Supplemental Information

ONLINE DATA SUPPLEMENT

Single *ABCA3* Mutations Increase Risk for Neonatal Respiratory Distress Syndrome

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METHODS

Referred Infant Cohort

The referred infant cohort consisted of 48 infants (≥34 weeks' gestation) referred for evaluation of an extreme respiratory phenotype (prolonged ventilator course and oxygen requirement, need for chronic pulmonary therapies, or death) to 2 of the authors (Drs Hamvas and Cole) from locations outside Washington University Medical Center. Their clinical characteristics are presented in the following table (Supplemental Table 5).

Next-Generation Sequencing

The goal was to optimize selection of significance thresholds for detection of rare variants in each sequencing run. We added a polymerase chain reaction–amplified 1934 base pair oligonucleotide from the single-stranded DNA M13mp18 vector backbone with no variation confirmed by using Sanger sequencing, and a 335 base pair, polymerase chain reaction–amplified product of a synthetically engineered pGEM T-Easy DNA vector library containing 15 known insertions, deletions, and substitutions mimicking a range of minor allele frequencies in the minor allele frequencies in the patient DNA pool with a majority of the mutations present at <1 allele per pool. To optimize pool size, the European-descent referred-infant samples were pooled with the European-descent RDS infant samples; similarly, the African-descent referred-infant samples were pooled with the African-descent RDS infant samples.

Rare Variant Selection and Validation

After aligning the individual raw reads to the annotated reference sequence consisting only of target regions, rare variants were identified with SPLINTER, a computational algorithm to detect rare variants. SPLINTER permits accurate detection and quantification of short insertions, deletions, and substitutions of up to 4 base pairs. Nonsynonymous variants were characterized as mutations if predicted by using both SIFT and PolyPhen to be "not tolerant" and "damaging," respectively. We also included 2 mutations that had known associations with pediatric respiratory disease (p.R288K [c.863G>A]) and an intronic mutation (c.3863-98 C>T) that results in a 50 amino acid insertion. All rare mutations were subjected to validation by using an independent genotyping strategy. Sequenom assays were initially used for validation of the pools in which the variant was detected. However, some Sequenom assays were unable to be designed for specific variants or could not be multiplexed efficiently. For these variants, as well as those that could not be reliably evaluated with the data obtained from the Sequenom assay, we designed a TaqMan assay. However, some TaqMan assays were unable to be designed for specific variants and were then validated by using Sanger resequencing. The following tables summarize the discovery and validation process for the disease-based and population-based cohorts.

SUPPLEMENTAL TABLE 5 Clinical Characteristics of Infants Referred for Extreme RDS Phenotype

Race	
African descent	8 (0.17)
European descent	40 (0.83)
Gender	
Female	19 (0.40)
Male	29 (0.60)
Birth weight, mean ± SD, kg	3.2 ± 0.5
Gestational age, mean ± SD, wk	38.2 ± 2.2
Route of delivery	
Vaginal	19 (0.39)
Cesarean	18 (0.38)
Unknown	11 (0.23)

SUPPLEMENTAL TABLE 6 Next-Generation Sequencing Primers

Gene	Exon	Forward Primer	Reverse Primer
CHPT1	1a	CTGTGTTCTCACACGAAAACCCCCA	CTGGGGGCAGGGAGAGGTGGA
	1b	GGTGAGTCCAGCCCGGCAGTC	GGGATAGTGGCGTGACAGTCTCCAG
	2+3	CATTGTGGACTTCTGTGACCCTCTACC	GGTGTAGCATTTATGAGTACTCCGACGA
	4	CCCTCTGCATATACCGTTACCTATGTGTGA	GGGAAATGAGGTTGTCTTCTTCTATGTGGC
	5	CCCCCTCACTACTGTACTTGGCTAGTCT	CCTTCCCCATTCTTGAACTTTGGCA
	6	CACCCTCAGGTTCTATGAAACCTTGGAA	CCTTCTCAAATTCACAACTGGGTAGC
	7	GCTACCCAGTTGTGAATTTGAGAAGG	TGAGTCACCACGCCTGGCCTAGACT
	8	TCCAGAGGACTACAGTGAGGTCCAA	TGATGATACAATGGGGGCTTCA
	9	CCTTCTATACTTGGTTTTCTACCTTTTGGG	TTCCATGAAACAGCAGCAGCAAGAG
LPCAT1	1	GCGGGAGGCGAGGCTTCCA	CCTCCCTGGCCCCAGCATC
	2	GCTGCCTGGTGTCCTACATGGATTC	GAAGGGAAGGACAGATGGGCTAGGG
	3	AGGAAGCCCGTGGCCTGGAC	TTCTCAGCAGCAGGGGGATCTCTCAC
	4	GTGCTGTTCCCGTTCCCTCAGTCAG	AGGAAGGGCCCCAGTTTCTTTCTCC
	5	GAGCGGAAATCAGTGTGTGCTTCCA	CCTCTAAGAACCCCAGCACACAGGA
	6	ATAGTCCAGGCATGGAGTGCCCTTG	GGCGCAGATAAAGGGTGTGGAGAGA
	7	TGGAGCTCTAGCACCCTCCTCCCTT	CTAACGGCTGTCCCACACCTGCTTT
	8	GGAGGTTTCACCCCAGAATTGGGAG	TCTGCATCAACCACTGTGAAGAGCG
	9	GGTGTTGCTGAGGTCAGCGTCTTGT	AGCACTCTGGGAGACACCCCTGACT
	10	ATGAATGTGTGACTGCCCCACCAAC	CGGCATCACGGGTTCTAGCTAATGC
	11	CGCACCTTCCAATTAGGAGAATGCCT	TCAAAAAGCAGGAGGTTTTGCCGAC
	12	CAGATGTTCCCGAGTGAGAGTGGGA	TGAACAATGGTGCTGACGTGACTGC
	13	ATGGCTCCACATGGAAGTTCGAGTC	CACGGAGACTCGCCCCACACTCT
	14	CACCAGGGCCGTGGGAAAAGAG	CCTGGAACTCGGGCTGAAGACAGTG
PCYT1B	1	GGGTGGAGGGTAGAGGGGAGAAAAA	GGATTAGGCGGTAGCGCTCTTCTCATT
	2	CTTCAGAGGAAGTTAGTGCCAAAGGA	GCCCGGCCCCTTTCTGATACTTTA
	3	TGGGATTCCTCATTCTGCAGTTCTGT	GGCAGTGAACAAGCGAGCCAGTATG
	4	GGCTGTGCTTCTTTTGTCCTGCTGA	TTTGGGCTTTCCGTAGTCTTGGCTG
	5	AGACATGCACATCCATCCCAGTGGT	ATACACTGCAGATGTCCCAGGGCAG
	6	CTGAGGGTGGAAGAGTCACTTGGGG	GTGGCATGGTGGTCTTCAGAGGGTT
	7	TGCCTTTCCATCAACCTAGCCCTTG	TGCAGTGGATCTGAGATAAGGGGCA
	8	TGGGCTCAGGAGTTTTGGGGTAGTG	TGGTCCCAAGACCTTCCCTTAGCAG
ABCA3	1	GGGGTGGACCGGGCACCTG	GCGGCACTCACCGAGCCTG
	2	CACTCAAACACCTTCCATCTGTCCAA	CAGGGCTGGGAGAGAAGGTCAGAAA
	3	CGTGCATCTTAACCTGGCTGATGGA	AAGGAACACAGACACTGAACCCAGA
	4+5	CGTGTTTCTATTGCGCACCCACACT	TTTACTCGCAGGCAGGCAGAGGTTT
	6	CCGTCTTTCATCTGCCAGTGACCTG	TGACTTGCAGGCAGGCAGAGGTTTA
	7	AGGGACCACTCAGTGTGACATTCCG	GGCTGGTAACACGAACCCTAACCGA
	8	TGAGCTGAAGTCACTCTGTTGCCCC	ACAGCGCGGTTTCTAGAGTGTTGGG
	9	CTGCTGGGACAGTCGGACTCAGG	CACCGAGAGGAGTGGGACATTGACA
	10	GGGCCCTCTTGGGAAGAACTTTGTG	CGCTGACTTTCCTCCTTCCAGTCCA
	11	GTGCTGGAGCTTGTGTCCCGTGTAG	ACAGGCTGGACAAGGCAAACACTCA
	12	GGGCCACTTTCCTGATGTGTCTTCC	GGTACTGGGGACACCTCTGCACTCA
	13+14	TTCTCCACAGCAGTGCCTCGAAGTC	CTGGCGCTGAGATGGTGTTAAAGGG
	15	GTGTCGTGGGTTTCTCCTCCCTGAC	GAGCACATCAGTGGAAACACCCCTG
	16	ATCTCCCTGCGTCCCCTGTCGTT	GGCTTGAGTCCTCCAAGGATGGTGA
	17	GACAAGGCCATCACCATCCTTGGAG	CTAGAAAAGGCCACCCCTGCCTGAT
	18	CTGGGGGATCAGCCAAAGATCTCAC	CCATGGGGATCCCATCTTGGATGTA
	19	ACTGTGCCTGGCCGAGGGG	AGCCCAGTCCTAGGTGGACGGAAAT
	20	TGCTCCCCTCAGTGCCTCTAACCAT	CTGCATGGGCTTACATGAGGCGTTT
	21	AGAGTCTGCACAGGTGACCCTGCC	GGCGAACTCTGGCTGCAGGACT
	22	CCGGTTCCTGACTGGCATTCAAAGT	TTGGGAGGGCAGACACAATGCTCTA
	23	GTGCTCCGTCCCTGACCTTCTCTGT	ACCCTCTCTGTCCCAGTTTCCCCTC
	24	AGGGGTCTGAGGACCTCCAAATGCT	CAGAGGGGCTGGTGAGCATGAACT
	25	CCCTCACTCCACACAGCACGGATAA	AAGGCGGTACAGAGGAACGCACCAG
	26	TCGAGAGGCAGCTGTGACCTACTGG	CTGAGGCCGTACAGTGGGAGACCAT
	27+28	CATGCGGTCTTTGTCCTGGTCAATC	CTTGTCTCGCTGTCCAGAGGCATGT
	29	TGTGTCCCTGTTCCAAGAGCTTCCA	GAGCGGTCACTCCCAGCTCTATGCT
	30	TTTCCAGGTGCACACACAGCTCCTT	CTCTGCACCAGATGCTGATGGGTCT
	31+32	ATCAGGAACAGCCTGATCGGAGAGC	AAAACCCCCAAACCAGCACGTATCA
	33a	CCCTATTGCCAGAGGACTCCCAGGT	TCACAGTCAGCAGCTTCCCTCCACT
	33b	CTGAGCTGGGGGTTGAATTTCTCCA	GCGTTGATCTAGCCGGCTTTTTCCT
SFTPC	1	TGGGAGGAGGCAAGGTAAGGGAAAG	GGGTCCCTGGCTTAGTGGTCTTGGT

SUPPLEMENTAL TABLE 6 Continued

Gene	Exon	Forward Primer	Reverse Primer
	2	GACAGCCCTGAGTCAGAAGCCATGA	GCCTCTTTCCTTCTAGCTGTGCCCC
	3+4	CAACCCAGCTCAGGCTTTTCCACAA	AGTCGTGCAGGGGAATAGGAGAGGG
	5	CCGAATGGTGGCTATTTGTCACCTG	GATGACCCCGCTTCAGTGGACG
	6	CGGTACTTCCCACTCCCCTGATTCTC	GCCCTGCAGGAAGACAGAAGCAGAC

SUPPLEMENTAL TABLE 7 European-Descent Disease-Based Cohort: Rare Variant Discovery and Validation

Gene	Synonymous Variants	Nonsynonymous Variants	Predicted Deleterious by SIFT or PolyPhen	Predicted Deleterious by SIFT and PolyPhen	Assay Design Failed	Confirmed by Independent Strategy
SFTPC	1	3	0	1	0	0
ABCA3	13	36	6	14	0	9
CHPT1	1	0	NA	NA	NA	NA
LPCAT	6	7	4	2	0	0
PCYT1B	2	3	2	0	0	0

SUPPLEMENTAL TABLE 8 European-Descent Population-Based Cohort: Rare Variant Discovery and Validation

Gene	Synonymous Variants	Nonsynonymous Variants	Predicted Deleterious by SIFT or PolyPhen	Predicted Deleterious by SIFT and PolyPhen	Assay Design Failed	Confirmed by Independent Strategy
SFTPC	6	8	4	1	1	1
ABCA3	23	52	19	15	1	8
CHPT1	4	8	0	1	NA	NA
LPCAT	7	14	3	4	2	1
PCYT1B	4	5	0	4	NA	NA

SUPPLEMENTAL TABLE 9 African-Descent Disease-Based Cohort: Rare Variant Discovery and Validation

Gene	Synonymous Variants	Nonsynonymous Variants	Predicted Deleterious by SIFT or PolyPhen	Predicted Deleterious by SIFT and PolyPhen	Assay Design Failed	Confirmed by Independent Strategy
SFTPC	1	4	1	2	0	0
ABCA3	8	34	9	7	0	3
CHPT1	0	0	NA	NA	NA	NA
LPCAT	9	13	5	1	1	0
PCYT1B	1	4	3	0	NA	NA

SUPPLEMENTAL TABLE 10 African-Descent Population-Based Cohort: Rare Variant Discovery and Validation

Gene	Synonymous Variants	Nonsynonymous Variants	Predicted Deleterious by SIFT or PolyPhen	Predicted Deleterious by SIFT and PolyPhen	Assay Design Failed	Confirmed by Independent Strategy
SFTPC	1	2	1	0	NA	NA
ABCA3	12	17	1	5	0	2
CHPT1	0	0	NA	NA	NA	NA
LPCAT	6	8	2	1	0	0
PCYT1B	0	2	0	0	NA	NA

SUPPLEMENTAL TABLE 11 Estimated Gestational Age and Birth Weight Among European-Descent Infants With and Without *ABCA3* Mutations

ABCA3 Mutation	European Descent, RDS			European Descent, Non-RDS		
	Present ($n = 16$)	Absent ($n = 96$)	*P* Value	Present ($n = 6$)	Absent ($n = 155$)	*P* Value
Estimated gestational age, mean ± SD, wk	37.6 ± 1.7	36.8 ± 1.7	.12	38.3 ± 2.1	38.2 ± 1.5	.92
Birth weight mean ± SD, kg	3.0 ± 0.6	3.1± 0.6	.75	3.3 ± 0.7	3.1 ± 0.7	.49

SUPPLEMENTAL TABLE 12 Disease Severity Measurements Among European-Descent RDS Infants With and Without *ABCA3* Mutations

ABCA3 Mutation	Present ($n = 16$)	Absent ($n = 96$)	*P* Value
Ventilation duration, mean ± SD, d	10.2 ± 11	10.5 ± 15	.93
Oxygen duration, mean ± SD, d	17.1 ± 16	17.4 ± 22	.95
Pneumothorax, *n* (%)	4 (0.25)	32 (0.33)	.58
ECMO, *n* (%)	1 (0.06)	5 (0.05)	1.0
Home oxygen, *n* (%)	2 (0.13)	10 (0.10)	.68
Death, *n* (%)	0	7 (0.07)	.59

ECMO, extracorporeal membrane oxygenation.

SUPPLEMENTAL TABLE 13 Validated Rare Mutations Among Referred Infants

Gene	Variant	European Descent, Referred RDS ($n = 40$)	African Descent, Referred RDS ($n = 8$)
ABCA3	L212M	1	0
	R280C	0	1
	R280H	1	0
	R288K	1	0
	E292V	3[a]	0
	G378R	1	0
	P933L	1[a]	0
	E1364K	1	0
	c.3863-98C>T	3	0
Infants with variant, *n* (%)		11 (27.5)	1 (12.5)

[a] Includes 1 compound heterozygous individual (p.E292V, p.P933L).