Pulmonary Surfactant and the Genetic Basis of Lung Disease: What’s New in Neonates and Infants

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Financial Interest Disclosure

Lawrence M. Nogee, M.D.

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Learning Objectives:

• Name different components of surfactant and explain their role in surfactant function.

• Recognize clinical presentations (phenotypes) of children with single gene disorders causing surfactant dysfunction.

• Give examples of how rare disorders may provide insights into normal metabolism and pathophysiology of more common diseases.
Common disease: Neonatal RDS
Major cause of morbidity for prematurely born infants

\[ P \approx \frac{\text{Surface Tension}}{r} \]
**Benefits of Surfactant Replacement Therapy**

Polin, Carlo and COFAN, *Pediatrics* 133:156, 2014

### TABLE 1 Meta-analyses of Surfactant Replacement: Prophylaxis and Rescue Treatment With Animal-Derived and Synthetic Surfactant$^{2,3,8,11}$

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Prophylactic Surfactant</th>
<th>Rescue Surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animal Derived</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Neonatal mortality</td>
<td>8.00 (0.47–0.77)</td>
<td>7.00 (0.58–0.85)</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>9.04 (0.29–0.54)</td>
<td>6.07 (0.65–0.90)</td>
</tr>
<tr>
<td>PIE</td>
<td>6.46 (0.36–0.59)</td>
<td>2.68 (0.50–0.93)</td>
</tr>
<tr>
<td>BPD$^a$</td>
<td>8.01 (0.79–1.05)</td>
<td>4.06 (0.83–1.36)</td>
</tr>
<tr>
<td>BPD/death$^a$</td>
<td>8.08 (0.72–0.88)</td>
<td>4.89 (0.77–1.03)</td>
</tr>
</tbody>
</table>

**N**, number; **PIE**, pulmonary interstitial emphysema.

$^a$ Defined at 28 d.

### TABLE 3 Levels of Evidence$^{58}$

<table>
<thead>
<tr>
<th>Recommendation LOE</th>
<th>LOE</th>
<th>Grade of Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm infants born at &lt;30 wk of gestation who need mechanical ventilation because of severe RDS should be given surfactant after initial stabilization.</td>
<td>1</td>
<td>Strong Recommendation</td>
</tr>
<tr>
<td>Using CPAP immediately after birth with subsequent selective surfactant administration should be considered as an alternative to routine intubation with prophylactic or early surfactant administration in preterm infants.</td>
<td>1</td>
<td>Strong Recommendation</td>
</tr>
<tr>
<td>Rescue surfactant may be considered for infants with hypoxic respiratory failure attributable to secondary surfactant deficiency (e.g., meconium aspiration syndrome or sepsis/pneumonia).</td>
<td>2</td>
<td>Recommendation</td>
</tr>
</tbody>
</table>
Atypical RDS in Preterm

Hx: 30 wk GA female, 2\textsuperscript{nd} twin, C/S maternal PEC, +ANS

Surfactant x 3 doses

More severe disease than expected from GA, history
Persistent RDS in near-term infant

2200 gm, 37 6/7 wks gestation A-A female. SVD, Apgars 6¹, 8⁵. Respiratory distress in hours.

Epidemiology wrong for RDS. Disease persisted / worsened with time
Variability explained by genetic mechanisms?

Full-term RDS more likely to reflect genetic mechanisms
Composition of Pulmonary Surfactant

- **PG**: 8%
- **Chol**: 8%
- **Protein**: 11%
- **PE**: Other

**Dipalmitoyl or Disaturated Phosphatidylcholine (PC)**
- Adsorbs poorly
- Not lung specific

**Serum derived**
- **SP-A**
- **SP-B**
- **SP-C**
- **SP-D**

**Sphingomyelin**

**Other**
Hydrophobic Surfactant Proteins

Chromosome 2 → mRNA → PreProSP-B
SP-B

Chromosome 8 → mRNA → ProSP-C
SP-C

Weaver & Whitsett, NEJM, 2002
Developmentally Regulated
Surfactant Protein B (SP-B) Deficiency

Epidemiology: Very rare, < 1 in 1 million live births

Clinical: Neonatal RDS
Persistent disease

Genetic
Autosomal Recessive
Bi-allelic loss-of-function SFTPB mutations

Mature SP-B:

ProSP-C:

Control

SP-B Def.

Outcome: Usually fatal < 3 months

Treatment: Compassionate/Palliative care

Lung transplantation
Surfactant Protein B (SP-B) Deficiency

Partial Deficiency (splicing mutation)

Implication: Critical level of SP-B for normal lung function

Genetically engineered mice: Lung disease at 20 – 25% of control levels.
Pulmonary Function in Adults Heterozygous for SFTPB 121ins2

Screened 47,600 individuals for SFTPB 121ins2
85 heterozygous (1 in 560)
Pulmonary function:

Gene-environment Interaction
SP-C Dysfunction

Epidemiology: Rare, incidence & prevalence unknown

Clinical: Neonatal RDS, chILD, Pulmonary Fibrosis, no Sx

Genetic: Autosomal Dominant, sporadic
Mono-allelic coding SFTPC mutations
Common mutation (p.Ile73Thr)

Pathophysiology: SP-C deficiency – dominant negative
Abnormal routing of proSP-C
Toxic gain of function
Aggregation misfolded protein in ER

Outcome: Highly variable
Outcome with *SFTPC* Mutations

<table>
<thead>
<tr>
<th>Current Age</th>
<th>Alive</th>
<th>Transplanted</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3m – 1y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – 5y</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5 – 10y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 – 21y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21y + w/o Sx</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
UPR and Epithelial ER Stress in IPF

Lawson et al., AJP Lung, 2008.
ABCA3

Member A3 of ABC transporter family: hydrolyze ATP to moves substances across membranes.

ABCA3 located on lamellar body limiting membrane.

Small, dense lamellar bodies in ABCA3 deficient lung (“fried –egg”).

ABCA3 transports lipids essential for surfactant function into lamellar bodies.

Decreased surfactant PLs in BALF

Garmany et al., Pediatr Res, 2006
Outcome with *ABCA3* Mutations

Autosomal recessive; mutations needed on both *ABCA3* alleles.

Correlation of Genotype with Outcome (Phenotype)

Null = nonsense or frameshift
**NKX2.1**  
(TTF-1, TITF-1, T/EBP)  
Homeodomain-containing transcription factor  
Expressed in thyroid, CNS (basal ganglia), lung (Clara, AEC2)  
Lung: CCSP, SP-A, SP-B, SP-C, ABCA3, LAMP3, CEACAM6, T1α

**Brain**  
Chorea  
Ataxia  
Hypotonia  
Dev Delay

**Thyroid**  
Agenesis  
Hypothyroid

**Lung**  
RDS  
Infection  
ILD \ DLD

Pulmonary phenotype mediated by target gene(s) affected
Surfactant Production and Metabolism

Alveolar Type II cell

ABCA3

TTF1

Alveolar Macrophage

GM-CSF

Type I cell
Phenotype: Lung Histology and Surfactant Genes

CPI

ABCA3

SFTPC

DIP

SFTPB

NKX2-1
Genetic Testing for Surfactant Dysfunction

Advantages:
- Noninvasive means to establish specific diagnosis.
- Potentially avoid lung biopsy in unstable child.
- Appropriate counseling re: prognosis, recurrence risk.

Potential Problems:
- Expensive (but cheaper than biopsy).
- Turn-around-time in weeks.
- Interpretation of results not always straight-forward. Missense variants (VUCS), heterozygotes.
- Not 100% sensitive.
Heterozygous *ABCA3* variants and RDS

Wambach, J et al., Pediatrics, 2012

- Case control study of infants > 34 wks GA with/without RDS.
- Next Generation Sequencing to identify all variants in cohort.
- Overrepresentation of single *ABCA3* variants in RDS group
  
  14.3% vs 3.7 % ($p = 0.002$) (European descent)
  
  4.5% vs 1.5 % ($p = 0.23$) (African descent)
  
  (Predicted incidence 1 in 4,300 – 20,000)
RDS morbidity and mortality greatly reduced.

Still some non-responders, outliers:

- Full-term infants with RDS Phenotype
- Persistent Disease – DLD \ ILD

Relatively older prematures with severe RDS

Likely to have genetic mechanisms.

Genetic testing clinically available.

While rare, these disorders have implications for lung diseases beyond the neonatal period.
Surfactant Replacement Therapy in 2014

Current preparations:

- Reduce air leak in neonatal RDS
- Reduce mortality in neonatal RDS

But:

- No change in BPD \ CLD.
- Ineffective in ARDS.
- Non-responders.
Newer Surfactants
Synthetic Analogs of Surfactant Proteins

SP-B  FPIPLPYCWLCRALIKRIQAMIPKagalava...llattllgrmlpqlvcrllvlrcs
S-MB  FPIPLPYCWLCRALIKRIQAMIPKGRMLPQLVCRLLVLRCs

SP-C  FGIPCCPVHLKRLIVVVVVVLIVVVIVGALLMGL
SP-C33  IPSSPVHLKRLKLLLLLLLLLLLLLLILGALLMGL

Seehase et al. PLOS One, 2012.
Genetic Risk for RDS

- Earlier study had demonstrated increased RDS risk in subsequent pregnancy with first-born with RDS (Nagourney et al., J Pediatr 1996; 129:591-6)

- Twin study, 2 center, GA < 32 weeks, N = 332 twin pairs

<table>
<thead>
<tr>
<th></th>
<th>Both</th>
<th>One</th>
<th>Neither</th>
<th>p = 0.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ</td>
<td>44</td>
<td>12</td>
<td>14</td>
<td>observed</td>
</tr>
<tr>
<td>DZ</td>
<td>138</td>
<td>89</td>
<td>35</td>
<td>vs. expected</td>
</tr>
</tbody>
</table>

49.7% variance due to genetic factors

<table>
<thead>
<tr>
<th>Product</th>
<th>Source</th>
<th>Protein</th>
<th>[PL] mg/ml</th>
<th>Dose ml/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beractant (Survanta)</td>
<td>Bovine mince</td>
<td>SP-C, SP-B</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Calfactant (Infasurf)</td>
<td>Calf lavage</td>
<td>SP-B, SP-C</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>Poractant (Curosurf)</td>
<td>Porcine mince</td>
<td>SP-B, SP-C</td>
<td>80</td>
<td>2.5, 1.25</td>
</tr>
<tr>
<td>Lucinactant (Surfaxin)</td>
<td>Synthetic</td>
<td>KL4</td>
<td>30</td>
<td>5.8</td>
</tr>
<tr>
<td>Colfosceril (Exosurf)</td>
<td>Synthetic</td>
<td>None</td>
<td>13.5</td>
<td>5</td>
</tr>
</tbody>
</table>
Surfactant Proteins

Hydrophilic

SP-A
(SFTP A1, SFTP A2)
- Large glycoproteins.
- Multigene locus Chr. 10q
- Pulmonary collectins:
  Innate Immunity.
- Limited role in surfactant metabolism & function.

SP-D
(SFTP D)

Hydrophobic

SP-B
(SFTP B, Chr. 2p)
- Small, partition with lipids.
- Augment ST lowering properties of surfactant lipids.
- Essential components of animal derived surfactants used clinically to treat RDS.

SP-C
(SFTP C, Chr. 8p)
Surfactant Production

**Common Variants**

**SP-B**
- C,A
- C,T
- (CA)$_n$
- A,G

**SP-C**
- TTF1
- 121ins2
- I73T

**ABCA3**
- TTF1
- E292V

**Rare (damaging) Variants**

**Lamellar Body Assembly**

**Post-translational Processing**

**Transcription**

**Translation**

**Surfactant Production**

**Surfactant Production**
<table>
<thead>
<tr>
<th>OMIM</th>
<th>SMDP1</th>
<th>SMDP3</th>
<th>SMDP2</th>
<th>“B-T-L”</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Locus / Protein</strong></td>
<td>SFTPB 2p11.2 SP-B</td>
<td>ABCA3 16p13.3 ABCA3</td>
<td>SFTP C 8p23 SP-C</td>
<td>Nkx2.1 14q13.3 TTF-1</td>
</tr>
<tr>
<td><strong>Phenotype</strong></td>
<td>RDS</td>
<td>RDS &gt; ILD</td>
<td>ILD &gt; RDS</td>
<td>RDS, ILD CNS (Chorea) Hypothyroid</td>
</tr>
<tr>
<td><strong>Inheritance</strong></td>
<td>Recessive</td>
<td>Recessive</td>
<td>Dominant/sporadic</td>
<td>Sporadic/Dominant</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Fatal without lung transplant</td>
<td>Severe/variable</td>
<td>Variable</td>
<td>Variable</td>
</tr>
</tbody>
</table>
Acknowledgements

Collaborators:

Trey Dunbar
Scott Cameron
Janine Bullard
Adam Gower
Amit Agrawal
Fred Askin

Aaron Hamvas
Sesh Cole
Jennifer Wambach
Tammy Garmany
Fran White
Harvey Colten

Mike Dean

Bill Hull
Susan Wert
Machiko Ikegami
Jim Bridges
Tim Weaver
Jeff Whitsett
Bruce Trapnell

Leland Fan
Megan Dishop
Robin Deterding

Lisa Young
Gail Deutsch
Claire Langston

U of Cincinnati &
Cincinnati Children’s Hospital

Children, families and their physicians; chILD Foundation.

Funding: NHLBI, chILD Foundation-ATS,Eudowood Board, MOD.
The Good News: Mortality from RDS, 1987 - 95

RDS mortality decreased; lower infant mortality
Neonatal RDS and Surfactant Deficiency

$P \approx \frac{\text{Surface Tension}}{r}$

Normal

RDS