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Candidate Gene Analysis of the Surfactant Protein D Gene (*SFTPD*) in Pediatric Diffuse Lung Disease

W. Adam Gower, M.D.¹ and Lawrence M. Nogee, M.D.²

¹Section of Pediatric Pulmonology and Sleep Medicine, Department of Pediatrics and Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, NC USA 27157

²Eudowood Neonatal Pulmonary Division, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD USA 21287

Abstract

Mutations in surfactant-associated genes cause childhood diffuse lung disease. Mice lacking surfactant protein D (SP-D) develop lung disease with age. However, we identified no novel SP-D gene (*SFTPD*) coding or splice region variants in 73 unrelated children with diffuse lung disease from a cohort enriched for genetic surfactant dysfunction.

Suggested Keywords

SP-D; pulmonary surfactant; diffuse parenchymal lung diseases; interstitial lung diseases; human genetics

Pulmonary surfactant is the mixture of lipids and proteins synthesized and secreted by type II alveolar cells (AEC2s). Surfactant protein D (SP-D) is encoded by *SFTPD*, located at 10q22.2-23.1. SP-D has important roles in innate immunity and regulation of inflammation and is also expressed in tissues other than the lung [1]. Common *SFTPD* variants have been associated with chronic obstructive pulmonary disease and asthma, as well as susceptibility to respiratory syncytial virus infection and tuberculosis [2].

Transgenic mice engineered to lack SP-D production develop emphysema and pulmonary lipoproteinosis [3]. These changes occur in litters raised in a sterile environment, implying that they are not a response to an infectious stimulus [4]. The phenotype is not observed in the first two weeks of life, suggesting that SP-D does not play a critical role in pulmonary morphogenesis or perinatal adaptation [5-7]. SP-D also regulates fibrotic lung remodeling in response to injury in mice [8].

Mutations in genes encoding surfactant-related proteins, including ABCA3, SP-B, SP-C, and NKX 2.1 cause diffuse lung disease (DLD). The phenotypic spectrum of DLD due to

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Address correspondence to: Adam Gower, Meads Hall, Medical Center Boulevard, Winston-Salem, NC 27157, Fax: (336) 716-9229, wgower@wakehealth.edu.

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surfactant dysfunction is wide, ranging from fatal neonatal respiratory failure to mild chronic lung disease [9]. Mutations in one of two genes encoding SP-A, *SFTPA2*, cause adult-onset idiopathic pulmonary fibrosis and lung cancer [10]. Lung disease due to altered surfactant production may be caused by either deficiency of a critical component, as with SP-B, *ABCA3*, or *NKX2.1* gene variants, or toxic gain of function resulting in damage to AEC2s, as occurs with SP-A and SP-C gene mutations [11].

We hypothesized that rare damaging variants in the coding or splice regions of *SFTPD* cause DLD in children, based upon the putative roles of SP-D in the lung, the observation that genetically-altered surfactant production causes DLD in children, and the pulmonary phenotype observed in SP-D null mice. To test this hypothesis we examined the *SFTPD* sequence in a cohort of subjects with idiopathic childhood DLD enriched for genetic causes of lung disease.

METHODS

Subjects were drawn from an IRB-approved prospective study to identify inborn errors of surfactant metabolism in subjects with DLD of unknown etiology [12]. Enrollment criteria include either a histological specimen showing a pattern plausibly consistent with surfactant dysfunction or, in the absence of a biopsy, at least 2 of the following clinical findings: (1) chronic hypoxemia; (2) chronic respiratory symptoms; and (3) an abnormal chest radiograph. This clinical definition, in the absence of a known diagnosis, is suggestive of DLD in children [13]. The parents of all subjects provided written informed consent.

Of 404 subjects enrolled from January 1995 through December 2010, 130 (32%) had an identified mutation, indicating enrichment of this population for genetic mechanisms (Figure). From the remaining 274 subjects, we selected for testing 73 unrelated individuals enrolled between 5 months and 19 years of age with a phenotype consistent with surfactant dysfunction or plausibly consistent with altered SP-D function. We selected subjects with phenotypes other than severe neonatal respiratory failure based upon the later age of onset in SP-D null mice, although 6 subjects had a history of less severe neonatal respiratory disease. 24 subjects had a family history of first-degree relatives with similar lung disease, 10 with a dominant and 14 with a recessive inheritance pattern. An additional 9 subjects had a first-degree relative with a history of asthma.

We amplified the 7 coding exons and associated intron-exon boundaries for all subjects, using standard polymerase chain reaction techniques (Table I). Sequencing was performed via chain terminator methods at one of two Johns Hopkins Core Sequencing Facilities. We then compared the resulting sequence data with the known primary sequence for *SFTPD*, using Sequencher™ (Gene Codes, Ann Arbor, MI) and Mutation Surveyor® (Softgenetics, State College, PA) software. All sequence variants, including single nucleotide polymorphisms (SNPs), were recorded.

RESULTS

In our final study cohort of 73 subjects, 24 subjects (33%) analyzed had a positive family history of similar lung disease, and 18 (25%) had lung biopsies plausibly consistent with surfactant dysfunction. Clinical diagnoses suggested by the referring physician included interstitial lung disease, recurrent pneumonia, bronchiectasis, and bronchiolitis obliterans. We identified no novel sequence variants in the *SFTPD* coding regions or splice sites from any subjects. Allele frequencies of non-synonymous *SFTPD* SNPs are shown in Table II.

DISCUSSION

We screened a population of individuals with idiopathic DLD enriched for genetic surfactant dysfunction, but did not identify any *SFTPD* sequence variants that could have accounted for lung disease. We conclude that exonic and splice-site mutations in *SFTPD* are not a common cause of DLD in children. These findings do not exclude the possibility that *SFTPD* mutations cause lung disease. Even with an enriched population, our study may have been underpowered to detect mutations if they occur very rarely. Our approach would also not have identified functionally significant variants in regulatory or other untranslated regions that could alter SP-D expression. Large deletions on one allele that span a distance greater than the length of the amplicon, inversions, or other gross rearrangements would have been missed by our approach.

Alternatively, it is possible that *SFTPD* mutations cause lung disease, but that the resulting phenotype(s) differ from those in our cohort. Such mutations may be lethal *in utero* or cause rapid neonatal demise, although this seems unlikely as mouse models do not exhibit lung disease until 6 weeks of life. Alternatively, it is possible that humans with *SFTPD* mutations, like those with *SFTPA2* mutations (13), may not develop lung disease until adulthood. Such individuals would not have been represented in our cohort. Finally, it is possible that mutations in *SFTPD* do not cause a pulmonary phenotype in humans. Rare potentially damaging *SFTPD* variants have been reported in population-based genetic databases, including dbSNP and the NHLBI Exome Sequencing Project (ESP), but any associated pulmonary phenotypes are not reported.

In summary, our study provides support that mutations in *SFTPD* are not a frequent cause of DLD in children. SP-D therefore remains the only major surfactant protein not associated with genetic lung disease. Newer methodologies such as next-generation sequencing approaches that allow cost-effective evaluation of multiple genes in a large numbers of subjects, and techniques that allow exploratory evaluation of the entire genome or exome may provide new insights into genetic mechanisms underlying DLD in children.

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Figure. Selection of study cohort

Numbers next to *SFTPB* and *ABCA3* indicate individuals with disease-causing mutations on both alleles of those genes. Numbers next to *SFTPC* and *NKX2.1* indicate individuals with disease-causing mutations on one allele of those genes. *ABCA3/SFTPB* indicates individuals with a disease-causing mutation on one allele of each gene.

Table 1

PCR primers used for amplification of exons and intron-exon boundaries of *SFTPD*.

<u>Exon(s)</u>	<u>Amplicon</u>	<u>Sequence (5' → 3')</u>
1	1 sense	AAG TCC TAA ACC ATG TCC
	antisense	AAA GTA CCC AGA GTT GCT GG
2,3	2 sense	TGG AAG TCT CAT GCT CTG
	antisense	TCC TTT CCC TTC TCA GAC
4,5	3 sense	AAC CAC ACC TGC TGA ACG
	antisense	GCC TTT CCT GAG TTC CAC
6	4 sense	ATG TGG GCT TGC TGT GGG TCA G
	antisense	AGC CAA AGG CCA AAC CAA GG
7	5 sense	CTG ACT TTC TTC TTC TCC
	antisense	CCT CTG GCC AAA CTC CTG

Table 2

Non-synonymous single nucleotide polymorphisms (SNPs) within the regions sequenced.

Exon	Variant SNP reference #	Domain	Study Subjects	Population (dbSNP)	Population (NHLBI ESP)
1	Met31Thr rs721917	NT	0.51	0.47	0.41
4	Leu123Val rs17878336	CL	0.07	0.02	0.03
5	Ala180Thr rs2243639	CL	0.36	0.26	0.28
7	Ser290Thr rs3088308	CR	0.12	0.06	0.06
7	Glu309Lys rs4469829	CR	0.01	0.03	0.04

NT = N-terminal, CL = collagen-like, CR = carbohydrate recognition

Minor allele frequencies are shown for our study population and from two large SNP databases National Center for Biotechnology Information dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) and National Heart Lung and Blood Institute Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>). Both last accessed May 10, 2013. Comparisons should be approached with caution, however, as our cohort may not reflect the racial composition of the populations used to determine reference allele frequencies.