Curr Opin Pediatr. Author manuscript; available in PMC 2016 November 10.

Published in final edited form as:

Curr Opin Pediatr. 2010 April ; 22(2): 134–138. doi:10.1097/MOP.0b013e328336eb85.

Genetics of bronchopulmonary dysplasia in the age of genomics

Pascal M. Lavoie[1,2] and **Marie-Pierre Dubé**[3,4]

[1]Child & Family Research Institute, Vancouver Canada

[2]Department of Pediatrics, University of British Columbia, Vancouver Canada

[3]Montreal Heart Institute, Montreal Canada

[4]Université de Montréal, Montréal Canada

Abstract

Purpose of review—According to recent evidence, susceptibility to bronchopulmonary dysplasia (BPD) in preterm infants is predominantly inherited. The purpose of this review is to discuss current published genetic association studies in light of the accumulated knowledge in genomics.

Recent findings—Major advances in the development of next generation genotyping and sequencing platforms, statistical methodologies, inventories of functional outcome of some common genetic polymorphisms and large scale cataloguing of genetic variability among many of the world's ethnic populations have greatly facilitated the study of polygenic conditions. For BPD, genetic association studies have primarily focused on components of innate (e.g. first-line) immune and anti-oxidant defences, mechanisms of vascular and lung remodelling, and surfactant proteins. However, studies have been limited in sample size and therefore fraught with a high probability of false-positive and false-negative associations. Nonetheless, candidate gene associations have indicated some novel biological pathways and provide a conceptual framework for the design of larger, collaborative population-based studies.

Summary—Although studies to date have not been able to identify reproducible genetic risk markers for BPD, they have directed us towards new, promising research avenues.

Keywords

Bronchopulmonary dysplasia; genetic association studies; heredity; pulmonary disease; premature infant

Introduction

Over the last few decades, advances in neonatal care have greatly improved pulmonary outcomes and reduced mortality in preterm infants. In spite of these efforts, BPD remains a serious and common complication of prematurity. Over 10,000 infants are affected by BPD annually in North America and consequently, are at higher risk of respiratory morbidity (up

Correspondence to: Dr. Pascal Lavoie, Child & Family Research Institute, 950 West 28th Avenue, Vancouver BC V5Z 4H4. Tel.: (604) 875-2135. FAX: (604) 875-3106. plavoie@cw.bc.ca.

to 50% risk of hospitalization in the first year of life) and mortality in early childhood [1]. Children with BPD are also at a 2 to 3-fold higher risk of long-term neurodevelopmental disability [2].

Recent studies have highlighted the contribution of heredity to BPD susceptibility which led to an important paradigm shift in the field. It was originally believed that the disease was mainly due to the lung damaging effects of life-saving respiratory support therapies. However, recent observations have demonstrated that BPD nowadays occurs predominantly in most immature preterm infants and appears to arise relatively independent of exposure to supplemental oxygen and endotracheal ventilation [3]. A major implication of these findings is that practice changes aiming to minimize environmental stressors are unlikely to be sufficient to eradicate BPD or perhaps even to substantially impact on its incidence [4]. This model greatly enhances the relevance of more fundamental research aimed at identifying key genetic and biological pathways implicated in lung injury and development, with the promise that illumination of these pathways will guide the development of innovative therapies to reduce BPD.

Complexity of the human genome

The human genome includes over 20,000 genes encoding proteins and several non-coding, regulatory elements. The vast majority of this genetic information is identical among individuals, although about 0.1% of DNA base pairs differ, and such variability likely affects disease susceptibility. Genetic variation can take multiple forms but the most common are single nucleotide polymorphisms (SNP; pronounced “snip”), which occur at a frequency of approximately 1/300 base pairs (totaling approximately 10,000,000 SNPs in the human genome). Although the majority of SNPs are believed not to have a direct physiological outcome, they are responsible for 68% of the 85,558 human inherited disease polymorphisms according to recent data from the Human Gene Mutation Database [5].

SNPs differ from traditional disease-causing *mutations* in that they tend to be far more common (i.e. allelic frequency >1% in general population) and are expected to have only relatively modest effects on disease susceptibility. The extent of this genetic variability raises issues related to our understanding of their combined biological impact and interactions with environmental factors. This led to considerable technological and statistical developments along with recently improved high-throughput genotyping [6] and next generation DNA sequencing platforms [7], to the creation of a large catalogue of common human polymorphisms across three ethnic groups (The HapMap Project; http://www.hapmap.org/) [8]. The identification of reference SNPs capable of capturing the majority of genomic variability (“tag-SNPs”) has also contributed to the optimization of the number of SNPs that are needed to conduct genome-wide association studies [9]. However, obstacles remain, specifically regarding access to sufficiently large and well characterized at-risk cohorts to conduct well-powered analyses.

Recent advances in genomics and application to polygenic conditions

Familial pedigrees have traditionally provided a powerful tool for gene discovery of highly penetrant genetic traits [5]. However for the analysis of complex diseases, interests have shifted toward population-based association studies. Two main approaches can be used when searching for the genetic determinants of disease by association studies. C*andidate-gene* studies use a set of polymorphisms selected based on existing knowledge of their biological mechanism of action, hinting at their possible implication in the disease of interest. This approach has the advantage of focusing resources on a manageable number of polymorphisms that are likely to be important. This increases detection power and reduces the false positive rate, which may be critically advantageous in the study of small size preterm populations.

G*enome-wide association* studies (GWAS) typically rely on a large (>300,000) number of SNPs to search anonymously throughout the entire genome, without *a priori* assumptions about the mechanism of disease. Although GWAS are powerful gene discovery tools, they are also limited in the spectrum of genetic variation they can survey. They depend on the assumption that an underlying disease marker will be correlated (i.e. in linkage disequilibrium) with one or a few of the SNPs being tested, in a sufficiently strong and detectable way. The success in GWAS has been seen in nearly all complex human phenotypes, for either continuous or discrete traits. A public database of the National Human Genome Research Institute (http://www.genome.gov/gwastudies/) currently lists 421 published GWAS involving 1935 SNPs. Typically, the effect sizes of the majority of disease risk variants identified by genetic association tend to be very small, increasing liability by ~10–30% [10].

The size of the study population has great impact on the success of genetic association studies and for obvious reasons, has greatly limited the study of conditions affecting preterm infants. Altshuler and colleagues have shown that for GWAS, 1500 cases and an equal number of controls provide 90% statistical power to detect an allele (which has a 30% population frequency) for a variant conveying a relative 50% increase in disease risk. In contrast, in GWAS using exon sequencing data, 330 cases and an equal number of controls would provide 90% power to detect a gene in which rare polymorphisms (population frequency of 1%) convey a relative 8-fold increase in disease risk [11].

When testing many polymorphisms in an association study, it is necessary to adjust the threshold of statistical significance to control for the false positive rate from multiple comparisons. Dense genome-wide scans of common polymorphisms typically involve the equivalent of approximately 1 million independent hypotheses [12]. A significance level of $P = 5\times10^{-8}$ thus represents a finding expected by chance once in 20 GWAS. In candidate gene studies, some polymorphisms tested are in linkage disequilibrium with one another and as such, the Bonferroni method frequently overestimates the correction for multiple testing [13]. Common procedures to address this problem have been developed: Monte Carlo procedures [14], global multivariate corrections [13] and empirical Bayes shrinkage estimates [15]. Meta-analyses have also become a popular and successful approach to

identify genes of modest effect size [16,17]. To this end, the use of conventional, validated case definitions is imperative.

Genetic associations for BPD: current state of knowledge

Heritability of BPD was first inferred from evidence of racial disparities in disease susceptibility [18,19]. Parker first suggested a genetic contribution to BPD by demonstrating higher concordance within pairs of affected siblings, although their analysis could not account for potential unidentified familial factors shared within sibling pairs [20]. The concept of a hereditary susceptibility to BPD was strengthened by recent twin studies demonstrating that at least half of the observed susceptibility to BPD could be attributed to genetic factors [21,22]. These findings were also corroborated using surrogate case definitions having better long-term prognostic value and therefore enhanced the clinical relevance of genetic associations [22].

Based on current proposed mechanisms of BPD, association studies have mainly focused on candidate genes encoding components of innate (e.g. first-line) immune and anti-oxidant defences, mechanisms of vascular and lung remodelling, as well as on genes coding for surfactant proteins. However, published case-control comparisons have small samples sizes often marginally powered to detect genetic effects of an anticipated modest penetrance, producing a potentially large number of false positive results. Furthermore, due to the small size of the cohorts relative risks attributable to genetic polymorphisms, in most cases, could not be precisely estimated.

Polymorphisms in the tumour necrosis factor-α (TNF-α) gene have been most studied. TNF-α is mainly produced by mononuclear phagocytes and is a potent mediator of acute inflammation. Levels of TNF-α are increased early on in tracheal aspirates of preterm infants who develop BPD (generally defined here as supplemental oxygen-dependence at 36 weeks of post-menstrual age) and pilot trials indicated a possible clinical therapeutic benefit from TNF-α inhibitors [23]. The *TNF-α* gene is located in the most polymorphic region of the human genome: the human leukocyte antigen (HLA) locus on chromosome 6. Two best studied nucleotide substitutions in the TNF-α gene gained particular attention due to their functional impact on cytokine levels *in vitro* [24,25]. In preterm infants, initial studies revealed a potential role of a hypofunctional allele for the −308 SNP (in reference to its position upstream of the gene transcription initiation site) on BPD susceptibility. However, this interaction did not remain significant (OR 1.04; 95%CI 0.85 to 1.25) when 804 at-risk preterm infants were combined in a meta-analysis [17]. Similarly, another −238 *TNF-α* hypofunctional allele was significantly underrepresented in preterm infants with more severe BPD [26]. However, this observation also failed to be replicated in two independent cohorts, suggesting it may have occurred by chance [27,28].

Surfactant proteins are essential to lung physiology by lowering the surface tension in the terminal air space and preventing alveolar collapse. In addition, surfactant proteins play an important role in innate immune defences. Previous studies reported positive associations between surfactant protein gene polymorphisms and respiratory distress syndrome in preterm infants [29], prompting additional investigations in BPD. In explorative analyses in

a Finnish cohort of singletons and first born twins, a length variation in the surfactant protein-B gene (SP-B), consisting of a variable number of CA nucleotide-rich microsatellite repeat sequences (termed SP-B i4Δ), appeared to have a modest influence on BPD [30]. Interestingly, variable deletions of this CA-rich sequence lead to abnormal splicing of the SP-B mRNA, and possibly also abnormal protein expression in cell lines [31]. In another study, which included 71 infants with mild or moderate BPD, family-based tests were used to determine associations with microsatellite markers within the surfactant protein-A, B, C and D gene loci [32]. Several associations were detected in this study [32]. However, this study lacked sufficient details to evaluate potential population biases. Also, an association with an allele in the glutathione-S-Transferase-P1 anti-oxidant defence gene was reported, but this association did not remain significant after correcting for confounders [33].

Mannose-binding lectin (MBL2) is a serum protein involved in innate immunity, which binds carbohydrate moieties at the surface of microbes where it can activate the complement system and facilitate opsonisation. As neonates critically depend on their innate immune defences for fighting micro-organisms and as infection is a strong risk factor for the development of BPD, a role for genetic variants in *MBL2* appeared plausible in BPD. In humans, three common SNPs in exon 1 of the *MBL2* gene significantly reduce serum protein levels, affect protein oligomerization and have been linked to an increased risk of infections [34]. In preterm infants, *MBL2* gene variants predicted to decrease protein function were shown to be associated with an increased risk of BPD, but were not corroborated in independent studies [35,36]. Although studies may indicate a role for SP-B or MBL in susceptibility to BPD, further studies are required to determine whether alterations in these protein can be detected in preterm infants with BPD [37].

Gene-targeted replacement of vascular endothelial growth factor (VEGF) promotes lung angiogenesis and prevents alveolar damage due to hyperoxia in animal models, indicating a potential role in BPD [38]. In a Caucasian at-risk Polish preterm cohort, a VEGF −460C promoter allele was associated with a dose-dependent reduction in risk of BPD, although it lacked evidence of effect on serum VEGF levels in preterm infants [39]. Matrix metalloproteinases (MMP) also play a role in lung embryogenesis and repair following injury. Recently, a role for MMP16 in BPD pathogenesis has been proposed based on lung alterations in the relative abundance of alternatively spliced isoforms of the protein following exposure to hyperoxia [40]. Interestingly, minor alleles for two SNPs co-segregating with other polymorphisms located in the MMP16 hemopexin protein domain (potentially involved in alternate splicing of the MMP16 mRNA), were shown to be underrepresented in preterm infants with BPD [40]. These SNPs were also associated with a significant reduction in MMP16 protein levels in tracheal aspirates of preterm infants with BPD [40]. The MMP16 endopeptidase catalyses activation of gelatinase A which is an important down-stream mediator of vascular remodelling. Consistent with a role for MMPs in the pathogenesis of BPD, the activity of gelatinase A also appears reduced in preterm infants with BPD [41]. Additional studies are required to determine the direct functional impact of these two SNPs on MMP16 activity and lung remodelling.

In one of the largest neonatal genetic studies aimed at identifying homeostasis variants influencing the risk of brain injury in a cohort of 1008 very low birth weight infants, a

significant association was detected between an insertion/deletion variant at position 323 in the coagulation factor VII gene promoter and BPD, in post-hoc analyses [42]. Although this polymorphism appears to affect factor VII coagulant activity in healthy subjects, the significance of this surprising association is obscure [43]. Similarly, an intronic repeat sequence variant within the angiotensin-converting enzyme gene was shown to influence BPD susceptibility [44], but again findings could not be replicated [45]. Other associations with polymorphisms in genes encoding for cytokines critical to the development of adaptive immune responses (i.e. interferon-γ and interleukin-12) as well as in the extracellular matrix receptor dystroglycan gene have been reported, but the functional relevance of these observations is unclear [46,47].

Altogether, these studies illustrate the enormous challenge faced in trying to identify robust genetic contributions to multi-factorial diseases such as BPD. Undetected or unaccounted heterogeneity in population structure may result in biases, which may be particularly relevant when the magnitude of the genetic effect is expected to be small. Ethnic heterogeneity may also contribute to discrepancies observed among studies as implied from recent data from The HapMap Project, which identifies substantial differences in haplotypes (i.e. blocks of larger genome areas defined by sets of highly co-segregating allelic variants) among individuals of Asian, Caucasian and African descent [48]. Racial admixture can be controlled by statistically matching groups according to the subjects' ethnicity. Heterogeneity in clinical practices among neonatal intensive care units may also affect the relative contribution of genetic factors and therefore complicates the replication of findings.

In order to ascertain bias and correct for population substructure, one can use relatives of cases in family-based methods such as the transmission disequilibrium test (TDT) [49]. Additional methods have been proposed, including principal component analyses (a technique which makes inferences solely on the basis of clustering within the genetic data [50]), the method of genomic control, which relies on control polymorphisms having "null" effects randomly chosen across the genome [51], as well as other methods of clustering [52] and latent population stratification [53]. In most cases, independent replication of results in association or family-based samples is crucial [54].

Concluding remarks

Most candidate genes associations studies reported to date with BPD require independent confirmation. However, genetic studies remain powerful, relatively non-invasive approaches for elucidating disease processes [55]. Identification of gene markers in future large multi-centric, well-characterized populations may help more judiciously select at-risk infants who are most likely to benefit from interventions and therefore avoid harmful side-effects in lower-risk groups [56].

Acknowledgments

PML is supported by a Child & Family Research Institute (BC) Clinician-Scientist Salary Award. MPD is supported by the Fonds de la Recherche en Santé du Québec (FRSQ).

Abbreviations

BPD	bronchopulmonary dysplasia
GWAS	genome wide association study
HLA	human leukocyte antigen
MBL	Mannose-binding lectin
MMP	Matrix metalloproteinases
SNP	single nucleotide polymorphism
SP-B	Surfactant protein-B
TDT	transmission disequilibrium test
TNF-α	Tumour necrosis factor-α
VEGF	vascular endothelial growth factor

References

1. Kinsella JP, Greenough A, Abman SH. Bronchopulmonary dysplasia. Lancet. 2006; 367:1421–1431. [PubMed: 16650652]
2. Schmidt B, Asztalos EV, Roberts RS, et al. Impact of bronchopulmonary dysplasia, brain injury, and severe retinopathy on the outcome of extremely low-birth-weight infants at 18 months: results from the trial of indomethacin prophylaxis in preterms. Jama. 2003; 289:1124–1129. [PubMed: 12622582]
3. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001; 163:1723–1729. [PubMed: 11401896]
4. Bhandari A, Bhandari V. Pitfalls, problems, and progress in bronchopulmonary dysplasia. Pediatrics. 2009; 123:1562–1573. [PubMed: 19482769]

5*. Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database: 2008 update. Genome Med. 2009; 1:13. The Human Gene Mutation Database constitutes the largest inventory of genetic variants. The database currently includes over 85,000 lesions detected in 3,253 genes, accumulated through an international consortium of scientists. Reported entries include either disease-causing mutations, disease-associated polymorphisms as well as some genetic polymorphisms that have clear physiological effects without necessarily having been associated to a specific human disease. [PubMed: 19348700]

6. McCarroll SA, Kuruvilla FG, Korn JM, et al. Integrated detection and population-genetic analysis of SNPs and copy number variation. Nat Genet. 2008; 40:1166–1174. [PubMed: 18776908]
7. Shendure J, Ji H. Next-generation DNA sequencing. Nat Biotechnol. 2008; 26:1135–1145. [PubMed: 18846087]
8. Frazer KA, Ballinger DG, Cox DR, et al. A second generation human haplotype map of over 3. 1 million SNPs. Nature. 2007; 449:851–861. [PubMed: 17943122]
9. Johnson GC, Esposito L, Barratt BJ, et al. Haplotype tagging for the identification of common disease genes. Nat Genet. 2001; 29:233–237. [PubMed: 11586306]
10. Hindorff LA, Sethupathy P, Junkins HA, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A. 2009; 106:9362–9367. [PubMed: 19474294]
11. Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. Science. 2008; 322:881–888. [PubMed: 18988837]

12. Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genetic Epidemiology. 2008; 32:381–385. [PubMed: 18348202]

13. Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genet Epidemiol. 2008; 32:361–369. [PubMed: 18271029]

14. Dudbridge F, Koeleman BP. Efficient computation of significance levels for multiple associations in large studies of correlated data, including genomewide association studies. Am J Hum Genet. 2004; 75:424–435. [PubMed: 15266393]

15. Thomas D, Langholz B, Clayton D, et al. Empirical Bayes methods for testing associations with large numbers of candidate genes in the presence of environmental risk factors, with applications to HLA associations in IDDM. Ann Med. 1992; 24:387–392. [PubMed: 1418924]

16. Zeggini E, Ioannidis JP. Meta-analysis in genome-wide association studies. Pharmacogenomics. 2009; 10:191–201. [PubMed: 19207020]

17*. 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:F257–259. This is a well conducted meta-analysis and largest study reporting a genetic association with BPD. Associations with nucleotide substitutions at position −308 in the TNF-α gene did not remained significant after combining studies. [PubMed: 19066187]

18. Avery ME, Tooley WH, Keller JB, et al. Is chronic lung disease in low birth weight infants preventable? A survey of eight centers. Pediatrics. 1987; 79:26–30. [PubMed: 3797169]

19. Horbar JD, McAuliffe TL, Adler SM, et al. Variability in 28-day outcomes for very low birth weight infants: an analysis of 11 neonatal intensive care units. Pediatrics. 1988; 82:554–559. [PubMed: 3273485]

20. 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]

21*. Bhandari V, Bizzarro MJ, Shetty A, et al. Familial and genetic susceptibility to major neonatal morbidities in preterm twins. Pediatrics. 2006; 117:1901–1906. This is the first report demonstrating a genetic contribution to bronchopulmonary dysplasia by comparing concordance for the disease in pairs of twins genetically identical versus genetically non-identical twins. [PubMed: 16740829]

22*. Lavoie PM, Pham C, Jang KL. Heritability of bronchopulmonary dysplasia, defined according to the consensus statement of the national institutes of health. Pediatrics. 2008; 122:479–485. Classic twin analyses confirmed the substantial (>79%) genetic contribution to BPD susceptibility, as well as to persistence of the ductus arteriosus. The conclusion is strengthened by the use of two validated case BPD definitions, including the National Institute of Child Health and Human Development consensus definition. In this study population, mild BPD which generally has low long-term prognostic value was mainly attributable by shared environmental effects. In contrast, moderate or severe BPD was attributable to strong genetic factors and therefore indicates a valid outcome to use in genetic association studies. [PubMed: 18762515]

23. Lauterbach R, Szymura-Oleksiak J, Pawlik D, et al. Nebulized pentoxifylline for prevention of bronchopulmonary dysplasia in very low birth weight infants: a pilot clinical study. J Matern Fetal Neonatal Med. 2006; 19:433–438. [PubMed: 16923699]

24. Wilson AG, Symons JA, McDowell TL, et al. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A. 1997; 94:3195–3199. [PubMed: 9096369]

25. Kaluza W, Reuss E, Grossmann S, et al. Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. J Invest Dermatol. 2000; 114:1180–1183. [PubMed: 10844563]

26. Kazzi SN, Kim UO, Quasney MW, Buhimschi I. Polymorphism of tumor necrosis factor-alpha and risk and severity of bronchopulmonary dysplasia among very low birth weight infants. Pediatrics. 2004; 114:e243–248. [PubMed: 15286263]

27. 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]

28. Lee, K., Whalen, M., Speert, D., et al. Single Nucleotide Polymorphism in the Tumor Necrosis alpha (TNF-α) gene promoter and severity of bronchopulmonary dysplasia in preterm infants. 2009 Pediatric Academic Societies' Annual Meeting; May 2009; Baltimore, US. 2009: Poster presentation Abstract #751359

29. Hallman M, Haataja R. Surfactant protein polymorphisms and neonatal lung disease. Semin Perinatol. 2006; 30:350–361. [PubMed: 17142161]

30. Rova M, Haataja R, Marttila R, et al. Data mining and multiparameter analysis of lung surfactant protein genes in bronchopulmonary dysplasia. Hum Mol Genet. 2004; 13:1095–1104. [PubMed: 15102713]

31. Lin Z, Thomas NJ, Wang Y, et al. Deletions within a CA-repeat-rich region of intron 4 of the human SP-B gene affect mRNA splicing. Biochem J. 2005; 389:403–412. [PubMed: 15790313]

32. Pavlovic J, Papagaroufalis C, Xanthou M, et al. Genetic variants of surfactant proteins A, B, C, and D in bronchopulmonary dysplasia. Dis Markers. 2006; 22:277–291. [PubMed: 17264398]

33. Manar MH, Brown MR, Gauthier TW, Brown LA. Association of glutathione-S-transferase-P1 (GST-P1) polymorphisms with bronchopulmonary dysplasia. J Perinatol. 2004; 24:30–35. [PubMed: 14726935]

34. Casanova JL, Abel L. Human Mannose-binding Lectin in Immunity: Friend, Foe, or Both? J Exp Med. 2004; 199:1295–1299. [PubMed: 15148331]

35. Capoluongo E, Vento G, Rocchetti S, et al. Mannose-binding lectin polymorphisms and pulmonary outcome in premature neonates: a pilot study. Intensive Care Med. 2007; 33:1787–1794. [PubMed: 17653692]

36. Hilgendorff A, Heidinger K, Pfeiffer A, 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]

37. Agbeko RS, Peters MJ. Mannose-binding lectin and pulmonary morbidity in premature infants. Intensive Care Med. 2008; 34:777. author reply 778. [PubMed: 18214435]

38. Thebaud B, Ladha F, Michelakis ED, et al. Vascular endothelial growth factor gene therapy increases survival, promotes lung angiogenesis, and prevents alveolar damage in hyperoxia-induced lung injury: evidence that angiogenesis participates in alveolarization. Circulation. 2005; 112:2477–2486. [PubMed: 16230500]

39. Kwinta P, Bik-Multanowski M, Mitkowska Z, et al. Genetic risk factors of bronchopulmonary dysplasia. Pediatr Res. 2008; 64:682–688. [PubMed: 18614962]

40. Hadchouel A, Decobert F, Franco-Montoya ML, 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]

41. Danan C, Jarreau PH, Franco ML, et al. Gelatinase activities in the airways of premature infants and development of bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol. 2002; 283:L1086–1093. [PubMed: 12376362]

42. Hartel C, Konig I, Koster S, et al. Genetic polymorphisms of hemostasis genes and primary outcome of very low birth weight infants. Pediatrics. 2006; 118:683–689. [PubMed: 16882823]

43. Atac FB, Ince DA, Verdi H, et al. 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. 2009; 29:13–18.

44. Kazzi SN, Quasney MW. Deletion allele of angiotensin-converting enzyme is associated with increased risk and severity of bronchopulmonary dysplasia. J Pediatr. 2005; 147:818–822. [PubMed: 16356438]

45. Yanamandra K, Loggins J, Baier RJ. The Angiotensin Converting Enzyme Insertion/Deletion polymorphism is not associated with an increased risk of death or bronchopulmonary dysplasia in ventilated very low birth weight infants. BMC Pediatr. 2004; 4:26. [PubMed: 15610555]

46. Bokodi G, Derzbach L, Banyasz I, et al. 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:F25–29. [PubMed: 16754651]

47. Concolino P, Capoluongo E, Santonocito C, et al. Genetic analysis of the dystroglycan gene in bronchopulmonary dysplasia affected premature newborns. Clin Chim Acta. 2007; 378:164–167. [PubMed: 17196572]
48. Conrad DF, Jakobsson M, Coop G, et al. A worldwide survey of haplotype variation and linkage disequilibrium in the human genome. Nat Genet. 2006; 38:1251–1260. [PubMed: 17057719]
49. Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. Am J Hum Genet. 1996; 59:983–989. [PubMed: 8900224]
50. Price AL, Patterson NJ, Plenge RM, et al. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–909. [PubMed: 16862161]
51. Bacanu SA, Devlin B, Roeder K. The power of genomic control. Am J Hum Genet. 2000; 66:1933–1944. [PubMed: 10801388]
52. Gao X, Starmer J. Human population structure detection via multilocus genotype clustering. BMC Genet. 2007; 8:34. [PubMed: 17592628]
53. Satten GA, Flanders WD, Yang Q. Accounting for unmeasured population substructure in case-control studies of genetic association using a novel latent-class model. Am J Hum Genet. 2001; 68:466–477. [PubMed: 11170894]
54. Igl BW, Konig IR, Ziegler A. What do we mean by ‘replication’ and ‘validation’ in genome-wide association studies? Hum Hered. 2009; 67:66–68. [PubMed: 18931511]
55. Hirschhorn JN. Genomewide association studies--illuminating biologic pathways. N Engl J Med. 2009; 360:1699–1701. [PubMed: 19369661]
56*. Doyle LW, Halliday HL, Ehrenkranz RA, et al. Impact of postnatal systemic corticosteroids on mortality and cerebral palsy in preterm infants: effect modification by risk for chronic lung disease. Pediatrics. 2005; 115:655–661. In this meta-analysis of randomized controlled trials of post-natal corticosteroid use, the authors demonstrate how the combined risk of death and cerebral palsy is modified with variations in risk of BPD. Their model identifies that a risk of BPD greater than 65% would tip the balance in favor of using corticosteroids for this outcome. Therefore, predictive genetic markers able to identify infants who have a >65% risk of BPD may be useful clinically to identify a higher group of infants who may potentially benefit from corticosteroids. [PubMed: 15741368]