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Role of Genetic Susceptibility in the Development of Bronchopulmonary Dysplasia

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Abstract

Objective: To assess heritable contributions to bronchopulmonary dysplasia (BPD) risk in a twin cohort restricted to gestational age (GA) at birth <29 weeks.

Study design: 250 twin pairs [192 dichorionic (DC), 58 monochorionic (MC)] born <29 weeks GA with known BPD status were identified. Three statistical methods applicable to twin cohorts (Chi-squared tests (χ^2), intra-class correlations (ICC) and ACE modeling (additive genetic (A), common environmental (C) and unique environmental (E) components)) were applied. Heritability was estimated as percent variability from A. Identical methods were applied to a subcohort defined by zygosity and to an independent validation cohort.

Results: χ^2 analyses comparing whether neither, one, or both of MC (23, 19, 16) and DC (88, 56, 48) twin pairs developed BPD revealed no difference. Although there was similarity in BPD outcome within both MC and DC twin pairs by intra-class correlation (ICC) [MC ICC = 0.34, 95% CI (0.08, 0.55); DC ICC = 0.39, 95% CI (0.25, 0.51)], MC twins were not more likely than DC twins to have the same outcome (p=0.70). ACE modeling revealed no contribution of heritability to BPD risk [%A = 0.0%, 95% CI (0.0%, 43.1%)]. Validation and zygosity based cohort results were similar.

Conclusions: Our analysis suggests that heritability is not a major contributor to BPD risk in preterm infants <29 weeks GA.

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Keywords

Bronchopulmonary Dysplasia; Genetics; Disease risk; Heritability; Twin study; ACE modeling; Extremely Low Gestational Age Newborns

Bronchopulmonary dysplasia (BPD), defined as receiving supplemental oxygen (O_2)[1] or by a severity-stratified NIH consensus definition[2] at 36 weeks postmenstrual age (PMA) remains a major morbidity among preterm infants. A BPD diagnosis acknowledges both current respiratory status and risk of chronic respiratory morbidity that is associated with higher BPD severity and lower gestational age (GA)[3,4,5]. BPD etiology is considered multifactorial, with hyperoxia, barotrauma, volutrauma and inflammation-based injury and impaired development and repair of the lung.[6]

Because only a subset of infants with similar degrees of prematurity and early illness severity develop BPD, it is reasonable to consider the role of genetic predisposition. The contributions of DNA sequence variants to BPD pathogenesis, particularly in genes involved with lung development, inflammation and repair of injury, are proposed to contribute to BPD pathogenesis[7] result from associated protein dysfunction[7,8].

Twins are logical subjects for the epidemiologic study of heritable disposition to disease. Conventional statistical methods that assume independent observations (as would apply with singletons) are subject to error in twin cohort analyses. Although simple pair concordance may be used, advanced statistical modeling approaches can quantify the proportions of heritable vs environmental influences responsible for disease risk and provide for statistical tests and confidence intervals.

Prior analyses of preterm twin cohorts, using a variety of statistical methods, suggest that heritable factors significantly contribute to BPD risk[9,10,11]. BPD incidence varies widely in these cohorts. Strong associations exist between BPD risk and both GA and birth weight [12]. When BPD was first described, the mean GA at birth of diagnosed infants was 33 weeks[13]. With advancements in perinatal and neonatal care practices, BPD risk among infants born at GA > 29 weeks has decreased. Recent Vermont Oxford Network data suggest that < 10% of infants born at GA between 29 – 32 weeks develop BPD[14] and infants at highest BPD risk are born below 29 weeks GA [5,14].

This study questioned whether, with the evolution of BPD definition and cohort characteristics over time, prior conclusions derived from twin studies regarding BPD heritability held firm. We evaluated BPD heritability within a preterm infant cohort (<29 weeks GA) whose GA distribution reflects those currently at highest BPD risk.

Methods

We collected clinical data including pulmonary and other outcomes on all infants born prior to 29 weeks gestation from two Boston high-risk perinatal centers (from 1997 through 2015 at Brigham and Women’s Hospital (BWH) and from 2004 through 2015 at Beth Israel Deaconess Medical Center (BIDMC)). We received IRB approval for medical

record review from both hospitals to study morbidities and outcomes. Data collected included demographics, course of respiratory support and development of common major co-morbidities of extreme prematurity, such as patent ductus arteriosus, early onset sepsis (diagnosed at ≤ third postnatal day), late onset sepsis (diagnosed at > third postnatal day) and necrotizing enterocolitis.

Each infant was assigned a diagnosis of BPD by three definitions: 1) need for supplemental oxygen at 36 weeks PMA, 2) need for supplemental oxygen, CPAP, high flow nasal cannula (with or without oxygen), or positive pressure ventilation (with or without oxygen) at 36 weeks PMA or 3) criteria set by an NIH consensus panel for either moderate or severe BPD[2] (for infants born at <32 weeks GA, Moderate BPD was defined as a need for supplemental oxygen for >28 days plus treatment with <30% O_2 at 36 weeks PMA, and severe BPD as oxygen for >28 days plus >30% O_2 and/or positive pressure at 36 weeks PMA). Definition #1 was considered our primary definition and definitions #2 and #3 were considered alternative definitions for secondary analyses.

Twins were characterized as monochorionic (MC) or dichorionic (DC) based on placental pathology or, when not available, prenatal sonography reports. A pilot study was performed comparing the accuracy of sonographic-based to placental pathology-based determination of chorionicity for an estimate of the magnitude of sonographic chorionicity misclassification. MC twins were considered monozygous (MZ). Dichorionic twins that were discordant for sex or blood type were considered dizygous (DZ). Dichorionic twins that were same sex and same blood type were not assigned a zygosity and were excluded from zygosity-based analyses. Analyses were conducted comparing MC to DC twins as well as MZ to DZ twins. We thus describe the univariate profile of the chorionicity determined primary cohort that yields a larger sample size with avoidance of confounding selection biases and acknowledges a presumed ~9% DZ misclassification.

The twin pairs defined by chorionicity comprised the primary study population. For confirmation, we applied an identical analytic approach to a validation cohort of similar twin pairs enrolled in the multicenter ELGAN study[15]. Although the ELGAN study investigated contributors to injury in the developing brain, respiratory outcome data were available. After exclusion of ELGAN subjects who had been born at our institutions, 105 twin pairs (29 MC, 76 DC) were available for analysis in the validation cohort. Chorionicity was determined by placental pathology.

Statistical Analyses

Demographic and clinical characteristics were compared between infants who did and did not develop BPD as defined by oxygen support at 36 weeks PMA (primary definition), and between MC and DC infants. We used a mixed logistic model with a random twin pair effect to account for the clustered nature of the data (twin pairs representing clusters). These analyses were performed using the GLIMMIX procedure in SAS/STAT software[16]. For comparisons where the variables analyzed were completely concordant (same value in both members of every twin pair) the pair was considered the unit of analysis and the Fisher exact test was used.

Our analysis of heritability was designed to consider how the BPD outcome varies among and between twin pairs. When twin siblings tend to have the same outcome, this suggests the influence of common environmental and/or genetic effects. Using chorionicity as a proxy for zygosity, a genetic effect is suggested if MC twins are more concordant for the outcome than DC twins. This analysis was also performed on a subcohort for which zygosity was confirmed.

Twin pairs were cross-classified by chorionicity (MC or DC) and by the number of individuals affected with BPD (0, 1, or 2). From this 2×3 table, we calculated the Pearson chi-squared test to test whether the within-family BPD distribution is associated with chorionicity. We measured twin similarity with the intra-class correlation (ICC) of the BPD phenotype separately within MC and DC pairs[17]. We also report confidence intervals for the ICCs using a goodness-of-fit approach and tested whether the two ICCs differ[17]. A high ICC could be due to shared environmental effects and/or due to genetic effects, but one would expect the ICC in MC pairs to be larger if there is a genetic contribution. To quantify the heritability of BPD, we used the ACE model which decomposes the total variation in "liability" of disease into additive genetic (A), common or shared environmental (C) and unique or unshared environmental (E) effects[18]. The model was fit with SAS/STAT software using nonlinear mixed models (NLMIXED procedure) [16,19]. Heritability was estimated as the percent of the total variation in liability from the genetic component. We used the likelihood ratio test with appropriate mixture of chi-squared distributions (½ χ^2_0 + ½ χ^2_1) as the reference distribution to evaluate statistical significance and profile likelihood based confidence intervals (CI)[20]. ACE modeling was performed without adjusting for covariates but rather allowing any variation due to subject characteristics to contribute to the components of variation being modeled.

Results

Placental pathology was available for establishment of chorionicity for 92% of twin pairs (n=230). The remaining 8% (n=20) were determined by sonography. A pilot study in 121 twin pairs evaluating accuracy of chorionicity determination by sonography, as compared with placental pathology, revealed a < 3% discrepancy. Thus, use of sonography rather than pathology in 8% of our twin pairs would yield, at most, 0.3% chorionicity misclassification.

Of 2,198 total infants followed in our database, 733 were born of twin gestations. Death rates were the same whether an infant was part of a MC or DC pair (15.6% vs. 16.6%, p = 0.81). Sixty-eight percent (500 infants, 250 twin pairs (58 MC and 192 DC)) met the study inclusion criteria of documented chorionicity, survival of both twins to 36 weeks PMA and known supplemental O_2 and positive pressure support status (Figure; available at www.jpeds.com). The mean (± SD) GA was 26.7 (± 1.3) (range 23–28) weeks and mean birth weight was 950 (± 208) grams. Two hundred five (41%) were diagnosed with BPD. GA and birth weight were significantly lower in the BPD group. Of the 250 twin pairs, 58 (23%) were MC. There was no difference in BPD incidence between MC and DC twins, nor was there a difference in birth weight or GA (Table I). MC twins were presumed to be monozygotic (MZ). Of the 97 same sex DC twins, 67 could be not be confirmed as

DZ based on discordance of sex or blood type. Discarding these twin pairs from analysis reduced the size of the zygosity-based cohort to 183 twin pairs.

BPD was more likely to occur in infants of lower GA, lower birth weight, or with growth restriction. There was no difference in sex, race/ethnicity, or antenatal steroid use between infants with or without BPD. Infants with BPD were more likely to have had a patent ductus arteriosus and/or late-onset sepsis than infants without BPD ($P < .05$). Patient characteristics were similar in MC and DC twins. There was no difference between hospitals in the percent of MC twins. The percentage of infants developing BPD was significantly different between the two birth hospitals.

A comparison of the GA distributions between the study and validation cohorts is presented in Table 2. GA distributions of the study and validation cohorts differed in that only the study cohort included infants born between 28 0/7 and 28 6/7 weeks. Thus, the mean GA of the validation cohort was skewed slightly toward infants born at lower GA.

χ^2 analysis comparing whether neither, one, or both of MC and DC twin pairs developed BPD revealed no difference (p=0.71), suggesting heritability is not a major factor. This finding was confirmed in the validation population (p=0.32, Table 3).

Assessment of similarity in the BPD outcome by intraclass correlations (ICC) within twin pairs demonstrated significant familial aggregation within both MC and DC pairs: MC ICC = 0.34, 95% CI (0.08, 0.55); DC ICC = 0.39, 95% CI (0.25, 0.51). However, the difference between these correlations in MC vs. DC twins was not significant (p=0.70). This finding was replicated in the validation population: MC ICC = 0.51, DC ICC = 0.49, p=0.92 (Table 3).

The ACE model yielded a non-significant estimate of heritability. The estimated percentage of variance from additive genetic effects (%A) was 0.0% (95% CI: 0.0%, 43.1%; p=1.0). This finding was similar in the validation population, with %A 6.5% (95% CI: 0.0%, 67.4%; p=0.44). With combined cohorts, (355 twin pairs) %A = 0.0% (95% CI: 0.0%, 38.6%; p=1.0) (Table 3). The common environmental (%C) impact on BPD development was significant in both the study cohort (56.4%; 95% CI: 25.0, 68.0%; p=0.004) and the validation cohort (66.9%; 95% CI:17.2%, 83.7%; p=0.016) (Table 3). The %C in the combined cohort (n=355 pairs) was 61.2% (CI 33.0% – 70.4%, p<.001).

To address the concern that heritability might only be demonstrated in a comparison of twins selected by zygosity, we repeated the three statistical tests comparing MZ and DZ twins, excluding those DC twins with indeterminate zygosity status. Chi square, ICC and ACE modeling (%A) methods did not suggest BPD heritability (Table 4; available at www.jpeds.com). Given these results, all remaining comparisons were performed comparing twin pairs based on chorionicity to avoid bias introduced by discarding same sex twin pairs of indeterminate zygosity and to take advantage of the larger sample size, acknowledging the possibility of up to 10% misclassification of DC twins who are actually MZ.

We also assessed BPD heritability using multiple definitions [2–6]. In addition to categorizing infants as having BPD based on oxygen use at 36 weeks PMA (Table 3), we reclassified

infants by three additional BPD definitions: 1) Oxygen or positive pressure support (CPAP, high flow nasal cannula or ventilator) with room air at 36 weeks PMA, and 2) NIH consensus definition moderate or severe BPD categories[2] and 3) "death or BPD (oxygen use at 36 weeks PMA). None yielded evidence for BPD heritability using the described statistical methods (Table 5; available at www.jpeds.com).

To test the hypothesis that a genetic influence might not be dominant in babies of lowest gestational age, we combined the primary and validation cohorts, divided them into two GA strata (23 – 26 weeks and 27 – 28 weeks) and repeated the three statistical tests in each of the two strata (Table 6; available at www.jpeds.com). Chi square, ICC and ACE modeling (%A) analyses did not suggest BPD heritability by any method in either GA group.

To test the hypothesis that race may influence whether BPD is heritable, we divided the primary cohort into Caucasian vs. non-Caucasian strata and repeated the three statistical tests independently in each of the two strata (Table 7; available at www.jpeds.com). Chi square, ICC and ACE modeling (%A) analyses did not suggest BPD heritability by any method in either Caucasian or Non-Caucasian twins.

There are no standard power or sample size formulas for ACE modeling. We performed an approximate post hoc power calculation by using a confidence interval (CI) from our data analyses to estimate how statistical precision changes with sample size. Because our profile likelihood CIs are not symmetric, we used the half-width based on the estimate and the lower bound, as it is the lower bound that corresponds to a test of the null hypothesis of zero heritability. We used the CI for %C in the combined cohorts (Table 3) because we needed a CI whose lower bound was non-zero. Using the fact that CI widths in more standard analyses are proportional to $1/N^{½}$, we estimated the proportionality constant and calculated that if the heritability effect was 51.9% or higher, we would have had ≥80% power with our sample size of 355 twin pairs.

Discussion

Three independent twin cohort studies have suggested genetic factors contribute significantly to BPD risk[9,10,11] (Table 8). These findings launched genetics-focused research efforts including single gene, GWAS and whole exome sequencing (WES) searches for candidates or pathways responsible for BPD[21–25]. Although differences in single gene allele frequencies between large BPD and non-BPD cohorts have been reported, major genetic determinants have not been confirmed[6]. Although a large GWAS study was unsuccessful in identifying specific BPD associated loci, subsequent analysis suggested association of pathways with severe BPD[22, 23]. Most recently, WES of severely affected infants suggested new BPD gene candidates that may reveal further understanding of pathophysiology[24,25]. Expression profiling studies revealed differences in gene expression between BPD and non-BPD infants, but identification of associated genetic variants remains a challenge[26].

Over the past five decades, the BPD definition has evolved due to adoption of antenatal glucocorticoid therapy, improvements in NICU care, and resulting shifts in cohort characteristics [1–6, 27]. Currently, infants born above 1,250 grams birth weight or 29 weeks

GA are at relatively low BPD risk and their risk factors differ from lower GA and birth weight infants. Therefore, we focused on the highest risk population born before 29 weeks GA and applied appropriate statistical methodologies for twin pair assessment to tease out contributions of genetic and/or environmental factors to BPD development.

Differing approaches to statistical analysis, BPD definition, cohort inclusion criteria, and cohort size between this and prior studies may explain observed differences in results and conclusions (Table 8). Parker's[9] pre-surfactant era cohort was assembled when survival of infants below 28 weeks GA was low. Their inclusion criteria (<1,500g, survival beyond 28 days of life) were biased towards growth-restricted infants at more advanced gestational ages. Recent Vermont Oxford Network data suggest birth at 29 – 32 weeks GA carries a <10% risk of BPD[14]. Although Bhandari[10] and Lavoie[11] enrolled babies in the post-surfactant era and utilized contemporary BPD definitions, both of their cohorts include higher proportions of infants at low BPD risk due to higher mean GA (Table 8). The mean GA at birth of Bhandari's cohort was older than the GA of all infants in our primary and validation cohorts.

In our combined cohort ACE model of BPD, the estimate of heritability is small (%A = 0%). This finding is consistent with the results from the χ^2 and ICC analyses in our primary and validation populations, i.e. that MC twins do not have a higher concordance of outcome than DC twins, as would be expected if there is a genetic influence on development of BPD.

Thirty percent of all twins are MZ and 25–33% of these are DC[29,30]. Analyses focused on zygosity found no substantial differences compared with a cohort defined by chorionicity. Even though categorizing by zygosity is considered the gold standard, in the absence of universal zygosity determination (not performed in this or the prior studies[9,10,11]) a bias may be introduced when discarding large numbers of same sex DC twin pairs of unknown zygosity (69% of same sex DC twins in our primary cohort), as was done by Lavoie[11]. Our chorionicity based analysis accepted that ~9% of DC twins (used as a surrogate for DZ) are MZ. Analysis by chorionicity retains a larger cohort size; our cohort would have shrunk from 250 to 183 twin pairs if restricted by zygosity.

Biological evidence suggests that female infants have lower BPD risk.[5,28] Excluding same-sex pairs reduces same-risk pairs, which in turn reduces same-outcome (concordant) pairs and creates a bias towards including discordant sex pairs in the DZ group. Higher concordance of outcomes in "mono-" (MZ or MC) than in "di-" (DZ or DC) pairs is evidence of hereditability, so increasing the concordance of outcomes in "mono-" pairs or reducing concordance of outcomes in "di-" pairs creates a bias toward finding hereditability. Even with this potential bias toward finding hereditability, results in our chorionicity-based and zygosity-based cohorts were not different (Table 4). We used chorionicity as a proxy for zygosity given that the small zygosity misclassification may be less likely to introduce bias than eliminating same-sex DC twins whose zygosity can't be distinguished by blood type.

Our findings do not support the conclusions of prior twin studies (heritability estimates 53% – 82%, Table 8). Heritability should have been evident if the true magnitude of effect was of the size previously reported. Lavoie[11] reported a hereditability estimate from a

model assuming no common environmental contribution to BPD (%C = 0), because the C component was not significant in their data. The impact of environmental effects on BPD (including clinical practice differences) may vary between populations. By assuming %C = 0, their model reallocates any contribution from C to the A and E components. We believe this is an inappropriate application of the method unless there is strong evidence for no common environmental contribution. Bhandari[10] reported significant hereditability estimates from the full ACE model, both unadjusted and adjusted for predictors of BPD. In our analytic approach, we let patient characteristics contribute to the shared and unshared environmental components of the ACE model rather than removing their contributions before the ACE modeling. Nevertheless, Bhandari's[10] adjusted and unadjusted heritability estimates were similar, suggesting adjustment for patient characteristics doesn't affect conclusions. The wide confidence intervals we identified yielded limits approaching values reported in other studies with modest population size.

Lavoie[11] utilized multiple BPD definitions: 1) O_2 at ≥28 days, 2) O2 at ≥36 weeks PMA or, if earlier, at discharge, and 3) NIH consensus statement on BPD definition (mild/mod/severe)[2]. Although a model with BPD defined as O_2 at ≥ 28 days of age did not yield a substantial heritability estimate, the other two definitions generated %A of 82% and 79%, suggesting a significant contribution of heritability. In our cohort, the inclusion of babies on CPAP in RA or the use of NIH consensus definitions did not alter our results, nor could we demonstrate race or gestational age strata influenced heritability.

We studied a population of lower GA infants to reflect current epidemiologic patterns in BPD. Information on care practices, such as SpO_2 limits used to justify supplemental oxygen use, is missing from all studies making it difficult to quantify the possible contribution of differences in care practices. Even though stratification by GA did not identify obvious differences in heritability estimates at 23 – 26 weeks vs. 27 – 28 weeks, it is possible that a heritability factor could play a role in infants born at GA ≥ 29 weeks.

Based on findings contrary to those of Parker[9], Bhandari[10] and Lavoie[11], we sought a validation cohort. The ELGAN cohort was younger in GA than our primary study cohort. Because findings were similar in study and validation populations, we presented data both separately and combined. The combined sample size approximated that of the largest[10] prior study with a higher absolute number of affected infants.

A negative ACE model result doesn't preclude genetic variation contributing to BPD development. Genomic studies have also yielded mixed results[21–26], neither confirming nor disproving heritable factors. Genomic analyses on preterm infants are challenged by unavailable allele frequencies for unaffected premature survivors for comparison.

In conclusion, we found no evidence for a significant contribution of heritable factors to BPD risk in infants born <29 weeks gestation. We speculate that, for extremely preterm infants, the roles of immature lung structure and biochemical status trump the potential role of genetic factors in BPD pathogenesis, the latter of which may play a more significant role in the rarer instances in which BPD develops in infants born at or over 29 weeks GA. Possible factors contributing to differing results include our gestational-age restricted

population, specifics of model construction and analysis, and population characteristics. The greater gestational maturity of the populations of babies included in other studies suggest that if genetic predisposition to BPD exists, its role is more prominent among preterm infants born beyond 29 weeks GA.

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Abbreviations:

ACE	Additive genetic, Common environmental, unique Environmental
AGA	Appropriate for Gestational Age
BIDMC	Beth Israel Deaconess Medical Center
BPD	Bronchopulmonary Dysplasia
BWH	Brigham and Women's Hospital
ELGAN	Extremely low gestational age newborn
GA	Gestational age
GWAS	Genome Wide Association Study
ICC	Intraclass correlations
O_2	Oxygen
PDA	Patent Ductus Arteriosus
PMA	Postmenstrual age
NICU	Newborn Intensive Care Unit

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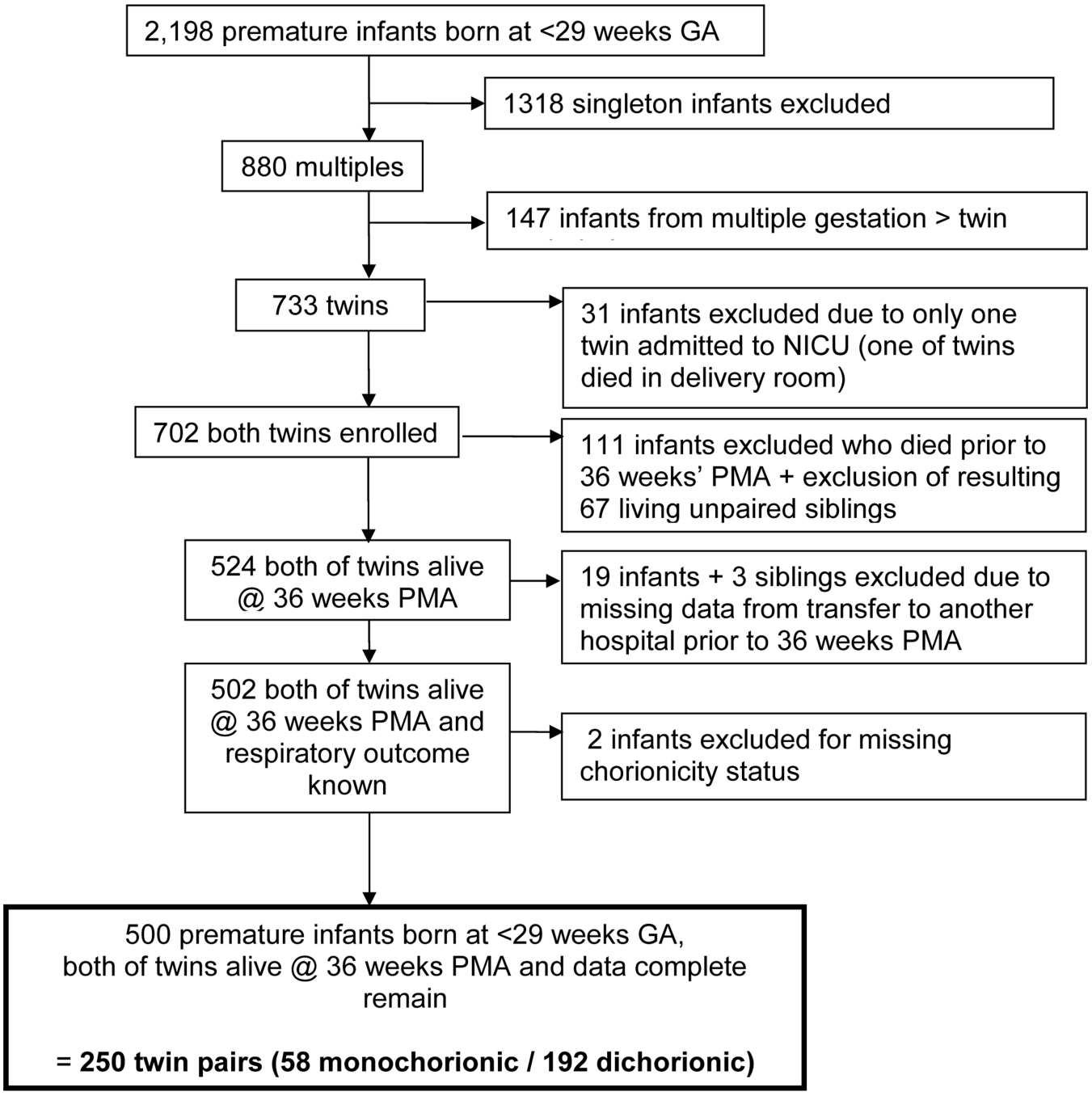

Figure 1(online).
Primary study population

Table 1.

Primary study population infant characteristics by respiratory status at 36 weeks PMA and by chorionicity. BPD is defined as need for supplemental oxygen at 36 weeks PMA. MC = monochorionic, DC = dichorionic

		Respiratory Status 36 weeks PMA			Chorionicity		
Characteristic	**Total (N = 500)**	**BPD (N = 203)**	**No BPD (N = 297)**	**p-value**	**MC (N = 116)**	**DC (N = 384)**	**p-value**
Respiratory Status at 36w PMA							
BPD	203 (41%)				51 (44%)	152 (40%)	.47
No BPD	297 (59%)				65 (56%)	232 (60%)	
Chorionicity							
Monochorionic	116 (23%)	51 (25%)	65 (22%)	.47			
Dichorionic	384 (77%)	152 (75%)	232 (78%)				
Gestational Age (w)							
23–24	44 (9%)	31 (15%)	13 (4%)	<.001	10 (9%)	34 (9%)	.94
25–26	146 (29%)	83 (41%)	63 (21%)		36 (31%)	110 (29%)	
27–28	310 (62%)	89 (43%)	221 (74%)		70 (60%)	240 (63%)	
mean(±SD)	26.7 (±1.3)	26.2 (±1.4)	27.0 (±1.2)		26.7 (±1.4)	26.6 (±1.3)	
Birth weight (g)							
mean(±SD)	950(±208)	855(±185)	1014(±198)	<.001	910(±194)	962(±211)	.13
Growth restriction (z score < −1)							.18
No	415 (83%)	151 (74%)	264 (89%)	<.001	88 (76%)	327 (85%)	.18
Yes	85 (17%)	52 (26%)	33 (11%)		28 (24%)	57 (15%)	
Gender							
Male	261 (52%)	113 (56%)	(50%)	.26	52 (45%)	209 (54%)	.25
Female	239 (48%)	90 (44%)	(50%)		64 (55%)	175 (46%)	
Race/Ethnicity [a]							
Caucasian	344 (70%)	138 (69%)	206 (70%)	.57	84 (76%)	260 (68%)	.20
African-American	60 (12%)	26 (13%)	34 (12%)		8 (7%)	52 (14%)	

		Respiratory Status 36 weeks PMA			Chorionicity		
Characteristic	**Total (N = 500)**	**BPD (N = 203)**	**No BPD (N = 297)**	**p-value**	**MC (N = 116)**	**DC (N = 384)**	**p-value**
Hispanic	38 (8%)	14 (7%)	24 (8%)		4 (4%)	34 (9%)	
Asian	22 (4%)	13 (6%)	9 (3%)		10 (9%)	12 (3%)	
Other	30 (6%)	10 (5%)	20 (7%)		4 (4%)	26 (7%)	
Hospital							
A	354 (71%)	131 (65%)	223 (75%)	.03	84 (72%)	270 (70%)	.87
B	146 (29%)	72 (35%)	74 (25%)		32 (28%)	114 (30%)	
Antenatal Steroids							
None	28 (6%)	10 (5%)	18 (6%)	.49	8 (7%)	20 (5%)	.53
Partial	140 (28%)	51 (25%)	89 (30%)		26 (22%)	114 (30%)	
Complete	322 (66%)	142 (70%)	190 (64%)		82 (71%)	250 (65%)	
Chorioamnionitis							
No clinical signs	454 (91%)	191 (94%)	263 (89%)	.07	110 (95%)	344 (90%)	.22
Clinical signs	46 (9%)	12 (6%)	34 (11%)		6 (5%)	40 (10%)	
Mode of Ventilation at 6–18 h							
None or CPAP	46 (9%)	8 (4%)	38 (13%)	.003	9 (8%)	37 (10%)	.93
Conventional ventilation	424 (85%)	175 (86%)	249 (84%)		100 (86%)	324 (84%)	
High-frequency ventilation	30 (6%)	20 (10%)	10 (3%)		7 (6%)	23 (6%)	
Patent Ductus Arteriosis							
None or untreated	163 (33%)	36 (18%)	127 (43%)	<.001	32 (28%)	131 (34%)	.70
Medical treatment only	255 (51%)	117 (58%)	138 (46%)		60 (52%)	195 (51%)	
Surgical ligation only	9 (2%)	3 (1%)	6 (2%)		4 (3%)	5 (1%)	
Medical and surgical ligation	73 (15%)	47 (23%)	26 (9%)		20 (17%)	53 (14%)	
Early-onset sepsis							
None	345 (69%)	128 (63%)	217 (73%)	.04	86 (74%)	259 (67%)	.44
Clinically suspected	145 (29%)	73 (36%)	72 (24%)		26 (22%)	119 (31%)	
Culture proven	10 (2%)	2 (1%)	8 (3%)		4 (3%)	6 (2%)	

Characteristic	Total (N = 500)	Respiratory Status 36 weeks PMA			Chorionicity		
		BPD (N = 203)	No BPD (N = 297)	p-value	MC (N = 116)	DC (N = 384)	p-value
Late-onset sepsis							
None	281 (56%)	80 (39%)	201 (68%)	<.001	70 (60%)	211 (55%)	.74
Clinically suspected	107 (21%)	61 (30%)	46 (15%)		20(17%)	87 (23%)	
Culture proven	112 (22%)	62 (31%)	50 (17%)		26 (22%)	86 (22%)	
Necrotizing enterocolitis							
None	453 (91%)	179 (88%)	274 (92%)	.11	108 (93%)	345 (90%)	.80
Medical	26 (5%)	16 (8%)	10 (3%)		4 (3%)	22 (6%)	
Surgical	21 (4%)	8 (4%)	13 (4%)		4 (3%)	17 (4%)	

[a] N=494

Table 2.

Gestational age (GA) distribution for primary study and validation cohorts. There was no significant difference in GA distribution between Primary Study and Validation cohorts for births below 28 weeks gestation (shaded area) by Fisher's exact test (p = .51).

	Number of Twin Pairs		
GA (weeks)	**Study**	**Validation**	**Combined**
23	3	1	4
24	19	15	34
25	25	22	47
26	48	26	74
27	70.5[a]	41	111.5
28	84.5[a]	N/A	84.5
Total	250	105	355
Mean(±SD)	26.7(±1.3)	25.9(±1.1)	26.4(±1.3)
Range	23–28	23–27	23–28

[a]The infants in one twin pair were born separately, at gestational ages 27 and 28 weeks.

Table 3.

Statistical analyses of for BPD heritability by X^2, intra-class correlation and ACE Modeling.BPD heritability by X^2, intra-class correlation (ICC) and ACE modeling in twin pairs of primary study, validation and combined cohorts by chorionicity.

Cohort	Chorionicity	Number in Twin Pairs Affected								ACE modeling Percent of Variability (95% CI)			
		Neither	One	Both	Total	χ^2	p	ICC (95% CI)	p	%A (Heritability)	p	%C (Common Environmental)	p
Study	MC	23	19	16	58	0.69	.71	0.34 (0.08,0.55)	.70	0.0% (0.0%, 43.1%)	1.0	56.4% (25.0%, 68.0%)	.004
	DC	88	56	48	192			0.39 (0.25,0.51)					
	Total	111	75	64	250								
Validation	MC	9	7	13	29	2.27	.32	0.51 (0.15,0.75)	.92	6.5% (0.0%, 67.4%)	.44	66.9% (17.2%, 83.7%)	.016
	DC	34	19	23	76			.49 (0.27,0.66)					
	Total	43	26	36	105								
Combined	MC	32	26	29	87	2.33	.31	0.40 (0.20,0.57)	.88	0.0% (0.0%, 38.6%)	1.0	61.2% (33.0%, 70.4%)	<.001
	DC	122	75	71	268			0.42 (0.30,0.52)					
	Total	154	101	100	355								

Table 4 (online).

BPD heritability by X^2, intra-class correlation (ICC) and ACE modeling in twin pairs of known zygosity (DC pairs with same gender and same blood type were excluded from this analysis). MZ = monozygotic, DZ = dizygotic

Zygosity	Number in Twin Pairs Affected								ACE modeling Percent of Variability (95% CI)			
	Neither	One	Both	Total	χ^2	p	ICC (95% CI)	p	%A (Heritability)	p	%C (Common Environmental)	p
MZ	23	19	16	58	1.14	.57	0.34 (0.08,0.55)	.36	0.0% (0.0%, 32.6%)	1.0	62.5% (35.8%, 74.6%)	.001
DZ	58	32	35	125			0.47 (0.30,0.61)					
Total	81	51	51	183								

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Table 5 (online).

BPD Heritability in Primary study population using alternate definitions of BPD. MC = monochorionic, DC = dichorionic.

Definition	Chorionicity	Number in Twin Pairs Affected								ACE modeling Percent of Variability (95% CI)			
		Neither	One	Both	Total	χ^2	p	ICC (95% CI)	p	%A (Heritability)	p	%C (Common Environmental)	p
O_2, CPAP or high flow nasal cannula (with or without O_2) or positive pressure ventilation (with or without O2) at 36 weeks PMA	MC	23	18	17	58	.50	.78	0.37 (0.12,0.58)	.87	0.0% (0.0%, 47.9%)	1.0	57.9% (23.3%, 69.3%)	.004
	DC	86	56	50	192			0.40 (0.26,0.52)					
	Total	109	74	67	250								
Moderate or Severe BPD by NIH criteria[2]	MC	24	17	17	58	.72	.70	0.41 (0.15,0.61)	.78	9.3% (0.0%, 62.2%)	.40	50.4% (11.5%, 67.7%)	.017
	DC	88	58	46	192			0.37 (0.23,0.49)					
	Total	112	75	63	250								
Composite BPD (O2 at 36 weeks) or Death	MC	27	23	33	83	.16	.92	0.44 (0.23,0.61)	.79	7.5% (0.0%, 49.0%)	.39	56.7% (26.6%, 70.3%)	.001
	DC	91	80	102	273			0.41 (0.30,0.51)					
	Total	118	103	135	356*								

*for composite definition “BPD or Death”, total number of pairs is increased due to inclusion of twin pairs previously excluded based on death of one or both twins died prior to 36 weeks PMA

Table 6 (online).

BPD heritability by X^2, intra-class correlation (ICC) and ACE modeling in MC vs. DC twin pairs in combined study and validation cohorts stratified by gestational age (23–26 weeks, 27–28 weeks). MC = monochorionic, DC = dichorionic

Gestational Age	Chorionicity	Number in Twin Pairs Affected								ACE modeling Percent of Variability (95% CI)			
		Neither	One	Both	Total	χ^2	p	ICC (95% CI)	p	%A (Heritability)	p	%C (Common Environmental)	p
23–26 wks	MC	7	15	17	39	1.01	.60	0.18 (−0.13,0.46)	.55	0.0% (0.0%, 58.5%)	1.0	40.5% (0.0%, 57.4%)	.06
	DC	31	42	47	120			0.29 (0.11,0.45)					
	Total	38	57	64	159								
27–28 wks	MC	25	11	12	48	2.07	.36	0.51 (0.22,0.71)	.67	10.6% (0.0%, 61.5%)	.38	61.4% (20.9%, 79.2%)	.008
	DC	91	33	24	148			.44 (0.27,0.58)					
	Total	116	44	36	196								

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Table 7 (online).

Heritability estimates of BPD stratifying the primary study population by race: Caucasian vs. Non-Caucasian. MC = monochorionic, DC = dichorionic

Race	Chorionicity	Number in Twin Pairs Affected								ACE modeling Percent of Variability (95% CI)			
		Neither	One	Both	Total	χ^2	p	ICC (95% CI)	p	%A (Heritability)	p	%C (Common Environmental)	p
Caucasian	MC	18	13	11	42	0.15	.93	0.36 (0.06,0.61)	.80	0.0% (0.0%, 53.6%)	1.0	58.6% (18.6%, 71.9%)	.011
	DC	60	37	33	130			0.41 (0.24,0.55)					
	Total	78	50	44	172								
Non-Caucasian	MC	3	6	4	13	2.23	.33	0.07 (−0.42,0.53)	.34	0.0% (0.0%, 68.6%)	1.0	47.9% (0.0%, 69.7%)	.06
	DC	28	19	15	62			.36 (0.11,0.57)					
	Total	31	25	19	75								

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Table VIII.

Comparison of studies estimating the heritability of BPD

		Parker et al[9]	Bhandari et al[10]	Lavoie et al[11]	Current study	ELGAN validation[15]
Years subjects accrued		1976–1990	1994–2004	1993–2006	1997–2015	2002–2004
Population size		1872	450 twin pairs	478	2198	1506
Number ot twin pairs analyzed		108	252	159	250	105
Restrictions		<1500 g birth weight survival >28 d	≤32 wk gestational age survrval ≥36 wk PMA	≤30 wk gestational age	≤28 wk gestational age survival ≥36 wk PMA	<28 wk gestational age survival ≥36 wk PMA
Chonomcity known		No	Yes	Yes	Yes	Yes
Zygosity known		No	Yes	Yes	Yes	No
BPD definition		O_2 at 28 d + abnormal CXR + symptoms	O_2 at 36 wk PMA + abnormal CXR	O_2 at 36 wk PMA or discharge NIH consensus criteria	O_2 at 36 wk PMA O_2 CPAP/RA at 36 wk PMA NIH consensus critena	O_2 at 36 wk
Sex (male)		57% of BPD 46.3% of cohort	54% of cohort	55.7% of cohort	56% of BPO 52% of cohort	
Gestational age (wk)	Overall		29.7 ± 2.5	27.9 ± 1.8	26.7 (±1.3)	25.9(±1.1)
mean ± SD	No BPD	30.4 ± 2.3			27.0 (±1.2)	26.2 (±1.0)
	BPD	27.7 ± 2.0			26.2 (±1.4)	25.5 (±1.2)
Birth weight (g)	Overall		1397 ± 443	1116 ± 314	950 (±208)	873 (±189)
mean ± SD	No BPD	1211 ± 209			1014 (±198)	925 (±169)
	BPD	929 ± 225			855 (±185)	813 (±193)
% cohort with BPD			17.5%	19.8% moderate, 7.5% severe	41%	
Analysis methods		OR of BPD in twin A and twin B	ACE model *	ACE model * assuming %C = 0 (AE model)	ACE model *	ACE model *
Heritability estimate		aOR 12.3 ($P < .01$)	unadjusted %A = 63.6% adjusted %A= 53% 95% CI (16%–89%)	unadjusted %A = 82% 95% CI (70%–97%)	unadjusted %A = 0.0% 95% CI (0%–43%)	unadjusted %A = 6.5% 95% CI (0%–67%)

CXR, chest radiograph; *RA*, room air.

*ACE model: decomposes the total varation in “liability” of disease into additive genetic (A), common or shared environmental (C), and unique or unshared environmental (E) effects.