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Single Mutations in *ABCA3* Increase the Risk for Neonatal Respiratory Distress Syndrome in Late Preterm Infants (Gestational Age 34–36 Weeks)

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To the Editor

Respiratory distress syndrome (RDS) was the eighth leading cause of death in infants during 2010 in the United States [National Center for Health Statistics, 2013]. Respiratory disease, including RDS, is the most common cause of morbidity and mortality in preterm infants. RDS occurs due to pulmonary surfactant deficiency and a lack of structural maturation of the lungs [Moss, 2006]. Pulmonary surfactant is produced by alveolar type II cells and stored in lamellar bodies. When released via exocytosis, pulmonary surfactant decreases surface tension by forming a lipid layer that allows for the proper inflation of the lungs [Shulenin et al., 2004]. Twin studies and surfactant associated gene mutations strongly support a role for genetics, particularly in severe forms of RDS in term and near term infants [van Sonderen et al., 2002; Shulenin et al., 2004; Hallman et al., 2007; Levit et al., 2009; Wambach et al., 2010; Ryckman et al., 2012].

In a recent study, single *ABCA3* missense mutations were associated with increased risk for neonatal RDS in term and late preterm (gestational age (GA) greater than or equal to 34 weeks) Caucasian infants [Wambach et al., 2012]. The two most common mutations found by the study were rs117603931 (p.R288K) in exon 8 and rs149989682 (p.E292V) in exon 9, both of which have a minor allele frequency of less than 1% in unaffected controls [Database of Single Nucleotide Polymorphisms (dbSNP), 2013].

We sought to follow up this study by strictly analyzing the coding exons for rs117603931 (p.R288K) in exon 8 and rs149989682 (p.E292V) in exon 9 of *ABCA3* in 224 Caucasian preterm infants ranging in GA from 29 to 36 weeks (Table I) with RDS. We then compare these frequencies to existing control and population based data. African-Americans were excluded due to a lack of sufficient sample size. DNA was extracted from cord blood or buccal swabs taken from the infant. Demographic information was obtained via an interview with the mother and by a review of medical records. All samples were collected with signed consent from family members and had IRB approval. Gestational age was estimated using the first day of the last menstrual period and checked against an obstetric exam and prenatal

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ultrasound. Individuals were excluded for congenital anomalies, sepsis, and pulmonary hypoplasia. One twin was randomly excluded where appropriate. RDS was defined via a chest radiograph and supplemental oxygen requirement for 2 or more hours.

Primers were designed for the aforementioned mutations in exon 8 and exon 9 using the UCSC Genome Browser (genome.ucsc.edu) and Primer3 (biotools.umassmed.edu/bioapps/primer3_www.cgi) with Sanger sequencing performed at Functional Biosciences (Madison, WI). The results were analyzed using PHRED, PHRAP, POLYPHRED, and CONSED (University of Washington, Seattle, WA). The R programming language (<http://www.r-project.org/>) with the package `exact2x2` was used to perform Fisher exact tests, odds ratios, and Student t-tests.

In total, 10 mutations were found (6 at rs117603931 in exon 8 and 4 at rs149989682 in exon 9, combined allele frequency of 2.5% and all but one were present as heterozygotes. Due to the nature of anonymized samples, we do not have additional clinical information on the homozygote). Five mutations were found within GA 34–36, and the other 5 mutations were within GA 29–33 group. The distribution of gender ($p = 0.57$) and mode of delivery ($p = 0.64$) was similar across the two studies (Table I). The GA ($p < 0.0001$) and birth weight ($p < 0.0001$), however, were different (this also holds true for subsets of our RDS data as shown in Table I). This is most likely due to the inclusion of term infants in the Wambach et al. data (when our RDS data is broken down into GA 29–33 and GA 34–36, both GA and birth weight are still statistically significantly different than the Wambach et al. data (i.e. p -value < 0.0001) for both GA and birth weight as shown in Table I).

A Fisher exact test between our RDS data for GA 34–36 versus a control sample (data from the Exome Variant Server (evs.gs.washington.edu/evs), the 1000 Genome Project (browser.1000genomes.org), the Wambach et al. non-RDS controls, and the Wambach et al. Missouri population controls) resulted in a p -value = 0.04, OR = 0.36 (0.15, 0.93) (Table II). When combined with the RDS data from Wambach et al. the p -value became more significant (p -value < 0.0001 , OR = 0.21 (0.13, 0.36), Table II). Furthermore, a Fisher exact test between our RDS data for GA 34–36 and the Wambach et al. RDS data was not significant (p -value = 0.14, OR = 2.33 (0.80, 7.05), Table II).

To increase our modest sample size ($n = 94$), we included 130 infants with GA 29–33 (for a total $n = 224$). The Fisher exact test between these individuals (our RDS sample for GA 29–33) and the Wambach et al. RDS data was significantly different (p -value = 0.03, OR = 3.30 (1.12, 9.84), Table II). The same is true when comparing our combined RDS data (both GA 29–33 and GA 34–36) to the Wambach et al. RDS data (p -value = 0.02, OR = 2.80 (1.18, 6.60), Table II). The lower frequency of mutations in the GA 29–33 population is the main driver behind the statistically significant difference between both our GA 29–33 data versus the Wambach et al. RDS data and between our combined GA 29–36 data versus the Wambach et al. RDS data.

In conclusion, our GA 34–36 data replicates the Wambach et al. findings while that of the GA 29–33 does not. A possible explanation may be that both studies have modest sample sizes. An additional factor could be that the Wambach et al. paper suffers from the winner's

curse phenomenon. More specifically, given their modest sample size and stringent requirements for significance, they may have arrived at a larger effect size than other studies because their sample contained more mutations than would be expected in the general population. Or perhaps the pathophysiology of RDS differs when it occurs in early versus late preterm infants. In either case, we believe that our study replicates their findings and that further studies with increased sample sizes could further validate their results.

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Comparison of characteristics between the two studies.

Table 1

Characteristic	Wambach et. al. RDS sample	Wambach et. al. non-RDS (control) sample	P-value (comparison between columns 2 & 3)	Our RDS sample (GA 29–36 weeks)	P-value (comparison between columns 2 & 5)	P-value (comparison between columns 3 & 5)	P-value (comparison between columns 2 & 6)	P-value (comparison between columns 3 & 6)	P-value (comparison between columns 2 & 7)	P-value (comparison between columns 3 & 7)
Sample size (n)	112	161		224	94	130				
Gender			0.51				0.56	1.0	0.89	0.43
Female	47 (42%)	74 (46%)		103 (46%)	38 (40%)	65 (50%)				
Male	65 (58%)	87 (54%)		121 (54%)	56 (60%)	65 (50%)				
GA (mean +/- SD, wk)	37.0 +/- 1.7	38.2 +/- 1.6	<0.001	32.7 +/- 2.2	34.9 +/- 0.8	31.2 +/- 1.4	<0.0001	<0.0001	<0.0001	<0.0001
BW (mean +/- SD, kg)	3.1 +/- 0.6	3.1 +/- 0.7	0.37	2.1 +/- 0.6	2.6 +/- 0.45	1.7 +/- 0.37	<0.0001	<0.0001	<0.0001	<0.0001
Route of Delivery			0.54				0.64	0.21	0.09	0.014
Vaginal	55 (49%)	73 (45%)		117 (52%)	58 (62%)	59 (45%)				
Cesarean	57 (51%)	88 (55%)		107 (48%)	36 (38%)	71 (55%)				

RDS = respiratory distress syndrome, GA = gestational age, BW = birth weight

Table II

P-values from Fisher Exact Tests.

Fisher Exact Test		A	B	P-value	Odds Ratio (95% Confidence Interval)
All * control data (A) vs. our RDS data (GA 34–36) (B)	# individuals with mutations	196	5	0.04	0.36 (0.15, 0.93)
	# individuals without mutations	9791	89		
Wambach et. al. RDS data + our RDS data (GA 34–36) (A) vs. All * control data (B)	# individuals with mutations	18	196	<0.0001	0.21 (0.13, 0.36)
	# individuals without mutations	188	9791		
Wambach et. al. RDS data (A) vs. our RDS data (GA 34–36) (B)	# individuals with mutations	13	5	0.14	2.33 (0.80, 7.05)
	# individuals without mutations	99	89		
Wambach et. al. RDS data (A) vs. our RDS data (GA 29–33) (B)	# individuals with mutations	13	5	0.03	3.3 (1.12, 9.84)
	# individuals without mutations	99	125		
Wambach et. al. RDS data (A) vs. our RDS data (B)	# individuals with mutations	13	10	0.02	2.80 (1.18, 6.6)
	# individuals without mutations	99	214		
All * control data (A) vs. our RDS data (B)	# individuals with mutations	196	10	0.02	0.43 (0.22, 0.84)
	# individuals without mutations	9791	214		
All * control data (A) vs. our RDS data (GA 29–33) (B)	# individuals with mutations	196	5	0.12	0.50 (0.20, 1.30)
	# individuals without mutations	9791	125		
Wambach et. al. control data (A) vs. our RDS data (B)	# individuals with mutations	18	10	0.02	0.38 (0.16, 0.88)
	# individuals without mutations	1014	214		
Wambach et. al. control data (A) vs. our RDS data (GA 34–36) (B)	# individuals with mutations	18	5	0.04	0.32 (0.11, 0.91)
	# individuals without mutations	1014	89		
Wambach et. al. control data (A) vs. our RDS data (GA 29–33) (B)	# individuals with mutations	18	5	0.17	0.44 (0.16, 1.27)
	# individuals without mutations	1014	125		

* All controls: Exome Variant Server data, 1000 Human Genome data, Wambach et. al. non-RDS controls, Wambach et. al. Missouri population controls