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Heritability of bronchopulmonary dysplasia, defined according to the consensus statement of the National Institute of Health

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Abstract

Objective—The goal was to determine the magnitude of genetic effects on susceptibility and risk factors for bronchopulmonary dysplasia by using the clinically validated National Institutes of Health consensus definition as a demonstrated proxy for long-term respiratory and neurodevelopmental outcomes in extremely low birth weight infants.

Methods—We analyzed clinical data from twin pairs born at ≤30 completed weeks gestation in British Columbia, Canada, between 1993 and 2006. Differences in correlations between monozygotic (MZ) and dizygotic (DZ) twin pairs and model-fitting approaches were used to quantify the relative contribution of genetic, shared environmental and non-shared environmental effects.

Results—Among 318 twins of known zygosity, monozygotic twin pair similarities were greater than those observed for dizygotic pairs, which suggest significant heritability for bronchopulmonary dysplasia. Model-fitting analyses confirmed that genetic effects accounted for 82% and 79% of the observed variance in bronchopulmonary dysplasia susceptibility, defined on the basis of the need for supplemental oxygen at 36 weeks or the National Institutes of Health consensus definition, respectively. Variations in rates of hemodynamically significant PDA were largely accounted for by genetic effects, whereas variance in susceptibility to blood-borne bacterial infections was largely attributable environmental factors both common and unique to each infant.

Conclusions—Susceptibility to BPD and persistence of PDA are both significantly heritable. Our study strengthens the case for investigating further genetic risk stratification markers useful for predicting the most significant long-term respiratory and neurodevelopmental consequences of bronchopulmonary dysplasia in premature neonates.

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Keywords

Bronchopulmonary dysplasia; infant; premature; twin study; heritability; patent ductus arteriosus

Introduction

Bronchopulmonary dysplasia (BPD) is a multifactorial disease, characterized by impaired alveolar and vascular lung development. Environmental factors, such as high levels of oxygen exposure, mechanical ventilation, or repeated infections play a major role in etiology, although recent studies have suggested an important genetic predisposition.[1] Infants with BPD are at increased risk of death, childhood respiratory complications and long-term neurodevelopmental problems.[2, 3] The lack of sustained clinical benefits from late postnatal therapeutic interventions and the undesirable harmful effects of post-natal corticosteroids have fueled interest in seeking early biological markers useful in targeting therapeutic interventions aimed at promoting long-term pulmonary and neurodevelopmental benefits.[4, 5]

Two previous studies reported genetic influences in the etiology of BPD. The first was a family study by Parker *et al.*, which showed a higher concordance for BPD within pairs of affected siblings. This study indicated that BPD has a familial component but was unable to differentiate whether the increased concordance in siblings was due to susceptibility genes shared between siblings, or was due to environmental factors to which both siblings were exposed.[6] Twin studies are a powerful tool to separate genetic and environmental effects by comparing the rates of disease occurrence in monozygotic (MZ) and dizygotic (DZ) twin pairs. Greater MZ than DZ within-pair similarity implies the presence of genetic influences, because of the two-fold greater genetic similarity in MZ twins. Using data combined from four neonatal centers in the United States, Bhandari *et al.* reported that 53% of the observed variability in incidence of BPD was directly attributable to genetic differences (termed h^2, or heritability). However, for practical reasons the investigators used an arbitrary definition of BPD (need for supplemental oxygen at 36 weeks) that is known to correlate relatively weakly with later neurodevelopmental outcome, particularly in the at-risk ELBW population.[7]

To address this issue, we applied the National Institute of Child Health and Human Development (NICHD)/National Heart, Lung, and Blood Institute Workshop definition for BPD developed in 2000.[8] The main advantages of this definition is that it recognizes different levels of disease severity and more accurately predicts the spectrum of risk for adverse neurodevelopmental outcomes in early infancy compared with other definitions.[7]

The purpose of the present study is to determine the magnitude of genetic effects on susceptibility and risk factors for BPD, comparing clinically validated proxy definitions for long-term respiratory and neurodevelopmental outcomes.

Methods

Subjects and database

The sample consisted of 478 twins who were born between November 15, 1993 and December 31, 2006 and admitted to the Children's & Women's Health Centre of British Columbia (BC) in Vancouver, Canada. This centre is the main provincial Level III-IV NICU, admitting about 600 neonates annually, including the majority of extremely low birth weight infants born in the province of BC. Data were recorded prospectively on all infants from admission to discharge or death using pre-structured objectively defined criteria. Information collected included demographics, maternal pre- and peri-natal history, delivery room and neonatal course, and main neonatal outcomes. The accuracy of the information in the database records was confirmed as being over 95%, as established by secondary review of a majority of the medical charts for infants in this study. The study population included all infants less than or equal to 30 completed weeks gestation. Patients who died within 28 days of life or before 36 weeks PMA were excluded from the BPD analyses at 28 days or 36 weeks, respectively (see definitions below). Zygosity was determined using chorionicity derived from early fetal ultrasounds or placental histological examination performed at the Children's & Women's Health Centre of British Columbia. Histological confirmation of chorionicity took precedence over fetal ultrasounds except in 2 cases where antenatal septostomy of the membranes had been performed. Dichorionic twins were only considered dizygous if they differed in blood group or gender, otherwise they were excluded from the analysis. Monochorionic twins were all considered monozygous as they are only extremely rare occurrences of dizygozity in such cases [9]. The study was approved by the University of British Columbia Clinical Research Ethics Board.

Definitions

Three widely used clinically validated definitions of BPD were included in the analysis: i) chronic supplemental oxygen needs for at least 28 days (BPD-28DAYS)[10]; ii) chronic supplemental oxygen needs at either 36 weeks post menstrual age (PMA) or discharge home whichever came first (BPD-36WKS)[11]; and iii) BPD as categorically defined (mild, moderate or severe) based on the NICHD consensus definition (BPD-NICHD).[8] In accordance with the NICHD consensus definitions "mild BPD" was defined as the need for supplemental oxygen for at least 28 days. "Moderate BPD" was defined as the need for supplemental oxygen at 36 weeks PMA without positive pressure support (e.g. NCPAP or mechanical ventilation). "Severe BPD" was defined as the need for positive pressure support (e.g. NCPAP or mechanical ventilation) for chronic lung disease at 36 weeks PMA. Information about the respiratory status of the infant up to the time of discharge home, was also obtained on all infants who were transferred elsewhere prior to 36 weeks PMA. Our institution did not use a physiologic test confirming the oxygen requirement at the assessment time.[12]

Gestational age was determined based on the first day of last menstrual period if cycle dates were considered to be accurate or by early ultrasound dating if not. Discordant intrauterine growth was defined as more than a 20% discrepancy in growth measurements between the fetuses. Respiratory distress syndrome (RDS) was defined according to clinical criteria of

symptoms of respiratory distress in the first 24h of life and/or need for surfactant administration. Patent ductus arteriosus (PDA) was defined by clinical signs supported by echocardiographic confirmation. However only those with hemodynamically significant PDA requiring treatment with either indomethacin (PDA-INDO) or a surgical ligation (PDA-SURG) were entered into the analysis.

Statistical analysis

Heritability is estimated by comparing the intra-pair similarity (indexed, for example, by a correlation coefficient) between MZ and DZ pairs. Significant genetic effects are indicated when the similarity of MZ twin pairs is greater than the similarity of DZ twin pairs reflecting the two-fold greater genetic similarity of MZ over DZ siblings. Unlike previously published reports, the present study uses structural equation models to produce estimates of the relative contribution of additive genetic (A), or shared (C) and non-shared (E) environmental influences. Shared environmental effects include any experiences that influence all infants within a family to the same degree (e.g., socioeconomic status, institutional practices, etc.). Non-shared environmental effects include events that have differential effects on individual family members (e.g., intercurrent illness, etc.). Structural equation model fitting methods have become the preferred and standard methods for estimating heritability primarily because they permit explicit statistical evaluation of significance for genetic and environmental effects using the calculation of confidence intervals which was not possible with previous methods.[13, 14]

Intra-pair similarities regarding each of the definitions were indexed using a range of polychoric correlations estimated with the PRELIS 2.3 statistical software.[15] This statistical program selects the appropriate correlation coefficient to take into account the level of measurement of each definition (e.g., interval or categorical data)[14]. For BPD-28 Days, BPD-36WKS, RDS, PDA-INO, and PDA-SURG (coded as 0 = absence or 1 = presence) canonical correlations were computed from contingency tables created between siblings for each zygosity. A Pearson product moment correlation was calculated for BPD-NICHD (multi-categorically defined as 0 = absence, 1 = mild, 2 = moderate and 3 = severe and number of positive blood cultures (interval level). The study used a structural equation model fitting approach that fit four separate models to the MZ and DZ intra-pair correlations for each definition to estimate the magnitude and test the statistical significance of the parameters A, C, and E by the method of weighted least squares using the Mx statistical software (Virginia Commonwealth University, http://views.vcu.edu).[14,16] The primary means to assess the relative fit of each model is by comparing the magnitude of likelihood ratio (χ^2) computed for each model against one another in which models with smaller values of χ^2 indicate a superior fit to models with greater values of χ^2. Differences in χ^2 between models are tested at $p<0.05$. Model 1 is the baseline model that estimates the magnitude of A, C, and E influences (ACE model). Model 2 (AE model) tests the statistical significance of shared family environment, C, in each BPD definition by allowing the model to only estimate additive genetic (A) and non-shared environmental effects (E). The significance of C is tested by subtracting the value of χ^2 from the baseline model (Model 1) to the value of χ^2 associated for the AE model (Model 2) and tested against a χ^2 distribution alone can account for the observed variability (AE model). Absence of significant changes in χ^2

between models indicates that deleted parameter had no effect and can be zero. Conversely, a significant change in χ^2 indicate that the parameter significantly contributes to the observed variability and must be retained. Similarly, Model 3 tests the significance of genetic influenced by specifying only shared and nonshared environmental influences (CE); whereas Model 4 (E only) tests if events or circumstances unique to each individual can satisfactorily account for the data.

In addition to χ^2, model fit is also assessed using Akaike's Information Criterion (Akaike's Information Criterion = $\chi^2 - 2df$).[17] The AIC is used because it factors into magnitude of likelihood ratio χ^2 the principle of parsimony in which models that can account for the data with fewer parameters are considered superior to models that specify more parameters. In summary, the most satisfactory model in the present study is one that does not significantly increase χ^2, yields the smallest value of AIC, and accounts for the variance with the fewest number of parameters. The estimates of the A, C, and E parameters from the best fitting are squared to yield the familiar standardized proportions (%) of the variance attributable to each effect h^2_A, c^2, and e^2, respectively.

Results

Clinical characteristics were similar between the entire cohort of 478 neonates and the subset of 318 neonates with known zygosity used for the analysis (see Table 1). Within the group with known zygosity, MZ and DZ neonates were similar for potential confounding variables with the exception that there was a higher incidence of RDS and lower incidence of bacteremia in DZ twins (Table 2).

In the initial analyses, intra-pair correlations within MZ and DZ twins were determined for the three definitions of BPD: BPD-28DAYS, BPD-36WKS and BPD-NICHD (see detailed definitions in methods). As shown in Table 3, similarities in correlation coefficients (r) within MZ pairs were greater for BPD-36WKS and BPD-NICHD, suggesting the presence of a significant genetic influence on the need for supplemental oxygen at 36 weeks.

MZ correlations were also greater for patent ductus arteriosus (PDA) requiring either indomethacin (PDA-INDO) or surgical treatment (PDA-SURG), implying genetic factors influence the risk of hemodynamically significant PDA. Little genetic influence is suggested for BPD-28DAYS, respiratory distress syndrome or the number of positive blood cultures (Table 3).

Independent model fitting analyses presented in Table 4 confirmed the findings of the twin correlations in Table 3. Regarding PDA-INDO, PDA-SURG, BPD-36WKS, and BPD-NICHD a model specifying A and E effects provided the most satisfactory fit to the data (Table 4). Heritability estimates were high for BPD-36WKS and BPD-NICHD, ranging from 79% to 82% (Table 5). The remainder of the effects was entirely accounted for by non-shared (E) environmental effects (denoted as e^2). Regarding RDS, the number of positive blood cultures and BPD-28DAYS a model specifying only C and E effects provided the most satisfactory fit, again indicating little genetic effects on these outcomes (see Table 4).

Discussion

The present study indicates that susceptibility to BPD is significantly heritable in neonates ≤ 30 weeks gestational age using clinically validated definitions for long-term pulmonary and neurodevelopmental outcomes. We also report for the first time significant genetic effects on hemodynamically significant PDA, a risk factor for BPD. In contrast to previous studies, the methodological approach used in our analysis also allows delineation of the role of common environmental stressors and additionally those unique to each infant (i.e. non-shared environmental effects). This also has particular importance in understanding the multifactorial etiology of BPD. For BPD-36WKS and BPD-NICHD, heritability estimates were somewhat higher than previously reported, perhaps due to differences in the relative magnitude of environmental stressors, the inclusion of lower gestational age neonates in our study or differences in the methods used.[1]

It is of particular interest that the relative contribution of genetic and environmental effects appears to vary depending on the severity of BPD. Variations in BPD-28DAYS were completely attributable to environmental effects, and predominantly to shared environmental effects. Dependency on supplemental oxygen at 28 days likely reflects an early phase in the disease process, one largely determined by factors common to both twins such as gestational age, whereas the condition at 36 weeks appears to better reflect underlying biological susceptibility.[8]

Although this study indicates a significant genetic component in the susceptibility to BPD, it cannot address the molecular nature of this effect. In common with other complex diseases, environmental influences likely interact to alter genetic susceptibility. Positive pressure ventilation inducing alveolar stretch, and oxygen trigger systemic and local inflammatory responses that can be damaging to the lungs of the immunologically immature preterm infant.[18] Animals genetically engineered to have altered surfactant protein levels, excessive cytokine production (e.g. interleukin-6 or 13), or deficiency in Toll-like receptor 4, which is a critical receptor component for gram-negative bacteria's lipopolysaccharide inflammatory response, show increased susceptibility to hyperoxia-induced lung injury.[19–23] In humans, polymorphisms in surfactant protein A & B, TNF-α, angiotensin-converting enzyme and gluthathione-S-transferase-P1 genes, genes, and having known altered biological functions, have been linked in small studies with variations in risk of BPD or its severity,[24–27] although others have failed to show similar associations in independent populations.[28, 29]

PDA persistence was also largely heritable in our analysis, a finding that is consistent with a significant contribution of individual's ethnic background to susceptibility.[30] PDA closure mainly depends on circulating levels of endogenous prostaglandins, produced by cyclo-oxygenase (COX), in addition to nitric oxide (NO) which mediates ductal relaxation in highly immature neonates.[31] So far, only one study has reported an association between an A1166C polymorphism in the angiotensin II type 1 receptor gene and PDA persistence, although the mechanism underlying this association is unclear.[32] In humans, several COX genetic variants have been described, which potentially may account for inter-individual differences in pharmacological response to COX inhibitors.[33]

A limitation to all retrospective twin analyses, including ours, is the potential for disease misclassification. This can significantly affect heritability estimates (see [13] for a review). This is best illustrated by our findings of strong shared environmental but no significant genetic effects on RDS, in sharp contrast to previous reports.[34] The RDS definition used in our cohort did not specifically incorporate radiological criteria, and disease classification may have been biased by our practice of routinely administering surfactant early to neonates weighing less than 1000 g, potentially masking the ability to measure genetic effects. Nonetheless, this observation highlights the sizeable impact of institutional variations in clinical practice, disease incidence or issues of disease misclassification can have on estimates of heritability. In relation to BPD, a somehow arbitrarily defined disease, we believe our methodology of rigorously verifying the accuracy of the clinical information and using validated long-term proxy maximized our ability to detect most clinically relevant genetic effects.

Advances in human genome mapping now open the exciting possibility that knowledge of gene variants may be used to target therapy based on risk stratification. Our results provide essential independent corroboration of a significant contribution of heritability in the etiology and severity of BPD, as well as persistent PDA. In addition, we further delineated the relative contribution environmental effects potentially amendable to changes in clinical practice. The use of the NICHD consensus definition greatly strengthens the rationale for identifying genetic markers useful for stratifying risk and targeting interventions to prevent BPD and its most undesirable life-long neurodevelopmental consequences in prematurely born children.

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Abbreviations

RDS	Respiratory distress syndrome
BPD	Bronchopulmonary dysplasia
ELBW	Extremely low birth weight
MZ	Monozygotic
DZ	Dizygotic
NICHD	National Institute of Child Health and Human Development
PMA	Post-menstrual age
PDA	Patent ductus arteriosus
GA	Gestational age

BW	Birth weight
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit

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Table 1

Clinical characteristics for reference population and for infants of known zygosity.

	Entire cohort[&]	Infants of known zygosity[&]	95% CI[*]
n =	478 neonates	318 neonates	
GA (wks ± SD)	28,0 ± 1,9	27,9 ± 1,8	-
BW (g ± SD)	1136 ± 314	1116 ± 314	-
Male gender	262 (54.8)	177 (55.7)	−6.2, 7.9 %
Death ≥28d and ≤36wks PMA	6 (1.3)	3 (0.9)	−1.1, 1.8 %
Discordant intrauterine growth	29 (6.1)	26 (8.2)	−5.8, 1.6 %
RDS	371 (77.6)	246 (77.4)	−5.7, 6.2 %
PDA requiring indomethacin treatment	142 (29.7)	96 (30.2)	−7.0, 6.0 %
PDA requiring surgical ligation	61 (12.8)	36 (11.3)	−3.2, 6.0 %
≥1 positive blood culture	123 (25.7)	88 (27.7)	−8.2, 4.4 %
BPD[a]	86 (18.0)	63 (19.8)	−7.4, 3.8 %
Severe BPD[b]	29 (6.1)	24 (7.5)	−5.1, 2.1 %
Duration of supplemental of O2 (days)	10 [1 – 43]	12 [1 – 44]	-
Duration of mechanical ventilation	5 [1 – 22]	6 [1 – 23]	-

[&]Data is expressed as mean ± SD, n (%) or n [interquartile range].

[*]none of the differences were significant within a 95% CI;

[a]BPD is defined as the need for supplemental oxygen at 36 weeks;

[b]Severe BPD is defined as the need for continuous positive pressure support (e.g. NCPAP or mechanical ventilation) at 36 weeks.

Table 2

Comparison of sibling pairs of known zygosity.

	MZ[&]	DZ[&]	95% CI[*]
n =	70 pairs	89 pairs	
GA (wks ± SD)	27.8 ± 1,9	27.9 ± 1,9	-
BW (g ± SD)	1104 ± 326	1129 ± 311	-
Male gender	84 (60.0)	93 (52.2)	−3.2, 19 %
Death ≥28d and ≤36wks PMA	1	2	−2.5, 1.7 %
Discordant intrauterine growth	9 (6.4)	17 (9.6)	−9.0, 2.8 %
Cesarean delivery	44 (62.9)	62 (70.0)	−21, 8.0 %
RDS	99 (70.7)	147 (82.6)	**2.5, 21 %**
PDA requiring indomethacin treatment	35 (25.0)	61 (34.3)	−0.7, 19 %
PDA requiring surgical ligation	14 (10.0)	22 (12.4)	−4.6, 9.3 %
≥1 positive blood culture	47 (33.6)	41 (23.0)	**0.5, 20 %**
NEC requiring surgery	2 (1.4)	4 (2.2)	−3.8, 2.1 %
BPD[a]	33 (23.6)	30 (16.9)	−2.2, 16 %
Severe BPD[b]	13 (9.3)	11 (6.2)	−2.9, 9.1 %
Duration of supplemental of O2 (days)	12 [1 – 55]	14 [2 – 41]	-
Duration of mechanical ventilation (days)	7 [2 – 24]	5 [2 – 22]	-
Use of oral diuretics	31 (21.4)	31 (17.4)	−3.5, 14 %

[&]Data is expressed as mean ± SD, n (%) or n [interquartile range].

[*]significant differences are bolded;

[a]BPD is defined as the need for supplemental oxygen at 36 weeks;

[b]Severe BPD is defined as the need for continuous positive pressure support (e.g. NCPAP or mechanical ventilation) at 36 weeks.

Table 3

Sibling pair similarities.

Outcome analyzed	r_{MZ}	r_{DZ}
BPD-28DAYS	0.87 (0.79; 0.92)	0.87 (0.80; 0.91)
BPD-36WKS	0.81 (0.70; 0.88)	0.53 (0.36; 0.67)
BPD-NICHD	0.78 (0.66; 0.86)	0.51 (0.34; 0.65)
RDS	0.71 (0.57; 0.81)	0.67 (0.54; 0.77)
PDA-INDO	0.93 (0.88; 0.95)	0.57 (0.41; 0.70)
PDA-SURG	0.99 (0.98; 1.00)	0.76 (0.66; 0.84)
# positive blood cultures	0.25 (0.02; 0.46)	0.27 (0.06; 0.45)

r = Monozygotic (MZ) or dizygotic (DZ) intra-pair correlation coefficients with 95% confidence intervals denoted in parentheses. Greater correlations for monozygotic pairs, compared with dizygotic pairs ($r_{MZ} > r_{DZ}$) implies sizable additive genetic effects (h^2_A), which was further tested in the structural equation models (detailed in methods).

Table 4

Model fitting results.

	χ^2		
	Model 1	**Model 2**	**Model 3**
Parameters in model	ACE	AE	CE
Degree of freedom	3	4	4
BPD-28DAYS	0.000	153.7	0.011
BPD-36WKS	0.000	3.20	12.4
BPD-NICHD	0.000	2.21	10.2
RDS	1.000	6.5	0.058
PDA-INDO	0.000	1.4	15.2
PDA-SURG	0.000	57.6	45.0
# positive blood cultures	0.009	n/a	0.009

Model 1 was used as the baseline model. The parameters specified in each model are indicated (A: shared environmental effects; C: common environmental effects; E: non-shared-environmental effects). No non-shared environmental effects-only model provided a fit to the data; therefore, this model is not shown. N/A = Not tested as the twin correlation suggested no genetic effects ($r_{DZ}>r_{MZ}$). Best fitting model are underlined.

Table 5

Heritability estimates.

Outcome/dependent variable	h^2_A	c^2	e^2
BPD-28DAYS		.868 (.754, 1.00)	.132 (.000, .246)
BPD-36WKS	.822 (.704, .973)	-	.178 (.027, .296)
BPD-NICHD	.790 (.672, .934)	-	.210 (.061, .328)
RDS	-	.692 (.532, .880)	.308 (.120, .468)
PDA-INDO	.927 (.809, 1.00)	-	.073 (.000, .191)
PDA-SURG	.479 (.332, .616)	.519 (.375, .658).	.002 (.000, .126)
No. positive blood cultures	-	.260 (.077, .454)	.740 (.564, .923)

Correlation are denoted by h^2_A = additive genetic effects; c^2 = shared environmental effects; e^2 = non-shared environmental effects, and were calculated by squaring the parameters obtained from the structural equations. Numbers in parenthesis indicate 95% confidence intervals.