Supplemental table 1

Patients with homozygous ABCA3 mutations

Patient ID	Sex	Mutation (c.DNA)	Mutation (p.protein)	Provean	SIFT	Polyphen-2	Presentation (age[y])	Follow-up (age[y])	Therapy (response)	Mutation previously described by
other/other										
1	m	c.578C>G	p.P193R	-7.575	0.002	1.000	RDS, PAP^{x} (neonatal)	dead (0.15)	surf (ni), ster (ni), hcl (ni)	[1]$^{\Delta}$
2	f	c.578C>G	p.P193R	-7.575	0.002	1.000	RDS, PAP^{x} (neonatal)	dead (0.32)	surf (ni), ster (ni), hcl (ni)	[1]$^{\Delta}$
3	m	c.643C>A; c.863G>A	p.Q215K; p.R288K	-3.733; 0.670	0.000; 1.000	1.000 0.000	RDS (neonatal)	dead (0.06)	ster (ni), surf (ni)	[2]$^{\Delta}$
4	f	c.922C>T	p.W308R	-8.367	0.000	1.000	RDS (neonatal)	LTX (1.5), stable (3.3)	ster (mi), surf (mi), hcl (mi)	[3, 4]
5	f	c.4164G>C	p.K1388N	-4.810	0.000	0.999	RDS (neonatal)	dead (0.20)	surf (ni), ster (mi), hcl (ni)	*
6	m	c.3960G>A	p.G1314R	-7.70	0.000	1.000	RDS (neonatal)	dead (0.66)	surf (mi), ster (ni), hcl (mi), azt (ni)	*
7	m	c.4060G>A	p.E1364K	-3.85	0.000	1.000	RDS, PAP^{x} (neonatal)	dead (0.4)	ster (ni)	[4]
8	m	c.4195G>A	p.V1399M	-2.85	0.001	1.000	RDS (neonatal)	dead (0.29)	surf, ster, hcl	[4, 5]
9	m	c.3134A>G	p.Q1045R	-3.00	0.003	0.992	RDS (neonatal)	alive with oxygen 1l/min, sick-better (0.46)	ster (gi), hcl (gi)	[4]
10	m	c.1076_1078delCCT	p.S359del	-9.32	na	na	RDS (neonatal)	dead (0.06)	surf (mi), ster (mi)	*
11	f	c.2891G>A	p.G964D	-6.03	0.003	0.998	cough, PAP^{x} (2)	restrictive lung disease, sick-same (16)	ster (mi)	[6]$^{\Delta}$
12	m	c.2891G>A	p.G964D	-6.03	0.003	0.998	cough, dyspnea (52)	restrictive lung disease, sick-same (57)	ster (mi), azt (mi)	[6]$^{\Delta}$
13	m	c.2891G>A	p.G964D	-6.03	0.003	0.998	none (52)	restrictive lung disease, sick-same (57)	none	[6]$^{\Delta}$
								dead 62%$^{+}$ (median age 0.25^{+})		
null/null										
14	m	c.4681C>T	p.R1561X	truncated protein			RDS (neonatal)	dead (0.13)	surf (mi), ster (ni), hcl (ni)	[2, 4, 7]
15	f	c.4681C>T	p.R1561X	truncated protein			RDS (neonatal)	dead (0.39)	surf (mi), ster (ni), azt (ni)	[2, 4, 7]

16	m	c.4681C>T	p.R1561X	truncated protein	RDS (neonatal)	dead (0.19)	surf (mi), hcl (mi), ster (ni)	[2, 4, 7]
17	m	c.4681C>T	p.R1561X	truncated protein	RDS (neonatal)	dead (0.08)	surf (mi), ster (ni)	[2, 4, 7]
18	m	c.4681C>T	p.R1561X	truncated protein	RDS (neonatal)	dead (0.07)	surf (mi), ster (ni)	[2, 4, 7]
19	f	c.1897-1G>C	del Ex16	loss of exon 16	RDS, PAP[x] (neonatal)	worsening (0.3), interstitial lung disease diagnosis (0.4), LTX (1.5), dead (4.13)	ster (ni), surf (ni)	[2][Δ]
20	m	c.2429-2430delTT	p.F810Cfs*2	truncated protein	RDS (neonatal)	dead (0.06)	surf (mi), hcl (ni), ster (ni)	*
21	f	c.3005-1G>A	del Ex22	loss of exon 22	RDS (neonatal)	dead (0.22)	surf (ni), ster (ni)	[2][Δ]
22	m	c.4877-8delAG	p.E1626Vfs*16	truncated protein	RDS (neonatal)	dead (0.04)	surf (ni), ster (ni)	[2][Δ]
						dead 100%[+] (median age 0.11[+])		

Provean: < -2.5 deleterious; > 2.5 neutral; SIFT: ≤ 0.05 damaging, > 0.05 tolerated; Polyphen-2 Score: ≤ 0.1 probably damaging; 0.1 ≤ 0.2 possibly damaging; > 0.2 benign

Abbreviations: [x] reminiscent of PAP according to the records, * previously undescribed mutations, [+] of patients without LTX, [Δ] patient previously published by, azt azithromycin, f female, gi good improvement, hcl hydroxychloroquine, LTX lung transplantation, m male, mi moderate improvement, na information not available, ni no improvement, PAP pulmonary alveolar proteinosis, RDS respiratory distress syndrome, surf surfactant, ster systemic steroids, y years

Supplemental table 2

Patients with compound heterozygous ABCA3 mutations

Patient ID	Sex	Mutation (c.DNA)	Protein (p.protein)	Provean	SIFT	Polyphen-2	Presentation (age[y])	Follow-up (age[y])	Therapy (response)	Mutation previously described by
other/other										
23	f	c.622C>T c.3863-98C>T	p.R208W intronic	-5.285	0.002	0.997	RDS (neonatal)	intermittant oxygen with infection, sick-same (2.7)	surf (mi), hcl (mi)	[8] *
24	f	c.577C>T c.4261G>A	p.P193S p.G1421R	-6.721 -7.696	0.014 0.000	1.000 1.000	RDS (neonatal)	LTX (0.6), dead (0.7)	surf (mi), hcl (ni), ster (ni)	* *

25	m	c.577C>T c.4261G>A	p.P193S p.G1421R	-6.721 -7.696	0.014 0.000	1.000 1.000	RDS (neonatal)	dead (0.5)	surf (mi), ster (ni)	* *
26	m	c.806_807ins GCT c.4157T>C	p.L268_L269insL p.L1386P	-7.743 -6.168	Na 0.001	Na 1.000	RDS, PAP^{x} (neonatal)	dead (0.4)	surf (ni), whole lung lavages (ni)	[4] [4]
27	m	c.127C>T c.622C>T	p.R43C p.R208W	-6.043 -5.285	0.000 0.002	1.000 0.997	cough, tachydyspnea, failure to thrive (0.9), PAP^{x}, clubbing, repeated pneumonia	dead (4.5)	surf (ni), ster (mi)	[4] [9, 10]
28	f	c.94C>T c.3941G>A	p.P32S p.G1314E	-2.963 -7.696	0.029 0.000	0.902 1.000	cough, cyanosis, retractions, wheezing, failure to thrive (0.5), clubbing	dead (3.6)	ster (gi), hcl (mi)	* *
29	f	c.742C>T c.3358G>T	p.P248S p.V1120F	-5.80 -4.55	0.018 0.010	1.000 1.000	RDS, PAP^{x} (neonatal)	dead (0.25)	surf (ni), ster (ni)	* *
30	f	c.839G>A c.3914G>T	p.R280H p.R1305L	-2.70 -4.89	0.016 0.004	1.000 0.260	RDS (neonatal)	dead (day 0)	ster (ni), surf (ni)	[4] *
31	f	c.2125C>T c.3579C>G	p.R709W p.I1193M	-3.055 -2.642	0.003 0.001	0.716 0.934	dyspnea at exercise, PAP^{x} (33)	dyspnea at exercise, sick-same (36)	repeated whole lung lavages (mi)	[8] *
32	f	c.2857G>C c.3229T>A	p.D953H p.F1077I	-1.23 -1.41	0.109 0.369	0.968 0.002	mycoplasm pneumonia, clubbing, hypoxemia (2.2)	SaO2 88-90%, sick-same (7.4)	ster (mi)	* [4]
33	f	c.2888A>G c. 875A>T	p.Y963C p.E292V	-8.27 -6.53	0.0002 0.0000	1.000 0.989	na	repeated pneumonia, sick-same (3)	na	[8] [4, 9-13]

34	f	c.2393T>C c.4853G>C	p.L798P p. R1612P	-6.60 -2.41	0.000 0.091	1.000 0.322	RDS, PAP[x] (neonatal)	dead (0.28)	surf (mi), ster (ni), hcl (ni)	[14][Δ] [14][Δ]
								dead 64%[+] (median age 0.4[+])		
null/other										
35	f	c.2905C>T c.4311_12insG	p.P969S p.D1439Gfs*11	-7.033	0.036	0.999	RDS (neonatal), then better, cough from 6 weeks	ventilated with tracheostomy, short time intervals without ventilation (8.8)	surf, ster, hcl (gi), azt, whole lung lavages	* *
36	f	c.4360-1G>C c.3137C>A c.4012G>A	Del Ex29 p.A1046E p.A1338T	loss -4.200 -0.554	of 0.000 0.554	exon 29 1.000 0.146	RDS (neonatal), tracheotomia (0.1)	respiratory insufficiency, dead (0.2)	surf (ni), ster (mi), hcl (mi)	* * COSM4059368
37	f	c.875A>T c.2293G>T	p.E292V p.E765X	-6.533 Truncated protein	0.000	0.999	RDS (neonatal)	intermittant oxygen and infections, sick better (7)	surf, ster, hcl (gi)	cf pt 33 *
38	f	c.1736T>C c.3812delG	p.L579P p.R1272Gfs*73	-6.650 Truncated protein	0.000	1.000	RDS, PAP[x] (neonatal)	respiratory insufficiency, dead (0.1)	ster (ni), surf (ni)	[2][Δ]
39	f	c.4751delT c.128G>T c.863G>A	p.L1584Rfs*49 p.R43L p.R288K	na -4.51 0.67	0.002 1.000	1.000 0.000	RDS (neonatal)	respiratory insufficiency, dead (0.13)	surf (ni), ster (ni)	[2][Δ]
								dead 60% (median age 0.13)		
null/null										
40	f	c.1601_1604dup ACCT c. 3907delG	p.S536Pfs*10 p.V1303Sfs*43	Truncated protein Truncated protein			RDS (neonatal)	respiratory insufficency, dead (0.19)	surf (ni), ster (ni), hcl (ni)	* *
								dead 100%		

Provean: < -2.5 deleterious; > 2.5 neutral; SIFT: ≤ 0.05 damaging, > 0.05 tolerated; Polyphen-2 Score: ≤ 0.1 probably damaging; 0.1 ≤ 0.2 possibly damaging; > 0.2 benign

Abbreviations: [X] reminiscent of PAP according to the records, * previously undescribed mutations, [+] median death age of patients without LTX, [Δ] patient previously published by, azt azithromycin, BAL bronchoalveolar lavage, f female, gi good improvement, hcl hydroxychloroquine, LTX lung transplantation, m male, mi moderate improvement, na information not available, ni no improvement, RDS respiratory distress syndrome, surf surfactant, ster systemic steroids, y years

Supplemental table 3

Primers used for analysis of ABCA3. The sequence of all PCR primers is given in the 5´ to 3´ direction.

ABCA3 gene

A3-4-1 CCAAATCCCCACTCTGCGTG
A3-5-2 CAGCTGCTTCGCACATCCTG
A3-6-1 CAAAGCCCTAGAGGATTTGCC
A3-6-2 CAGACCCAAAGGAGTGACTGC
A3-7-1 CTCTCCCACTCCACCCTGTTG
A3-7-2 CTGCTATAAGGACACATGCACACG
A3-8-1 TCTCATTTGCTGTCAGTGTGTGG
A3-8-2 CTAAAACACCAAGCCTTTGGACATG
A3-9-1 CTGCTGGGACAGTCGGACTC
A3-9-2 CTGACCATCCCTGGTCACAGG
A3-10-1 CTCTTGGGAAGAACTTTGTGGTCAG
A3-10-2 GCTGACTTTCCTCCTTCCAGTCC
A3-11-1 GTGTAGATGGCAAGTGCCAGGAG
A3-11-2 CAGCTATCCAGCCCACACTCAG
A3-12-1 CATGCCAACCAAGCAGTGG
A3-12-2 CTCTCTCTGAACCAGTCCCAAGG
A3-13-1 CTGCATGGCTGTGTGCATCTAG
A3-13-2 CTATGAGGTCTCACTGCCGTGC
A3-14-1 CTAGGCTTGGTTCCTTCTGAGACG
A3-14-2 GTGCATCTCCTGCCGCTGTG

A3-15-1 CAGGGTCCTCAGAGGAAATTAGG
A3-15-2 CTCAGAACCCTGGCTCCTGC
A3-16-1 CAGCTACGTCAAGGAGAGGTTCC
A3-16-2 GCTCGTCCAGTATCAGCACCTG
A3-17-1 CCATCCTTGGAGGACTCAAGC
A3-17-2 CAGAGGCAACAGACAGGAAGTCTAG
A3-18-1 CAAGACACATTCATTCTGCTTCAGC
A3-18-2 GCAGATTCATCTGGGCTGATG
A3-19-1 GTTCAAGTGTTCTCCTGCCTCTG
A3-19-2 CTGGGCAACTAGAGTGAAACTCC
A3-20-1 CATAAGCAGATGCATGAGCAAGC
A3-20-2 CTCGCAGACTCTCCTCTGCATG
A3-21-1 GCTGGCGTCACACAGAACAG
A3-21-2 CATTCGGAACAGCCAAGAACC
A3-22-1 GTCCCTGATTAGCCATGCTCAG
A3-22-2 GCAGACACAATGCTCTATCTATGGG
A3-23-1 GTGCTTGTGCTCTCCCATAAGC
A3-23-2 CAGCTGGTTCCGGTTCTGC
A3-24-1 GTCTGAGGACCTCCAAATGCTC
A3-24-2 CATGAACTGGGCCCATTGC
A3-25-1 CTCCACACAGCACGGATAAGG
A3-25-2 CCACTCAGACGCAGAGGAGC
A3-26-1 GTCTGCCATGTCGCTCATGG
A3-26-2 GAGACCATCTGGTGCAGGAGC
A3-27-1 GATTGGGACGAGAAGCCTTG
A3-27-2 GCAAAGCAGAGCAGTCTGAGC
A3-28-1 CTGATTATCAAGGAGCTCTCCAAGG
A3-28-2 CAAGCCACAGATGCAGCAGC
A3-29-1 CACTGGCAGGAACCACAG
A3-29-2 CTCCATCCTGGAGCCACAAG
A3-30-1 CTGTTCTGCAATTGCTGGGTG
A3-30-2 GACTCTGCACCAGATGCTGATG
A3-31-1 GAGAGCCAATGCCTTCCTGTC

A3-31-2 GTGCTCAGCACTGGAGTCCTC
A3-32-1 GAGGACTCCAGTGCTGAGCAC
A3-32-2 GTGACTCCTCTGTGGAAAGAGCC
A3-33-1 CTATTGCCAGAGGACTCCCAGG
A3-33-2 GAGTGCCTGGAGAAATTCAACC

Supplemental table 4 - Clinical characteristics of 22 patients with homozygous ABCA3 mutations

Characteristic	Number of patients
n	22
Sex	15m, 7f
Family history	8 families 1-2 abortions and/or 1-2 siblings or cousins or other family members died due to lung disease age 0-11 months, 1 healthy family, 8 na
Mother mutation	12 heterozygous, 10 na
Father mutation	13 heterozygous, 9 na
Consanguinity	17 yes, 1 no, 4 na
Ethnic group	14 Caucasian, 2 African, 1 Indian, 5 na
Gestational age	13 mean 40 weeks (36-42), 9 na
Neonatal mechanical ventilation	17 yes, 4 no, 1 na
Other organs problems	1 hyperbilirubinemia, 1 arteria cerebri media infarction/hepatomegaly, 1 nephromegaly/seizures, 1 PFO, 1 pyelonephritis/VSD/cholecystitis, 1 ASD/VSD, 1 chronic ischemic heart disease (57y), 9 none, 6 na

Abbreviations: f female, m male, na no information available, PFO persistent foramen ovale, VSD ventricular septal defect, y years

Supplemental table 5

Clinical characteristics of 18 patients with compound heterozygous ABCA3 mutations

Characteristics	Number of patients
N	18
Sex	15f; 3m
family history	2 siblings in one family death age 0.5 and 0.7; 2 siblings in 1 family postpartal RDS then healthy, 1 sibling in one family death due to neonatal RDS, 2 siblings in same family healthy, 1 atopic dermatitis, 5 families healthy, 7na
mother mutation	12 het, but healthy, 6 na
father mutation	11 het, but healthy, 1 no, 6 na
Pregnancy	1 IVF, 1 PROM, 1 gestosis, 1 placental insufficiency, 1 oligohydramnion and SGA, 12 na, 1 none
consanguinity	10 no, 8 na
ethnic group	16 Caucasian, 1 na, 1 family African/Asian
gestational age	mean 37.3 (33-42); 6 na
neonatal oxygen	14 yes, 2 no, 2 na
neonatal mechanical ventilation	12 yes, 4 no, 2 na
failure to thrive	8 yes of whom 1 PEG, 10 na
other organ problems	1 cor triatriale sinistrum/atrium septal defect II, 1 PFO/PDA one cleft palate, 1 systemic inflammatory response syndrome (age 3.5y), 1 gastroesophageal reflux, 1 benign occipital epilepsia/microcephaly, 13 na

Abbreviations: f female, LTX lung transplantation, m male, na no information available, PFO persistent foramen ovale, PDA persistent ductus arteriosus, RDS respiratory distress syndrome, y years

Supplemental table 6

Hydrophobic surfactant proteins (SP) C and B. Semiquantitative levels assessed by Western blotting in bronchoalveolar lavage fluid of patients with homozygous ABCA3 mutations

Pt ID	Age at BAL (years)	SP-C mol wt. (kDa)		SP-B mol. wt. (kDa)		
		4	8	8	16	24
1	0.1	0	0	+	++	0
4	0.1	+++*	0	++	+++	+++
6	0.1	0	0	0	+++	0
5	0.1	0	+++**	+++	+++	+++**
10	0.04	0	0	0	+++	+++
11	13.1	0	0	0	++	0
12	52	0	0	0	++	0
15	0.11	0	0	0	+++	+++
16	0.01	0	0	0	+	0
17	0.01	0	0	0	+	0
18	0.07	0	0	0	++	+
19	0.21	0	0	++	0	0
22	0.01	0	0	+	0	0

Immune reactive bands were semiquantitatively expressed in relation to standard run concurrently (Griese et al 2015). 0 no band, + > 0 but below reference range, ++ within reference range, +++ above reference range
Reference values SP-C (4-5 kDa 116 ± 14 ng/ml; 8 kDa 140 ± 81 ng/ml), SP-B (8kDa 68 ± 22 ng/ml; 16-18kDa 580 ± 53 ng/ml; 24-38 kDa 288 ± 89 ng/ml)

underscored = patients with p.R1561X
* likely that sample was taken after exogenous surfactant application
** abundant higher molecular forms of SP-B and SP-C
Abbreviations: mol wt molecular weight

Supplemental table 7

Hydrophobic surfactant proteins (SP) C and B. Semiquantitative levels assessed by Western blotting in bronchoalveolar lavage fluid of patients with compound heterozygous ABCA3 mutations

Pt ID	Age at BAL (years)	SP-C mol. wt. (kDa)		SP-B mol. wt. (kDa)		
		4	8	8	16	24
23	0.7	0	+	0	++	0
25	0.3	++	0	0	++	+
27	1.7	+++	+++	+++	+++	+++**
28	2.9	+	+	++	+++	++
31	33	++	+++	+	+++	+**
36	0.2	+	+		+++	0
38	0.05	+*	0*		+	+++

Immune reactive bands were semiquantitatively expressed in relation to standard run concurrently (Griese et al 2015). 0 no band, + > 0 but below reference range, ++ within reference range, +++ above reference range; Reference values SP-C (4-5 kDa 116 ± 14 ng/ml; 8 kDa 140 ± 81 ng/ml), SP-B (8kDa 68 ± 22 ng/ml; 16-18kDa 580 ± 53 ng/ml; 24-38 kDa 288 ± 89 ng/ml)
*repeated analysis with 20µg protein, very little SP-C detected, mainly at higher molecular weight;
** abundant total protein, relative to SP-B normal SP-C, resembles PAP, surfactant uptake error
Abbreviations: mol wt molecular weight

Supplemental table 8

Semiquantitative immunohistochemical score of ABCA3 protein expression in different lung tissues of 12 patients with ABCA3 mutations mutations

Patient ID	Mutation	Follow-Up (age [y])	Intensity of ABCA3 Staining	ABCA3-Aggregates	ABCA3 staining pattern
patients with homozygous ABCA3 mutations					
other/other					
7	p.E1364K	dead (0.4)	2	1	1
5	p.K1388N	dead (0.2)	1	1	1
null/null					
17	p.R1561X	dead (0.08)	0	0	0
14	p.R1561X	dead (0.13)	0	0	0
20	p.F810Cfs*	dead (0.06)	0	0	0
patients with compound heterozygous ABCA3 mutations					
other/other					
34	p.L798P p.R1612P	sick-worse (0.15)	1	0	1
24	p.P193S p.G1421R	dead (0.7)	1	0	1
28	p.P32S p.G1314E	dead (3.6)	2	1	1
27	p.R43C p.R208W	dead (4.5)	2	1	1
null/ other					
36	p.A1046E p.A1338T Del Ex29	dead (0.2)	1	0	1
39	p.L1584Rfs*49 p.R43L	dead (0.13)	1	0	1

38	p.R288K p.L579P p.R1272Gfs*73	dead (0.1)	1	0	1
controls					
c1			3	2	2
c2			3	2	2
c3			3	2	2
c4			3	2	2
c5			3	2	2
c6			2	1	2
c7			3	2	2
c8			3	2	2
c9			3	2	2
c10			3	2	2

Stained slides were blinded and rated twice. Intensity of ABCA3 staining was rated as 0 no, 1 weak, 2 moderate 3 strong staining, ABCA3 aggregates as 0 no, 1 small, 2 big aggregates and ABCA3 staining pattern as 0 no, 1 diffuse, 2 ring-like staining.

Supplemental table 9

Chest X-rays (CXR) in patients with homozygous ABCA3 mutations

age range	≤ 0.1 years		0.1 – 1 years	
CXR finding	# of positive patients/total patients	mean score of finding in positive patients	# of positive patients/total patients	mean score of finding in positive patients
ground glass opacity	4/4	1.0	2/2	1.0
cysts	1/4	0.8	0/2	
bronchial wall thickening	4/4	0.8	2/2	1.3
peripheral bronchial dilatation	4/4*	0.6	2/2*	1.2
reticular pattern	4/4	1.4	2/2	1.8
hyperinflation	2/4	0.3	2/2	0.6
nodular pattern	0/4		1/2	0.4
lobe retraction	0/4		0/2	
consolidation	3/4	0.2	2/2	0.2
mean age at CXR (range [years])	0.02 (0.01-0.04)		0.3 (0.28-0.34)	
# of total investigations/age range	33		17	

CXRs over time with sufficient rating quality were available in 5 homozygous patients (IDs 6, 7, 17, 18, 20); CXRs were rated in six areas (apex to carina, carina to lower pulmonary vein, below pulmonary vein on left and right side) for the indicated findings as 0=none, 1= discrete, 2 = diffuse and 3 = very strong; mean patient values were grouped in the indicated age ranges; mean values were then calculated per finding

* radiological presentation resembles bronchiectasis as defined by the Fleischner society [15]

Supplemental table 10

Chest X-rays (CXR) in patients with compound heterozygous ABCA3 mutations

age range	≤ 0.1 years		0.1 - 1 years		≥ 1 year	
CXR finding	# of positive patients/total patients	mean score of finding in positive patients	# of positive patients/total patients	mean score of finding in positive patients	# of positive patients/total patients	mean score of finding in positive patients
ground glass opacity	4/4	1.4	5/5	1.7	3/3	0.9
cysts	0/4		3/5	0.3	3/3	0.2
bronchial wall thickening	4/4	1.0	5/5	1.2	3/3	1.3
peripheral bronchial dilatation	0/4		0/5		2/3*	0.5
reticular pattern	4/4	1.5	5/5	2.1	3/3	1.7
hyperinflation	2/4	0.3	4/5	1.1	3/3	0.9
nodular pattern	2/4	0.5	3/5	0.8	1/3	0.2
lobe retraction	0/4		0	0	0/3	
consolidation	2/4	0.1	3/5	0.2	3/3	0.3
mean age at CXR (range [years])	0.02 (0.01-0.04)		0.3 (0.13-0.53)		2.35 (1.58-3.5)	
# of total investigations/age range	27		18		36	

CXRs over time with sufficient rating quality were available in 7 compound heterozygous patients (IDs 23, 27, 28, 35, 36, 37, 40); CXRs were rated in six areas (apex to carina, carina to lower pulmonary vein, below pulmonary vein on left and right side) for the indicated findings as 0=none, 1= discrete, 2 = diffuse and 3 = very strong; mean patient values were grouped in the indicated age intervals; mean value were then calculated per finding

* radiological presentation resembles bronchiectasis as defined by the Fleischner society [15]

Supplemental table 11

Chest computed tomography (CT) in patients with homozygous and compound heterozygous ABCA3 mutations

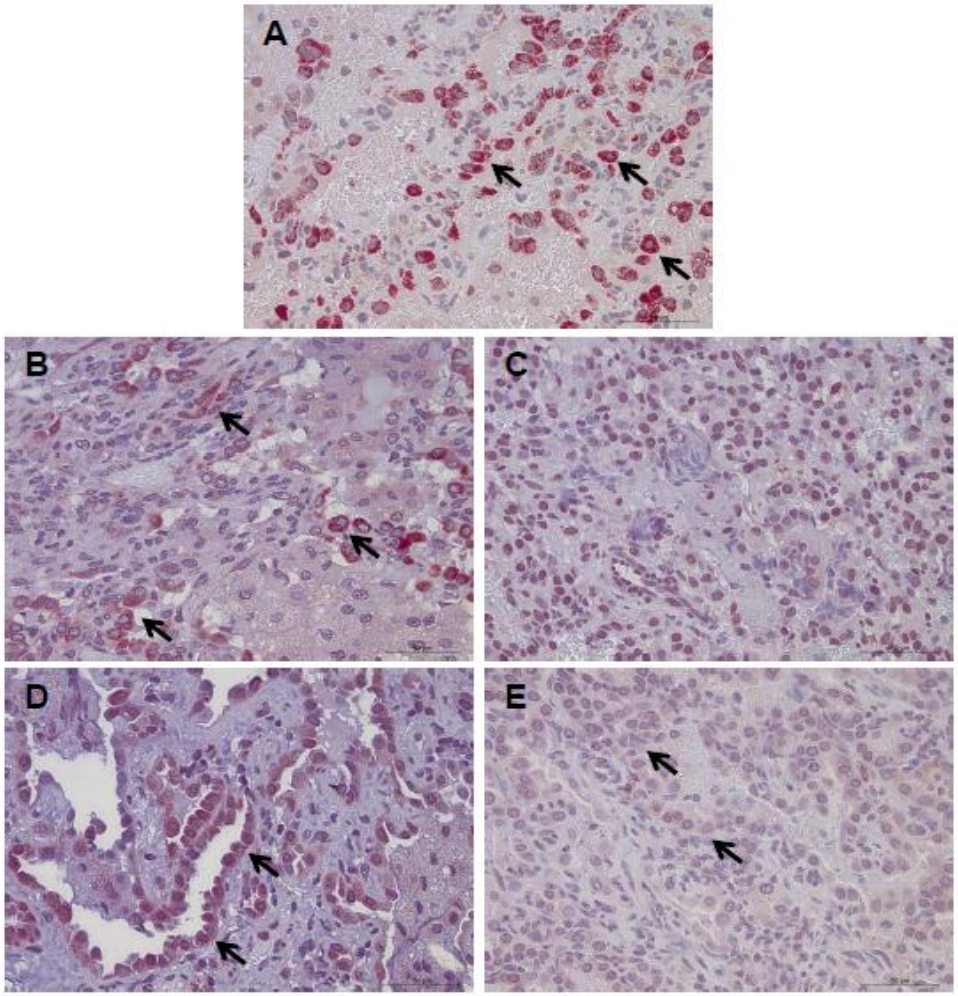
	homozygous		compound heterozygous					
age range	≤ 0.15 years		0.15 – 1 years		1-3 years		7 years	
CT finding	# of positive patients/ total patients	mean score of finding in positive patients	# of positive patients/ total patients	mean score of finding in positive patients	# of positive patients/ total patients	mean score of finding in positive patients	# of positive patients/ total patients	mean score of finding in positive patients
ground glass	6/6	2.8	2/2	1.4	2/2	1.3	1/1	1.3
cysts	4/6	0.7	2/2	1.2	1/2	0.2	1/1	1.2
bronchial wall thickening	2/6	0.6	2/2	0.5	2/2	0.8	1/1	0.7
peripheral bronchial dilatation	4/6*	1.8*	1/2*	0.3	0/2		1/1	0.7
honey combing	1/6	0.7	2/2	0.6	1/2	0.3	0/1	0
reticular pattern	6/6	2.1	2/2	2.1	2/2	0.7	1/1	2
paraseptal emphysema	0/6		1/2	0.8	0/2		1/1	1
nodular pattern	0/6		1/2	0.3	0/2		0/1	0
lobe retraction	0/6		0/2	0	0/2		0/1	0
consolidation	3/6	0.7	1/2	0.3	0/2		0/1	0
crazy paving	1/6	1.3	1/2	1.2	0/2		0/1	0

mean age at CT (range [years])	0.07 (0.03-0.13)	0.55 (0.48-0.62)	2.11 (1.58-2.63)	7.4

CCTs with sufficient rating quality were available in 6 homozygous patients (patients IDs 6, 8, 9, 14, 17, 21) and 4 compound heterozygous patients (patient IDs 23, 27, 32, 35). CTs were rated in six areas (apex to carina, carina to lower pulmonary vein, below pulmonary vein on left and right side) for the indicated findings as 0=none, 1= discrete, 2 = diffuse and 3 = very strong; the results were grouped in the indicated age intervals, mean values calculated per finding

* radiological presentation resembles bronchiectasis as defined by the Fleischner society [15]

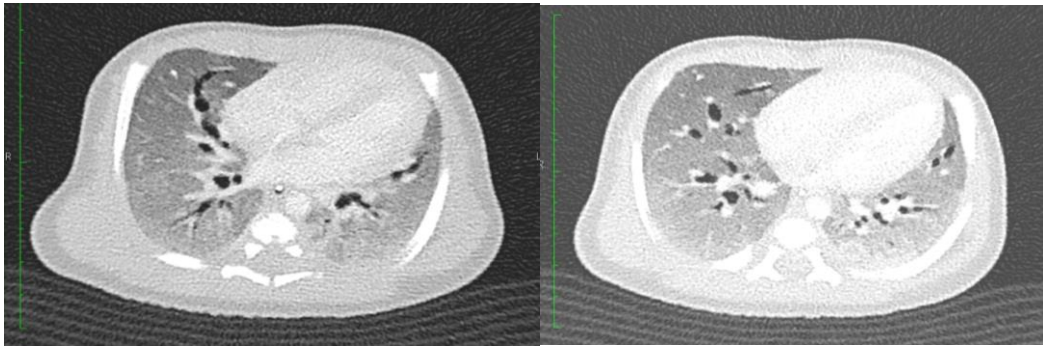
Supplemental figure 1:



Legend to supplemental figure 1:

Immunohistochemical staining of ABCA3 protein in lung tissues. A: Control tissue showing strong cytoplasmatic ring-like staining of ABCA3 protein in type II pneumocytes (arrow, patient c1). B: Tissue with homozygous other/other ABCA3 mutations displaying diffuse or/and almost absent staining of ABCA3 protein (arrow, patient 7). C: Tissue with homozygous null/null ABCA3 mutation showing no protein expression (arrow, patient 20). D: Tissue with compound heterozygous other/other ABCA3 mutations showing weak and diffuse staining of ABCA3 protein (arrow, patient 27). E: Tissue with compound heterozygous null/other ABCA3 mutations displaying diffuse and very weak staining of ABCA3 protein (arrow, patient 38). Scale: 50µm.

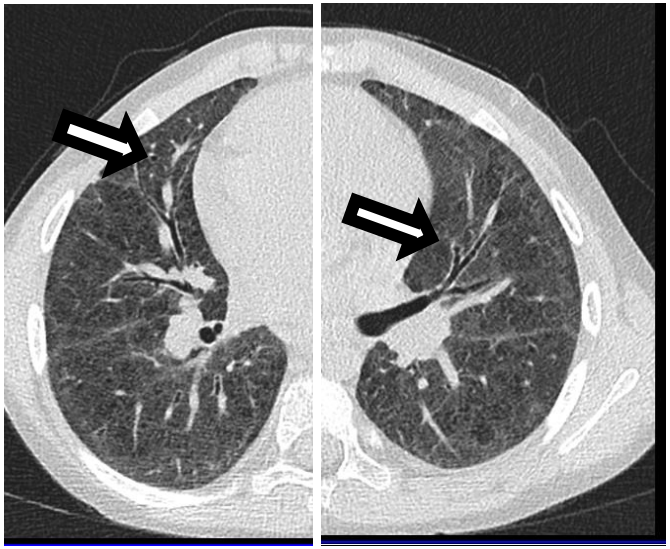
Supplemental figure 2:



Legend to supplemental figure 2:

CT of patient 6 (0.03 years): peripheral bronchial dilatation, dorsal consolidation, reticular pattern

Supplemental figure 3:



Legend to supplemental figure 3:

CT patient 32 (7.4 y): reticular pattern, beginning bronchiectasis (arrows)

Supplement A:

Clinical course of 5 patients with compound-heterozygous ABCA3 mutations and prolonged survival

Patient 27 presented at the age of 0.9 years with dry cough; at the age of one year he suffered two bronchopneumonias and has been since then oxygen dependent. His weight percentile was < 3. CXR exhibited lymphoreticular interstitial thickening and hilar lymphadenopathia. At age 1.4 years, he had cyanosis with excitement and subcostal retractions, at the age of 1.8 years discrete clubbing. A lung biopsy at the age of 1.5 years showed interstitial lung disease with combination of DIP, NSIP and PAP, focal intraalveolar cholesterol granulomas, pneumocyte type II hyperplasia. HRCT at the age of 1.8 showed diffuse ground glass, micronodular attenuations, interlobular and interlobar septal thickening and hilar lymphadenopathia. Treatment with systemic steroids yielded little effect; he was discharged with home oxygen. At the age of 4.5 years he died due to respiratory insufficiency.

Patient 28 presented at the age of 0.5 years with failure to thrive; at the age of one year she suffered cough attacks, cyanosis and clubbing, and repeated pneumonias. Surfactant analysis at the age of 2.9 years showed a SP-C deficiency. Lung biopsy at the age of 3 years showed NSIP and DIP and unusual SP-C staining, however, normal ABCA3 staining. At the age 3.5 years she was diagnosed with interstitial pneumonia, treatment with systemic steroids showed initial good improvement, and hydroxychloroquine moderate improvement. She was discharged home with oxygen, and died at 3.6 years due to RDS after a viral infection.

Patient 31 presented first at the age of 33.5 years with slight dyspnea and dry cough with strong exercise as a top athlete. CT demonstrated a reticular-nodular pattern. Diagnostic lavage and serum investigations demonstrated the typical findings of GMCSF autoantibody positive, adult autoimmune pulmonary alveolar proteinosis (PAP). She had 10 fold elevated levels of autoantibodies. Clinically and radiologically she responded well to two treatments with whole lung lavages (WLLs). However she had persistent impaired diffusion capacity for CO (about 60% of predicted), exercise intolerance and some ground glass opacities on CT scan over several years, whereas her PAP was in remission. We hypothesized that the patient is suffering from both, autoimmune PAP and DPLD due to the ABCA3 mutations.

Patient 32 first presented at the age of 2.5 years with a mycoplasm pneumonia, cyanosis and tachydyspnea. She exhibited finger clubbing. She has since then been steroid dependent and suffered recurrent respiratory infections. Due to gastro-esophageal reflux a Thal and a Nissen surgery was performed. Today, at the age of 5 years her SaO2 at rest is 88-90% and CXR reveals interstitial pulmonary fibrosis with ground glass and interstitial thickening. Treatment with steroid yields moderate improvement.

Patient 37 was born at 39 weeks, developed primary respiratory distress syndrome and was intubated on day 2. She was treated with systemic steroids and surfactant, and was severely ventilated, developed several pneumothoraces. Radiologically, she presented with ground glass and marked interstitial thickening. After a short period of extubation she was reintubated at the age of one month, again requiring HFO. Hydroxychloroquine was started at the age of one month with delayed but moderate improvement. She was in- and extubated several times, was oxygen dependent and lastly ventilated at the age of 5 months. Since then she has been hospitalized several times due to viral infections and was intermittently without oxygen. At the age of 4 years, her CXR shows only mild interstitial thickening. Today, at the age of 7.5 years she is still oxygen dependent, remains on hydroxychloroquine (4mg/kg/d), and attends school. Her FVC is

39%pred; FEV1 48%pred; SaO2 98% at rest, after exercise: 83-85%. HRCT today shows diffuse ground glass both sides and multiple cysts up to 2-3 cm. She has a PEG for failure to thrive.

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