Microbiol Rev 35:147–200. https://doi.org/10.1111/j.1574-6976.2010.00243.x
11. Clemmensen I (1981) Fibronectin and its role in connective tissue diseases. Eur J Clin Invest 11(3):145–146
12. Hynes RO (1986) Fibroentins. Sci Am 254(6):42–51. https://doi.org/10.1177/2333794X16668773
13. Fischer HS, Schmolzer GM, Cheung PY, Bührer C (2018) Sustained inflations and avoiding mechanical ventilation to prevent death or bronchopulmonary dysplasia: a meta-analysis. Eur Respir Rev. https://doi.org/10.1183/16000617.0083-2018
14. Jin R et al (2020) IL-33-induced neutrophil extracellular traps degrade fibronectin in a murine model of bronchopulmonary dysplasia. Cell Death Discov. https://doi.org/10.1038/s41420-020-0261-2
15. Isayama T, Lee SK, Yang J, Lee D, Daspal S, Dunn M, Shah PS (2017) Revisiting the definition of bronchopulmonary dysplasia. JAMA Pediatr 171:271. https://doi.org/10.1001/jamapediatrics.2016.4141
16. Avila JJ, Lympamy PA, Pantelidis P, Welsh KI, Black CM, Du Bois RM (1999) Fibronectin gene polymorphisms associated with fibrosing alveolitis in systemic sclerosis. Am J Respir Cell Mol Biol 20:106–112. https://doi.org/10.1165/arcmb.20.1.3232
17. Murat M, Ackerer P, Yuan LY, Alim T, Du GJ, Abdusamat A, Wu GW, Aniwer Y (2015) Correlation between the development of calcium oxalate stones and polymorphisms in the fibronectin gene in the Uighur population of the Xinjiang region of China. Genet Mol Res 14:13728–13734. https://doi.org/10.4238/2015.October.28.35
18. Natarajan G, Shankaran S (2016) Short- and long-term outcomes of moderate and late preterm infants. Am J Perinatol 33:305–317. https://doi.org/10.1055/s-0035-1571150
19. Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, Aschner JL, Davis PG, McGrath-Morrow SA, Soll RF et al (2019) Bronchopulmonary dysplasia. Nat Rev Dis Prim. https://doi.org/10.1038/s41572-019-0127-7
20. Kalikko Thekkeveedu R, Guaman MC, Shivanna B (2017) Bronchopulmonary dysplasia: a review of pathogenesis and pathophysiology. Respir Med 132:170–177
21. Dani C, Corsini I, Berti G, Pratesi S, Barp J, Rubaltelli FF (2011) Effect of multiple INSURE procedures in extremely preterm infants. J Matern Fetal Neonatal Med 24(12):1427–1431
22. Gortner L, Misselwitz B (2011) Rates of bronchopulmonary dysplasia in very preterm neonates in Europe: results from the MOSAIC cohort. Neonatology 99:112–117. https://doi.org/10.1159/000313024

23. Islam JA, Keller RL, Aschner JL, Hartert TV, Moore PE et al (2015) Understanding the short- and long-term respiratory outcomes of prematurity and bronchopulmonary dysplasia. Am J Respir Crit Care Med 192(2):134–156. https://doi.org/10.1164/rccm.201412-2142PP

24. Carraro S, Giordano G, Pirillo P, Maretti M, Reniero F, Cogo PE, Periolo G, Stocchero M, Baraldi E et al (2015) Airway metabolic anomalies in adolescents with bronchopulmonary dysplasia: new insights from the metabolomic approach. J Pediatr 166(2):234–239. https://doi.org/10.1016/j.jpeds.2014.08.049

25. Korhonen PH, Suursalmi P, Kopeli T, Nieminen R, Lehtimäki L, Laukkaala T, Korppi SA, Moilanen E, Tammela OK et al (2015) Inflammatory activity at school age in very low birth weight bronchopulmonary dysplasia survivors. Pediatr Pulmonol 50(7):683–690. https://doi.org/10.1002/ppul.23038

26. Shahzad T, Radajevski S, Chao CM, Bellusci S, Ehrhardt H (2016) Pathogenesis of bronchopulmonary dysplasia: when inflammation meets organ development. Mol Cell Pediatr 3(1):23. https://doi.org/10.1515/mcp-2015-00019

27. Mao Y, Schwarzbaumer JE (2005) Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. Matrix Biol 24:389–399. https://doi.org/10.1016/j.matbio.2005.06.008

28. Sinkin RA, Roberts m, LoMonaco MB, Sanders RJ, Metlay LA (1998) Fibronectin expression in bronchopulmonary dysplasia. Pediatr Dev Pathol 1:494–502. https://doi.org/10.1002/s100 49900068

29. Mižíková I, Morty RE (2015) The extracellular matrix in bronchopulmonary dysplasia: target and source. Front Med 2:91. https://doi.org/10.3389/fmed.2015.00091

30. Duan J, Zhang X, Zhang S, Hua S, Feng Z (2017) MiR-206 inhibits FN1 expression and proliferation and promotes apoptosis of murine lung. Development 125(18):3615–3623

31. de Vasconcellos JF, Jackson WM, Dimtchev A, Nesti LJ (2020) A microRNA signature for impaired wound-healing and ectopic bone formation in humans. J Bone Joint Surg Am 102:1891–1899. https://doi.org/10.2106/JBJS.19.00896

32. Kallapur SG, Fryhuber GS (2015) Bronchopulmonary dysplasia: the search for answers continues. Clin Perinatol 42:xix–xx. https://doi.org/10.1016/j.clp.2015.09.001

33. Onaran M, Yilmaz A, Şen I, Ergun MA, Çamtosun A, Küpelı B, Meneye S, Bozkırli I (2009) A HindIII polymorphism of fibronectin gene is associated with nephrolithiasis. Urology 74:1004–1007. https://doi.org/10.1016/j.urology.2009.05.010

34. Lee CS, Fu H, Baratang N, Rousseau J, Kumra H, Sutton VR, Nicketa M, Ciolfi A, Yamamoto G, Bertola D et al (2017) Mutations in fibronectin cause a subtype of spondylometaphyseal dysplasia with “corner fractures.” Am J Hum Genet 101:815–823. https://doi.org/10.1016/j.ajhg.2017.09.019

35. Yang HY, Su SL, Peng YJ, Wang CC, Lee HS, Salter DM, Lee CH (2014) An intron polymorphism of the fibronectin gene is associated with end-stage knee osteoarthritis in a Han Chinese population: two independent case: control studies. BMC Musculoskelet Disord 15:1–9. https://doi.org/10.1186/1471-2474-15-173

36. Qin S, Zhang B, Xiao G, Sun X, Li G, Huang G, Gao X, Li X, Wang H, Yang C et al (2016) Fibronectin protects lung cancer cells against docetaxel-induced apoptosis by promoting Src and caspase-8 phosphorylation. Tumor Biol 37:13509–13520. https://doi.org/10.1007/s13277-016-2506-8

37. Siemianowicz K, Gminksi J, Francuz T, Syz dol M, Polanska D, Machalski M, Brulinski K, Magiera-Moleldowska H (2001) Fibronectin gene polymorphism in patients with lung cancer. Oncol Rep. https://doi.org/10.3892/or.8.6.1289

38. Sun Y, Zhao C, Ye Y, Wang Z, He Y, Li Y, Mao H (2020) High expression of fibronectin 1 indicates poor prognosis in gastric cancer. Oncol Lett 19:93–102. https://doi.org/10.3892/ol.2019.11088

39. Libring S, Shinde A, Chanda MK, Nuru M, George H, Saleh AM, Abdullah A, Kinzer-Ursem TL, Calve S, Wen dt MK et al (2020) The dynamic relationship of breast cancer cells and fibroblasts in fibronectin accumulation at primary and metastatic tumor sites. Cancers. https://doi.org/10.3390/cancers12051270

40. Nakata K, Ujike H, Sakai A, Takaki M, Imamura T, Tanaka Y, Kuroda S (2003) Association study between the fibronectin gene and schizophrenia. Am J Med Genet Neuropsychiatr Genet 116:41–44. https://doi.org/10.1002/ajmg.b.10796

41. Szpecht D, Al-Saad SR, Karbows IG, Miskik K, Kurzawińska G, Szymankiewicz M, Dreks K, Seremak-Mrozikiewicz A (2020) Role of fibronectin-1 polymorphism genes with the pathogenesis of intraventricular hemorrhage in preterm infants. Child Nerv Syst 36:1729–1736. https://doi.org/10.1007/s00381-020-04598-3

42. Rezvani M, Wilde J, Vint P, Mailaparambil B, Grychotd K, Krueger M, Heinzmann A (2013) Association of a FGFR-4 gene polymorphism with bronchopulmonary dysplasia and neonatal respiratory distress. Dis Mark 35:633–640. https://doi.org/10.1155/2013/932356

43. Weinstein M, Xu X, Ouyama K, Deng CX (1998) FGFR-3 and FGFR-4 function cooperatively to direct alveogenesis in the murine lung. Development 125(18):3615–3623

44. Floros J, Lendon D, Gordon D, Silveyra P, Diangelo SL, Viscardi RM, Worthen GS, Shenberger J, Wang G, Lin Z et al (2012) IL-18R1 and IL-18RAP SNPs may be associated with bronchopulmonary dysplasia in African-American infants. Pediatr Res 71:107–114. https://doi.org/10.1038/pr.2011.14

45. Winters AH, LeVan TD, Vogel SN, Chesko KL, Pollin TI, Viscardi RM (2013) Single nucleotide polymorphism in toll-like receptor 6 is associated with a decreased risk for ureaplasm respiratory tract colonization and bronchopulmonary dysplasia in preterm infants. Pediatr Infect Dis J 32:898–904. https://doi.org/10.1097/INF.0b013e3182f15f93

46. Yang H, Su S, Peng Y, Wang C, Lee H, Salter D, Lee C (2014) An intron polymorphism of the fibronectin gene is associated with end-stage knee osteoarthritis in a Han Chinese population: two independent case: control studies. BMC Musculoskelet Disord 15:1–173

47. Murat M, Akeper A, Yuan L, Alim T, Du G, Abdusamat A et al (2015) Correlation between the development of calcium oxalate stones and polymorphisms in the fibronectin gene in the Uighur population of the Xinjiang region of China. Genet Mol Res 14(4):13728–13734

48. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3—new capabilities and inter-}

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