Supplement Files

Supplement table 1. Characteristics of patients with *ABCA3* variants and surviving beyond age 1 year of life from the kids lung register.

Pt_ID	Age at Last FU_Year	Mutations	Variant Type	Poly Phen-2	PROVEAN	ClinVar	Freq. of Variant	Geno type	Age at death/LTX*	Ref
Hypo/Hypo										
P1	1.2	p.V1399M (c.4195G>A)	Missense	1.000	-2.853	-	7.97E-06	Homo	1.2*	
P2	1.3	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Homo		
P3	1.3	p.G202R (c.604G>A)	Missense	0.742	-7.254	-	1.59E-05	Homo		
P4	1.8	p.W308R (c.922T>C)	Missense	1.000	-8.367	-	-	Homo	1.8*	[1, 2]
P5	2.3	p.K537R (c.1610A>G)	Missense	1.000	-2.850	-	-	Homo		
P6	2.5	p.R43H (c.128G>A)	Missense	0.000	-3.494	Pathogenic	1.77E-05	Homo		
P7	2.8	p.R280C (c.838C>T)	Missense	0.973	-5.467	Conflicting[#]	1.60E-04	Compt		
		p.A90D (c.269C>A)	Missense	0.544	-3.400	-	-			
P8	3.6	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt		[2]
		p.Y963C (c.2888A>G)	Missense	1.000	-8.267	-	-			
P9	3.6	p.P32S (c.94C>T)	Missense	0.902	-2.963	-	-	Compt	3.6	[2]
		p.G1314E (c.3941G>A)	Missense	1.000	-7.696	-	-			
P10	4.2	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Homo		
P11	4.4	p.R43C (c.127C>T)	Missense	0.998	-6.043	-	7.07E-06	Compt	4.4	[2]
		p.R208W (c.622C>T)	Missense	0.846	-5.285	-	2.40E-05			
P12	4.7	p.L798P (c.2393T>C)	Missense	1.000	-6.603	-	-	Compt		[2]
		p.R1612P (c.4835G>C)	Missense	0.322	-2.406	-	-			

P13	5.0	p.R288K (c.863G>A)	Missense	0.001	0.670	Conflicting	6.13E-03	Compt		
		p.S693L (c.2078C>T)	Missense	0.945	-5.895	Uncertain[ε]	2.59E-04			
P14	5.0	p.R208W (c.622C>T)	Missense	0.846	-5.285	-	2.40E-05	Compt	5*	[3]
		p.M760R (c.2279T>G)	Missense	0.989	-4.667	-	2.48E-05			
P15	6.0	p.A348P (c.1042G>C)	Missense	0.506	-2.113	-	-	Compt		
		c.1285+4_1285+7del AGT	Intron	UK	UK	-	-			
P16	6.9	p.R288K (c.863G>A)	Missense	0.001	0.670	Conflicting	6.13E-03	Compt		
		p.N124S (c.371A>G)	Missense	0.243	-1.600	Conflicting	1.03E-03			
P17	7.0	p.Q1045R (c.3134A>G)	Missense	0.992	-3.000	-	-	Homo		[2]
P18	7.3	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt		
		p.G571R (c.1711G>C)	Missense	1.000	-7.600	-	1.59E-05			
P19	9.4	p.P766S (c.2296C>T)	Missense	0.080	-5.578	Conflicting	1.66E-03	Compt		
		p.T1472R (c.4415C>G)	Missense	1.000	-5.672	-	-			
P20	10.5	p.R208W (c.622C>T)	Missense	0.846	-5.285	-	2.40E-05	Compt		[2]
		c.3863-98C>T	Intron	UK	UK	Pathogenic	-			
P21	10.7	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt		
		p.G571R (c.1711G>C)	Missense	1.000	-7.600	-	1.59E-05			
P22	11.0	p.R43H (c.128G>A)	Missense	0.995	-3.494	Pathogenic	1.77E-05	Homo		
P23	11.4	p.A348P (c.1042G>C)	Missense	0.506	-2.113	-	-	Compt		
		c.1285+4_1285+7delAGT	intron	UK	UK	-	-			
P24	11.7	p.M363I (c.1089G>T)	Missense	0.675	-3.013	-	-	Compt		
		p.H626Y (c.1876C>T)	Missense	1.000	-5.700	-	-			
P25	12.0	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt	12*	
		p.T1114M (c.3341C>T)	Missense	0.959	-2.703	-	1.74E-05			

P26	12.4	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt	
		p.P248L (c.743C>T)	Missense	0.997	-8.000	-	7.97E-06		
P27	13.4	p.P248S (c.742C>T)	Missense	1.000	-5.800	-	-	Homo	
P28	13.6	p.D953H (c.2857G>C)	Missense	0.968	-1.232	-	-	Compt	[2]
		p.F1007I (c.3091T>A)	Missense	0.205	-4.067	-	-		
P29	13.5	p.P246L (c.737C>T)	Missense	0.969	-9.333	-	7.98E-06	Compt	
		p.L1104R (c.3311T>G)	Missense	0.993	-5.370	Uncertain	-		
P30	16.2	p.R208W (c.622C>T)	Missense	0.846	-5.285	-	2.40E-05	Compt	
		p.S411Y (c.1232C>A)	Missense	0.984	-4.183	Uncertain	-		
P31[*1]	20.3	p.R20L (c.59G>T)	Missense	1.000	-6.165	-	8.00E-06	Compt	
		p.G961= (c.2883C>T)	Synonymous	UK	UK	Likely pathogenic	-		
P32[*2]	21.2	p.F245L (c.733T>C)	Missense	0.897	-5.533	-	-	Compt	
		p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06		
P33[*3]	27.7	p.G964S (c.2890G>A)	Missense	0.995	-5.100	-	4.09E-06	Compt	
		p.R1482W (c.4444C>T)	Missense	1.000	-7.563	-	-		
P34[*4]	29.2	p.G964D (c.2891G>A)	Missense	0.998	-6.033	-	-	Homo	[2, 4]
P35[*5]	44.6	p.R709W (c.2125C>T)	Missense	0.339	UK	Conflicting	1.37E-03	Compt	[2]
		p.I1193M (c.3579C>G)	Missense	0.895	-2.642	-	1.59E-05		
P36[*6]	57.0	p.G964D (c.2891G>A)	Missense	0.998	-6.033	-	-	Homo	[2, 4]
P37[*7]	70.3	p.R43C (c.127C>T)	Missense	0.998	-6.043	-	7.07E-06	Compt	
		p.G1002S (c.3004G>A)	Missense	0.826	-3.650	-	4.14E-06		
Hypo/null									
P38	2.2	p.L579P (c.1736T>C)	Missense	1.000	-6.650	-	-	Compt	[2]

		p.R1272GfsX73 (c.3812delG)	Frameshift	Truncated Protein		-	-			
P39	4.5	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt		
		p.V1548GfsX31 (c.4643dupG)	Frameshift	Truncated Protein		-	-			
P40	6.6	p.P969S (c.2905C>T)	Missense	0.999	-7.033	-	-	Compt		[2]
		p.D1439GfsX11 (c.4311-4312insG)	Frameshift	Truncated Protein		-	-			
P41	6.6	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt		
		p.R998PfsX11 (c.2993delG)	Frameshift	Truncated Protein		-	-			
P42	11.8	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt		[2]
		p.E765X (c.2293G>T)	Nonsense	Truncated Protein		-	-			
null/null										
P43	1.1	p.R1333GfsX24 (c.3997_3998delAG)	Frameshift	Truncated Protein		Pathogenic	-	Homo	1.1	
P44	1.5‡	c.1897-1G>C	Intron	del Ex16		-	-	Homo	4.1/1.5*	[5]

'Age at Last FU_Year' was the age of patients at their last clinical visit. Age marked with '‡' indicated the patient was diagnosed retrospectively (death before 2006), otherwise prospectively if not marked. All variants in compound heterozygous patients were confirmed to be in trans by analysis of the parents. Variants pathological prediction was conducted with PROVEAN v1.1.3 [6] and PolyPhen-2 [7]. The PolyPhen score ranges

from 0.0 (tolerated) to 1.0 (deleterious) according to the developer. When the PROVEAN score is less than -2.5, the variant is predicted to be deleterious. According to records from 'ClinVar', 'Conflicting[#]' in 'ClinVar' column represented 'Conflicting interpretations of pathogenicity', 'Uncertain[ε]' represented 'Uncertain significance' and '-' represented 'not recorded'. Freq. (frequency) of variants were documented from gnomAD (v2.1.1). Genotype of patients were grouped into homozygous (Homo) and compound heterozygous (Compt). The column 'Age at death/LTX*' was formatted in year; LTX*: lung transplant. The 'Ref' column recorded published literature(s) by our and co-operated groups from Kids Lung Register. Patients P31 to P37 were marked with asterisk and number, indicating they are seven adult patients. P32 had co-morbidity of failure of thrive, and P33 had para-psoriasis and retinopathy after hydroxychloroquine therapy, the rest 5 adult patients had no co-morbidities. UK: unknown.

Supplement table 2. Genetic information of patients with *ABCA3* variants who died, got lung transplanted or lost follow up before 1 year old.

Age at last visit_Year	Mutations	Variant Type	Poly Phen-2	PROVEAN	ClinVar	Freq. of Variant	Genotype	Age at death/LTX*	Ref
Hypo/Hypo									
0.28	p.V1399M (c.4195G>A)	Missense	1.000	-2.850	-	7.97E-06	Homo	0.28	[2]
0.38	p.E1364K (c.4090G>A)	Missense	1.000	-3.850	-	-	Homo	0.38	[2]
0.58	p.G1314R (c.3940G>C)	Missense	1.000	-7.700	-	-	Homo	0.58	[2]
0.07	p.G1314R (c.3940G>C)	Missense	1.000	-7.700	-	-	Homo	0.07	[2]
0.06	p.F810CfsX2 (c.2429-2430delTT)	Frameshift	-	-	-	-	Homo	0.06	
0.15	p.P193R (c.578C>G)	Missense	1.000	-7.580	-	-	Homo	0.15	[2]
0.30‡	p.P193R (c.578C>G)	Missense	1.000	-7.580	-	-	Homo	0.30	[2]
0.05	p.S359del (c.1076_1078delCCT)	Deletion	-		-	-	Homo	0.05	[2]
0.01	p.R280H (c.839G>A)	Missense	1.000	-2.700	Conflicting	8.27E-04	Compt		[2]
	p.R1305L (c.3914G>T)	Missense	0.260	-4.890	-	7.11E-06			
0.40‡	p.L1386P (c.4157 T>C)	Missense	1.000	-6.170	-	-	Compt	0.40	[2]
	p.L268_L269insL (c.806insGCT)	Insertion	1.000	-7.740	-	-			
0.50	p.G964S (c.2890G>A)	Missense	1.000	-5.100	-	4.09E-06	Compt		
	p.R709W (c.2125C>T)	Missense	0.716	-3.060	Conflicting	1.37E-03			
0.14	p.G1421R (c.4261G>A)	Missense	1.000	-7.700	-	1.19E-05	Compt	0.14	
	p.Q1045R (c.3134A>G)	Missense	0.992	-3.000	-	-			
0.01‡	p.Q215K (c.643C>A)	Missense	1.000	-3.730	-	-	Compt		[2]

	p.R288K (c.863G>A)	Missense	0.000	0.670	Conflicting	6.13E-03			
0.55‡	p.P193S (c.577C>T)	Missense	1.000	-6.720	-	-	Compt	0.55	[2]
	p.G1421R (c.4261G>A)	Missense	1.000	-7.700	-	1.19E-05			
0.55	p.P193S (c.577C>T)	Missense	1.000	-6.720	-	-	Compt	0.55*	[2]
	p.G1421R (c.4261G>A)	Missense	1.000	-7.700	-	1.19E-05			
0.25	p.G202R (c.604G>A)	Missense	1.000	-7.250	-	1.59E-05	Compt	0.25	
	p.C611R (c.1831T>C)	Missense	1.000	-11.400	-	7.95E-06			
0.21	p.K1388N (c.4164G>C)	Missense	0.999	-4.810	-	-	Homo	0.21	[2]
0.80	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt		
	p.E1364K (c.4090G>A)	Missense	1.000	-3.850	-	-			
0.13‡	p.R43L (c.128G>T)	Missense	1.000	-4.860	-	-	Compt	0.13	[2]
	p.R288K (c.863G>A)	Missense	0.000	0.670	-	6.13E-03			
0.54	p.E292V (c.875A>T)	Missense	0.990	-6.533	Likely-pathogenic	3.98E-06	Compt	0.54	
	p.L130P (c.389T>C)	Missense	1.000	-6.030	-	-			
0.01	p.P248S (c.742C>T)	Missense	1.000	-5.800	-	-	Compt	0.01	[2]
	p.V1120F (c.3358G>T)	Missense	1.000	-4.550	-	-			
Hypo/null									
0.11	p.N104TfsX47 (c.309delC)	Frameshift	Truncated Protein		-	-	Compt	0.11	
	p.P246L (c.737C>T)	Missense	1.000	-9.330	-	7.98E-06			
0.22	p.Q233X (c.697C>T)	Nonsense	Truncated Protein		-	-	Compt	0.22	
	p.R280C (c.838C>T)	Missense	1.000	-5.470	Conflicting	1.60E-04			
0.16	p.A1046E (c.3137C>A)	Missense	1.000	-4.200	-	-	Compt	0.16	[2]
	p.A1338T (c.4012G>A)	Missense	0.146	-0.550	Uncertain	4.09E-04			

	c.4360-1G>C	Intron	-	-	-	-			
null/null									
0.04‡	p.E1626VfsX16 (c.3997_3998del)	Frameshift	Truncated Protein		-	-	Homo	0.04	[2, 5]
0.14	p.R1561X (c.4681C>T)	Nonsense	Truncated Protein		-	1.21E-05	Homo	0.14	[2, 5, 8, 9]
2.50	p.R1561X (c.4681C>T)	Nonsense	Truncated Protein		-	1.21E-05	Homo	0.85*	[2]
5.00	p.R1561X (c.4681C>T)	Nonsense	Truncated Protein		-	1.21E-05	Homo	0.59*	[2]
0.07	p.R1561X (c.4681C>T)	Nonsense	Truncated Protein		-	1.21E-05	Homo	0.07	[2, 5, 8, 9]
0.39	p.R1561X (c.4681C>T)	Nonsense	Truncated Protein		-	1.21E-05	Homo	0.39	[2, 5, 8, 9]
0.19‡	p.R1561X (c.4681C>T)	Nonsense	Truncated Protein		-	1.21E-05	Homo	0.19	[2, 5, 8, 9]
0.08	p.R1561X (c.4681C>T)	Nonsense	Truncated Protein		-	1.21E-05	Homo	0.08	[2, 5, 8, 9]
0.25	p.R1333GfsX24 (c.3997_3998delAG)	Frameshift	Truncated Protein		Pathogenic	-	Homo	0.25	
0.22‡	c.3005–1G >A	Intron	-		-	-	Homo	0.22	[2]
0.19	p.S536PfsX10 (c.1601_1604depACCT)	Frameshift	Truncated Protein		-	-	Compt	0.19	[2]
	p.V1303SfsX43 (c.3907delG)	Frameshift	Truncated Protein		-	-			

In the column 'Age at last visit_Year', age marked with '‡' indicated the patient was diagnosed retrospectively, otherwise prospectively. According to records from 'ClinVar', 'Conflicting#' in 'ClinVar' column represented 'Conflicting interpretations of pathogenicity', 'Uncertainε' represented 'Uncertain significance' and '-' represented 'not recorded'. Freq. (frequency) of variants were documented from gnomAD (v2.1.1). Genotype of

patients were grouped into homozygous (Homo) and compound heterozygous (Compt). The column 'Age at death/LTX*' was formatted in year; LTX*: lung transplant. The 'Ref' column recorded published literature(s) by our and co-operated groups from Kids Lung Registery. UK: unknown.

Supplement table 3. Patients with ILD not analyzed for *ABCA3* because of lack of clinically plausible reason, no material or no consent.

All ILD patients in data base and not assessed for *ABCA3*	1436
Lung – diffuse	363
Adult idiopathic pulmonary fibrosis (IPF)	22
Alveolar microlithiasis	4
Bronchiolitis obliterans, BOOP, OP, emphysema	34
Chronic pneumonitis of infancy, lipoid pneumonitis, desquamative interstitial pneumonitis	13
Eosinophilic pneumonitis	7
Nonspecific interstitial pneumonia	29
Pulmonary alveolar proteinosis	48
Undefined ILD, diffuse alveolar damage	206
Lung – developmental	356
ACD, acinar dysplasia, CAD, unclear developmental disorder, lung hypoplasia, pulmonary interstitial glycogenosis, cellular interstitial pneumonitis	79
NEHI / PTI	165
Premature birth	112
Systemic - auto-inflammatory	16
Systemic - immune dysregulated	60
Systemic – immunocompetent	235
Systemic – immunodeficient	169
Exposure - non-infectious	79
Vascular	158

BOOP: bronchiolitis obliterans combined organizing pneumonia. OP: organizing pneumonia. ACD: alveolar capillary dysplasia. CAD: congenital acinar dysplasia. NEHI / PTI: neuroendocrine hyperplasia of infancy / Persistent tachypnea of infancy.

Supplement table 4. Difference of basic characteristics between patients with homozygous and compound heterozygous *ABCA3* variants (patients from KLR database)

	Total (n=44)	**Homo (n=13)**	**Compt (n=31)**	***P* value**
Gender				
Female	63.6%	57.1%	66.7%	0.5068
Male	34.1%	42.9%	30.0%	
Unknown	2.3%		3.3%	
Last Follow Up Age_Year				
Median	9.3	2.5	8.3	0.0202*
25% Percentile	4.1	1.3	4.7	
75% Percentile	15.0	11.6	15.0	
Gestational Age				
Term	93.2%	100.0%	90.3%	0.5402
Preterm	6.8%	0.0%	9.7%	
Birth Weight_g				
Mean	2959.0	2915.0	2982.0	0.5127
SD	674.4	775.1	635.1	
Neonatal_Oxygen				
Need	38.6%	42.9%	36.7%	0.7477
No need	61.4%	57.1%	63.3%	
Neonatal_Mechanical Ventilation				
Need	20.5%	42.9%	16.7%	0.1317
No need	79.5%	57.1%	83.3%	

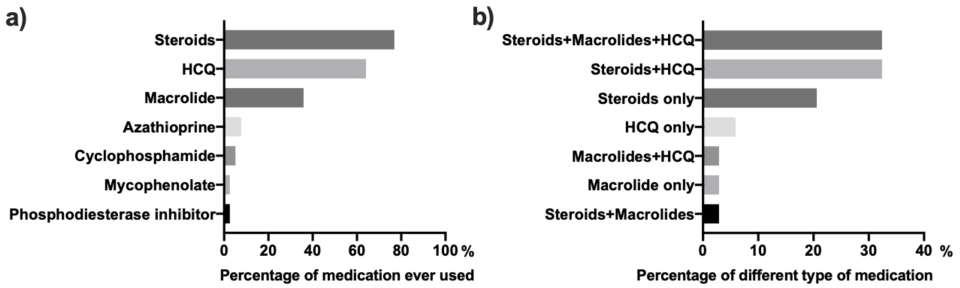
*Mann-Whitney test two-tailed exact *P* value.

Supplement table 5. Additional information on patients with *ABCA3* variants with conflicting or uncertain significance.

Pt_ID	Mutations	Age at Last FU_Year	Clinical phenotype	Pathology of the lung	Surfactant (lavage)	In vitro data on variant
P7	p.R280C p.A90D	2.8	Diffuse ILD, high flow treatment	Not available	Reduced SP-C (30% of normal)	70% of normal ABCA3+ vesicle volume [10] Not available
P13	p.R288K p.S693L	5.0	RDS, mature neonate, recurrent infections, clinically no symptoms at last follow up	Not available	Not available	Normal ABCA3+ vesicle volume, impaired doxorubicin detoxification and defective ATPase activity [11, 12] Not available
P16	p.R288K p.N124S	6.9	Chronic ILD, partial respiratory insufficiency, obstruction, clinically stable	Not available	Lacking SP-C	Normal ABCA3+ vesicle volume, impaired doxorubicin detoxification and defective ATPase activity [11, 12] Not available
P19	p.P766S p.T1472R	9.4	Chronic ILD, extensive ground glass opacity in both lungs, interlobular and intra-lobular septal and irregular thickening	Usual interstitial pneumonitis, pneumocyte type 2 hyperplasia, increased alveolar macrophages and focally abundant proteinaceous fluid in the alveoli	Not available	Not available Not available

P29	p.P246L p.L1104R	13.5	Chronic ILD, partial respiratory insufficiency, crackles, scoliosis	Prominent lobular remodeling with cystic dilated airspaces. Widened alveolar septa with mild diffuse chronic inflammation. Infiltrate and mild fibrosis (NSIP like). Focal PAP, increased macrophages, hyperplasia type II pneumocytes.	Not available	Not available Not available
P30	p.R208W p.S411Y	16.2	Chronic ILD, DCLO 36% (age 15y), 6MWT desaturation	Biopsy (age 1y) chronic pneumonitis of infancy; biopsy (age 15 y) simplification of alveolar architecture, marked pneumocyte type 2 hyperplasia, foamy macrophages, eosinophilic interalveolar material	Not available	88% of normal ABCA3+ vesicle volume [11] Not available
P31	p.R20L p.G961=	20.3	Chronic ILD with partial respiratory insufficiency	Fibrotic non-specific interstitial pneumonitis	Not available	Not available Not available
P35	p.R709W p.I1193M	44.6	Chronic ILD, reduced FVC, and DLCO	Not available	Reduced proSP-C at 12.3 kDa, mature SP-C present	Not available Not available

Supplement figure 1.



Supplement figure 1. Medication of patients. **a).** Percentage of patients who were ever treated with the medication indicated. **b).** For patients who ever treated with steroids, hydroxychloroquine (HCQ) and macrolides, percentage of different medication methods (alone or combination) were indicated.

References

1. Parappil, H., et al., *Respiratory distress syndrome due to a novel homozygous ABCA3 mutation in a term neonate.* BMJ Case Rep, 2011. **2011**.

2. Kroner, C., et al., *Lung disease caused by ABCA3 mutations.* Thorax, 2017. **72**(3): p. 213-220.

3. Doan, M.L., et al., *Clinical, radiological and pathological features of ABCA3 mutations in children.* Thorax, 2008. **63**(4): p. 366-73.

4. Campo, I., et al., *A large kindred of pulmonary fibrosis associated with a novel ABCA3 gene variant.* Respir Res, 2014. **15**: p. 43.

5. Brasch, F., et al., *Alteration of the pulmonary surfactant system in full-term infants with hereditary ABCA3 deficiency.* Am J Respir Crit Care Med, 2006. **174**(5): p. 571-80.

6. Choi, Y. and A.P. Chan, *PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels.* Bioinformatics, 2015. **31**(16): p. 2745-7.

7. Adzhubei, I.A., et al., *A method and server for predicting damaging missense mutations.* Nat Methods, 2010. **7**(4): p. 248-9.

8. Edwards, V., et al., *Ultrastructure of lamellar bodies in congenital surfactant deficiency.* Ultrastruct Pathol, 2005. **29**(6): p. 503-9.

9. Wambach, J.A., et al., *Genotype-phenotype correlations for infants and children with ABCA3 deficiency.* Am J Respir Crit Care Med, 2014. **189**(12): p. 1538-43.

10. Weichert, N., et al., *Some ABCA3 mutations elevate ER stress and initiate apoptosis of lung epithelial cells.* Respir Res, 2011. **12**: p. 4.

11. Schindlbeck, U., et al., *ABCA3 missense mutations causing surfactant dysfunction disorders have distinct cellular phenotypes.* Hum Mutat, 2018. **39**(6): p. 841-850.

12. Wittmann, T., et al., *Increased Risk of Interstitial Lung Disease in Children with a Single R288K Variant of ABCA3.* Mol Med, 2016. **22**: p. 183-191.