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The genetic predisposition to bronchopulmonary dysplasia

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Abstract

Purpose of review—Bronchopulmonary dysplasia (BPD) is a prevalent chronic lung disease in premature infants. Twin studies have shown strong heritability underlying this disease; however, the genetic architecture of BPD remains unclear.

Recent findings—A number of studies employed different approaches to characterize the genetic aberrations associated with BPD, including candidate gene studies, genome-wide association studies, exome sequencing, integrative omics analysis, and pathway analysis. Candidate gene studies identified a number of genes potentially involved with the development of BPD, but the etiological contribution from each gene is not substantial. Copy number variation studies and three independent genome-wide association studies did not identify genetic variations significantly and consistently associated with BPD. A recent exome-sequencing study pointed to rare variants implicated in the disease. In this review, we summarize these studies' methodology and findings, and suggest future research directions to better understand the genetic underpinnings of this potentially life-long lung disease.

Summary—Genetic factors play a significant role in the development of BPD. Recent studies suggested that rare variants in genes participating in lung development pathways could contribute to BPD susceptibility.

Keywords

bronchopulmonary dysplasia; copy number variations; exome-sequencing; genetic predispositions; genome-wide association studies

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a common severe chronic lung disorder in extremely premature infants and leads to significant morbidity and mortality [1]. The clinical features, pathogenesises, and causes of BPD have evolved since Northway first described the disease in 1967 [2]. Before the widespread use of surfactant, infants with ‘old BPD’ were often born with serious respiratory distress syndrome (RDS) and received prolonged mechanical ventilation with associated pulmonary edema, inflammation, fibrosis, and smooth muscle hypertrophy in the airways [3]. With advances in therapy, including antenatal glucocorticoids, exogenous surfactant, and improved ventilation techniques, BPD now occurs much less frequently in infants born mildly or moderately premature. Infants who are born extremely prematurely now survive, but these extremely low birth weight infants have a high incidence of ‘new BPD’. New BPD is not only seen in extremely low birth weight infants who initially have significant RDS but also in others who showed little or no respiratory distress at birth but developed signs of hypoxia weeks later [4]. Tachypnea with shallow breathing, retractions, and paradoxical breathing patterns are generally observed in patients with BPD [5]. BPD patients often suffered from hypoxemia or hypercapnia, or both, as well as long-term complications, including frequent lower respiratory tract infections [6], pulmonary hypertension [7], sleep hypoxia [8], delayed growth and development [9], and cognitive impairments [10].

KEY POINTS

- Two independent twin studies have shown strong heritability underlying BPD; however, the genetic architecture of BPD remains unclear.
- Candidate gene studies identified a number of genes potentially involved with the development of BPD, but the etiological contribution of each gene is not substantial.
- No CNV regions were significantly associated with BPD, and three GWASs did not identify common genetic variations significantly and consistently associated with BPD.
- A recent exome-sequencing study pointed to rare variants implicated in BPD; the genes harboring these rare variants were enriched for lung development pathways.
- Future studies are needed to characterize the epigenetic changes and mutations in noncoding regions implicated with BPD.

Twin studies have suggested strong heritability in BPD [11,12]. A variety of strategies have been used to map out the genetic architecture underlying this disease, including investigating candidate gene in mouse models and clinical studies [13], genome-wide association studies (GWASs) [14,15,16], and exome sequencing [17]. However, different approaches pointed to different sets of mutations in a number of genes, and the results were not always consistent [13].

In this review, we summarize the main strategies that have been used to investigate the genetic basis of BPD, outline the potential genetic mechanisms, and suggest future research directions to better understand this disease.

ESTIMATING THE HERITABILITY OF BRONCHOPULMONARY DYSPLASIA THROUGH TWIN STUDIES

Bhandari *et al.* [11] investigated the genetic component of major neonatal morbidities, including BPD, in preterm twins from four medical centers in the United States. They studied 63 monozygotic and 189 dizygotic twin pairs, including 30 monozygotic BPD and 58 dizygotic BPD infants. Since this was a retrospective study, DNA confirmation of zygosity was not possible. Among the 18 monozygotic twin pairs with more than one BPD member, 12 of them had both members suffering from this disease, whereas only 3.69 was expected. From their analysis, genetic factors explained 53% of the variance in susceptibility for BPD after controlling for covariates.

In a prospective study, Lavoie *et al.* [12] collected 70 monozygotic and 89 dizygotic twin pairs born at less than or equal to 30 weeks of gestational age in British Columbia, Canada. Zygosity was determined using fetal ultrasound and placental histological examinations. When using the National Institutes of Health consensus definition of BPD, they found that the genetic component accounted for 79% of the observed variance in BPD susceptibility. Genetic factors explained 82% of the observed variance in moderate-to-severe BPD, defined as needing supplemental oxygen at 36 weeks gestational age, whereas there was no genetic influence on the occurrence of mild BPD (supplemental oxygen at 28 days after birth but not at 36 weeks post menstrual age).

These twin studies showed that a genetic component contributes substantially to BPD susceptibility. Although slightly different definitions of BPD were used and cohorts with different gestational ages were included, the estimated heritability was consistently greater than 50% and approached 80%. These twin studies have led to a number of investigations aiming to identify the genetic architecture underlying BPD.

HYPOTHESIS-DRIVEN APPROACHES TO UNCOVER BRONCHOPULMONARY DYSPLASIA CANDIDATE GENES

Researchers have attempted to identify candidate genes and pathways implicated in BPD for decades. Early investigations focused on a few genes that likely participated in the biological processes associated with BPD pathogenesis. Specifically, a number of researchers prioritized genes related to lung maturation in the search of the genetics of the ‘new BPD’ [4]. With the availability of high-throughput experimental methods, researchers were able to investigate thousands of genes and variants simultaneously, and identified a few novel candidate genes associated with BPD [18]. Reported gene candidates included those encoding vascular endothelial growth factor [19,20], dystroglycan [21], matrix metalloproteinases [22], macrophage migration inhibitory factor [23], interleukins [18], tumor necrosis factor [24,25], as well as toll-like receptors [26]. Shaw and O’Brodoovich [13]

have provided a detailed overview of the genes and mutations identified by the candidate gene approach.

COPY NUMBER VARIATIONS IN BRONCHOPULMONARY DYSPLASIA

As in the investigation of many other complex human diseases, the above hypothesis-driven approaches typically focus on individual genes. On the genome-wide scale, genetic abnormalities could be revealed by large-scale deletion and duplication events affecting genic regions. Therefore, focused studies have been performed to correlate such genome-wide copy number variation (CNV) events with BPD. Hoffmann *et al.* [27^{*}] analyzed CNVs in 1666 infants, including 866 BPD cases and 800 controls. After quality control procedures, 899 BPD patients and 827 controls were successfully genotyped. Their analyses showed that BPD cases did not have a larger number of CNVs than preterm birth controls who did not develop BPD. Further, no CNV regions showed significant correlation with BPD after applying multiple test correction.

There are a number of possible explanations for this lack of findings. For instance, both cases and controls in this study underwent greater or equal to 3 days of mechanical ventilation. This inclusion criterion may have decreased the environmental differences between cases and controls; however, it was different from the criteria used in the twin studies revealing high heritability, thus the results may not be entirely comparable. In addition, BPD is a complex disease with multiple genetic factors. Different patients may have different genetic disruptions in the biological pathways related to the lung development. The locus-by-locus approach described here could only identify the associations between individual CNVs and BPD, but not the collective effects of multiple CNVs that participated in the BPD-related pathways. Furthermore, it is also possible that, given the lower mutation rate of CNVs relative to point mutations, BPD could be better explained by small-scale genetic disruptions rather than large-scale CNV events, which requires GWASs to reveal the loci related to disease susceptibility.

GENOME-WIDE ASSOCIATION STUDIES

rs1245560 single nucleotide polymorphism of *SPOCK2* gene in Caucasian and African premature infants

Hadchouel *et al.* [14] assessed 418 premature infants born at less than 28 weeks gestational age in three French medical centers. They divided their cohort into two discovery sets (infants in one set have both parents of Caucasian descent; infants of the other sets have both parents of black African descent) and one validation set (infants with one Caucasian parent and one black African parent). They identified that single nucleotide polymorphisms (SNPs) in the sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2 (*SPOCK2*) gene were associated with BPD in both discovery sets. As rs1245560 was the only SNP with similar minor allele frequency in Caucasian and African controls, they further performed individual genotyping for rs1245560 in both the discovery and validation sets, and found that allele C of this SNP was significantly associated with BPD. They replicated this finding in a cohort from Finland, and demonstrated that the mRNA level of *SPOCK2* was significantly increased during the alveolar stage of the development of rat lungs. These

results suggested the role of *SPOCK2* and alveolarization in BPD, and showed that GWAS could identify common variants implicated in BPD.

No significant single nucleotide polymorphism identified in a California cohort

To investigate the genetic causes of moderate-to-severe BPD, Wang *et al.* [15] conducted a GWAS on very low birth weight infants of 25–30 gestational age. They studied 899 infants with moderate-to-severe BPD and an additional 827 infants of similar gestational age without BPD. Results did not reveal any SNP statistically significantly associated with BPD, even for the ones reported in previous studies. This lack of results was also observed in another cohort (replication set) with 371 BPD cases and 424 controls. Joint analysis of discovery and replication sets also did not reveal any statistically significant association. Further, pathway analyses did not show any significantly enriched pathway after correcting for multiple tests.

No significant single nucleotide polymorphism identified by the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network

In an attempt to characterize genetic factors underlying BPD, Ambalavanan *et al.* [16*] conducted an integrative study of the SNPs and pathways associated with BPD. Their cohort included 834 infants, with 751 infants with adequate BPD phenotype information and call rate greater than 97% in genotyping. Among the 751 infants, 428 developed BPD or died. As infants who die early cannot develop BPD, they hypothesized that death is a competing outcome of BPD, and performed GWAS for three outcome analyses: [1] BPD or death vs. control; [2] severe BPD or death vs. control; and [3] severe BPD in survivors only vs. control. GWAS analysis revealed no statistically significant SNP at the whole genome level for any of the three comparisons. Although some SNPs in adenosine deaminase, RNA-specific, B2, CD44, among others had *P* values of 10^{-6} – 10^{-7} , they did not pass the criteria of multiple test correction. Gene Set Enrichment Analysis with a false discovery rate less than 0.1 and *P* less than 0.001 showed 75 significantly enriched pathways for BPD or death, 95 for severe BPD or death, and 90 for severe BPD in survivors. MicroRNA 219 (miR-219) targets and phosphorous oxygen lyase activity were among the pathways with the lowest false discovery rate. They further conducted gene expression analysis in mouse models and in the human lung. Results showed that exposure to hyperoxia was associated with increased expression of miR-219 and CD44. Upregulation of these two genes was also identified in the lungs of BPD patients compared with preterm or term lung.

Their results also suggested that pathways associated with severe BPD or death are different from mild or moderate BPD, indicating that the pathophysiology of mild or moderate BPD may be different from severe BPD. In addition, some marked differences by race/ethnic groups were observed in the associated pathways, suggesting that patients with different race/ethnicity may have different genetic mechanisms driving the development of BPD.

Genome-wide association study summary

GWASs have been successfully performed to identify risk loci involved in many complex human diseases, including type 1 and type 2 diabetes, Crohn's disease, rheumatoid arthritis, coronary artery disease, and bipolar disorder [28]. In contrast, GWAS for BPD has been less

effective. In addition to many technical reasons (e.g., sample sizes), biological considerations underlying GWAS should be an important factor. A preassumption of GWAS is the ‘common disease, common variants’ hypothesis [29,30]. Such a hypothetical underpinning likely holds in many diseases, such as type 2 diabetes or cancers, where at-risk alleles could transmit to patients’ off-spring, reaching moderate or even high population frequency in human populations over the course of human evolution. However, RDS, the usual precursor for BPD and BPD itself, are major causes for infant mortality, where severe natural selection would greatly suppress the at-risk alleles in human populations, thereby these risk alleles are expected to have very low frequency. As GWAS methods target common variants, it is likely that GWAS will have insufficient power to identify disease-associated loci of BPD. Nevertheless, the advent of whole-exome sequencing technologies could provide an opportunity to test the role of rare variants in BPD risk. The recent development of pathway analysis algorithms also made it possible to investigate the functional convergence of rare variants.

EXOME SEQUENCING OF BRONCHOPULMONARY DYSPLASIA PATIENTS AND CONTROLS

Li *et al.* [17²²] studied the exomes of 50 pairs of twins sequenced from newborn blood spot samples to investigate rare nonsynonymous variants for their contribution to BPD. Owing to the historical high mortality for RDS and BPD patients, genetic mutations contributing to BPD likely underwent severe purifying selection, resulting in a likely low frequency of mutations in the population. Li *et al.* investigated rare variants residing in highly conserved regions. They performed exome sequencing on the newborn blood spot samples from 50 pairs of twins, and focused on the rare variants not observed in the 1000 genome data set [31] and that also possessed a high conservation score.

Based on their analysis, 258 genes with rare nonsynonymous mutations were identified in BPD infants. Pathway analysis revealed that these genes were highly enriched for processes involved in pulmonary structure and functions, such as collagen fibril organization, morphogenesis of embryonic epithelium, and regulation of the wingless-int signaling pathway. They further demonstrated that these mutations in mice were enriched for several lung development related phenotypes, including embryonic development and cyanosis. These results were consistent with the clinical phenotypes of BPD. Expression analysis in human lung tissue and hypoxic rat pups showed significantly increased expression levels in the candidate genes. This study elucidated the role of rare variants in BPD, which complemented the analyses on common variants targeted by GWASs. Through pathway analysis, this study provided biological insights into the molecular cause of BPD. Although differing from one human to another, ‘rare’ single nucleotide variations are very common in an individual human’s genome; they have been estimated to occur in one of every 17 base pairs [32,33].

The study had a few limitations. First of all, information from the discordant twins was not utilized in their analysis. They did not use the twin relationships since there were only four monozygotic discordant twins and five dizygotic discordant twins. It would be very helpful

to gather a larger patient set and investigate the rare variants associated with disease susceptibility in discordant twin pairs. Additionally, a large number of potential candidate genes and mutations are identified, but the biological implications of these rare variants need to be further elucidated.

Another pilot study in Italy sequenced the exomes of 26 unrelated patients with severe BPD and analyzed potentially detrimental variants [34]. Their results reinforced the role of rare mutations in BPD susceptibility.

CONCLUSION

BPD is a complex disease with a strong genetic component in its suspected cause. Although none of the aforementioned studies is conclusive, they pointed to various variants, genes, or pathways associated with the susceptibility of BPD. It is likely that each genetic variant contributes a little to the development of BPD, and together these variants contribute to significant dysregulation in important biological pathways in the developing lung of premature infants.

A number of common variants and rare mutations showed associations with BPD with various strengths. Future studies are needed to characterize the effect size in of each mutant, to investigate the role of *de novo* mutations, and to map potential mutational convergence on BPD-related pathways.

From a methodologic standpoint, these BPD genetic studies provide some insight into the evolving research paradigm of complex human diseases. The pursuit of BPD-related genes started with selecting a few candidate genes based on information gained by experimental model systems or biological hypotheses, and gradually evolved into high-throughput methods that have become more familiar and accessible to researchers as genomic tools. The bioinformatics analytic approaches have also progressed from investigating the implications of individual mutations to global pathway studies, and from single omics analysis to multiomics integration.

With the recent availability of whole genome sequencing [35,36], bisulfite sequencing [37], and novel methods in identifying chromatin accessibility [38,39], it is possible to characterize epigenetic changes and mutations in noncoding regions implicated with BPD susceptibility. This might help disentangle the complex phenotypes of BPD and uncover the missing heritability of this deadly disease.

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