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Progress in understanding the genetics of Bronchopulmonary Dysplasia

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Abstract

Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease of infants. Its treatment imposes considerable health care burden and costs in the perinatal and early childhood period and patients are usually left with life-long deficits in lung function. Evidence exists for different pathophysiologic pathways that can promote the structural changes that characterize BPD, including the impairment in alveolarization; however, there is increasing interest regarding heritable factors that may predispose very low birth weight infants to BPD. Our review focuses on recent publications that have investigated genetic factors that may potentially contribute to such reported heritability. These publications point us towards some possible genomic candidates for further study, but certainly do not identify any particular gene or gene pathway that would be inferred to be contributing substantially to the underlying etiology of BPD.

I). Introduction

Bronchopulmonary dysplasia (BPD) remains a leading cause of morbidity and mortality in premature infants¹ and the risk of developing BPD rises with decreasing gestational age (GA) and birth weight (BW)²³. Although much research has been conducted to discover pathophysiologic mechanisms responsible for BPD², and there is evidence for specific mediators and pathways³, there has been little progress in decreasing the incidence of BPD in very low birth weight infants (VLBW) who have a BW < 1500 gm. As discussed in further detail below, twin studies⁴⁵⁶ have suggested that genetic factors are the major risk for developing BPD. Accordingly, investigators have used a variety of strategies to identify the heritable factors. With the exception of previous twin studies, our review focuses on research that has been published from 2006 onwards. We refer readers to a previous review⁷ for earlier work (e.g.⁸).

II). Approaches to Discover Heritable Factors

In reviewing the extant literature on genetic factors influencing the risk of human BPD, one finds reference to a variety of approaches, including studies involving familial aggregation, twins, candidate genes, and genome-wide association (GWA) studies. A brief description of each of these approaches is provided below.

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Familial Aggregation

Traits or diseases that are observed to occur more frequently in some families compared to other families are often suspected of having an underlying genetic etiology. However, familial aggregation of a trait or a disease may not only signal the inheritance of genes but also the inheritance of lifestyle factors and shared environmental factors. Thus, the challenge in interpreting the results from family studies is to try to disentangle these various heritable elements of disease risk. The basic premise of such studies has been to conduct what is known as a linkage analysis of the genome. The idea being that genetic markers widely spaced across the genome are evaluated in family members where 2 or more individuals may be affected by the disease phenotype of interest. Even in the circumstance where a particular genotype may be 100% present in a family (complete penetrance), its associated phenotype may vary (expressivity).

Twin Studies

Studies of twins are useful to explore genetic versus environmental etiologies of complex human diseases. The premise underlying such studies is that monozygotic twins share 100% of their genetic information, dizygotic twins share 50% of their genetic information, and both share the intrauterine environment. Thus, if the concordance of a disease is observed to be substantially higher in monozygotic twins than in dizygotic twins, it is reasonable to infer that genetic components underlie the disease etiology. However, the field of epigenetics⁹, traditionally defined as the study of heritable changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence, provides a caveat. Specifically, it had previously been thought that all epigenetic signals were “erased” during the formation of the zygote, however, there is some evidence that epigenetic modifications may be passed down from parents to the fetus¹⁰.

Genome Wide Association (GWA) Studies

GWA studies afford an agnostic evaluation of millions (currently upwards of 5 million in some assay platforms) of single nucleotide polymorphisms (SNPs) for their frequency differences among a “case” (disease phenotype) population compared to an appropriate control population (those without the disease phenotype). Such studies provide an investigator the tools to cast a broad net thereby permitting an examination of much of the common variation across the entire genome. The importance of evaluating the entire genome, and not just the exome, was recently illustrated by the ENCODE project which demonstrated that 80% of the genome contains elements linked to biochemical functions, disproving the long held view that the human genome is mostly ‘junk DNA’¹¹. Sequencing of the human genome in the last decade has provided the basis of such discovery tools for common genomic variation. It was demonstrated that genetic variation, SNPs, across the genome could be captured by what are known as tag SNPs. A tag SNP is chosen in such a way to be representative of numerous SNPs in the genome that are in high linkage disequilibrium (this refers to the association between two alleles that are inherited together more frequently than expected by chance). Thus, it is possible to interrogate regions of the entire genome without genotyping every single SNP in that particular region. However, the assumption is that unknown genetic disease markers being sought are sufficiently correlated with the tagSNPs being tested to be detected.

Candidate Gene Studies

Identification of candidate genes or pathways for study can derive from information gained by studies in experimental systems (e.g., cells and animals), by previous human studies, or by biologically-based theory as to why certain genes or their coded proteins might be functionally involved in the structural changes, such as under-development of the distal lung

unit, that characterize BPD. Such studies have clear hypothesis-driven advantages, but are also disadvantaged because such studies are targeted to specific areas of the genome and therefore may overlook critical genes or regions in the genome that could be etiologically relevant to a complex disease phenotype.

These different approaches are extremely valuable for identifying genetic etiologies for complex human disease. From the perspective of causal inference, no one study or study design can establish that a gene or a variant in a gene is the *sine qua non* underlying a disease phenotype. A series of observations from different study designs, different populations, supporting biological functional information, and experimental evidence are typically required to establish a causal connection. For genetic studies of BPD, critical aspects of study design and conduct such as source population case definition, control definitions, analytic treatment of covariates, choice of genotyping platforms, genotyping quality, etc. can all conspire to produce somewhat different results.

III). Familial Aggregation

During the era of Northway's old BPD, Nickerson et al ¹² evaluated premature infants with respiratory distress syndrome (RDS), some of whom developed BPD and some who did not develop BPD. They reported an increased incidence of BPD in patients with a family history of asthma, a disorder that is known to have familial aggregation.

Surfactant Proteins

The family-based study, carried out by Pavlovic et al ¹³, included 71 Greek neonates from 60 families who were born < 30 weeks GA and either had mild BPD (n = 52, supplemental oxygen at 28 d postnatal age (PNA)) or moderate-severe BPD (n = 19, supplemental oxygen at 36 weeks PMA). They examined transmission of individual SNPs from 5 surfactant associated protein genes and Surfactant Associated Protein-B (SP-B) linked microsatellites using two available measures to assess allelic transmission: using Transmission Disequilibrium Test (TDT) and Family Based Association Test (FBAT) they found significant associations ($p < 0.01$) for alleles of SP-B and SP-B-linked microsatellite markers, and haplotypes of SP-A, SP-D, and SP-B. Specifically, SP-B marker locus B-18 (A/C) within the 5' UTR of SP-B was associated with susceptibility in moderate – severe BPD. That is, the C allele at this locus was observed to be transmitted approximately 3.5 times more than expected ($p=0.02$). Microsatellite marker AAGG_6 was associated with susceptibility in the moderate-severe BPD group ($p=0.01$). However, it is unknown whether the susceptibility to BPD relates to SP-B or to another gene or variant located in or close to SP-B. Haplotype analysis revealed 10 susceptibility and 1 protective haplotypes for SP-B and SP-B-linked microsatellite markers and two SP-A-SP-D protective haplotypes. The interpretation of these findings is influenced by the large proportion of mild BPD patients with the study including only 19 moderate-severe BPD cases. Also, they only analyzed relative to prenatal steroids, post-natal steroids and use of surfactant, so it is unknown whether the associations would have remained if they had included other potential covariates such as GA and BW that are also strongly associated with BPD. Indeed patent ductus arteriosus (PDA), which is associated with BPD, has been shown in a twin study to have a genetic linkage ¹⁴.

IV). Twin Studies

Parker et al ⁴ evaluated BPD, defined as supplemental oxygen at 28 days PNA, in twins having BW < 1,500 grams and demonstrated if the first twin had BPD the odds ratio was 20.9 in unadjusted and 12.3 in adjusted analyses for the second twin to have BPD. Although this suggested that genetic factors were present, since the investigators did not determine

zygosity, they could not attempt to exclude the role of shared environmental risk factors. Indeed, the analyses by “same-sex” of the twin pair did not reveal a greater concordance of BPD than those of unlike sex but the sample sizes for these analyses were small. This study was pivotal in suggesting that BPD might have an underlying genetic etiology but indicated that further investigations were warranted.

In a USA-based study, Bhandari et al ⁵ conducted a retrospective study of twins who had GA < 32 weeks and were born from 1994 – 2004. Zygosity was evaluated by placental examination and sex of the neonate. The concordance of BPD, defined as the need for supplemental oxygen at 36 weeks PMA with compatible radiographic findings, was higher in monozygotic (MZ) than in dizygotic (DZ) twins. This study had 192 twin pairs with BPD, and those with zygosity information included 30 MZ and 58 DZ twin pairs with BPD. Their analysis showed that the variance in genetic liability, after adjusting for co-variables, was 53%. This suggested that genetic factors contributed substantially to the occurrence of moderate-severe BPD. They did not provide information regarding infants who had mild BPD (oxygen supplementation at 28 days PNA but who were on room air at 36 weeks GA).

In a Canadian-based study, Lavoie et al ⁶ studied twin pairs with GA < 30 weeks born between 1993 and 2006. Zygosity was primarily determined by ultrasound or placental examination with secondary assessments using sex or blood group. Their study population consisted of 70 MZ and 89 DZ twin pairs. They used three different definitions of BPD ¹⁵¹⁶, one of which was the NICHD consensus definition for mild, moderate and severe BPD. They used structural equation models to produce estimates of the relative contributions of additive genetic, shared environmental, and non-shared environmental influences. For their study population they found that genetic effects accounted for 79% of the observed variance in BPD susceptibility for moderate to severe BPD as defined by the NICHD. In contrast, the variability in mild BPD (oxygen requirement at 28 days PNA but not at 36 weeks PMA) could be explained by environmental effects with no evidence for heritability.

Taken together, these three twin studies implicate genetic factors as playing an important role in the susceptibility to BPD. It is notable that the heritability that was estimated to be between 50-80%, for moderate – severe BPD is at least as large, and probably greater than, that for systemic hypertension (~30%) ¹⁷, cancer (~40%) ¹⁸, and psychiatric disorders (~60%) ¹⁹. This comparison of heritability for BPD and adult onset disease may be even more interesting when one considers that twin studies outside the newborn period become more susceptible to shared environment or behaviors during the life course. Thus, one might view the heritability estimates for these adult diseases as overestimates.

V). GWA Study

Spock2

Hadchouel et al ²⁰ studied 418 Caucasian-French and African-French premature infants who were born < 28 weeks GA in 3 centers in France from 2002 – 2009. Ethnicity was determined by the geographic origin of each parent. BPD was diagnosed using “physiologic test” to assess the need for supplemental oxygen at 36 weeks PMA ²¹; there was a 22% incidence of BPD. The authors first did a GWA study on some of the patients (43 BPD and 162 controls) but used “pooled DNA” for each of the case and control groups. The “controls” (non-BPD) infants used as referents were not defined in detail. For the Caucasian samples they used the Illumina Hap300 chip with 318,237 SNPs, whereas they used the Hap 650 chip with 660,918 SNPs for the African-French samples. They found that the rs1245560 SNP of SPOCK 2 gene was associated with BPD ($p = 1.66 \times 10^{-7}$). The authors then went back and performed individual genotyping for that SNP which was found in both the African-French and Caucasian-French infants. They then were then able to “replicate” these

findings in both an internal replication cohort (49 BPD and 163 controls) and an external cohort consisting of 213 Finnish infants who were born between 1997-2010 and had a GA < 30 weeks. The Finnish infants had a 26% incidence of BPD which was similar to the incidence in the French NICUs. The C allele for rs1245560 SNP of SPOCK2 was associated with risk of moderate – severe BPD in both Caucasian-French (OR=3.0 (1.4-6.4)) and African-French patients (OR=5.0 (1.9-12.6)), but it was not associated with mild BPD which is consistent with the findings of Lavoie et al ⁶. The authors then did fine mapping of the SPOCK2 region and reconfirmed the CC genotype risk in both Caucasian-French and African-French patients. They found other SNPs, but they were all in linkage disequilibrium with rs1245560. They also replicated the CC and CG genotypes in the Finnish cohort. Their subsequent studies in rat pups demonstrated that the level of mRNA coding for SPOCK2 increased during stage of alveolar development. SPOCK2 was expressed at the mRNA level in both fibroblasts and epithelial cells and increased when rats were exposed to hyperoxia. The authors did not determine if SPOCK2 was expressed at the protein level in a similar fashion to their mRNA findings.

SPOCK2 (SPARC/osteonectin, CWCV, and Kazal-like domains proteoglycan 2), is also known as Testican-2 and is a member of the testican group of extracellular chondroitin and heparin sulfate proteoglycans. SPOCK 2 interacts with matrix metalloproteinase-14 (MMP-14) and MMP-16 which, together with the mRNA expression data, supports their speculation that their findings may have biologic importance.

VI. Candidate Genes, TAG SNPs, Haplotypes

Several decades of research have identified potential pathophysiologic pathways or molecules that may be responsible for the acute and chronic characteristics of BPD. Accordingly, investigators have evaluated whether specific alterations in the sequence of candidate genes, as illustrated by SNPs, might be associated with a change in the risk of developing BPD. Below, we discuss different recent publications. For further details regarding the relevance of these genes to potential pathophysiologic processes for BPD we refer you to review articles ²³ and their references to the original scientific literature.

Surfactant proteins (SFTPA2, SFTPB, SFTPC, SFTPD), mannose-binding lectin (MBL2), tumor necrosis factor (TNF), interferon gamma (IFNG) and angiotensin converting enzyme (ACE)

In a USA – based study on Caucasian infants, Rykman et al ²² evaluated previous reports regarding 8 genes associated with increased risk for BPD. They were surfactant proteins (SFTPA2, SFTPB, SFTPC, SFTPD), mannose-binding lectin (MBL2), tumor necrosis factor (TNF), interferon gamma (IFNG) and angiotensin converting enzyme (ACE). Although they looked at more babies and multiples, the report focuses on 134 singleton BPD infants born between 2000-2009 with a GA < 32 weeks at the University of Iowa. BPD was defined as needing supplemental oxygen at 36 weeks GA. They compared the findings to 255 control infants without BPD. In general, tagSNPs were selected to provide the most adequate coverage of each gene including markers both upstream and downstream of the given region. Emphasis was often given to SNPs in coding regions and promoter elements. Several SNPs from each gene were used yielding a total of 34 SNPs and thus requiring a Bonferonni corrected p value of $p < 0.001$. None of the SNPs reached this threshold, but the authors do provide information on SNPs that reached a lower statistical threshold (p values < 0.01) and also found that the ACE SNP, rs8066114, was associated with an increased risk of BPD, OR=4.3 (1.3-14.3).

Interleukin-18 (IL-18)

IL-18 is a cytokine that belongs to the IL-1 superfamily, is produced by macrophages and other cells and is an important regulator of innate and acquired immune responses. There have been two recent studies, described below, which studied its relationship to BPD.

A high throughput approach was used by Floros et al ²³ to evaluate 601 genes with 6,324 SNPs in 1091 Caucasian and African American (AA) infants who were born in the USA between 1989-2008 with a GA < 35 weeks. These authors selected these candidate genes based on previous knowledge regarding the role of these genes in immune response, inflammation, tissue repair, or on biologic plausibility to lung disease development. They used ancestral markers to determine ethnicity rather than the typical approach of having subjects self-report their race - ethnicity. This is of special importance when study lung (patho)physiology; a recent publication has demonstrated that ancestral markers can vary between subjects who self-report the same race ethnicity and, at least in the case for African Americans (AA), that their related lung function correlates with the “dose” of ancestral genes ²⁴. BPD was defined as the need for supplemental oxygen at 28 days PNA and therefore the cohort included mild, moderate and severe BPD and likely some babies from the pre-surfactant era. As discussed above, heritability for BPD has been demonstrated for moderate – severe, but not mild BPD ⁶.

Both nonsynonymous and tag SNPs were studied. Associations were observed only for rs3771150 (IL-18RAP) and rs3771171 (IL-18R1) in AA with mild/moderate/severe BPD (n=17) vs. AAs without BPD (n = 98); $q < 0.05$). The SNP markers were in the introns of these genes and are likely markers for other polymorphisms conferring risk; this was supported by their haplotype analyses that showed other markers with high linkage disequilibrium. Marker rs3771150 had C and T alleles with an odds ratio for BPD of 5.7 (2.5-13.2) for the T allele. Marker rs3771171 had A and G alleles with the G allele being associated with an odds ratio of 5.9 (2.4-13.9). These SNPs were not associated with BPD in the Caucasian patients with mild/moderate/severe BPD. This ancestral dependency of the clinical phenotype is consistent with another report ²⁵.

Genetic markers were used to stratify cohorts to either AA (n=169), Northern European Caucasians (n = 897) or Southern European Caucasians (n = 25) but all Caucasians were reported together. The authors chose a replication set that consisted of self-reported AA with 88 BPD and 102 no BPD. In their replication cohort they evaluated the specific SNPs that had been identified during their discovery phase. Whole genome amplified DNA was used for the replication cohort study and they found that the p values in their replication study were 0.012 for rs3771150 and 0.07 for rs3771171. The p-values were smaller in the replication data set, but oddly the minor allele frequency (MAF) for these two SNPs in the replication set was much lower than the MAF in the discovery set, particularly among cases.

In a German study ²⁵, Krueger et al recruited Caucasian infants and genotyped them for six different SNPs within IL-18. They compared 228 infants who were born < 32 weeks GA with adult controls who had not been born prematurely. They found a strong association of IL-18 with preterm birth ($p < 0.001$) but not with BPD. They could not replicate this association with premature birth when they studied the most significant SNP in a second population of 346 preterm infants where the controls were infants born at term (n=56 with BPD). No association with any lung condition of prematurity, including BPD, was observed. BPD was defined as the need for supplemental oxygen or positive pressure at 36 weeks PMA.

Surfactant, Growth Factors, Vascular Activity, Inflammation, and Immune Function

This German study²⁶ included 155 preterm (<29 weeks) infants born 1996-2005. Among the 155, 108 were considered to have no or mild BPD and 47 to have moderate/severe BPD. RFLP genotyping was employed to assess 37 SNPs in 16 genes. These were IL-8, SFTPC, SFPTD, TNF α , TNF β , IL-4, IL-13, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF β), TGF α , IL-10, SELE (codes for E-selectin), connective tissue growth factor (CTGF), intercellular adhesion molecule (ICAM), IL-8, and toll-like receptor10 (TLR10). The interrogated genes are involved with surfactant, growth factors, vascular activity, inflammation, and immune activity. Three SNPs in 3 genes were statistically significant in analyses unadjusted for multiple comparisons, but not after such adjustment. The 3 were TNF α (rs=1799724), VEGFA (rs=699947), and TLR10 (rs=11096955).

Superoxide dismutases (SOD)

Guisti et al²⁷, in a retrospective study conducted in Italy, recruited 152 premature infants who were born between 2004 and 2008 and had a GA < 28 weeks. They report genotypes on 43 (28%) who developed BPD (supplemental oxygen at 36 weeks PMA) and 108 neonates without BPD who were considered controls. They evaluated 10 SNPs in 4 candidate genes because of the genes' demonstrated or putative function related to oxidant stress: SOD1 (rs17880135 and rs204732), SOD2 (rs4880 Ala16Val and rs5746136), SOD3 (rs8192287, rs2536512 Thr40Ala and rs1700895 Arg213Gly) and CAT(rs1001179 -262C/T, rs1049982 -20T/C and rs769217 C22348T). SOD3 (rs2536512) was marginally associated with a reduced risk of BPD, OR=0.49 (0.21-1.1) adjusted for BW and GA. Two haplotypes showed statistically significant associations for BPD; one with SOD2 (rs4880 and rs5746136) and SOD3 (rs8192287, rs2536512 and rs1799895). None of the 10 SNPs had a p value sufficiently small to be associated with BPD when adjusted for BW and GA. Superoxide dismutases play an important role in the defense against oxidant stress.

Toll Like Receptors (TLR)

Sampath et al²⁸ investigated toll like receptors because of their known relationship to inflammation in both humans and animal models. They used a pathway-intensive approach to examine impact of 9 functional TLR pathway SNPs [targeting TLR2, TLR4, TLR5, TLR9, IRAK1, MAL(TIRAP), NFKB1, and NFKBIA] on BPD susceptibility and severity in a VLBW infant cohort recruited from 4 USA centers between 2006 – 2009. They report on moderate (n =66) or severe (n =32) BPD and compare to 214 VLBW infants without BPD. The authors found that BPD occurrence was influenced by smaller BW, earlier GA, male sex, the use of assisted ventilation, the presence of a patent ductus arteriosus (PDA), and late onset sepsis. There was no significant association for TLR2, TLR4, TLR9, nuclear factor kappa B 1(NFKB1), and nuclear factor kappa B inhibitor alpha (NFKBIA), a trend towards significance for toll-interleukin 1 receptor domain-containing adaptor protein (TIRAP) 2054 C>T (rs8177374) and interleukin receptor-associated kinase 1 (IRAK1) 6435T>C (rs1059703), and one statistically significant (TLR5 1174 C>T, rs5744168, which encodes a stop codon) association with BPD. TLR5 was associated with an OR=2.6 (1.1-6.0) for BPD, OR=3.8 (1.5-9.9) for severe BPD, and OR=3.6 (1.3-10.1) among Caucasians only (approximately 2/3 of the infants were Caucasians). TLR 5 is essential for recognition of a flagellin, a cell wall component of gram negative bacteria.

TLR4

Since previous studies indicated that the TLR4 polymorphisms Asp299Gly (rs4986790) and Thr399Ile (rs4986791) predisposed older infants to respiratory syncytial virus (RSV) infection, Lavoie and colleagues²⁹ investigated whether these TLR4 SNPs were associated

with BPD. In two Canadian centers they recruited 269 infants who were born between 2006 – 2008 and had a GA < 30 weeks. Study infants were either singletons or the first born of multiples, 65% were Caucasian and 38% were of moderate or severe BPD. The TLR4-299 heterozygous genotype was significantly under-represented in infants without BPD (1.6% of infants versus 12% in infants with severe BPD) after adjusting for twinning, ethnicity, GA, BW and sex ($p = 0.014$). These adjusted estimates should be viewed with caution due to the limited sample size; only 1 or 2 control infants with the genotype were involved in these analyses. This association was not replicated in a Finnish cohort ($n = 434$) of premature singletons or first-born siblings of Caucasian descent of < 32 weeks GA where incidence of moderate – severe BPD was only 15%. Similarly, as described above, Sampath et al ²⁸ did not find an association of TLR4 with BPD. TLR4 is an important component of the innate immune system and it plays a role in pathogen recognition by detecting lipopolysaccharide from gram negative bacteria.

Macrophage Migration Inhibitory Factor (MIF)

Prencipe and colleagues ³⁰ recruited 103 Caucasian Italian infants who had been born at a GA of 24 - 34 weeks and had RDS. Twelve infants died and 25 developed BPD as defined by the need for supplemental oxygen at 36 weeks PMA. The -173 G/C MIF promoter polymorphism in the cytokine macrophage migration inhibitory factor (MIF), which is associated with higher MIF expression, was found in two of the 25 BPD (8%) and 20 of the 66 (30%) who did not develop BPD ($p = 0.04$). Univariable analysis showed C allele compared to the GG genotype had odds ratio of 0.20 (0.04-0.9). The small sample size in these analyses raises questions regarding the validity of the multivariable results. MIF favors innate immune response, promotes angiogenesis, induces VEGF and is up-regulated by hypoxia.

Human Leukocyte Antigens

In a Portuguese study that involved two NICUs ³¹, Rocha et al identified 400 Caucasian infants born 2000 – 2009 and GA < 32 weeks. After exclusion criteria, the investigators had 156 neonates who were alive at 36 weeks PMA. BPD was defined using standard NIH guidelines for mild, moderate/severe BPD. The investigators measured class I molecules HLA-A, -B, -C and class II, HLA-DR for their association with BPD and claim some were significantly related to BPD. However, this publication has internal inconsistencies in the reporting of the data and some methodological approaches that are unusual (e.g., relative risk calculations and odds ratio calculations from the same data). Accordingly, additional study would be required to assess their hypotheses and findings.

Factor X111, Factor VII and TGF β polymorphism

Between 2006 and 2008 Atac et al ³² recruited Caucasian Turkish infants to evaluate the relation between BPD and Factors XIII and VII, genes involved in the coagulation cascade, and TGF β , a cytokine involved in a plethora of cellular processes with BPD. BPD was defined as a state of supplemental oxygen requirement at 28 days PNA or 36 weeks PMA. They classified their infants as either mild/moderate BPD ($n=42$) or severe BPD ($n = 56$). Ninety four infants did not have BPD. Their cohort included infants < 37 weeks GA and the controls and BPD had different GA and BW, differences that should have been considered analytically, but were not. They did not detect any difference in frequency of specific polymorphisms (FXIIIVal34Leu, FVII-323 del/ins, and TGF β 1 (915G=T) gene polymorphisms) between the BPD and no BPD infants. However, the limitations of this study; specifically that the control and BPD infants were not similar, there was aggregation of mild/moderate and inclusion of some quite late GA and high BW babies may explain their negative findings regarding FVII-323 which disagrees with the association described by Hartel et al ³³ as outlined below.

Factor VII

Hartel et al³³ combined results from two separate German multicenter trials, carried out from 2000 through 2005, so they could evaluate the potential association of specific hemostasis genes to morbidity, including BPD. In the approximately 765 VLBW babies who were genotyped, there were approximately 120 infants with BPD as defined as the need for supplemental oxygen at 36 weeks PMA. Genotypes of factor V Leiden mutation, prothrombin G20210A mutation, the factor VII-323 del/ins polymorphism, and the factor XIII-Val34Leu polymorphism were determined and none were related to their primary outcome of IVH or PVL. These investigators did observe that the presence of Factor VII-323 del/ins polymorphism was associated with a reduced risk of BPD ($p = 0.01$). It is not clear whether potential covariates were considered in assessment of these genotypes and risk of BPD.

TNF

This systematic review³⁴ and meta-analysis of 6 previous studies investigated the relation of the multi-functional pro-inflammatory cytokine tumor necrosis factor (TNF) -308A polymorphism and BPD. These authors concluded that there is no association between TNF -308A polymorphism and BPD, a conclusion consistent with other work²²³⁵.

Vascular Endothelial Growth Factor (VEGF)

During 2003-2006 this prospective Polish study³⁶ Kwinta et al recruited 181 Caucasian infants who were born between 24 - 32 week GA, weighed <1,500 gm BW and required respiratory support during first 3 days after birth. Some infants developed mild (58, 32%), moderate (3, 13%) and severe (37, 20%) BPD using standard definitions (total = 118 BPD infants).

Polymorphisms in VEGF, TGF β 1, 5,10- Methylene tetrahydrofolate reductase (MTHFR), and insulin-like growth factor -1 (IGF-1) genes were evaluated. Newborns with the polymorphic VEGF allele -460T were over represented in the BPD group. Multivariable analysis revealed that carrying T allele increased the risk of BPD by 9% (95%CI: 2–14%) above the baseline risk established for given GA, length of oxygen therapy, and sex. Multivariable analysis showed the CC genotype for the -460C gene was associated with a substantially lower risk of BPD, odds ratio = 0.20 (0.05-0.85). The VEGF -460CC homozygotes had a lower risk than babies with -460TT or -460TC genotypes and the authors speculate that this may arise because the -460 polymorphism increases the promoter activity of VEGF. However, they could not identify different serum levels of VEGF in the respective infants. No differences in allelic frequencies for the other genes investigated, TGF-B1, 5,10-MTHFR, and IGF-1, were observed.

Matrix Metalloproteinase (MMP) polymorphisms

From 2002 – 2006 Hadchouel et al³⁷ carried out a prospective study in two different French NICUs and recruited 284 neonates with a GA < 28 weeks. Forty five infants developed BPD as defined as a supplemental oxygen requirement at 36 weeks using the physiologic test²¹. Nine SNPs within MMP2, MMP14 and MMP16 genes, and previously linked to other respiratory diseases, were used as tag SNPs. After adjustment for BW and ethnic origin, the heterozygous and homozygous genotypes for both MMP16 C/T (rs2664352) and MMP16 A/G (rs2664349) were found to be associated with a reduced risk of BPD (odds ratios ranging from 0.2-0.7). These genotypes were also associated with a smaller active fraction of MMP2 and with a 3-fold-lower MMP16 protein level in tracheal aspirates collected within 3 days after birth. MMP16, a matrix metalloproteinase, is also called MT3-MMP, increases

expression during the latter parts of intrauterine lung development and in models of arrested alveolarization its mRNA expression decreases.

Dystroglycan

An Italian study carried out by Concolino et al³⁸ enrolled Caucasian patients in 2005 and 2006. The study population consisted of 33 premature newborns who had a GA < 34 weeks (range 24 – 33 weeks) and underwent endotracheal intubation at birth. Eleven babies developed BPD (supplemental oxygen at 36 weeks PMA) and their DAG1 exons and introns were sequenced and compared with both the other 22 infants who did not develop BPD and with 20 adult blood donors. They investigated 8 polymorphisms, one of which (N494H homozygous genotype) was found to be associated with BPD ($p=0.033$) based on 4 BPD infants and 1 control infant with the homozygous genotype. Dystroglycan is highly expressed in the lung where it plays a role in the maintenance of membrane stability by serving as an extracellular matrix receptor for extracellular matrix molecules and supports morphogenesis, adhesion and wound repair. It is unknown whether the N494H SNP has functional significance.

Interferon gamma (IFNG)

In a retrospective study Bokodi et al³⁹ studied Hungarian infants who were born between 2000 and 2003 and had BW < 1500 g ($n=153$). These infants were compared to healthy term neonates ($n=172$). Stepwise logistic regression analysis showed that carriers of the IFNG T⁺⁸⁷⁴ A allele were protected against BPD, OR=0.35, $p=0.049$, (95% CI 0.12 to 0.99). The specific number of BPD patients was not provided in this report. Interferon-gamma, in addition to having an anti-viral activity, coordinates a diverse array of cellular programs through transcriptional regulation of immunologically relevant genes.

Mannose-binding Lectin

Mannose-binding lectin (MBL) is a collectin that plays a role in the innate immune system. MBL1 is a pseudogene and MBL-2 is the functional gene. There have been two different reports investigating MBL SNPs and their relation to the susceptibility to BPD.

In a German study by Hilgendorff et al⁴⁰ 284 newborn infants with a GA <32 weeks were recruited. Presumably these infants were predominately German and Caucasian. Of the 284 infants, 1 had mild BPD and 32 had moderate/severe BPD giving an overall 12% frequency of BPD as defined by usual criteria. The authors evaluated 3 SNPs in the coding region (rs5030737 (codon 52), rs1800450 (codon 54) and rs1800451 (codon 57)) and 1 SNP in the promoter region rs7096206 (-221 G>C) of MBL2. After adjusting for GA, RDS severity, and days on mechanical ventilation, there was a significant association between BPD at 36 weeks PMA and SNPs in exon 1 (rs1800450, codon 54) and in the promoter region (rs7096206, -221). Using a dominant inheritance model, carriers of the A allele in codon 54 (B variant; coding for asparagine) had a higher risk of BPD compared to those who were homozygous for the G allele (odds ratio (OR) =3.59, 95%, CI = 1.62–7.98) while carriers of the C allele at position -221 (X variant) also revealed a higher risk of BPD compared to those who were homozygous for the G allele (OR = 2.40, 95% CI = 1.16– 4.96). The association of putative MBL haplotypes with BPD, independent of the factors GA and days on mechanical ventilation, differed across the estimated haplotypes ($P=0.0087$). The MBL haplotypes MBL*03 (YB) (frequency =14.32%) and MBL*02 (XA; frequency = 21.38%) revealed positive associations with the diagnosis of BPD ($P=0.0048$ and 0.0216). In contrast, the wild-type haplotype MBL*01 (YA; frequency = 55.97%) showed a negative association with BPD ($P<0.0001$).

An Italian study conducted from 2005 – 2006 by Capoluongo and colleagues⁴¹ enrolled 75 Caucasian infants whose GA ranged from 24–36 weeks. All study infants had RDS and required surfactant therapy but had no other associated pulmonary disorders. They compared known polymorphisms in the MBL2 gene and found that the polymorphism -550 G>C ($p=0.02$) was associated with a reduced risk and the R52C polymorphism was associated with an increased risk of BPD ($p = 0.04$). This study, which only included 13 BPD cases and carried out multiple comparisons, has a sample size that is too small to derive valid inferences about risk.

TNF-alpha Polymorphisms

Between 2002 to 2004 Strassberg et al³⁵ enrolled, at a single center in New York State, 105 infants who had a BW < 1 kg and survived to at least 36 weeks PMA or discharge. Among the 105 infants, 89 had BPD (mild, $n=27$, moderate, $n=32$ and severe, $n=28$) while 18 infants did not have BPD. Their study included Caucasians, African Americans, Hispanics and “other”. BPD was classified as mild, moderate and severe using standard criteria at 28 days PNA and at 36 weeks PMA. The authors evaluated the TNF α SNPs: -1,031, -863, -857, -308, and - 238, with the objective of detecting differences across phenotype groups of BPD severity. They did not find any relation with BPD severity but this is not surprising since the study size was so small, particularly with only having 18 infants in the control group. Only very large risks would have been observable with such a sample size.

Selectin

In a retrospective Hungary-based study Derzbach et al⁴² identified 125 LBW infants who were born between 1995 – 1999 and had a GA ranging from 24 – 36 weeks and BW 510–1500 grams. They were compared to 156 full term control infants. Twenty four infants had BPD, defined as the need for supplemental oxygen at either 28 d PNA or 36 weeks PMA. No sub-division between mild and moderate BPD was provided. They evaluated nonsynonymous SNPs for E-selectin Ser128Arg, P-selectin Thr715Pro and L-selectin Pro213Ser. The first two, but not the last, SNPs have been shown to alter function of their respective gene. Neither E nor P selectin SNPs were associated with the combined mild-moderate BPD cohort. After adjustment for variables (GA and “other perinatal complications”), the authors suggested that carriers of L Selectin were at greater risk of BPD (OR 2.45, CI 1.01 ~ 5.95, $p = 0.04$). However, the L Selectin mutant allele was the same frequency among the BPD patients (0.185) and the non-BPD LBW patients (0.195), but both were higher than the term controls used for comparison (0.12). Perhaps the L Selectin is a marker for preterm birth rather than for BPD.

VII. Summary

The literature published from 2006 onwards shares many characteristics. Studies in general have been small in sample size with nearly all studies including fewer than 100 moderate/severe BPD patients. The criteria defining BPD, and the groupings for mild, moderate and severe BPD, has been somewhat variable across studies. A surprising number of studies have compared BPD infants to late term or term infants and in some cases even to adults. Associations that are observed in such studies could be potentially attributable to prematurity rather than BPD. Many studies have relied on single clinic populations and therefore the generalizability of their observations is limited owing to clinic service area considerations. Finally, although many genes have been investigated, few studies have investigated the same gene or sets of genes. Thus, we have a body of information since 2006 that points us towards some possible candidates for further study, but certainly does not illuminate any particular gene or gene pathway that would be inferred as the etiologic smoking gun of BPD.

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References

- Walsh MC, Szeffler S, Davis J, Allen M, Van ML, Abman S, Blackmon L, Jobe A. Summary proceedings from the bronchopulmonary dysplasia group. *Pediatr.* 2006; 117:S52–S56.
- Baraldi E, Filippone M. Chronic lung disease after premature birth. *N Engl J Med.* 2007; 357:1946–1955. [PubMed: 17989387]
- Bhandari A, Bhandari V. Pitfalls, problems, and progress in bronchopulmonary dysplasia. *Pediatr.* 2009; 123:1562–1573.
- Parker RA, Lindstrom DP, Cotton RB. Evidence from twin study implies possible genetic susceptibility to bronchopulmonary dysplasia. *Semin Perinatol.* 1996; 20:206–209. [PubMed: 8870123]
- Bhandari V, Bizzarro MJ, Shetty A, Zhong X, Page GP, Zhang H, Ment LR, Gruen JR. Familial and genetic susceptibility to major neonatal morbidities in preterm twins. *Pediatr.* 2006; 117:1901–1906.
- Lavoie PM, Pham C, Jang KL. Heritability of bronchopulmonary dysplasia, defined according to the consensus statement of the national institutes of health. *Pediatr.* 2008; 122:479–485.
- Bhandari V, Gruen JR. The genetics of bronchopulmonary dysplasia. *Semin Perinatol.* 2006; 30:185–191. [PubMed: 16860158]
- Rova M, Haataja R, Marttila R, Ollikainen V, Tammela O, Hallman M. Data mining and multiparameter analysis of lung surfactant protein genes in bronchopulmonary dysplasia. *Hum Mol Genet.* 2004; 13:1095–1104. [PubMed: 15102713]
- Yang IV, Schwartz DA. Epigenetic control of gene expression in the lung. *Am J Respir Crit Care Med.* 2011; 183:1295–1301. [PubMed: 21596832]
- Czyz W, Morahan JM, Ebers GC, Ramagopalan SV. Genetic, environmental and stochastic factors in monozygotic twin discordance with a focus on epigenetic differences. *BMC Med.* 2012; 10:93. [PubMed: 22898292]
- Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M. An integrated encyclopedia of DNA elements in the human genome. *Nature.* 2012; 489:57–74. [PubMed: 22955616]
- Nickerson BG, Taussig LM. Family history of asthma in infants with bronchopulmonary dysplasia. *Pediatr.* 1980; 65:1140–1144.
- Pavlovic J, Papagaroufalos C, Xanthou M, Liu W, Fan R, Thomas NJ, Apostolidou I, Papathoma E, Megaloyianni E, DiAngelo S, et al. Genetic variants of surfactant proteins A, B, C, and D in bronchopulmonary dysplasia. *Dis Markers.* 2006; 22:277–291. [PubMed: 17264398]
- Bhandari V, Zhou G, Bizzarro MJ, Buhimschi C, Hussain N, Gruen JR, Zhang H. Genetic contribution to patent ductus arteriosus in the premature newborn. *Pediatr.* 2009; 123:669–673.
- Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med.* 2001; 163:1723–1729. [PubMed: 11401896]
- Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA, Wraage LA, Poole K. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. *Pediatr.* 2005; 116:1353–1360.
- Agarwal A, Williams GH, Fisher ND. Genetics of human hypertension. *Trends Endocrinol Metab.* 2005; 16:127–133. [PubMed: 15808811]
- Pharoah PD, Dunning AM, Ponder BA, Easton DF. Association studies for finding cancer-susceptibility genetic variants. *Nat Rev Cancer.* 2004; 4:850–860. [PubMed: 15516958]
- Tandon K, McGuffin P. The genetic basis for psychiatric illness in man. *Eur J Neurosci.* 2002; 16:403–407. [PubMed: 12193182]
- Hadchouel A, Durrmeyer X, Bouzigon E, Incitti R, Huusko J, Jarreau PH, Lenclen R, Demenais F, Franco-Montoya ML, Layouni I, et al. Identification of SPOCK2 as a susceptibility gene for bronchopulmonary dysplasia. *Am J Respir Crit Care Med.* 2011; 184:1164–1170. [PubMed: 21836138]

21. Walsh MC, Yao Q, Gettner P, Hale E, Collins M, Hensman A, Everette R, Peters N, Miller N, Muran G, et al. Impact of a physiologic definition on bronchopulmonary dysplasia rates. *Pediatr*. 2004; 114:1305–1311.
22. Ryckman KK, Dagle JM, Kelsey K, Momany AM, Murray JC. Genetic associations of surfactant protein D and angiotensin-converting enzyme with lung disease in preterm neonates. *J Perinatol*. 2012; 32:349–355. [PubMed: 21960125]
23. Floros J, Londono D, Gordon D, Silveyra P, Diangelo SL, Viscardi RM, Worthen GS, Shenberger J, Wang G, Lin Z, et al. IL-18R1 and IL-18RAP SNPs may be associated with bronchopulmonary dysplasia in African-American infants. *Pediatr Res*. 2012; 71:107–114. [PubMed: 22289858]
24. Kumar R, Seibold MA, Aldrich MC, Williams LK, Reiner AP, Colangelo L, Galanter J, Gignoux C, Hu D, Sen S, et al. Genetic ancestry in lung-function predictions. *N Engl J Med*. 2010; 363:321–330. [PubMed: 20647190]
25. Krueger M, Heinzmann A, Mailaparambil B, Hartel C, Gopel W. Polymorphisms of interleukin 18 in the genetics of preterm birth and bronchopulmonary dysplasia. *Arch Dis Child Fetal Neonatal Ed*. 2011; 96:F299–F300. [PubMed: 20971720]
26. Mailaparambil B, Krueger M, Heizmann U, Schlegel K, Heinze J, Heinzmann A. Genetic and epidemiological risk factors in the development of bronchopulmonary dysplasia. *Dis Markers*. 2010; 29:1–9. [PubMed: 20826912]
27. Giusti B, Vestrini A, Poggi C, Magi A, Pasquini E, Abbate R, Dani C. Genetic polymorphisms of antioxidant enzymes as risk factors for oxidative stress-associated complications in preterm infants. *Free Radic Res*. 2012; 46:1130–1139. [PubMed: 22574884]
28. Sampath V, Garland JS, Le M, Patel AL, Konduri GG, Cohen JD, Simpson PM, Hines RN. A TLR5 (g.1174C > T) variant that encodes a stop codon (R392X) is associated with bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2012; 47:460–468. [PubMed: 22058078]
29. Lavoie PM, Ladd M, Hirschfeld AF, Huusko J, Mahlman M, Speert DP, Hallman M, Lacaze-Masmonteil T, Turvey SE. Influence of common non-synonymous Toll-like receptor 4 polymorphisms on bronchopulmonary dysplasia and prematurity in human infants. *PLoS One*. 2012; 7:e31351. [PubMed: 22348075]
30. Prencipe G, Auriti C, Inglese R, Devito R, Ronchetti MP, Seganti G, Rava L, Orzalesi M, De BF. A polymorphism in the macrophage migration inhibitory factor promoter is associated with bronchopulmonary dysplasia. *Pediatr Res*. 2011; 69:142–147. [PubMed: 21045753]
31. Rocha G, Proenca E, Areias A, Freitas F, Lima B, Rodrigues T, Alves H, Guimaraes H. HLA and bronchopulmonary dysplasia susceptibility: a pilot study. *Dis Markers*. 2011; 31:199–203. [PubMed: 22045425]
32. Atac FB, Ince DA, Verdi H, Gokmen Z, Yazici AC, Gulcan H, Tarcen A, Taneri A, Sezgin E, Ozbek N. Lack of association between FXIII-Val34Leu, FVII-323 del/ins, and transforming growth factor beta1 (915G/T) gene polymorphisms and bronchopulmonary dysplasia: a single-center study. *DNA Cell Biol*. 2010; 29:13–18. [PubMed: 19778283]
33. Hartel C, Konig I, Koster S, Kattner E, Kuhls E, Kuster H, Moller J, Muller D, Kribs A, Segerer H, et al. Genetic polymorphisms of hemostasis genes and primary outcome of very low birth weight infants. *Pediatr*. 2006; 118:683–689.
34. Chauhan M, Bombell S, McGuire W. Tumour necrosis factor (–308A) polymorphism in very preterm infants with bronchopulmonary dysplasia: a meta-analysis. *Arch Dis Child Fetal Neonatal Ed*. 2009; 94:F257–F259. [PubMed: 19066187]
35. Strassberg SS, Cristea IA, Qian D, Parton LA. Single nucleotide polymorphisms of tumor necrosis factor-alpha and the susceptibility to bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2007; 42:29–36. [PubMed: 17123322]
36. Kwinta P, Bik-Multanowski M, Mitkowska Z, Tomasik T, Legutko M, Pietrzyk JJ. Genetic risk factors of bronchopulmonary dysplasia. *Pediatr Res*. 2008; 64:682–688. [PubMed: 18614962]
37. Hadchouel A, Decobert F, Franco-Montoya ML, Halphen I, Jarreau PH, Boucherat O, Martin E, Benachi A, Amselem S, Bourbon J, et al. Matrix metalloproteinase gene polymorphisms and bronchopulmonary dysplasia: identification of MMP16 as a new player in lung development. *PLoS One*. 2008; 3:e3188. [PubMed: 18784838]

38. Concolino P, Capoluongo E, Santonocito C, Vento G, Tana M, Romagnoli C, Zuppi C, Ameglio F, Brancaccio A, Sciandra F. Genetic analysis of the dystroglycan gene in bronchopulmonary dysplasia affected premature newborns. *Clin Chim Acta*. 2007; 378:164–167. [PubMed: 17196572]
39. Bokodi G, Derzbach L, Banyasz I, Tulassay T, Vasarhelyi B. Association of interferon gamma T +874A and interleukin 12 p40 promoter CTCTAA/GC polymorphism with the need for respiratory support and perinatal complications in low birthweight neonates. *Arch Dis Child Fetal Neonatal Ed*. 2007; 92:F25–F29. [PubMed: 16754651]
40. Hilgendorff A, Heidinger K, Pfeiffer A, Bohnert A, Konig IR, Ziegler A, Merz C, Frey G, Chakraborty T, Gortner L, et al. Association of polymorphisms in the mannose-binding lectin gene and pulmonary morbidity in preterm infants. *Genes Immun*. 2007; 8:671–677. [PubMed: 17898783]
41. Capoluongo E, Vento G, Rocchetti S, Giardina E, Concolino P, Sinibaldi C, Santonocito C, Vendettuoli V, Tana M, Tirone C, et al. Mannose-binding lectin polymorphisms and pulmonary outcome in premature neonates: a pilot study. *Intensive Care Med*. 2007; 33:1787–1794. [PubMed: 17653692]
42. Derzbach L, Bokodi G, Treszl A, Vasarhelyi B, Nobilis A, Rigo J Jr. Selectin polymorphisms and perinatal morbidity in low-birthweight infants. *Acta Paediatr*. 2006; 95:1213–1217. [PubMed: 16982492]