Original article

Genetic susceptibility to neonatal lung diseases

Marco Somaschini[1], Emanuela Castiglioni[1], Silvia Presi[2], Chiara Volonteri[3], Maurizio Ferrari[1, 2, 3], Paola Carrera[1, 2]

[1] Unità di Genomica per la Diagnostica delle Patologie Umane, Centro di Genomica Traslazionale e Bioinformatica, Istituto Scientifico San Raffaele, Milano; [2] Diagnostica e Ricerca San Raffaele, Milano; [3] Università Vita Salute San Raffaele, Milano

Abstract. Advances in molecular genetics have enabled improvement of knowledge in pathogenesis and diagnosis of either monogenic or multifactorial neonatal lung diseases. Variants in genes regulating surfactant function and metabolism are implicated in some rare and common respiratory diseases. Congenital surfactant deficiencies are rare diseases due to mutations in genes encoding surfactant proteins and cause significant and often lethal respiratory failure in newborns and interstitial lung disease in older children. Diagnosis is made by molecular analysis and eventually confirmed by histological analysis of lung tissue. A multifactorial contribution, resulting from interaction between multiple genes and environmental factors, has been supposed for respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD). Several potential candidate genes, especially regarding surfactant proteins and cytokines, have been shown in association with these diseases. Genetic variants predisposing to RDS or BPD are usually polymorphisms which are not causative, but can increase susceptibility to the disease. Identification of infants at risk of disease can be useful to provide them individualized therapies. (www.actabiomedica.it)

Key words: newborn, surfactant proteins, Respiratory Distress Syndrome (RDS), Bronchopulmonary Dysplasia (BPD)

Introduction

Impact of genetics on clinical medicine has become incisive in the last years. The Human Genome Project and the Hap Map Project provided new technologies and tools for molecular analysis of DNA and allowed us to improve our knowledge in the pathogenesis and diagnosis of disease, to identify markers of susceptibility with the aim of providing individualized treatments.

In the lung, physiologic transition from fetal to neonatal period requires production of pulmonary surfactant, a complex mixture of phospholipids and proteins that reduces alveolar collapse by decreasing surface tension within the alveoli. Surfactant proteins represent a small percentage in surfactant composition but they are essential for surfactant function and metabolism. In particular surfactant proteins A and D (SP-A and SP-D) are important for local immunity and host defence, whereas surfactant protein B and C (SP-B and SP-C) are important for their tensioactive properties. ATP-binding cassette 3 (ABCA3) is another protein which participates in the transport of phospholipids to lamellar bodies, the organelles where surfactant is stored before secretion in the alveolus.

Mendelian Diseases

Deficiency of pulmonary surfactant due to prematurity is the most important cause of respiratory distress syndrome (RDS) in newborns (1). This deficiency may be also due to variants in genes encoding surfactant proteins, which can cause disruption of sur-

factant metabolism and cause respiratory distress in neonates and children (2,3).

Recessive mutations in SFTPB (MIM: 178630) and ABCA3 (MIM: 601615) genes have been recently identified in full-term infants with unexplained distress that clinically and radiographically resembles RDS in preterm infants. In these cases, a family history of unexplained neonatal death or parents' consanguinity is often present (4). In these subjects, clinical symptoms of severe respiratory distress are evident in the immediate newborn period and may result in death in the first months of life (5-7). To date, mutations mainly located in exons or immediate intron-exon boundaries of SFTPB or ABCA3 have been reported as causative mutations. Recently, it has been demonstrated that mutation in non coding region may result in aberrant ABCA3 mRNA splicing causing an altered ABCA3 expression (8).

Mutations of ABCA3 are associated with either lethal respiratory distress in neonates or interstitial lung disease (ILD) in infants and children (7, 9).

More frequently ILD in older infants and children is caused by mutations in SFTPC (MIM: 178620) gene (10). Hereditary SFTPC disorder is an autosomal dominant inherited disease with a variable phenotype regarding severity and age of onset.

Patients with lung disease similar to ABCA3 deficiency with only one or no mutation have been reported, thus indicating either the possibility of a low sensitivity of molecular testing methods or suggesting a different underlying disease mechanism (11, 12).

Moreover, it has been shown that heterozygosity for ABCA3 mutations can modify the severity of lung disease associated with a surfactant protein C gene mutation (13).

In table 1 the principal features of neonatal and paediatric monogenic diseases related to lung surfactant metabolism are summarized.

Multifactorial diseases

Mendelian disorders can only explain a well defined group of monogenic lung diseases. However a multifactorial inheritance has been proposed. Multifactorial or complex diseases result from interaction between multiple genes and environmental factors. Since congenital surfactant deficiency is the cause of RDS in term neonates, genes encoding surfactant proteins become the logical candidates for studying RDS also in preterm neonates in which prematurity could influence the morbidity and severity of genetic condition. Association between alleles of SFTPA (MIM: 178630) and development of RDS has been demonstrated in premature infants: alleles as 6A2 and 1A0 are associated with increased risk of RDS even after treatment with antenatal steroids, whereas allele 6A3 should have a protective effect. SNP Thr131Ile of SFTPB could act in association with alleles of SP-A in increasing susceptibility to RDS (14, 15). SNP rs13332514 (F353F) in ABCA3 is mainly represented in premature infants with RDS and is significantly as-

Table 1. Genes involved in paediatric and neonatal disorders.

Protein (Gene)	Inheritance	Clinical presentation/ Diagnosis	Onset/Disease
Surfactant Protein B (SFTPB)	AR	Cyanosis, RDS, hypoxic respiratory failure. Radiographic opacification typical of RDS	Full term infants during the first hours or days
Surfactant Protein C (SFTPC)	AD	Variable respiratory symptoms in neonatal and pediatric age. Radiographic diffuse alveolar damage, interstitial feature of inflammation typical of ILD	Newborns, infants and children
Adenosine triphosphate (ATP)-binding cassette transporter A3 (ABCA3)	AR	Cyanosis, RDS, hypoxic respiratory failure in newborns. ILD in infants and children. Radiographic finding of alveolar disease and atelectasia.	Newborns, infants and children

AR: Autosomic Recessive. AD: Autosomic Dominant. RDS: Respiratory Distress Syndrome. ILD: Interstitial Lung Disease

sociated with chronic lung disease with prolonged oxygen-dependency (16). Recently, a complex surfactant homeostasis disorder caused by TTF-1 genetic defect combined to a novel heterozygous ABCA3 mutation in an infant with severe neonatal RDS has been described (17).

Rather than being causative, different DNA variations have been reported to give a genetic contribution to multifactorial diseases as Bronchopulmonary Dysplasia (BPD), where immature lungs are exposed to environmental injurious factors such as infection, inflammation, hyperoxia, and mechanical ventilation. Interaction between environmental and genetic factors should determine the susceptibility to the disease, severity, response to therapies and outcome (18, 19). Genetic involvement in BPD susceptibility has been suggested by two unrelated twins studies (20, 21).

Since BPD results from the interaction between different biological pathways, many candidate genes have been interrogated for a possible role in BPD through genetic association studies (22-26). Most of these studies, however, involved small number of subjects and few have been replicated.

Table 2 shows the results of selected studies where variants have been associated with BPD risk.

Table 2. Candidate genes for BPD

Genes	DNA variation	Population	p value	Risk	References
Glutathione-S-Transferase-P1 (GST-P1)	p.Val105Ile	133 newborns (35 MOD and SEV BPD vs 98 controls)	p=0.05	↑ BPD	Manar MH, Brown MR, Gauthier TW and Brown LAS. J Perinatol 2004; 24: 30-35
Surfactant Protein–B (SP-B)	Intron 4 deletion	245 newborns <=32 wk (67 MOD and SEV BPD vs 178 controls)	P=0.008	↑ BPD	Rova M, Haataja R, Marttila R, Ollikainen V, Tammela O, Hallman M. Hum Mol Genet 2004; 13:1095-104
Tumor Necrosis Factor alpha (TNFα)	c.-238 G/A	120 newborns <1250 g (51 MOD and SEV BPD vs 69 controls)	p=0.026	↓ BPD	Kazzi SNJ, Kim UO, Quasney MW, and Buhimschi I. Pediatrics 2004; 114 (2): 243-248
Angiotensin Converting Enzyme (ACE)	intron 16 deletion	120 newborns <1250 g120 (51 MOD and SEV BPD vs 69 controls)	p=0.025	↑ BPD	Kazzi SN and Quaesny MW. L Pediatr 2005. 147:818-22
Vascular Endothelial Growth Factor (VEGF)	c.-460 T>C	181 newborns 24-32 wk (118 MILD, MOD and SEV BPD vs 63 controls)	p=0.013	↓ BPD	Kwinta O, Bik-Multanowski, Mitkowska Z, et al. Pediatr Res 2008; 64: 682-688
Matrix Metalloproteinase16 (MMP16)	rs2664352 rs2664349	263 newborns <28 wk (45 MOD and SEV BPD vs 218 controls)	p=0.013 (T/T) p=0.047 (G/G)	↓ BPD ↓ BPD	Hadchouel A, Decobert F, Franco-Montoya ML, et al. PLoS ONE 2008; 3(9): 1-10
Testican-2 (SPOCK2)	rs1245560	390 newborns <28 wk (87 MOD and SEV BPD vs 303 controls)	p=1.66x10-7	↑ BPD	Hadchouel A, Durrmeyer X, Bouzigon E, et al. Am J Respir Crit Care Med 2011; 15; 184(10):1164-70.
Toll-Like Receptor 5 (TLR-5)	p. Arg392X	280 newborns <=1500 g (66 MOD and SEV BPD vs 223 controls)	p=0.03	↑ BPD	Sampath V, Garland J, Le Min, et al. Pediatr Pneumol 2012; 47: 460-468

MOD: Moderate. SEV: Severe

Discussion

Phenotype of RDS is complex. In the past decade, many advances have been done, improving knowledge and diagnostic capability in the field, especially with respect to the monogenic forms. Mutations transmitted in a mendelian pattern are rare and with a high penetrance, as for the fatal respiratory distress in neonatal age and chronic interstitial lung disease in later infancy. In these cases, hystologic analysis of lung tissue is useful to confirm diagnosis, and electron microscopy shows abnormalities of lamellar bodies in infants with SFTPB and ABCA3 deficiency (27). Mutations in genes encoding surfactant proteins have been identified also in preterm newborn infants with a particularly severe and persistent respiratory distress (28). Molecular analysis may be more easily performed being a non-invasive method; it allows to make a diagnosis with identification of the specific mutation, and to offer genetic counselling to the family. In families at risk for fatal respiratory distress, it is possible to perform prenatal diagnosis. Identification of mutations can be performed on chorionic villous samples or amniocentesis and is rapid and reliable (29).

Whereas congenital surfactant deficiencies are rare and mutations have a high penetrance, RDS of premature infants and BPD are more frequent and they may behave as multifactorial disorders. For these diseases, a genetic basis has been demonstrated and numerous genes have been interrogated for a possible role as predisposing factors. Associated genetic variants are usually polymorphisms with a low impact on the expression or protein function, that can increase the susceptibility to the disease (30). A genetic component of RDS has been hypothesized for a long time because lung disease is more serious in males compared to females, in neonates of caucasian origin rather than afro-americans, and it is more frequent in both twins when they are monozygous.

A specific method to study genetic susceptibility to diseases is the case-control association study, which consists on comparison of the frequency of common variants in two groups of infants: when an allele has a significantly increased frequency in cases compared to controls, it is concluded that it is associated to that phenotype (19). Published association studies have identified several potential candidate genes, especially regarding surfactant proteins and cytokines, but many studies have reported small sample size, and the findings of most of them have not been replicated in subsequent cohorts (15, 31, 32). However association studies have succeeded in explaining only a modest fraction of genetic variance.

During the past five years, new high-throughput, massively parallel sequencing or next generation sequencing (NGS) technologies have emerged. NGS approach has the advantage to harvest both common and rare genetic variations (33) in a relatively short time and has affordable costs allowing a wide range of applications from sequencing a group of candidate genes to all the coding regions (exome sequencing) and/or the entire human genome (34).

Thus the technology of the next-generation sequencing represents a suitable study strategy for both mendelian RDS forms in which other genes, in addition to those already known, may confirm or define new genetic pathways involved in the pathology, as well as for the multifactorial forms of unexplained RDS and BPD in which common variants could have a role as modifiers of more rare penetrant variants exerting a higher contribution to the disease risk.

A better understanding of genetic mechanisms that regulate surfactant production and metabolism may allow us to develop new therapies and individualized treatments in the future.

References

1. Farrell PM, Avery ME. Hyaline membrane disease. *Am Rev Respir Dis* 1975; 111: 657-88.
2. Whitsett JA, Wert SE, Trapnell BC, et al. Genetic disorders influencing lung formation and function at birth. *Hum Mol Genet* 2004; 13: 207-15.
3. Nogee LM. Genetic mechanisms of surfactant deficiency. *Biol Neonate* 2004; 85 (4): 314-8.
4. Nogee LM, Garnier G, Dietz HC, et al. A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. *J Clin Invest* 1994; 93: 1860-3.
5. Nogee LM, Wert SE, Proffit SA, et al. Allelic heterogeneity in hereditary surfactant protein B (SP-B) deficiency. *Am J Respir Crit Care Med* 2000; 161: 973-81.

6. Somaschini M, Wert SE, Mangili G, et al. Hereditary surfactant protein B deficiency resulting from a novel mutation. *Intensive Care Med* 2000; 26: 97-100.
7. Shulenin S, Nogee LM, Annilo T, et al. 2004. ABCA3 gene mutation in newborns with fatal surfactant deficiency. *N Engl J Med* 350: 1296-303.
8. Agrawal A, Hamvas A, Cole FS, et al. An intronic ABCA3 mutation that is responsible for respiratory disease. *Pediatric Research* 2012; 1-5.
9. Doan ML, Guillerman RP, Dishop MK, et al. Clinical, radiological and pathological features of ABCA3 mutations in children. *Thorax* 2008; 63: 366-73.
10. Nogee LM. Alterations in SP-B and SPC expression in neonatal lung disease. *Ann Rev Physiol* 2004; 66: 601-23.
11. Gower WA, Wert SE, Ginsberg JS, et al. Fatal familial lung disease caused by ABCA3deficiency without identified ABCA3 mutation. *J Pediatr* 2010; 157: 62-8.
12. Somaschini M, Nogee LM, Sassi I, et al. Unexplained neonatal respiratory distress due to congenital surfactant deficiency. *J Pediatr* 2007; 150: 649-53.
13. Bullard JE, Nogee LM. *Pediatr Res* 2007; 62: 176-9.
14. Hallman M, Haataja R. Genetic influences and neonatal lung disease. *Semin Neonatol* 2003; 8: 19-27.
15. Hallman M, Haataja R. Surfactant proteins polymorphisms and neonatal lung disease. *Semin Perinatol* 2006; 30: 350-61.
16. Karjalainen MK, Haataja R, Hallman M. Haplotype analysis of ABCA3 : association with respiratory distress in very premature infants. *Ann Med* 2007; 40: 56-68.
17. Peca D, Petrini S, Tzialla C, et al. Altered surfactant homeostasis and recurrent respiratory failure secondary to TTF-1 nuclear targeting defect. *Respiratory Research* 2011; 12: 115-9.
18. Parton LA, Strassberg SS, Qian D, et al. The genetic basis for bronchopulmonary dysplasia. *Front Biosc* 2006; 11: 1854-60.
19. Maresso K, Broeckel U. The role of genomics in neonatal ICU. *Clin Perinatol* 2009; 36: 189-204.
20. Bhandari V, Bizzarro MJ, Shetty A, et al. Familial and genetic susceptibility to major neonatal morbidities in preterm twins. *Pediatrics* 2006; 117: 1901-06.
21. Lavoie PM, Phan C, Kang KL. Heritability of bronchopulmonary dysplasia defined per the consensus statement of the National Institutes of Health. *Pediatrics* 2008; 122 (3): 479-85.
22. Bhandari V, Gruen JR. The genetic of Broncopulmonary Displasia. *Semin Perinatol* 2006; 30: 185-91.
23. Kwinta P, Bik Multanowski M, Mitkowska Z, et al. Genetic Risk Factors of Bronchopulmonary Dysplasia. *Pediatric Research* 2008; 64 (6): 682-8.
24. 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 (9): e3188.
25. Hadchouel A, Durrmeyer X, Bouzigon E, et al. Identification of SPOCK2 as a susceptibility gene for bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2011; 184 (10): 1164-70.
26. Sampath V, Garland JS, Le M, et al. A TLR5 (g.1174C>T) variant that encodes a stop codon (R392X) is associated with bronchopulmonary dysplasia. *Pediatr Pulmonol* 2012; 47 (5): 460-8.
27. Edwards V, Cutz E, Viero S, et al. Ultrastructure of Lamellar Bodies in Congenital Surfactant Deficiency. *Ultrastruct Pathol* 2005; 29: 503-9.
28. Somaschini M, Carrera P, Presi S, et al. Variation in surfactant protein genes and severe respiratory distress in premature newborn infants. Abstract, 2011 PAS/ASPR Joint Meeting, Denver, April 29-May 3, 2011
29. Stuhrmann M, Bohnhorst B, Peters V, et al. Prenatal diagnosis of congenital alveolar proteinosis (surfactant protein B deficiency). *Prenat Diagn* 1998; 18: 953-5.
30. Meng H, Gruen JR. Genetic approach to complications of prematurity. *Front Biosc* 2007; 12: 2344-51.
31. Lavoie PM, Dubé MP. Genetics of bronchopulmonary dysplasia in the age of genomics. *Curr Opin Pediatr* 2010; 22: 1-5.
32. Abman SH, Mourani PM, Sontag M. Bronchopulmonary dysplasia: a genetic disease. *Pediatrics* 2008; 122: 658-59.
33. Cirulli E, Goldstein DB. Uncovering the roles of rare variants in common disease though whole- genome sequencing. *Nat Rev Genet* 2010; 11 (6): 415-25.
34. Zhang W, Cui H, Wong LJC. Application of Next Generation Sequencing to molecular Diagnosis of Inherited Diseases. *Top Curr Chem* 2012.

Correspondence:
Marco Somaschini
Unità di Genomica per la Diagnostica delle Patologie Umane,
Centro di Genomica Traslazionale e Bioinformatica,
Istituto Scientifico San Raffaele –Dibit 2
Via Olgettina 60, 20132 Milano
Tel. 00393407308431
Fax 003902 26434351
E-mail: masomaschini@gmail.com