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Genetic Factors Contribute to Risk for Neonatal Respiratory Distress Syndrome among Moderately Preterm, Late Preterm, and Term Infants

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Abstract

Objective—To determine the genetic contribution to risk for respiratory distress syndrome (RDS) among moderately preterm, late preterm, and term infants (estimated gestational age ≥ 32 weeks) of African and European-descent.

Study Design—We reviewed clinical records for 524 consecutive twin pairs ≥ 32 weeks gestation. We identified pairs in which at least 1 twin had RDS ($n=225$) and compared the concordance of RDS between monozygotic (MZ) and dizygotic twins (DZ). Using mixed effects logistic regression, we identified covariates that increased disease risk. We performed additive genetic, common environmental, and residual effects modeling to estimate genetic variance and used the ratio of genetic variance to total variance to estimate genetic contribution to RDS disease risk.

Results—Monozygotic twins were more concordant for RDS than dizygotic twins ($p=0.0040$). Estimated gestational age, European-descent, male sex, delivery by cesarean, and five minute Apgar score each independently increased risk for RDS. After adjusting for these covariates, genetic effects accounted for 58% ($p=0.0002$) of the RDS disease risk variance for all twin pairs.

Conclusions—In addition to environmental factors, genetic factors may contribute to RDS risk among moderately preterm, late preterm, and term infants. Discovery of risk alleles may be important for prediction and management of RDS risk.

Keywords

RDS; twins

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Respiratory distress syndrome (RDS) remains a leading cause of neonatal morbidity and mortality in the United States.^{1,2} RDS results from a deficiency of pulmonary surfactant, a phospholipid-protein complex that reduces surface tension and maintains alveolar expansion at end expiration. Pulmonary surfactant is synthesized exclusively by type II alveolar epithelial cells, and production increases with advancing gestational age.^{3,4} Historically, neonatal RDS has been attributed to a developmentally-regulated deficiency of surfactant, with an inverse correlation between RDS incidence and gestational age.^{5,6} However, studies demonstrating varying rates of RDS by ethnicity and sex,^{6–8} the persistence of racial disparities in disease risk despite widespread surfactant replacement therapy,^{1,9} the identification of biallelic or dominant mutations in surfactant protein-associated genes among infants with progressive RDS,^{10–12} and the contributions of monoallelic mutations in surfactant-associated genes to risk for reversible RDS¹³ suggest genetic mechanisms contribute to disease risk. Prior studies have demonstrated increased concordance for RDS among monozygotic (MZ) twins as compared with dizygotic (DZ) twins.^{14–16} However, these studies focused on premature infants (< 32–34 weeks gestation) at higher risk for concordance due to developmental deficiency of surfactant production.

In addition to gestational age, factors associated with RDS risk include maternal characteristics (age,¹⁷ assisted reproduction,¹⁸ diabetes¹⁹), intrapartum factors (antenatal steroids,²⁰ preeclampsia,²¹ delivery mode,^{22–25} chorioamnionitis²⁶), and neonatal characteristics including immature lung structure, sex,⁶ ethnicity,^{6–8} birth order,^{15, 27–31} and 5 minute Apgar score.¹⁶

To determine the genetic contribution to RDS disease risk, we focused on moderately preterm, late preterm, and term twin pairs (> 32 weeks gestation) with lower risk for developmental causes of RDS, and who may be enriched for RDS disease risk alleles. We compared concordance rates between MZ and DZ twins, performed statistical modeling to account for RDS risk factors, and used the ratio of genetic variance to total variance to estimate genetic disease risk.

Methods

We retrospectively reviewed clinical records for consecutive twin pairs > 32 weeks estimated gestation born between January 1, 2000, to April 25, 2014, and admitted to the Barnes-Jewish Hospital Special Care Nursery in St. Louis, Missouri. We defined RDS as need for supplemental oxygen ($\text{FiO}_2 > 0.3$) or continuous positive airway pressure or mechanical ventilation within the first 24 hours of life, need for respiratory support for greater than 24 hours, and chest radiograph consistent with respiratory distress syndrome.^{13, 32} Gestational age was based on best obstetrical estimate.³³ We excluded twin pairs with documented systemic infection, chromosomal anomalies, and congenital cardiopulmonary anomalies that could contribute to respiratory distress. Zygosity of the twin pairs was assigned based on discordant infant sex, discordant infant blood type, DNA samples when available, placental histology, gross placental examination, and/or prenatal ultrasound records.¹⁶ We did not include infants from gestations with more than 2 fetuses (triplets, etc). The Washington

University School of Medicine Human Research Protection Office reviewed and approved this study.

We collected pertinent clinical data from the infant and maternal records for all twin pairs. A full course of antenatal steroids was defined as 2 doses given 24 hours apart with the first dose given at least 48 hours prior to delivery. Premature rupture of membranes was defined as rupture of membranes prior to onset of labor. Prolonged rupture of membranes was defined as rupture of membranes more than 18 hours prior to delivery. Chorioamnionitis was based on histopathological examination of the placenta.¹⁶ Small for gestational age (SGA) status was defined as birth weight less than the tenth percentile according to standard premature infant growth charts.³⁴ Birth weight discordance was defined as $\geq 25\%$ discordance based on weight of the heavier twin.³⁵

Statistical analyses

Statistical analyses were performed using SAS® 9.2, Cary, NC. Demographic data were analyzed using Student's t-test or Chi-square. To determine concordance for RDS phenotype, we selected all twin pairs in whom at least 1 infant had RDS (n=225 pairs) and performed Chi-square analyses. Mixed effects logistic regression (MELR) was performed to identify clinical covariates that increased RDS risk. We used race-stratified additive genetic, common environmental, and residual effects (ACE) modeling to identify the impact of genetic factors on RDS risk.³⁶ We used covariates that were determined to be statistically significant with MELR in the ACE models. We determined genetic contribution to disease risk using the ratio of genetic variance to the total variance.¹⁶

Results

We identified 524 twin infant pairs, which included 212 MZ infants and 836 DZ infants (Table I). Zygosity of the twin pairs was assigned based on discordant infant sex (n=181 pairs), discordant infant blood type (n=80 pairs), DNA samples (n=4 pairs) placental histology (n=247 pairs), gross placental examination (n=2 pairs), and/ or prenatal ultrasound records (n=10 pairs). Most infants were of African or European-descent (Table I). MZ twins were more frequently observed among European-descent twins (25% v. 14%, $p<0.0001$) and more of the MZ twins were female (58% MZ v. 46% DZ, $p=0.0018$). There were no differences in estimated gestational age, birth weight, SGA status, birth weight discordance, delivery mode, 5 minute Apgar score, or incidence of RDS between the MZ and DZ twins. Mothers of DZ twins were slightly older than mothers of MZ twins (27.8 years v. 26.8 years, $p=0.032$), and DZ twins were more likely to have been conceived by assisted reproductive techniques (12% DZ v. 6% MZ, $p=0.012$). Although mothers of DZ twins were more likely to have had preeclampsia (27% DZ v. 19% MZ, $p=0.018$), there were no differences in maternal diabetes mellitus, antenatal steroid exposure, premature or prolonged rupture of membranes, maternal antibiotic exposure, or chorioamnionitis between the twin groups.

Using all twin pairs, MZ twins were more likely to be concordant for RDS phenotype than DZ twins ($p=0.0040$; Table II). We performed race-stratified analyses and found that European-descent MZ twins were more likely to be concordant for RDS than DZ twins (73% MZ v. 51% DZ, $p=0.031$; Table II). Even though more MZ twins of African-descent

were concordant for RDS than DZ twins, this did not reach statistical significance (70% MZ v. 39% DZ, $p=0.089$; Table II).

We performed mixed effects logistic regression (MELR) and found that race (European-descent), male sex, estimated gestational age, cesarean delivery, and Apgar score <7 at 5 minutes of life were significant predictors for RDS (Table III). Although the second-born twin was more likely to develop RDS than the first-born twin among the 111 twin pairs discordant for RDS (58% v. 42%, $p=0.023$), birth order was not statistically associated with RDS risk among all twin pairs ($n=524$) (Table III and IV; Table IV available at www.jpeds.com).

As male sex was significantly associated with RDS risk (Table III), and could confound concordance analyses among sex discordant twin pairs, we repeated concordance analyses using only same sex twin pairs. Using twins of all races, we found that same sex MZ twins were more likely to be concordant for RDS than same sex DZ twins (70% MZ v. 48%, $p=0.013$) (Table V; available at www.jpeds.com). We performed race-stratified analyses of same sex twins of European and African- descent. Among same sex twins of European-descent, MZ twins were more concordant for RDS than DZ twins (73% MZ v. 50% DZ, $p=0.042$, Table 5; online). Among the same sex African- descent twins, more of the MZ twins were concordant for RDS than DZ twins, however, this did not reach statistical significance (70% MZ v. 42% DZ, $p=0.16$) (Table V).

Using all twin pairs and accounting for significant covariates identified by MELR, we performed additive genetic, common environmental, and residual effects (ACE) modeling and determined that genetic effects accounted for 58% ($p=0.0002$) of the variance in RDS disease risk. By performing race-stratified analyses, we found that genetic effects accounted for 61% ($p=0.035$) of the variance in risk for RDS among European-descent infants and 72% ($p<0.0001$) of the variance among African-descent infants.

Discussion

Several previous studies have compared concordance rates for RDS among MZ and DZ twin pairs. Myrianthopoulos et al in 1971 compared 46 twin pairs and demonstrated increased concordance for RDS among MZ twins (85% MZ v. 44% DZ).¹⁴ Van Sonderen et al compared concordance rates for 80 premature (30–34 week) twin pairs and also found increased concordance for RDS among MZ twins (67% v. 29%).¹⁵ In contrast, using 13 years of data obtained from the Finnish Medical Birth Register, Marttila et al found similar concordance rates for RDS among same-sex and opposite-sex twin pairs ($n=638$ pairs), suggesting that environmental factors predominate over genetic effects for risk of RDS.³⁰ Levit et al studied 332 premature (< 32 weeks) twin pairs and found that MZ twins were more concordant for RDS and estimated that 49.7% of the variance in risk for RDS was due to genetic factors.¹⁶

As the incidence of RDS decreases dramatically after 30 weeks gestation,⁵ we included infants < 32 weeks (mean gestational age of 34 weeks, range 32–39 weeks) due to their lower risk for confounding developmental causes of RDS (Table VI; available at www.jpeds.com).

Similar to previous studies,^{14–16} we found that MZ twins were more likely to be concordant for RDS than DZ twins and that European-descent, male sex, estimated gestational age, cesarean delivery, and low 5 minute Apgar score were independent predictors of RDS risk.^{6–8, 16, 22–24} Among the 111 twin pairs who were discordant for RDS, similar to other groups,^{15, 27–30} we found that the second-born twin was more likely to develop RDS than the first-born twin (Table IV). We did not find differences in other clinical variables including sex, birth weight, delivery mode, or low 5 minute Apgar score between the twins discordant for RDS (Table IV).

Although more of the DZ twins were of African-descent with a lower incidence of RDS at each gestational age interval (Table VI), DZ twins were also more likely to be male and conceived via assisted reproduction, and their mothers were slightly older and more likely to have had preeclampsia, risk factors previously associated with neonatal RDS.^{6, 17, 18, 21} However, there was no difference in overall incidence of RDS or RDS severity between MZ and DZ twins (37% MZ v. 31% DZ, $p=0.12$), and zygosity was not a significant predictor of RDS status in our regression analysis ($p=1.00$) Table I, III, and VII; Table VII available at www.jpeds.com).

Although our study includes a large number of African-descent twin pairs ($n=293$), comparison of RDS concordance among African-descent MZ and DZ twin pairs did not reach statistical significance (70% MZ v. 39% DZ, $p=0.089$; Table II). Statistical power may have been limited due to fewer MZ African-descent twin pairs (10 MZ African-descent v. 30 MZ European-descent twin pairs), the lower incidence of RDS among African-descent infants at all gestational age intervals (Table VI), or the effects of other maternal, intrapartum, or neonatal factors not accounted for in the concordance analyses. However, using race-stratified ACE modeling that accounted for significant covariates, we determined that genetic factors contribute to RDS risk among African-descent infants (72%, $p<0.0001$).

There are several important limitations to our study. First, we assigned race based on maternal self-identification rather than genetic ancestry, as this study was retrospective and DNA samples were not available for all infants. Second, zygosity was assigned based on available data including infants' sex and blood types, DNA samples when available, placental examination, and prenatal ultrasound records, and it is possible that in some cases zygosity was incorrectly assigned. For example, we may have included dichorionic MZ twins within the dizygotic comparison groups. However, as this was a retrospective study, and DNA samples and sequence data are not available for most infants, we are not able to further delineate zygosity. Third, our definition of RDS included infants who required respiratory support for greater than 24 hours and whose chest radiograph was consistent with surfactant deficiency; however, it is possible that we could be including infants with transient tachypnea of the newborn. Of note, we did not find an association with birth weight and RDS risk (Table III), possibly related to the diverse factors that contribute to birth weight at later gestational ages.³⁷ We did not find an association with maternal antenatal steroid exposure and risk for RDS (Table III). Approximately one-half of the infants in the study were exposed to antenatal corticosteroids and the effects of antenatal steroids at later gestational ages are not well characterized.²⁰

Our data suggest that in addition to environmental factors, genetic factors contribute to RDS risk in moderately preterm, late preterm, and term infants. Discovery of risk alleles may provide insight about novel RDS disease mechanisms and may be important for prediction and management of RDS. For example, stratification of genetic disease risk may identify late preterm and term fetuses likely to respond to antenatal glucocorticoids. Multiple investigators have sought to determine the specific genes that contribute to risk for neonatal RDS in premature and term infants. Earlier studies of common variants within surfactant-associated candidate genes identified modest statistical associations with risk for neonatal RDS.^{38–43} However, common variants are likely to have smaller effect sizes than rare variants and likely to encounter significant selection pressure.^{44, 45} In previous work, we and others reported that rare, monoallelic, deleterious variants in the ATP-binding cassette transporter A3 gene (*ABCA3*) are associated with increased risk for reversible RDS among late preterm and term infants of European-descent.^{13, 46} Next generation exome and whole genome sequencing will permit identification of additional variants that contribute to heritability of neonatal RDS.⁴⁷

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Abbreviations

ACE	additive genetic, common environment, and residual effects
AD	African-descent
ED	European-descent
DZ	dizygotic
MELR	mixed effects logistic regression
MZ	monozygotic
RDS	respiratory distress syndrome

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Table 1

Demographic Data for Monozygotic and Dizygotic Twins

	Monozygotic Twins (MZ) N=212	Dizygotic Twins (DZ) N=836	p-value
Maternal Race or Ethnicity			
European-descent	104 (0.49)	306 (0.36)	<0.0001
African-descent	84 (0.39)	502 (0.60)	
Hispanic	14 (0.07)	16 (0.02)	
Asian	10 (0.05)	6 (0.01)	
Others	0	6 (0.01)	
Sex			
Male	90 (0.42)	455 (0.54)	0.0018
Female	122 (0.58)	381 (0.46)	
Estimated Gestational Age (mean weeks $\pm$ SD)	34.4 $\pm$ 11.7	34.6 $\pm$ 1.7	0.19
Birth Weight (mean kg $\pm$ SD)	2.1 $\pm$ 0.5	2.1 $\pm$ 0.4	0.31
Small for Gestational Age (SGA)	35 (0.17)	137 (0.16)	0.97
Birth Weight Discordance ($\geq$ 25%)	26 (0.12)	86 (0.10)	0.41
Delivery Mode			
Vaginal	58 (0.27)	222 (0.27)	0.81
Cesarean	154 (0.73)	614 (0.73)	
Apgar <7 at 5 minutes	9 (0.04)	50 (0.06)	0.33
Respiratory Distress Syndrome	78 (0.37)	261 (0.31)	0.12
Maternal Age (mean years $\pm$ SD)	26.8 $\pm$ 5.3	27.8 $\pm$ 6.2	0.032
Assisted Reproduction			
Any	12 (0.06)	96 (0.12)	0.012
In vitro Fertilization	10 (0.05)	88 (0.11)	0.0087
Maternal Diabetes Mellitus	16 (0.08)	88 (0.11)	0.20
Antenatal Steroids			
Any	122 (0.58)	422 (0.51)	0.076
Full course/ 2 doses	100 (0.47)	346 (0.41)	0.68
>1 week prior to delivery	56 (0.26)	214 (0.26)	0.30
Preeclampsia	40 (0.19)	224 (0.27)	0.018
Premature Rupture of Membranes	28 (0.13)	108 (0.13)	0.91
Prolonged Rupture of Membranes	12 (0.06)	60 (0.07)	0.44

	Monozygotic Twins (MZ) N=212	Dizygotic Twins (DZ) N=836	p-value
Maternal Antibiotics	72 (0.34)	292 (0.35)	0.79
Chorioamnionitis	2 (0.01)	20 (0.02)	0.28

Table 2

Concordance of RDS among Monozygotic and Dizygotic Twins

Concordance of RDS among Same Sex Monozygotic and Dizygotic Twins, All Races*				
	Both have RDS	One has RDS	Total	p-value
Monozygotic (MZ)	32	14	46	0.0040
Dizygotic (DZ)	82	97	179	
Total	114	111	225	
Concordance of RDS among Same Sex Monozygotic and Dizygotic Twins, European-descent				
MZ	22	8	30	0.031
DZ	42	41	83	
Total	64	49	113	
Concordance of RDS among Same Sex Monozygotic and Dizygotic Twins, African-descent				
MZ	7	3	10	0.089
DZ	34	54	88	
Total	41	57	98	

* Includes European-descent, African-descent, Hispanic, Asian, and other infants

Table 3

Mixed Effects Logistic Regression for RDS (n=1048)

	Odds Ratio Estimate	95% CI	p-value
Race (European-descent vs. African-Descent)	3.13	2.17–4.35	0.0008
Sex (Female vs. Male)	0.66	0.49–0.90	0.0090
Estimated Gestational Age (mean weeks $\pm$ SD)	0.58	0.49–0.68	<0.0001
Birth Weight (mean kg $\pm$ SD)	1.00	1.000–1.001	0.79
Small for Gestational Age (Y v. N)	0.92	0.51–1.69	0.80
Birth Weight Discordance ($\geq$ 25%, Y v. N)	0.84	0.51–1.39	0.50
Birth Order (1 v. 2)	0.83	0.62–1.12	0.22
Delivery Mode (cesarean vs. vaginal)	1.51	1.05–2.16	0.025
Zygosity (DZ v. MZ)	1.00	0.69–1.45	1.00
Apgar <7 at 5 minutes (Y v. N)	3.73	1.97–7.04	<0.0001
Maternal Age (mean years $\pm$ SD)	0.99	0.96–1.02	0.51
Assisted Reproduction			
Any	0.41	0.074–2.25	0.30
In vitro Fertilization	2.54	0.44–14.71	0.30
Maternal Diabetes Mellitus	1.00	0.61–1.64	0.99
Antenatal Steroids (Any)	1.05	0.73–1.50	0.80
Preeclampsia	0.99	0.70–1.39	0.95
Premature Rupture of Membranes	0.98	0.55–1.76	0.95
Prolonged Rupture of Membranes	0.55	0.25–1.22	0.14
Maternal Antibiotics	1.07	0.77–1.48	0.70
Chorioamnionitis	0.63	0.22–1.81	0.39

Table 4

Clinical Data for Twins Discordant for RDS

	No RDS n=111	RDS n=111	p-value
Sex			
Male	53 (0.48)	65 (0.59)	0.11
Female	58 (0.52)	46 (0.41)	
Birth Weight (mean kg $\pm$ SD)	2.1 $\pm$ 0.5	2.2 $\pm$ 0.5	0.057
Birth Order			
First	64 (0.58)	47 (0.42)	0.023
Second	47 (0.42)	64 (0.58)	
Delivery Mode			
Vaginal	34 (0.31)	32 (0.29)	0.77
Cesarean	77 (0.69)	79 (0.71)	
Apgar 5 minutes			
Less than 7	3 (0.03)	9 (0.08)	0.13
Greater or equal to 7	107 (0.96)	101 (0.91)	
Unknown	1 (0.01)	1 (0.01)	

Table 5

Concordance of RDS among Same Sex Monozygotic and Dizygotic Twins

Concordance of RDS among Same Sex Monozygotic and Dizygotic Twins, All Races*				
	Both have RDS	One has RDS	Total	p-value
Monozygotic (MZ)	32	14	46	0.013
Dizygotic (DZ)	48	53	101	
Total	80	67	147	
Concordance of RDS among Same Sex Monozygotic and Dizygotic Twins, European-descent				
MZ	22	8	30	0.042
DZ	24	24	48	
Total	46	32	78	
Concordance of RDS among Same Sex Monozygotic and Dizygotic Twins, African-descent				
MZ	7	3	10	0.16
DZ	20	28	48	
Total	27	31	58	

* Includes European-descent, African-descent, Hispanic, Asian, and other infants

Table 6

Incidence of RDS by Estimated Gestational Age

Incidence of RDS by Estimated Gestational Age, All Races*		
Estimated Gestational Age	No RDS n=709	RDS n=339
32 weeks	54 (0.38)	88 (0.62)
33 weeks	86 (0.54)	74 (0.46)
34 weeks	168 (0.65)	90 (0.35)
35 weeks	138 (0.75)	46 (0.25)
36 weeks	117 (0.82)	25 (0.18)
37 weeks	99 (0.88)	13 (0.12)
38 weeks	37 (0.93)	3 (0.07)
39 weeks	10 (1.0)	0 (0)
Incidence of RDS by Estimated Gestational Age, European-descent		
32 weeks	14 (0.24)	44 (0.76)
33 weeks	23 (0.36)	41 (0.64)
34 weeks	59 (0.55)	49 (0.45)
35 weeks	52 (0.68)	24 (0.32)
36 weeks	39 (0.81)	9 (0.19)
37 weeks	33 (0.82)	7 (0.18)
38 weeks	11 (0.79)	3 (0.21)
39 weeks	2 (1.0)	0 (0)
Incidence of RDS by Estimated Gestational Age, African-descent		
32 weeks	40 (0.50)	40 (0.50)
33 weeks	61 (0.71)	25 (0.29)
34 weeks	104 (0.73)	38 (0.27)
35 weeks	75 (0.80)	19 (0.20)
36 weeks	75 (0.85)	13 (0.15)
37 weeks	62 (0.94)	4 (0.06)
38 weeks	22 (1.0)	0 (0)
39 weeks	8 (1.0)	0 (0)

* Includes European-descent, African-descent, Hispanic, Asian, and other infants

Table 7

RDS Severity for Monozygotic and Dizygotic Twins

	Monozygotic Twins (MZ) N=212	Dizygotic Twins (DZ) N=836	p-value
Surfactant			
Any	53 (0.25)	195 (0.23)	0.61
Single Dose	50 (0.24)	179 (0.21)	0.77
Two Doses	3 (0.01)	16 (0.02)	
Mechanical Ventilation (Y/N)	25 (0.12)	72 (0.09)	0.15
Ventilation Duration (days)*	0.7±2.0	1.1±9.9	0.53
Positive Pressure (CPAP, HHNC) (Y/N)	67 (0.32)	248 (0.30)	0.58
Positive Pressure (CPAP, HHNC) Duration (days)*	2.2±2.3	2.0±2.6	0.66
Pneumothorax (Y/N)	2 (0.009)	6 (0.007)	0.67
Oxygen (Y/N)	100 (0.47)	402 (0.48)	0.81
Oxygen Dependency at 36 weeks corrected gestation**	7 (0.046)**	15 (0.025)**	0.18

* Only includes infants with RDS

** Denominator includes only infants born at ≥ 35 weeks gestation.