Genetic risk factors associated with respiratory distress syndrome

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Respiratory distress syndrome (RDS) among preterm infants is typically due to a quantitative deficiency of pulmonary surfactant. Aside from the degree of prematurity, diverse environmental and genetic factors can affect the development of RDS. The variance of the risk of RDS in various races/ethnicities or monozygotic/dizygotic twins has suggested genetic influences on this disorder. So far, several specific mutations in genes encoding surfactant-associated molecules have confirmed this. Specific genetic variants contributing to the regulation of pulmonary development, its structure and function, or the inflammatory response could be candidate risk factors for the development of RDS. This review summarizes the background that suggests the genetic predisposition of RDS, the identified mutations, and candidate genetic polymorphisms of pulmonary surfactant proteins associated with RDS.

Key words: Newborn respiratory distress syndrome, Genetic polymorphism, Pulmonary surfactant-associated proteins

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Introduction

Respiratory distress syndrome (RDS) is primarily due to the absence or transient deficiency of pulmonary surfactant (PS)[1]. RDS is a major acute postnatal pulmonary disease that especially affects the preterm newborns, and is caused by an insufficiency of PS metabolism. The disorder can progress to respiratory failure. Although the incidence of RDS is inversely related to gestational age, prematurity alone does not determine the risk of developing the disorder[2]. Besides surfactant deficiency, diverse risk factors such as gender, ethnicity, and maternal diseases, which have interactions with one another, influence the development and the severity of RDS[2-4].

According to empirical observation and epidemiologic studies concerning racial differences and familial tendency, genetic disposition has been suggested for the risk of RDS[5-7]. In spite of antenatal steroid administration, postnatal surfactant therapy, and optimal ventilator care, not all infants of the same gestational period respond equally to treatments. From the intractable RDS cases, a few mutations, including genes encoding surfactant proteins B (SP-B)[8], SP-C[9], and the adenosine triphosphate-binding cassette transporter A3 (ABCA3)[10], have been identified. Specific polymorphisms related to surfactant structure and function, pulmonary differentiation, and inflammatory response could be candidate genes for the development of RDS[2,11-13].

The present review summarizes the genetic aspect of RDS as follows. First, the histological and epidemiologic backgrounds that suggest the genetic predisposition for the development of RDS are introduced; these include studies on ethnic differences, and familial and twin studies. Then, the rare but revealed genetic causes of RDS are described, including the identified surfactant-associated mutations. Lastly, recent studies associating genetic

polymorphisms for SP with RDS are shown.

Historical studies that suggest a genetic predisposition to RDS

1. Ethnic/racial differences

A higher rate of neonatal death and RDS has been reported for white newborn infants compared with black newborns, in spite of the relatively lower birth weights of the black infants[5]. The study of the US national mortality statistics for RDS in 1968–1973 showed a higher mortality rate in male infants than in female infants, and a lower incidence of fatal RDS in black preterm infants[3]. On the other hand, there was a report that showed no racial differences in fetal lung maturation between the blacks and whites in the US; the influence of environmental factors, such as maternal body weight and smoking, for the development of RDS was suggested[14]. In spite of these few opposing results[14], regression analyses have shown that RDS occurs less frequently and less severely in black preterm infants, whereas white ethnicity is an independent risk factor of RDS in preterm and term infants[15-17].

To date, the underlying mechanisms related to the racial difference in the occurrence of RDS have not been definitively revealed. Olowe and Akinkugbe[18] suggested the earlier pulmonary maturation in Africans as being one of the mechanisms for the racial difference, based on the result of a higher amniotic fluid lecithin/sphingomyelin (L/S) ratio in African babies than in North American babies. Antenatal steroid treatment proved to be effective in non-Caucasians, whereas Caucasians showed little benefit from this intervention[19]. Richardson and Torday[20] stratified the data by L/S ratio to adjust for possible differences in maturational timing between races, and showed lower rates of RDS in the black infants at every level of the L/S ratio. They suggested an alternative hypothesis of qualitative differences in the surfactants between races, including differences of phospholipids and surfactant proteins, or anatomic differences in the alveolar size and structure. Genetic differences such as allelic variation may contribute to the protein structure and function[2,12,21].

2. Family studies

The increased risk of developing RDS with a history of previously affected infants has been suggested an important genetic or other familial tendency in its origin[6,7]. There should be other considerations for the results analysis of the family studies; that is, the possibility of including populations with a genetic predisposition to preterm birth, or the confounding environmental effects, as well as genetic influences[2,6]. Although most of the identified mutations of SP-B and *ABCA3* are family specific[22,23], and differences of SPs or maternal hormonal factor were suggested[6], the integrative precise mechanism to explain these results is not clear.

3. Twin studies

Genetic predisposition in the susceptibility to RDS has been suggested by twin studies. The concordance difference of RDS between monozygotic (MZ) and dizygotic (DZ) twins (viz., the higher frequency of RDS in MZ than in DZ twins) supports the genetic contribution to RDS[13,24]. On the other hand, there was a study showing no significant concordance difference between MZ and DZ twins[25]. Several reports pointed out that concordance twin studies need to consider the differences in the mean gestational age, birth weight, and gender between MZ and DZ twins, as well as birth order and intrauterine environmental factors[2,13,25].

Identified mutations of surfactant metabolism

SP-B and SP-C contribute to the surface tension-lowering activity of the PS. SP-B and SP-C are encoded by a single gene, located on chromosomes 2 and 8, respectively[26-28]. ABCA3, which is a transporter of phospholipid into lamellar bodies (LBs), the storage organelles for the surfactant complex, is critical for the proper formation of LBs and for surfactant function. Mutations in the genes encoding SP-B (*SFTPB*), SP-C (*SFTPC*), and ABCA3 (*ABCA3*) disrupt surfactant function and cause respiratory distress in the newborn[11,23,29]. Table 1 shows representative mutations of *SFTPB*, *SFTPC*, and *ABCA3*.

1. Hereditary SP-B deficiency

SP-B deficiency was first described in two siblings with severe lung disease in the newborn period[8]. Infants with hereditary SP-B deficiency, who are typically full-term, present severe respiratory failure shortly after birth, which is similar to RDS of preterm infants[30]. The most common mutation, found in 60%–70% of the cases of SP-B deficiency[31], is a 1 base-pair (C) deletion and 3 base-pair (GAA) insertion, which is a net 2 base-pair insertion at codon 121 in exon 4 of *SFTPB* (termed 121ins2)[32]. It causes a frame shift and premature termination signal of translation, which results in absence of mature SP-B production. Infants homozygous for this autosomal recessive loss-of-function mutation are refractory to surfactant replacement therapy, and the cases are mostly fatal without lung transplantation[33]. The frequencies of 121ins2 are rare: 0.4% of a cohort in Missouri, 0.1% in Norway, and 0% in Korea and South Africa, in a population-based study[34]. Besides the 121ins2 mutation, more than 30 loss-of function mutations in *SFTPB* have been identified to date[11,22,23,35-37]. Other loss-of-function mutations appear to be family specific and sometimes associated with the partial or transient deficiency of SP-B that results in a more gradually progressive and prolonged survival[38,39].

2. Mutations in *SFTPC*

The *SFTPC* gene encodes SP-C, a hydrophobic protein like SP-B, which functions to lower the surface tension of the PS[40]. The surfactant protein C precursor (proSP-C) is an essential endoplasmic reticulum (ER) membrane protein with an α-helical transmembrane domain. Various mutations in the ER-luminal C-terminal domain of proSP-C have been reported[41]. Mutations in *SFTPC* are thought to result in the production of misfolded proSP-C molecules that accumulate within the alveolar type II cells and cause cellular injury. Contrary to SP-B, mutations in *SFTPC* penetrate variably, and its expression ranges from severe RDS of the newborn to chronic interstitial lung disease of the adult or even asymptomatic conditions[9,42-44]. Presentation in full-term infants in the newborn period is associated with signs and symptoms typical of RDS, and may be fatal in the neonatal period[45]. Over half of the mutations are thought to arise spontaneously and result in sporadic cases[23]. About 35 dominantly expressed mutations in *SFTPC* have been identified, where threonine substitution for isoleucine in codon 73 (I73T) is the most common[43].

3. Mutations in *ABCA3*

Several mutations of *ABCA3* were first identified in 16 of 21 patients with severe neonatal surfactant deficiency[10]. Markedly abnormal LBs were observed by ultrastructural examination of lung tissue from four patients with different *ABCA3* mutations. The clinical manifestation and course of *ABCA3* mutations are variable: mutations in this gene can result in fatal surfactant deficiency in term newborn infants[29], which have similar clinical and radiologic findings to SP-B deficiencies, and chronic interstitial lung disease in older children[46,47]. More than 70 mutations have been reported, most of which are family specific[23]. The overall frequency of mutations and disease is unknown.

4. Transcription factors that regulate the gene expression of surfactant proteins: mostly animal experimental studies

A transcription factor is a protein that binds to specific DNA sequences to control transcription to messenger RNA. A number of transcription factors, including thyroid transcription factor-1 (TTF-1; also known as NK2 homeobox 1 [Nkx2.1] or thyroid-specific-enhancer-binding protein [T/EBP])[48,49], CCAAT enhancer binding protein-α (C/EBPα)[50], and forkhead box A2 (Foxa2)[51], have been

Table 1. Representative mutations of *SFTPB, ABCA3,* and *SFTPC*

Gene	Mutation		Predicted result		Reference
	Genetic change	Type	mRNA/protein	Clinical outcome	
SFTPB	1549C>GAA (121ins2)*				
	1549C>GAA homozygote (in exon 4)	Frameshift	Complete absence of mRNA and SP-B	Fatal respiratory failure in early newborn period	32
	1549C>GAA heterozygote				
	1549C>GAA/457delC (in exon 4/exon 2)	Frameshift	Complete absence of SP-B	Fatal respiratory failure in early newborn period	85
	1549C>GAA/4380C>T (236R>C) (in exon 4/exon 7)	Missense	Near normal content of mRNA, a low but detectable level of SP-B	Rapidly severe respiratory distress and persistent oxygen requirement (lethal)	86
	2479G>T (c.479G>T) homozygote (in exon 5)	Splice (a frameshift and a termination codon in exon 7)	Reduced amount of mature SP-B	Case 1: need lung transplantation Case 2: persistent oxygen requirement	38
	c.673-1248del2959 homozygote (in exon 7–8)	Deletion		Fatal respiratory failure in early newborn period	87
	2417G>A (G135S) heterozygote (in exon 5)	Missense	Transient absence of SP-B	Respiratory distress in early newborn period→continuous oxygen requirement	39
ABCA3	E292V (c.875A>T) heterozygote (in exon 9)	Missense (termination codon in exon 33)	Defects in phosphocholine transport	Less severe (interstitial lung disease)	46
	L1580P (4739T>C)/4552insT (in exon 31/exon 30)	Missense/frameshift	Trafficking defects	Rapidly fatal respiratory failure	10, 88
	G1221S(3661G>A)/ L982P(2945T>C) (in exon 24/exon 21)	Missense/missense	Trafficking defects	Rapidly fatal respiratory failure	10, 88
SFTPC	+128T>A heterozygote (in exon 5)		Misfolding and trapping of proSP-C	Familial pulmonary fibrosis	42
	p.I73T (218T>C) heterozygote (in exon 3)		Misfolding and trapping of proSP-C	Familial interstitial lung disease, asymptomatic parent of the patient	43
	p.L188Q		partially trafficked to lamellar bodies	Interstitial lung disease	89, 90
	p.P30L		Arrested proSP-C in the endoplasmic reticulum	Interstitial lung disease	90

SP-B, surfactant proteins B; proSP-C, surfactant protein C precursor.
*The most common mutation of *SFTPB*; 60%–70% of the cases of SP-B deficiency.

identified that influence lung formation and surfactant production as well as homeostasis in late gestation. TTF-1, C/EBPα, and Foxa2 interact reciprocally to regulate transcriptional targets of surfactant synthesis and peripheral lung maturation[50].

TTF-1, encoded by *NKX2-1,* is expressed in the thyroid gland, brain, and lung[48]. In the lung, it is an early marker of lung differentiation and an essential regulator for the expression of SP-A, SP-B, SP-C, and *ABCA3*[49]. Mutations in *NKX2-1* result in RDS and respiratory failure in newborn infants and in interstitial lung disease in older children[36,49,52].

C/EBPα, a member of a family of basic leucine zipper transcription factors, plays an important role in the synthesis and metabolism of surfactant lipids and proteins. Deletion of the *CEBPA* gene causes the inhibition of lung epithelial cell proliferation and differentiation[50].

Deletion of *FOXA2* inhibits branching morphogenesis and epithelial cell differentiation of the lung[51]. Foxa2 modulates gene expression by interacting with other transcription factors. An animal study showed that the mammalian sterile 20-like kinase 1 and 2 (Mst1/2) regulated Foxa2, and might be crucial factors in surfactant homeostasis[53].

Candidate genetic polymorphisms of SPs

1. SP-A

Among the three forms of PS[54] (viz., intracellular and extracellular LBs, extracellular tubular myelin [TM], and surface monolayer), TM was absent in the lung of newborns who had died of RDS[55]. Moreover, the expression of SP-A was very low[56]. The levels of SP-A in tracheal aspirates, cord blood, and serum of newborn infants with RDS were lower than in infants without RDS[57-60]. Owing to these significant findings and the important roles of SP-A in the pulmonary host defense mechanism and the formation of TM[61-63], the genes expressing SP-A have been thought to be strong candidates for susceptibility to RDS.

Two functional genes encoding SP-A (*SFTPA1* and *SFTPA2*) are located on chromosome 10q22-q23[64]. Various single nucleotide polymorphisms (SNPs) have been discovered in coding regions and untranslated regions of *SFTPA1* and *SFTPA2*[65-67], and many association studies with RDS have been reported[68-73]. Lee et al.[74] and Kim et al.[75] have presented the distribution and frequency of SP-A alleles in Korean newborns.

The SP-A1 $6A^2$, SP-A^2 $1A^0$ variant and SP-A1/SP-A2 $6A^2/1A^0$ haplotype have frequently been reported as being associated risk factors for the development of RDS, and the SP-A1 $6A^3$ variant as a protective factor for RDS, in Finnish and North American studies[12,68-70]. However, these association patterns were significantly different in a study of Korean preterm infants[71]. The SP-A2 $1A^0$ variant and

$1A^0/1A^0$ genotype were associated with protection from RDS, whereas SP-A1 $6A^2$ was not. The contrasting association of RDS with specific SP-A variants among different ethnic groups suggests that RDS is a multifactorial disease. In certain studies, associations between specific variants and RDS risk were confirmed under specific conditions or subgroups, such as infants born of twins or specific gestations, having the specific SP-B genotype or specific haplotypes of SP-A and SP-D[69,72,73]. Although various association studies of SP-A genetic polymorphisms and RDS have been reported, the precise mechanism of the cause-and-effect relationship between specific polymorphisms and RDS has not been revealed up to now[12]. Table 2 shows representative genetic polymorphisms of SPs associated with RDS.

2. SP-B

SP-B plays essential roles in forming LBs and maintaining the surface monolayer[76]. *SFTPB* has been thought to be an important candidate gene for RDS. Hereditary SP-B deficiency caused by mutations of *SFTPB* results in lethal neonatal respiratory disease. The reduction of SP-B concentration to <25% of normal levels caused respiratory failure in adult mice[77]. SP-B and SP-C levels of tracheal aspirate were correlated with surfactant function in premature infants[78]. The *SFTPB* gene on chromosome 2p12-p11.2 is approximately 9.7 kb long, with 11 exons and 10 introns, including a large untranslated region[26], and is polymorphic[79,80]. Hamvas et al.[80] reported 86 *SFTPB* polymorphic sites (9.8/1,000 base pairs of *SFTPB* reference sequence), including 81 SNPs and 5 small insertion/deletions. Several studies showed specific SNPs or length variation (insertion/deletion polymorphic changes) in intron 4 of *SFTPB* to be associated with RDS development[25,79,81].

3. SP-C

SP-C is expressed as a proSP-C that matures to SP-C, containing only 35 amino acids. *SFTPC* is located on the short arm of chromosome 8 (8p23.1)[27]. Mutations of *SFTPB* can disturb the proSP-C processing[28]. SP-C is thought to be a hydrophobic protein critical to lowering surface tension by enhancing the adsorption and spreading of surfactant phospholipids to the pulmonary surface monolayer; however, the exact functional roles of SP-C are currently not completely understood. Moreover, studies concerning the distribution of allelic variation in *SFTPC* and the associations with human disease are very rare. Lahti et al.[82] showed that SP-C polymorphisms were associated with RDS risk among infants born at <28 weeks of gestation.

4. SP-D

SP-D is an important component of the innate pulmonary immune system and, like SP-A, plays a role in PS homeostasis[61,62]. The *SFTPD* gene, encoding for SP-D, is assigned to chromosome 10q22-q23 near *SFTPA2*[64]. Specific polymorphisms of *SFTPD*

Table 2. Representative genetic polymorphisms of surfactant proteins associated with respiratory distress syndrome

Gene	Variants according to susceptibility		Specific condition/subgroups	Reference
	Risk	Protective		
SFTPA	$6A^2$, $6A^2/1A^0$	$6A^3$	Finnish	68
	$6A^2$, $1A^0$	$6A^3$	SFTPB 131 Thr/Thr, GP<32 wk, Finnish	69
	$6A^2$, $6A^2/1A^0$, $1A^0$		Caucasian, African-American	70
	$1A^1$	$1A^0$	Preterm infants, Korean	71
		$6A^2$, $6A^2/6A^2$, $6A^2/1A^0$	GP 32-36 twins, Finnish	72
		$1A^1$	SFTPD DA160 A	73
SFTPB	131 Thr		First-born preterm twins, Finnish	25
	Insertion/deletion of intron 4*		Caucasian, African-American	79
SFTPC	g.2772G>A (exon 5) or p.186 Asn		GP<34 wk, Finnish	82
SFTPD		rs1923537 G/G	GP<32 wk, German	84

GP, gestational period.
*The first half of intron 4 of the SFTPB gene is a CA-repeat-rich region that contains 11 motifs.
Modified from Jo HS. Neonatal Med 2013;20:311-7[91].

influence the structure, function, and serum concentration of SP-D[21,83]. Associations of protective haplotypes of SP-A/SP-D with RDS development[73] and specific genotypes of SP-D with the severity of RDS[84] have been reported.

Conclusions

Because RDS is thought to be a multifactorial and/or multigenetic disease, it is necessary to be well-informed of the genetic contributors for RDS before designing any studies to search for new factors associated with the disorder.

To date, the frequency of inherited mutations that result in defective surfactant metabolism has not been revealed in the Korean population. Although it seems to be rare, the clinical outcomes of such mutations are mostly fatal. Therefore, we need to investigate the possibility of these mutations and to try to diagnose for them using genetic sequencing in neonatal cases of RDS presenting with an unusual course of severe respiratory failure, especially those with a familial history.

Even though the direct association of genetic polymorphisms with the development of RDS has not been confirmed to date, there have been many meaningful association studies. Large-scaled association studies are needed, considering the low penetrance of common genetic variants. The results of association studies can provide basic information for high-throughput genomic and proteomic researches and help to target specific therapies in the future.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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