



Effect of Surfactant Protein B and D Genes Polymorphisms on Frequency and Severity of Acute Bronchiolitis

Surfaktan Protein B ve D Gen Polimorfizmlerinin Akut Bronşiolitin Sıklık ve Şiddeti Üzerine Etkileri

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Abstract

Aim: Acute bronchiolitis (AB) is the most common cause of hospitalization in infants. Varying bronchiolitis presentations in patients with similar demographic characteristics suggest genetic causes of individual differences.

Material and Method: The study included 106 infants diagnosed with AB and 107 healthy infants recruited from a pediatric outpatient clinic. Genotyping was conducted for intron 4 and C/A-18 in the SP-B gene, along with SP-D/160 (Ala160Thr) and SP-D/270 (Ser270Thr) polymorphisms in the SP-D gene.

Results: The SP-B intron 4 invariant polymorphisms of ins/ins and ins/del were 93.40% and 4.72% in the AB group and 84.11% and 15.89% in the control group, respectively ($p=0.012$). Frequency of polymorphism in the SP-D/270 (Ser270Thr) gene for Ser/Ser and Ser/Thr was 60% and 39.05% in the AB group and 79.44% and 18.69% in the control group, respectively ($p=0.004$). The frequencies of Ser and Thr alleles were 79.90% and 20.10% in the AB group and 88.79% and 11.21% in the control group, respectively ($p=0.012$). There was no observed correlation between the SP-B intron 4 and C/A-1, as well as the SP-D Ser270Thr and Ala160Thr polymorphisms, with the severity of AB ($p>0.05$).

Conclusion: SP-B gene polymorphisms were identified as risk factors for AB, while SP-D polymorphisms appeared to play a protective role. However, these polymorphisms were not linked to AB severity. Early identification of children at risk could enable close monitoring and preventive care.

Keywords: Acute bronchiolitis, surfactant protein, polymorphism, respiratory disease, infant

Öz

Amaç: Akut bronşiolit (AB), infantlarda en yaygın hastaneye yatış nedenidir. Benzer demografik özelliklere sahip hastalar arasında bronşiolitin farklı şekilde seyretmesi, bireysel farklılıklarda genetik faktörlerin rol oynayabileceğini düşündürmektedir.

Gereç ve Yöntem: Çalışmaya bir çocuk polikliniğinden alınan, AB tanısı almış 106 bebek ve sağlıklı 107 bebek dahil edilmiştir. SP-B genindeki intron 4 ve C/A-18, ayrıca SP-D genindeki SP-D/160 (Ala160Thr) ve SP-D/270 (Ser270Thr) polimorfizmleri için genotipleme yapılmıştır.

Bulgular: SP-B intron 4 sabit polimorfizmleri olan ins/ins ve ins/del oranları, AB grubunda sırasıyla %93,40 ve %4,72, kontrol grubunda ise %84,11 ve %15,89 olarak bulunmuştur ($p=0,012$). SP-D/270 (Ser270Thr) genindeki Ser/Ser ve Ser/Thr polimorfizmleri AB grubunda %60 ve %39,05, kontrol grubunda ise %79,44 ve %18,69 oranında tespit edilmiştir ($p=0,004$). Ser ve Thr allel frekansları AB grubunda sırasıyla %79,90 ve %20,10, kontrol grubunda ise %88,79 ve %11,21 olarak belirlenmiştir ($p=0,012$). SP-B intron 4, C/A-18 ile SP-D Ser270Thr ve Ala160Thr polimorfizmleri ile AB'nin şiddeti arasında herhangi bir ilişki bulunmamıştır ($p>0,05$).

Sonuç: SP-B gen polimorfizmleri, AB için bir risk faktörü olarak belirlenirken, SP-D polimorfizmleri koruyucu bir rol oynamaktadır. Bununla birlikte, bu polimorfizmler AB'nin şiddeti ile ilişkili bulunmamıştır. Risk altındaki çocukların önceden tespit edilmesi ve yakından izlenmesi AB morbiditesi açısından önemli olabilir.

Anahtar Kelimeler: Akut bronşiolit, surfaktan protein, polimorfizm, solunum yolları



INTRODUCTION

Acute bronchiolitis (AB) is characterized by bronchial obstruction with inflammation, edema, mucus, and cellular debris. AB is predominantly a viral disease. Respiratory syncytial virus (RSV) is responsible for >50% of cases.^[1] The severity of AB is associated with the degree of immune response.^[2] Research on genetic susceptibility in patients with lower respiratory tract (LRT) RSV infections has highlighted heterozygous surfactant protein (SP)-B-knockout mice exhibit decreased lung compliance and increased susceptibility to pulmonary infections and oxidative stress.^[3-5]

Pulmonary surfactant is a mixture of phospholipids and proteins synthesized, packaged, and secreted by alveolar type II cells, which lower surface tension and prevent atelectasis at end-expiration.^[6,7] Surfactants contain 80% lipids, 12% protein, and 8% neutral fats. They also contain serum proteins and SPs.^[8] SP-B, located on human chromosome 2, is approximately a 9.5-kb gene that encodes a 2-kb mRNA transcript. This transcript is translated into a 381-amino-acid proprotein, which is glycosylated and undergoes a series of proteolytic cleavage to produce the 79-amino-acid hydrophobic mature SP-B protein.^[9,10] SP-B enables the adsorption of phospholipids to the alveolar surface, provides surfactant stability, and is essential for tubular myelin formation.^[11] SP-B enables the surfactant to spread on the alveolar surface.^[6] Although not an acute respiratory distress syndrome (RDS), patients with AB have decreased amounts and functions of surfactant. Surfactants play a role in the opsonization of RSV.^[12,13] Mutations and polymorphisms of exon 4 of SP-B are common and cause significant functional disorders.^[6,14]

SP-D is a multimeric collectin that is a part of innate immunity and is expressed in pulmonary and extrapulmonary epithelia. SP-D exerts some antimicrobial effects and decreases inflammation through direct microbial interactions and modulation of inflammatory cell responses. SP-D increases phagocytosis of microbes and dying host cells.^[15] Recent studies have shown that SP-D exerts antimicrobial and anti-inflammatory effects on various nonpulmonary organs. An important function of SP-D is binding to bacteria, viruses, fungi, and even helminthic parasites for their phagocytosis through opsonization.^[16,17] SP-D/SP-A haplotypes, including the Met11 allelic SP-D variant, are protective against RDS development but detrimental to patients with bronchopulmonary dysplasia (BPD).^[18] The surfactant protein-D (SFTPD) gene is located at the genomic position 10q22.2-23.1. Lower serum SP-D levels were observed in individuals homozygous for the RS721917 minor allele (threonine/Thr11), which affects the predominance of the trimeric structure of SP-D and its immunological ability to bind microbes.^[19] The rs721917 major allele (methionine 11) has been associated with an increased risk of severe RSV bronchiolitis in infants.^[20]

The individual differences in susceptibility to AB prompted the investigation in this study. We aimed to determine the association of SP B (SP-B) intron 4 and SP-D SP-D/270 (Ser270Thr) and SP-D/160 (Ala160Thr) polymorphisms with AB in infants.

MATERIAL AND METHOD

This study was conducted on infants diagnosed with AB and healthy infants recruited from pediatric outpatient and emergency clinics between January 1, 2015, and January 1, 2016. Data on subjects were derived from a prior thesis study conducted by our team.^[21] Demographic, clinical, laboratory, and radiological data were collected. Infants in good health visiting the pediatric outpatient clinic for routine check-ups were enrolled in the study. A physician assessed patients for enrollment using a standardized questionnaire and clinical evaluation. In children aged <1 year, AB was diagnosed if at least one of the signs of increased respiratory effort such as wheezing, rhonchi, prolonged expiration, tachypnea, and intercostal or subcostal retractions and findings of upper respiratory tract infections such as fever, nasal discharge, and cough were present.^[1,2,22] Exclusion criteria included existing cardiopulmonary diseases, immunodeficiency, congenital anomalies, prematurity, cystic fibrosis, and BPD. We excluded these diseases from the study as they could influence the diagnosis and severity of AB. Parental consent was obtained for participation in the study, which adhered to the Declaration of Helsinki and received approval from the Gaziosmanpasa University School of Medicine ethics committee (15-KAEK-040). The clinical severity score (**Table 1**), based on respiratory rate, wheezing, retraction, and general condition (irritability, poor feeding, and lethargy), was assessed upon admission, categorizing patients into mild, moderate, and severe groups.^[2,23]

Table 1 Clinical severity scores(3)

Variables	Score			
	0	1	2	3
Respiratory rate (breaths/min)	<30	30–45	46–60	>60
Wheezing	None	Terminal respiratory or only with stethoscope	Entire expiration or audible on expiration without stethoscope	Inspiration and expiration without stethoscope
Retraction	None	Intercostal only	Tracheosternal	Severe with nasal flaring
General condition	Normal	Mild irritable	Irritable, poor feeding	Noon-feeding, alteration in consciousness

Genetic Analysis

Blood samples were collected, and DNA was extracted using a GeneAII® Exgene™ Blood SV Genomic DNA Kit. SP-B intron 4, C/A-18, and SP-D/270 (Ser270Thr), and SP-D/160 (Ala160Thr) polymorphisms were analyzed through the polymerase chain reaction (PCR)-based restriction fragment length polymorphism method. The PCR reaction was conducted in a total volume of 25 µL, following specific guidelines and using designated primers (Fermentas, Shenzhen, China). The PCR primers and product sizes are shown in **Table 2**.

Table 2. PCR primers and product sizes for the SP-B intron4, SP-B C/A-18 and SP-D/270 (Ser270Thr), and SP-D/160 (Ala160Thr) polymorphisms.

Polymorphism	Primers	Product Size
SP-B intron4	F;5'TGTGTGTGAGAGTGAGGGTGTAAAG3' R;5'CTGGTCATCGACTACTTCCA3'	604 bp (inv)
C/A-18	F;5'GTCCAGCTATAAGGGCCGTG3' R;5'GTGAGTGGAGCTGCCTA3'	168 bp
SP- DA1a160Thr	F;5'CTCGCAGGCCCTAAGGGAGAG3' R;5'CTGGACCCAGCCAGCCAG3'	107 bp
Ser270Thr	F;5'ACGGAGGCACAGCTGCTG3' R;5'GGAAAGCAGCCTCGTCT3'	115 bp

Statistical Analysis

Statistical analyses were performed using Epi Info Software and Openepi for genetic data comparison, with the χ^2 test applied for genotype distribution and Fisher's exact tests for allele distribution. A p-value of <0.05 was deemed statistically significant. Analyses, except for genetic tests, were performed using IBM SPSS Statistics for Windows version 19 (IBM Corp, Armonk, NY, USA).

RESULTS

The study included 106 infants with AB admitted to Gaziosmanpasa University School of Medicine and 107 healthy controls from outpatient clinics. The gender distribution showed 42.5% female among the AB group. The mean ages for the patient and control groups were 7.66 ± 2.80 months and 7.70 ± 3.02 months, respectively ($p=0.231$).

The respective genotype frequencies of the inversion (inv)/inv, inv/(insertion [ins]/deletion [del]), ins/ins, and del/del genotypes of SP-B intron 4 were 93.40%, 4.72%, 0.94%, and 0.94% in the AB group and 84.11%, 15.89%, 0%, and 0% in the control group ($p=0.012$) (Table 3). The inv/inv polymorphism presented an odds ratio for AB 3.74 (CI: 95%; 1.33-10.55; $p=0.029$).

A significant difference in the genotype frequencies of intron 4 polymorphisms in SP-B was found between the AB and control groups ($p=0.029$) (Table 3). However, no significant difference in SP-B C/A-18 polymorphisms was observed between the control and AB groups ($p=0.643$) (Table 3).

Table 3. Frequency of Intron 4 Polymorphism in the SP-B Gene

Polymorphism INT4 Genotypes	Patients n=106(%)	Control n=107(%)	P
Inv/inv	99 (93,40)	90 (84,11)	0.029
Inv/# (ins/del)	5 (4,72)	17 (15,89)	
Ins/ins	1 (0,94)	0 (0)	
Del/del	1 (0,94)	0 (0)	
SP-B 18 (C/A) Genotypes	n=104 (%)	n=103 (%)	0.643
CC	23 (22,12)	18 (17,48)	
CA	50 (48,08)	55 (53,40)	
AA	31 (29,80)	30 (29,12)	
Alleles			0.607
C	96 (46,15)	91 (44,17)	
A	112 (53,85)	115 (55,83)	

χ^2 (Chi-Square) test applied for Two-Way Tables in genotype distribution, INT 4: Intron 4, inv: invariant, ins: insertion, del: deletion, SP: Surfactant protein, A: Adenine, C: Cytosine, T: Thymine.

SP-B C/A-18 polymorphisms did not influence the risk for AB ($p=0.643$). The genotype frequencies of the CC, CA, and AA genotypes of SP-B C/A-18 are summarized in Table 3. The frequencies of the C and A alleles were 46.3% in the AB group and 32.4% in the control group ($p=0.607$).

The SP-D/270 (Ser270Thr) polymorphism in SP-D showed a statistically significant difference ($p<0.05$) between the two groups (Table 4). The Ser genotype presented an odds ratio for AB 0,50 (CI: %95; 0.29-0.86; $p=0.004$). However, when examining the SP-D/160 (Ala160Thr) polymorphism in SP-D, no statistically significant difference was noted ($p>0.05$) (Table 4).

Table 4. SP-D/270 (Ser270Thr) polymorphism in the SP-D gene

Polymorphism SP-D/270 (Ser270Thr) Genotypes	Patients n=105 (%)	Control n=107 (%)	P
Ser/Ser	63 (60.00)	85 (79.44)	0.004
Ser/Thr	41 (39.05)	20 (18.69)	
Thr/Thr	1 (0.95)	2 (1.87)	
Alleles			0.012
Ser	167 (79.90)	190 (88.79)	
Thr	42 (20.10)	24 (11.21)	

χ^2 (Chi-Square) test applied for Two-Way Tables in genotype distribution, SP: Surfactant protein, Ser: Serine, Thr: Threonine.

No relationship was found between clinical severity and gene polymorphism. The χ^2 and p-value could not be calculated because of the insufficient number of patients included in the study (Table 5).

Table 5. SP-D/160 (Ala160Thr) polymorphism in the SP-D gene

Polymorphism SP-D/160 (Ala160Thr) Genotypes	Patients n=105 (%)	Control n=106 (%)	P
Ala/Ala	37 (35.24)	35 (33.02)	0.933
Ala/Thr	50 (47.62)	53 (50.00)	
Thr/Thr	18 (17.14)	18 (16.98)	
Alleles			0.830
Ala	124 (59.05)	123 (58.02)	
Thr	86 (40.95)	89 (41.98)	

χ^2 (Chi-Square) test applied for Two-Way Tables in genotype distribution, SP: Surfactant protein, Ala: Alanine, Thr: Threonine.

Table 6. Distribution of gene polymorphisms according to clinical severity

	Clinical severity			X2	p	
	Mild N=58	Moderate N=32	Severe N=16			
Intron 4	del/del	-	1 (100)			
	ins/ins	1 (100)	-			
	inv/del	2 (66.67)	-	1 (33.33)	-	-
	inv/ins	2 (100)	-	-		
	inv/inv	53 (53.54)	31 (31.31)	15 (15.15)		
C/A-18	AA	18 (58.06)	9 (29.03)	4 (12.91)		
	CA	27 (54.00)	15 (30.00)	8 (16.00)	0.340	0.987
	CC	13 (56.52)	6 (26.09)	4 (17.39)		
SP-D 160	TA	30 (60.00)	14 (28.00)	6 (12.00)	1.903	0.754
	TT	19 (51.35)	11 (29.73)	7 (18.92)		
	AA	37 (58.73)	20 (31.75)	6 (9.52)		
SP-D 270	AT	20 (48.78)	11 (26.83)	10 (24.39)	1.903	0.754
	TT	-	1 (100)	-		

χ^2 (Chi-Square) test applied for genotype distribution, SP: Surfactant protein, del: deletion, ins: insertion, inv: invariant, A: Adenine, C: Cytosine, T: Thymine.

DISCUSSION

AB is the most common LRT disease in infancy, and viral agents play a significant role in its etiology. Despite the known risk factors for AB and its inflammatory nature, the absence of disease in patients with similar demographics suggests that genetic factors may contribute to its etiology.

This study established a significant link between SP-B and SP-D polymorphisms and the frequency of AB. The *inv/inv* polymorphism in SP-B intron 4 was more prevalent in the AB group, while the *Ser/Ser* polymorphism in SP-D was less common. However, the study could not confirm any association between the severity of AB and these polymorphisms.

Previous studies have highlighted associations between certain alleles and increased risks of RDS and severe RSV bronchiolitis.^[24] Moreover, the C allele of the SP-B 1580 site may act as a susceptibility factor for lung damage. Similarly, Cao et al.^[25] identified an association between the C allele of the SP-B 1580 locus and early mortality in mice with viral pneumonia. Additionally, the C allele was associated with markedly elevated concentrations of inflammatory cytokines, such as tumor necrosis factor- α , interleukin (IL)-1 β , IL-18, and IL-6, in the bronchoalveolar lavage fluid of mice affected by viral pneumonia. Another experimental study suggested that the A allele of C/A-18 is a risk factor for BPD.^[26] But, the present study did not find similar associations between these alleles and AB and its severity. Moreover, some SFTPB variants may increase the incidence or severity of diseases (e.g., RDS) in genetically vulnerable individuals.^[7] In combination with other variants and under appropriate conditions, individual variants in SFTPB may lead to sufficiently low SP-B expression and cause disease.

The C allele of rs1130866, located on chromosome 2 p11.2 in the exon region of the SP-B N region and associated with glycosylation at position 129 of the SP-B protein, is overrepresented in patients with severe influenza. Therefore, glycosylation may affect the function of this protein.^[27] In Chinese Han infants, the homozygous *del* variant genotype for intron 4 was significantly higher in the BPD group than in the control group. Therefore, the homozygous *del* variant genotype of intron 4 might be associated with BPD.^[28] The *ins/del* variants in intron 4 were more frequent in adult patients with RDS than in the control participants.^[29] Another study reported a high frequency of CT and TT genotypes at the C/T locus in patients with COVID-19, suggesting increased susceptibility to COVID-19.^[30] In this study, the *ins/del* polymorphism was more frequent in the control group than in the AB group. These results suggest that the *ins/del* polymorphism might offer protection against AB. However, the SP-B 18 (C/A) polymorphism did not influence the frequency of AB.

SP-D belongs to the C-type lectin superfamily and prevents lung collapse during expiration. SP-D plays a role in innate immunity and regulates inflammatory processes.^[31] SP-D is thought to be associated with immunomodulatory functions. It binds to pathogens such as bacteria, fungi, viruses, and

mycobacteria, facilitating their aggregation.^[32] One main effect of SP-D is the aggregation and enhancement of the phagocytosis of microbes and dying host cells.^[15]

According to the results of this study, the Thr polymorphism at SP-D/270 (Ser270Thr) can be considered a risk factor for bronchiolitis in the first year of life. Three SNPs have been identified in the coding region of SP-D, resulting in amino acid variations. The variation at codon 11 within the sequence encoding mature protein results in significantly different serum SP-D levels.^[33] The SP-D allele coding for Thr has been suggested to increase the susceptibility to tuberculosis.^[34,35] Most studies have suggested disease associations with the Thr11 allele.^[15,32,36] Unlike these studies, no association was found between disease severity and the polymorphisms and alleles examined in this study. We think the small sample size of the present study significantly contributed to the absence of a meaningful relationship.

Conversely, the SP-D/160 (Ala160Thr) polymorphism is not associated with AB in infants. Consistent with our study, a study reported that the individual polymorphisms of amino acid residue 160 had no detectable influence on the oligomeric state of SP-D and no significant differences in serum SP-D levels were found in the population with variant genotypes of SP-D/160.^[33]

Limitations and strengths of the study

Viral identification was not performed in this study. The immune responses and clinical courses of various viral infections differ significantly. So, it is difficult to suggest that the effects of these polymorphisms are valid in all AB patients. This is a limitation of this study. The roles of these polymorphisms in the pathogenesis of diseases caused by specific viruses remain unclear. Nevertheless, this prospective case-control study provides insights into whether these polymorphisms are risk factors for AB, regardless of the etiological agent.

CONCLUSION

The *inv/inv* polymorphism in SP-B intron 4 may serve as a risk factor for AB, while the *inv/del* polymorphism could provide a protective effect against the condition in infants. Additionally, the *Ser* allele in SP-D appears less frequently in infants with AB, potentially indicating a lower risk. Continued monitoring and proactive measures may facilitate early detection of infants genetically predisposed to AB, although a definitive relationship between AB severity and these polymorphisms was not established.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval was received for this study from the Gaziosmanpasa University Faculty of Medicine Ethics Committee (Decision No: 15-KAEK-040).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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