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Original Article

Correlations of *SP-D* genetic polymorphisms and protein expression with the pathogenesis and severity of respiratory distress syndrome in preterm infants

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Abstract: Objective: This study is designed to explore the correlations of *SP-D* genetic polymorphisms and protein expression with the pathogenesis and severity of respiratory distress syndrome (RDS) in preterm infants. Methods: From January 2013 to January 2015, a total of 170 RDS preterm infants were chosen as the case group, and 204 healthy infants were selected as the control group. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was used to detect the frequency distributions of rs721917 C>T and rs2243639 A>G polymorphisms in the *SP-D* gene. Enzyme-linked immune sorbent assay (ELASA) was used to measure the serum expressions of SP-D. Results: The frequency distributions of CC, CT and TT genotypes of rs721917 were significantly different in the case and control groups. Compared with the control group, the risk of RDS for the patients carrying the CT genotype was 2.071 times higher than that for the patients carrying the CC genotype. However, there were no statistical differences in genotype frequencies of rs2243639 between the case and control groups. Genotypes and frequency distributions of rs721917 in *SP-D* were different in the grading of RDS in the case group. Compared with the control group, the expression of *SP-D* was higher in the case group. As for rs721917 the expression of *SP-D* with TT genotypes was higher than that with CC and CT genotypes. The logistic regression analysis indicated that the TT genotype in rs721917, pregnancy-induced hypertension, abnormal umbilical cord and placental abnormalities were the risk factors of death in preterm infants with RDS (all $P<0.05$). Conclusions: These findings reveal that *SP-D* genetic polymorphisms and protein expression may be associated with the pathogenesis and severity of RDS in preterm infants.

Keywords: Respiratory distress syndrome, *SP-D,* polymorphism, preterm infants, pathogenesis, severity

Introduction

Neonatal respiratory distress syndrome (NRDS) is a condition of pulmonary insufficiency that initiates at or shortly after birth and increases in severity over the first 2 days of life in its natural course [1]. Evidence demonstrated that NRDS was the most common cause of neonatal mortality in the first year after birth in the United States [2]. It was estimated that 32% of neonatal respiratory failure was ascribed to NRDS in China, ranking first among relevant causes [3]. Clinically, RDS has early respiratory distress comprising cyanosis, retractions, grunting and tachypnea [1]. RDS occurs in nearly a half of preterm infants born at less than 30 weeks of gestation [4]. Preterm birth is defined as birth at less than 37 week of gestation and considered the most prominent cause of neonatal morbidity and mortality in developed countries [5]. EuroNeoStat data reported an incidence of 52% at 30~31 weeks' gestation, 74% at 28~29 weeks', 88% at 26~27 weeks', and 91% at 23-25 weeks' [6]. Also, it has been demonstrated that NRDS was associated with a deficiency in pulmonary surfactant (PS) [7, 8].

PS, a complex of lipids and proteins lining the alveolar surface, includes pulmonary surfactant protein A (SP-A), SP-B, SP-C, and SP-D and is responsible for lowering surface tension at the air-liquid interface thereby preventing alveo-

lar collapse at the end of expiration [9, 10]. SP-D, an important member of the collectin family in regulating innate immunity of the lung, is composed of monomers (43 kDa) and synthesized in type II pneumocytes and Clara cells [11, 12]. Human SP-D protein is encoded by the *SFTPD* gene and located on chromosome 10q22.2-23.1, which is 43 kDa in length and contains eight exons and seven introns [13]. It has been reported that SP-D plays an important role in mitigating pulmonary inflammation and infection and is also involved in the regulation of pulmonary surface proteins [14]. In addition, *SP-D* gene is related to many pulmonary diseases, such as chronic obstructive pulmonary disease and bronchopulmonary dysplasia [15, 16]. In view of the role of *SP-D* in maintaining the function of lung tissue, it can be a candidate gene for the study of neonatal lung disease. In this study, we explored the correlations of *SP-D* genetic polymorphisms and protein expression with the pathogenesis and severity of RDS in preterm infants.

Materials and methods

Study subjects

From January 2013 to January 2015, a total of 170 hospitalized RDS preterm infants were chosen as the case group. Among them, 110 were male, 60 were female, the mean gestational age was (32.04 + 2.36) W and the mean weight was (1.83 + 0.48) k. The diagnosis was in accordance with European consensus guidelines on the management of neonatal respiratory distress syndrome (NRDS) in preterm infants [1], specifically, (1) the cases began progressive dyspnea within 12 h after birth; (2) PaO_2<50 mmHg in room air, central cyanosis in room air, a requirement for supplemental oxygen to maintain PaO_2>50 mmHg; (3) chest X-ray indicated the specific performances like ground-glass opacity, air bronchogram and "white lung". The RDS related risk factors like maternal age, history of pregnancy (pregnancy induced hypertension or diabetes, etc.); delivery mode; premature rupture of membranes; time of birth; placental abnormalities (placental abruption, placental previa) and abnormal umbilical cord (abnormality of umbilical cord or around neck). Another 204 preterm infants born during the same period with similar gestational age and birth weight and with no obvious symptoms of infection were recruited as the control group. Inclusion criteria for the control group: (1) chest X-ray indicated no signs of NRDS or pulmonary infection; blood routine and C-reaction protein (CRP) indicated no clear signs of infection. Exclusion criteria for both the case and control group: (1) the patients with severe congenital disease, such as complex congenital heart disease, diaphragmatic hernia, cerebral dysplasia; (2) the patients with genetic metabolic diseases, such as phenylketonuria, congenital hypothyroidism and diabetes; (3) the patients with a clear infection in the late pregnancy and the IgM of the neonatal cases increased. The research was in accordance with the ethical standards and was approved by the Ethics Committee of The First Hospital of Jilin University; all of the parents or guardians of the study objects were informed consent.

Classification of frontal chest X-ray

According to the normal chest X-ray classification, the case group fell into four grades. (1) RDS grade I (n = 54): the cardiac silhouette was normal; the transparency of the lung decreased or there was diffuse granular or reticular shadow in the field of bilateral pulmonary. (2) RDS grade II (n = 58): the transparency of the lung decreased; diffuse granular shadow appeared; patchy shadow in high density was seen in partial lung field; texture of double lung was fuzzy and undistinguishable; heart border and part of the surface of diaphragm were fuzzy; air bronchogram was obvious. (3) RDS grade III (n = 32): the transparency of the lung decreased significantly; large particle shadow overlapped appeared in the lung; air bronchogram was widely observed; lung texture completely disappeared; heart and diaphragmatic surface were not clear. (4) RDS grade IV (n = 26): uniform and dense shadow was observed in the lung field; characteristics of air bronchogram were not clear or partially clear; heart and diaphragmatic surface were blurred, with all white images.

DNA preparation and genotyping

Venous blood samples extracted from all the study objects were anti-coagulated in ethylene diaminetetraacetic acid (EDTA) and then reserved in -80°C refrigerator. The frozen whole blood samples were taken out and placed on the ice and after melt mixed slightly, followed

Table 1. Primer sequence of rs721917 C>T and rs2243639 A>G polymorphisms in the *SP-D* gene

SNP	Primer sequence (5'-3')
rs721917 (C>T)	Forward: TCACCTCTCAGGCCATGCTGCTCTTCCTCC
	Reverse: GAGCTACACATGACCAGGGTGCAAGCACTGCGC
	Sequencing: ATGCTGCTCTTCCTCCGA
rs2243639 (A>G)	Forward: GTTCCTGTGTGTTCCTTCTTCAGGAGAAGTAGG
	Reverse: CCAGCTCTTTCCACTGCTCACCTGCTCACCCTG
	Sequencing: TCCTGTGTGTTCCTTCTTCA

Note: SNP, single nucleotide polymorphism.

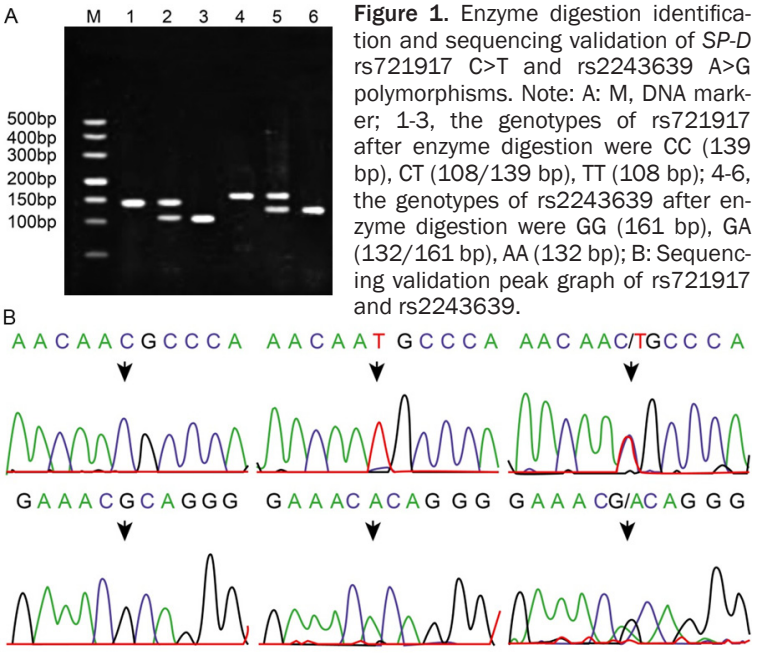

Figure 1. Enzyme digestion identification and sequencing validation of *SP-D* rs721917 C>T and rs2243639 A>G polymorphisms. Note: A: M, DNA marker; 1-3, the genotypes of rs721917 after enzyme digestion were CC (139 bp), CT (108/139 bp), TT (108 bp); 4-6, the genotypes of rs2243639 after enzyme digestion were GG (161 bp), GA (132/161 bp), AA (132 bp); B: Sequencing validation peak graph of rs721917 and rs2243639.

by centrifugation. An evenly mixed blood sample (200 μl) in a 1.5 ml centrifuge tube was placed on ice and labelled for further use, and the remaining were reserved at -80°C. Genomic DNA extraction was performed using the TIANamp Blood DNA Extraction Kit (Tiangen Biotech Co., Ltd., Beijing, China; centrifugal column type: DP-318). The information on *SP-D* gene SNPs was searched at NCBI (http://www.ncbi.nlm.nih.gov./snp/). We selected the SNPs rs721917 (Met11Thr) and rs2243639 (Ala-160Thr) for our study. Using Primer Premier 5.0 software, we designed and verified the polymerase chain reactor (PCR) primers for each SNP, which were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China); the primer sequences were shown in **Table 1**. The 9700 PCR instrument (Applied Biosystems, Inc., CA, USA) was applied for PCR amplification. The PCR reaction system (20 μl) included 2×PCR PLUS MIX (10 μl; DBI), DNA template (1.2 μl), forward and reverse primer (each 0.5 μl) and ultrapure water to fill the residual volume. PCR reaction conditions: predegeneration for 5 min at 95°C, degeneration for 30 s at 94°C, anneal for 1 min at 60°C, extension for 1 min at 72°C, a total of 45 cycles, and at the end, cycle for 10 min at 72°C. The PCR amplified product (6 μl) was digested respectively with FspI (Toyobo) and Dra III (NEB) enzymes at 37°C overnight; the reaction system (15 μl) contained Fsp Ienzyme (4 U) and Dra III (5 U). The enzyme-digested product (5 μl) and 6× sample buffer (3 μl) was separated by electrophoresis with agarose gel (3% concentration), and then observed and photographed under ultraviolet (UV) light. The genotype was determined according to the location of the electrophoresis strips and with reference to the molecular weight of the DNA standard (Takara Holdings Inc., Japan; DL500 DNA marker). The PCR amplified product mentioned above was sent to BGI for sequencing verification.

ELISA

The peripheral blood was centrifuged for 3 min at 5000 r/min using an 80-2 type low-speed centrifuge (Shanghai Anting Scientific Instrument Factory); the supernatant was reserved in a -80°C refrigerator. The protein level of *SP-D* was detected using enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Bioleaf Biotech Co., Ltd., Shanghai, China); the operation was performed completely according to the kit requirements.

Prognostic evaluation

According to the prognosis, the preterm infants were divided into two types. (1) The cases got

Table 2. The frequency distributions of rs721917 and rs2243639 of *SP-D* gene in the case and control groups

	The case group (n = 170)	The control group (n = 204)	OR (95% CI)[a]	*P*[a]	Adjusted OR (95% CI)[b]	*P*[b]
rs721917 (C>T)						
CC	52 (30.6)	98 (48.0)		ref		ref
CT	70 (41.2)	78 (38.2)	1.691 (1.061-2.696)	0.026	1.750 (1.065-2.877)	0.027
TT	48 (28.2)	28 (13.8)	3.231 (1.818-5.741)	<0.001	4.499 (2.251-8.990)	<0.001
C	174 (51.2)	274 (67.2)		ref		ref
T	166 (48.8)	134 (32.8)	1.951 (1.450-2.624)	<0.001	2.071 (1.490-2.878)	<0.001
rs2243639 (A>G)						
AA	108 (63.5)	122 (59.4)		ref		ref
AG	46 (27.1)	72 (35.2)	0.722 (0.459-1.134)	0.156	0.697 (0.409-1.190)	0.186
GG	16 (9.4)	10 (5.5)	1.807 (0.787-4.152)	0.158	1.854 (0.758-4.537)	0.176
A	262 (77.1)	316 (77.5)		ref		ref
G	78 (22.9)	92 (22.5)	1.023 (0.725 t-1.441)	0.899	1.062 (0.728-1.549)	0.755

Note: [a]the uncorrected OR (95% CI) and *P* value; [b]the corrected OR (95% CI) and *P* value after Logistic regression model was applied to correct the infant sex, gestational age, body weight, and other confounding factors.

improved or cured. Specifically, after standard treatment, symptoms and signs of respiratory distress disappeared gradually; characteristic changes of RDS observed from chest X-ray disappeared; the cases could be independent on oxygen therapy and ventilator-supporting treatment (not including the cases dependent on oxygen caused by complications); or vital signs got relatively stable and the disease was controlled. (2) The cases got worsened and gave up treatment or died. The cases got worsened and gave up treatment: the treatment result is unsatisfactory. Within 72 hours after birth, the vital signs were unstable; respiratory difficulties persisted; chest X-ray showed characteristic changes of RDS; out of economic consideration or prognosis, the family refused to continue treatment. The cases died. The treatment showed no effectiveness; the disease got progressively severe; vital signs disappeared and clinical death arrived.

Statistical analysis

The data was analyzed with SPSS 21.0 (SPSS Inc., Chicago, IL, USA) statistical software. Enumeration data were expressed in percentage or rate; the differences in genotype frequencies were validated using *chi* square test. Logistic regression analysis was used to correct baseline differences and calculate the risks of RDS in various genotypes which were expressed with odds ratio (OR) and 95% confidence interval (CI). Normal measurement data were expressed as mean ± standard deviation; comparison between two groups was verified with t test. Single factor variance analysis (one-way ANOVA) was used in comparison among three groups. Non normal distribution variables of non-normal distribution measurement data were expressed with quantile. If the data were normally distributed by logarithm transformation, the means of the transformed variables in each group were compared by independent sample t test or variance analysis. Shesis analysis software was applied to analyze the frequency of the haplotype of *SP-D*; Logistic regression model was used to analyze the effect of gene polymorphism and other factors on the prognosis of RDS patients. All the tests were two-sided tests. A $P<0.05$ was considered statistically significant.

Results

Identification of genotyping of rs721917 C>T and rs2243639 A>G polymorphisms in the SP-D gene

The rs721917 of *SP-D* gene was 139 bp in size after PCR amplification, and generated 3 genotypes after Fsp-1 enzyme digestion, namely, CC (139 bp), TC (108 bp, 139 bp) and TT (108 bp). The rs2243639 of *SP-D* gene was 161 bp in size after PCR amplification, and generated 3 genotypes after Dra III enzyme digestion, na-

Table 3. The frequency distribution of rs721917 and rs2243639 of *SP-D* and preterm infants with different RDS grades

SNP	RDS grade I	RDS grade II	RDS grade III	RDS grade IV	*P*
rs721917 (C>T)					
CC	20 (37.0)	17 (29.3)	9 (28.1)	6 (23.1)	0.002
CT	25 (46.3)	30 (51.7)	10 (31.3)	5 (19.2)	
TT	9 (16.7)	11 (19.0)	13 (40.6)	15 (57.7)	
C	65 (60.2)	64 (55.2)	28 (43.8)	17 (32.7)	0.005
T	43 (39.8)	52 (44.8)	36 (56.2)	35 (67.3)	
rs2243639 (A>G)					
AA	32 (59.3)	40 (69.0)	20 (62.5)	16 (61.5)	0.812
AG	18 (33.3)	12 (20.7)	8 (25.0)	8 (30.8)	
GG	4 (7.4)	6 (10.3)	4 (12.5)	2 (7.7)	0.904
A	82 (75.9)	92 (79.3)	48 (75.0)	40 (76.9)	
G	26 (20.1)	24 (20.7)	16 (25.0)	12 (23.1)	

Note: RDS, respiratory distress syndrome; SNP, single nucleotide polymorphism.

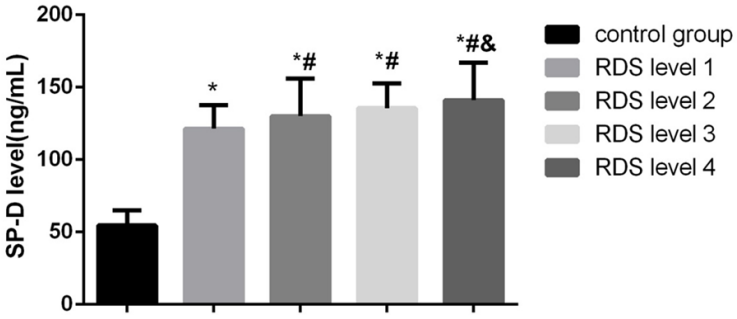

Figure 2. Comparison of the protein expression of SP-D between the control and case groups. Note: *, compared with the control group, *P*<0.05; #, compared with preterm infants with RDS level 1, *P*<0.05; &, compared with preterm infants with RDS level 2, *P*<0.05; RDS: respiratory distress syndrome.

mely, GG (161 bp), GA (132 bp, 161 bp) and AA (132 bp) (**Figure 1A**). The DNA sequencing results of rs721917 and rs2243639 of *SP-D* gene were in full agreement (**Figure 1B**).

The frequency distributions of genotypes and alleles of rs721917 C>T and rs2243639 A>G polymorphisms in the SP-D gene between the case and control groups

The genotypic distributions of *SP-D* gene were in accordance with the Hardy-Weinberg equilibrium (*P*>0.05), which can be considered representative of the equilibrium population.

Frequency distributions of the three genotypes CC, CT and TT of *SP-D* rs721917 were significantly different in the case and control group (*P*<0.05), the same as the frequency distributions of C and T alleles (both *P*<0.05). The risk of RDS for the patients carrying the CT genotype was 1.750 times higher than that for the patients carrying the CC genotype (OR = 1.750; 95% CI = 1.065-2.877); the risk of RDS for the patients carrying the TT genotype was 4.499 times higher than that for the patients carrying the CC genotype (OR = 4.499; 95% CI = 2.251-8.990); the risk of RDS for the patients carrying the T allele was 2.071 times higher than that for the patients carrying the C allele (OR = 2.071; 95% CI = 1.490-2.878). Frequency distributions of the genotypes and alleles of *SP-D* rs2243639 showed no significant differences in the case and control group (all *P*>0.05) (**Table 2**). Frequency distributions of the genotypes and alleles of *SP-D* rs721917 were significantly different in the grading of RDS (*P* = 0.002; *P* = 0.005); while the frequency distributions of the genotypes and alleles of *SP-D* rs2243639 showed no significant differences in the grading of RDS (all *P*>0.05) (**Table 3**).

Comparisons of the protein expression of SP-D among preterm infants with RDS with different genotypes and alleles of rs721917 C>T and rs2243639 A>G polymorphisms

The expressions of serum SP-D in the case group and the control group were 130.04 ± 22.58 (ng/ml) and 54.62 ± 10.25 (ng/ml); the difference was statistically significant (*P*< 0.001). The expression of SP-D of the patients in the case group was significantly higher than that of the patients in the control group (*P*<0.05); the expression of SP-D of the patients with RDS level 2/3/4 was significantly higher than that of the patients with RDS level 1 (*P*<0.05); the expression of SP-D of the patients with RDS level 4 was higher than that of the patients with RDS level 2 (*P*<0.05) (**Figure 2**).

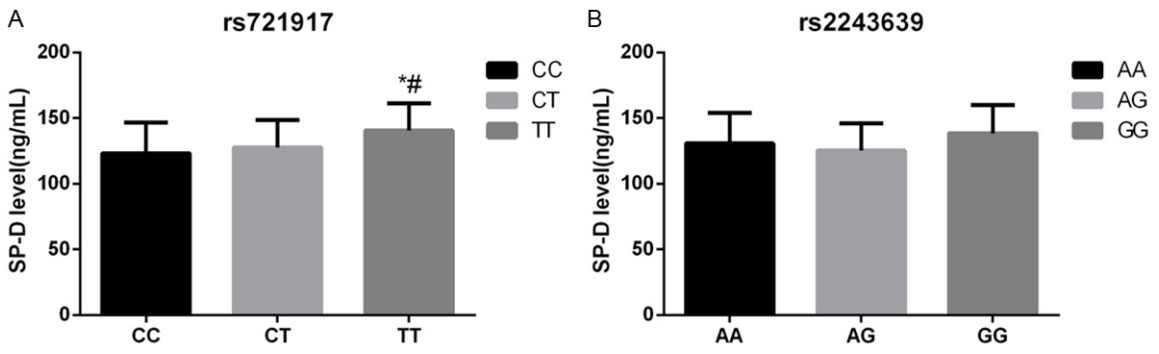

Figure 3. Comparison of the protein expression of SP-D in RDS preterm infants with various genotypes and alleles of rs721917 C>T and rs2243639 A>G polymorphisms. Note: A: Comparison of the protein expression of SP-Din RDS preterm infants with different genotypes of rs721917 C>T polymorphism; *, compared with the CC genotype, *P*<0.05; #, compared with the CT genotype; B: Comparison of the protein expression of SP-D in RDS preterm infants with various genotypes in rs2243639 A>G polymorphism; RDS: respiratory distress syndrome.

Table 4. The haplotype analysis of rs721917 C>T and rs2243639 A>G polymorphisms in the *SP-D* gene between the case and control groups

Haplotypes	Case (freq)	Control (freq)	χ^2	*P*	OR (95% CI)
CA	87 (0.512)	134 (0.655)	18.2	<0.001	0.525 (0.390~0.707)
TA	44 (0.259)	24 (0.119)	23.154	<0.001	2.530 (1.720~3.720)
TG	39 (0.229)	43 (0.209)	0.303	0.582	1.103 (0.779~1.561)

Note: freq, frequency; OR, odds ratio; CI, confidence interval.

The expressions of SP-D of the genotypes CC, CT and TT of rs721917 in the case group were 123.25 ± 23.42 ng/ml, 127.81 ± 20.76 ng/ml and 140.64 ± 20.79 ng/ml, respectively; the difference was statistically significant (F = 8.710, *P*<0.001). The expression of SP-D of the genotype TT was significantly higher than that of CC and CT genotypes (*P*<0.05). The expressions of SP-D of the genotypes CC, CT and TT of rs2243639 in the case group were 130.73 ± 23.33 ng/ml, 125.44 ± 20.46 ng/ml and 138.58 ± 21.41 ng/ml, respectively; the difference was not statistically significant (F = 2.200, *P* = 0.113) (**Figure 3**).

Haplotype analysis of rs721917 C>T and rs2243639 A>G polymorphisms in the SP-D gene

Shesis analysis software was applied for linkage disequilibrium (LD) analysis of the SNPs of *SP-D* and the haplotype analysis. The haplotypes with the frequency less than 3% were excluded. The results showed that the frequency of the haplotype CA was significantly different in the case and control group (*P*<0.001) and CA was possibly a protective haplotype for RDS (OR = 0.525, 95% CI = 0.390~0.707); that the frequency of the haplotype TA was significantly different in the case and control group (*P*<0.001) and the haplotype TA was possibly a risk factor for RDS (OR = 2.530, 95% CI = 1.720~3.720); and that the frequency of the haplotype TG was not significantly different in the case and control group (*P* = 0.582) (**Table 4**).

Logistic regression analysis for the risk factors of the prognosis of preterm infants with RDS

Of all the 170 preterm infants with RDS treated with standard treatment, 144 cases were significantly improved and cured, and 26 died. Whether patients died was set as the dependent variable, infant sex, gestational age, body weight, maternal age, gestational hypertension, gestational diabetes, premature rupture of membranes, time of birth, placental abnormalities and umbilical cord abnormality, the expression of SPD, rs721917 and rs2243639 were all set as independent variables into Logistic regression model. The results suggested that the TT genotype in rs721917, pregnancy-induced hypertension, abnormal umbilical cord and placental abnormalities were the risk factors of death in preterm infants with RDS (all *P*<0.05). However, the infant sex, gestational age, body weight, maternal age, gestational diabetes, premature rupture of membranes,

Table 5. Logistic regression analysis for the risk factors of the prognosis of preterm infants with respiratory distress syndrome

Factor	B	S.E,	Wald	df	Sig.	Exp(B)	Exp(B) 95% CI	
							Lower limit	Upper limit
Infant sex (male vs female)	0.458	0.475	0.931	1	0.335	1.581	0.623	4.008
Gestational age	-0.073	0.091	0.649	1	0.420	0.929	0.778	1.11
Body weight	0.179	0.448	0.159	1	0.690	1.196	0.497	2.879
rs721917 (CT vs CC)	-0.528	0.635	0.691	1	0.406	0.590	0.170	2.049
rs721917 (TT vs CC)	1.248	0.534	5.461	1	0.019	3.485	1.223	9.929
rs2243639 (AG vs AA)	0.197	0.803	0.06	1	0.806	1.217	0.252	5.874
rs2243639 (GG vs AA)	0.388	0.85	0.208	1	0.648	1.474	0.278	7.799
maternal age (≥30 vs <30)	0.047	0.438	0.012	1	0.914	1.048	0.445	2.472
Gestational hypertension (yes vs no)	2.703	0.815	10.986	1	0.001	14.919	3.018	73.757
Gestational diabetes (yes vs no)	0.896	0	3.275	1	0.070	2.450	0.928	6.465
Premature rupture of membranes (yes vs no)	0.182	0.536	0.116	1	0.734	1.200	0.419	3.434
Time of birth (≥2 vs <2)	0.573	0.935	0.376	1	0.540	1.773	0.284	11.074
Umbilical cord abnormality (yes vs no)	4.759	0.688	47.806	1	<0.001	5.679	2.002	10.733
Placental abnormalities (yes vs no)	3.237	0.575	31.671	1	<0.001	25.462	8.247	78.626
The expression of SPD	0.014	0.018	0.662	1	0.416	1.014	0.980	1.050

time of birth, the expression of SPD and rs2243639 were not correlated with the death risk of preterm infants in RDS (**Table 5**).

Discussion

NRDS, a disease caused by deficiency of PS, is subject to concurrent infection, chronic lung disease, pulmonary hemorrhage, intracranial hemorrhage, premature retinopathy etc., greatly influencing the survival and treatment of the neonates [17]. The present study is aimed at exploring the correlation of *SP-D* gene polymorphism and protein expression with the pathogenesis and severity of RDS in preterm infants.

The research results indicated that the expression of SP-D was higher in the preterm infants with RDS than the healthy infants. Studies demonstrated that high levels of plasma SP-Das a marker of pulmonary injury were associated with increased mortality [18, 19]. It was assumed that systemic circulating *SP-D* levels resulted from alveolar leakage into the bloodstream [20, 21]. In the lungs, *SP-D* has anti-inflammatory- and anti-oxidant capacities and protects against respiratory infections [19]. Increased *SP-D* levels were also detected in serum in acute or chronic lung injury and the finding that a quick up-regulation of SP-D in serum responding to acute airway inflammation might substantiate the notion that *SP-D* translocates from the airways into the vascular system, in favor of being synthesized systemically [22, 23].

From the present study, we also found that *SP-D* rs721917 polymorphism was correlated with the expression of *SP-D*, that the patients carrying the genotypes CT and TT had higher risk of incidence of RDS than those carrying the CC genotype, the same with the cases carrying the allele T to the ones with the C allele. According to the prognosis, the cases with the genotype TT had lower likelihood of recovery than the ones with the genotypes CC and CT. The rs721917 major allele (methionine 11) was associated with the risk of severe respiratory syncytial virus bronchiolitis in infants [18]. Individuals homozygous for the rs721917 minor allele (threonine 11) were reported to have lower levels of serum SP-D and display a predominance of the trimeric form of *SP-D* and the low level of higher oligomers affected the immunological ability of *SP-D* to bind microbes [24]. It was also demonstrated that *SP-D* could inhibit the growth of mycobacterium tuberculosis and can accelerate the decomposition of the bacteria [25]. Experiment done by Hartshor et al. reported that *SP-D* was put together with

albicans Saccharomyces, proved that *SP-D* may modulate host defense against bacteria and mycotic infection [26]. By binding to glycoconjugates and lipid moietis, *SP-D* can facilitate pulmonary clearance of bacterial and viral pathogens through multiple mechanisms (aggregation, opsonization, phagozytosis or lysis of pathogens) confirmed by several studies [27].

Furthermore, the *SP-D* expression analysis demonstrated that the higher the grade of RDS was, the higher level of *SP-D* was detected. In different grades of NRDS, alveolar type II cells were an indicator (AEC II) and damage, apoptosis and inflammatory mediators of AEC II, alveolar type II cells can decrease or increase the PS, resulting in severe loss of phospholipids and related proteins in the alveolar surface, which is one of the important mechanisms of NRDS [28]. *SP-D* is suggested to regulate surfactant uptake and catabolism by alveolar type II cells and influence the ultrastructure of surfactant in the alveolus [29]. *SP-D* is secreted by AEC II, which can identify and remove foreign bodies and apoptotic cells in the lung and when AEC II is severely damaged or disrupted, *SP-D* will further release into the alveolar cavity, resulting in a marked increase in *SP-D* levels in BAL fluid [22]. Todd et al. reported that the damage and proliferation of AEC II, the increase of the alveolar epithelium and capillary permeability may also lead to a pronounced increase in the level of *SP-D* in infants with NRDS [30]. As was shown in the grading criteria, RDS grade IV was defined as diffuse granularity or haziness of the lung, air bronchogram, obscured cardiac and diaphragm borders plus white lung [31]. When these signs were detected, it indicated increased pulmonary foreign bodies or decreased *SP-D* within the lung [32]. Previous studies also found that the deficiency or insufficiency of *SP-D* will lead to the susceptibility of body to pathogen infection, resulting in a more serious inflammatory reaction [32, 33].

In summary, we have demonstrated *SP-D* rs721917 polymorphism may be associated with the pathogenesis and severity of RDS in preterm infants. Therefore, *SP-D* gene might be used as a target for the diagnosis of RDS in preterm infants. However, further studies with a large sample size are required in order to validate our research results.

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Disclosure of conflict of interest

None.

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