



Oxidative stress and susceptibility of developing lungs

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Conflicts of Interest

- None
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Long-term Objective

- To understand the contribution of environmental and genetic modifiers on the development of lung disease in children born prematurely



Specific Objectives

- Who gets chronic lung disease of prematurity
 - Impact of prematurity on lung growth
 - Impact of oxidative stress and inflammation on neonatal lung growth
 - Neonatal mouse models
 - Registry for infants and children with chronic lung disease of prematurity registry
-

Prematurity in the U.S.

- In 2007- 4,317,119 live births in the U.S.
- 12.7% of all live births infants born prematurely (<37 weeks gestation)
- 6.7%- weighed, 1500-2499 grams- **low birth weight**
- 1.48% weighed <1500 grams (\approx 64,000)- **very low birth weight**

Survival of premature infants has increased over time

**Sarah Morris Hospital
1922-1940**

Survival

<1000 grams	13%
1000-1250 grams	38.5%

O'Donnell, Neonatology, 2008

**United States
2005**

Survival

500-749 grams	53.2%
750-999 grams	85%
1000-1250 grams	92.9%

MacDorman and Mathews, Public Health reports, 2009

Interventions that have improved survival

- Avoidance of mechanical ventilation when possible
 - Tolerance for lower oxygen saturations
 - Use of surfactant, prenatal steroids
 - Tertiary care centers
-



Medscape

Source: Neonatal Netw © 2009 Neonatal Netw



www.mercatornet.com/images/stories/bellieni2.jpg

High economic cost of caring for premature infants

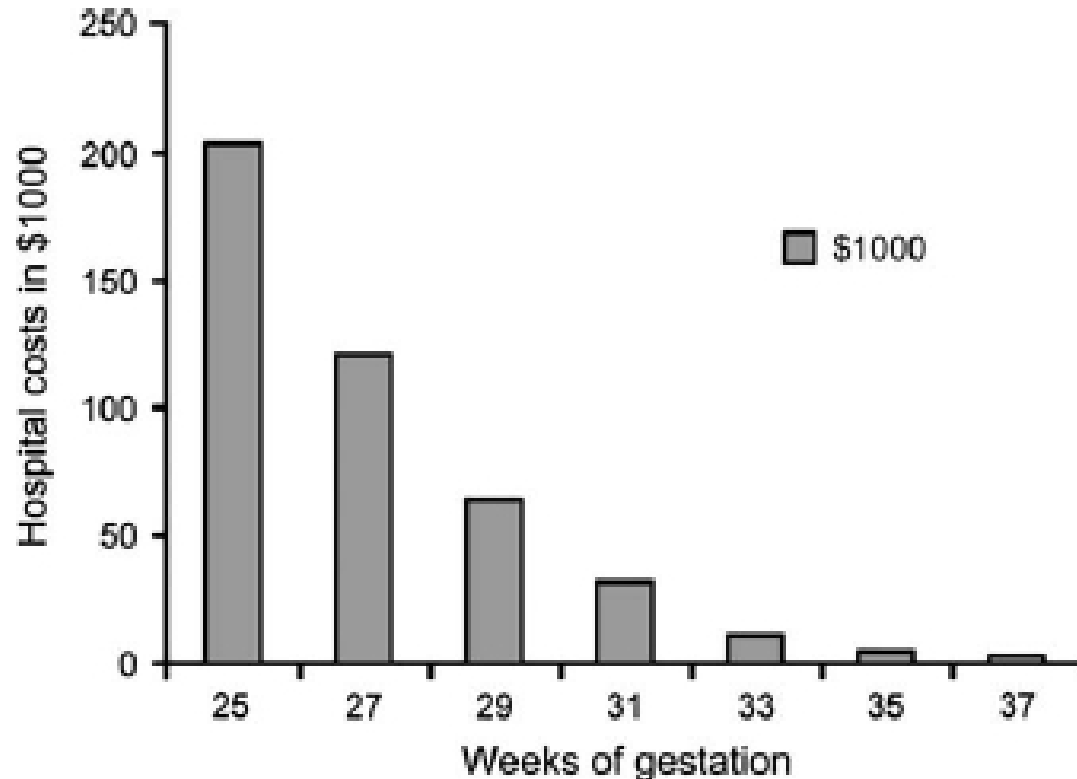


Figure 1. Hospital costs (in 1000 dollars) in 1996 of individual, surviving, premature newborns separated into weeks of gestation from 25 to 37 weeks. Reproduced with permission from Gilbert, et al. *Obstet Gynecol* 2003;102:488-92.⁵

Interventions that have improved survival

- Have shifted survival curves to earlier gestational ages
 - Co-morbidities are common in very preterm infants
-

Common long-term morbidities in preterm infants

- Neuro-cognitive deficits
 - **Chronic lung disease**
 - Gastrointestinal problems
 - Visual and hearing deficits
-

Definition of chronic lung disease of prematurity or BPD

- Premature infant with a need for supplemental oxygen/ventilatory support beyond 36 weeks gestation
-

What factors influence the severity of lung disease in premature infants?

Factors associated with chronic lung disease of prematurity

- **Gestational age** and weight at delivery
 - Respiratory distress at birth
 - Prenatal and postnatal infections
 - Initiation of mechanical ventilation
 - Exposure to high oxygen concentrations
 - Pre-eclampsia, poor systemic growth
-

Gestational age and BPD at one year

Gestational Age	22wks	23wks	24wks	25wks	26wks	<27wks (total)
Live-born infants	N=51	N=101	N=144	N=205	N=206	N=707
Birth weight (grams)	<u>508</u> (280-730)	<u>590</u> (320-808)	<u>674</u> (374-1070)	<u>784</u> (266-1235)	<u>920</u> (430-1500)	<u>730</u> (266-1500)
Survival at 1 yr	5 (9.8%)	53 (52%)	96 (67%)	167 (81%)	176 (85%)	497 (70%)
Severe BPD at 1 yr	2/5 (40%)	13/49 (26%)	27/87 (31%)	45/153 (29%)	26/158 (17%)	113/452 (25%)
Survivors at 1 yr without major morbidity	1/5 (20%)	9/53 (17%)	30/144 (21%)	75/205 (37%)	111/206 (54%)	226/707 (32%)

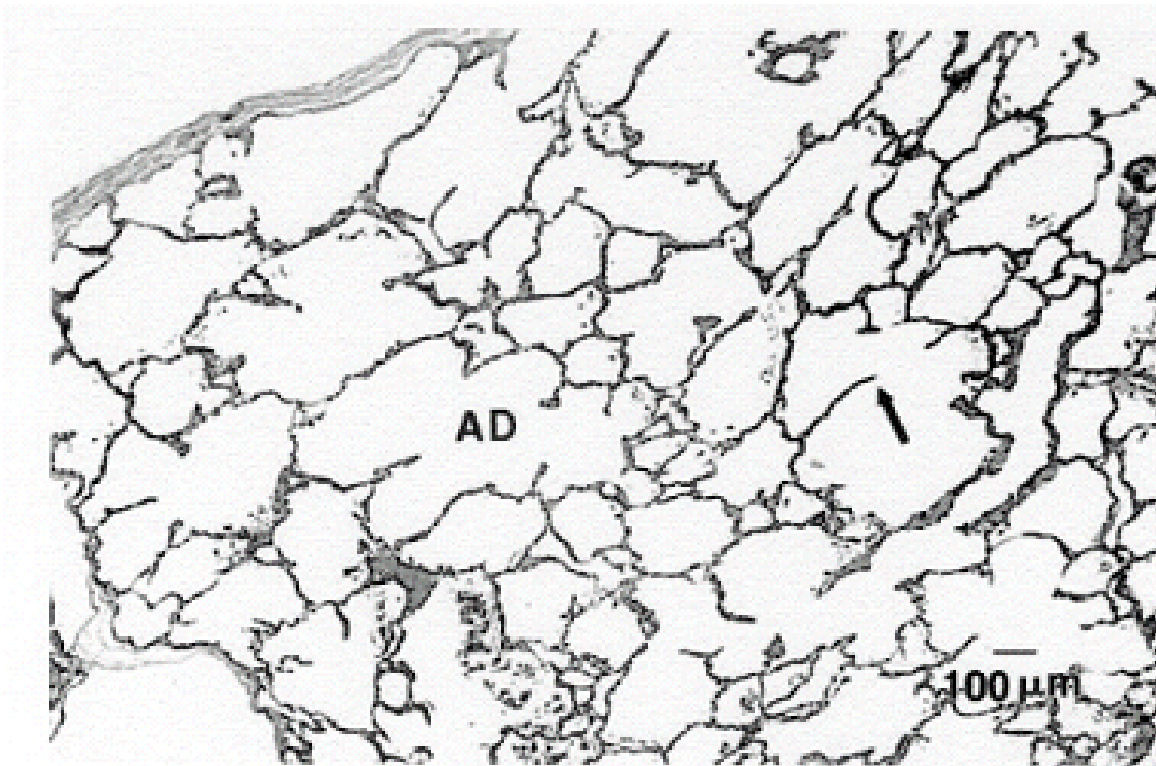
-
- 25 to 40% of infants with CLDP/BPD will be re-hospitalized in the first two years of life
 - Higher rate of asthma-like symptoms
 - CLPD/BPD in very low birth weight infants is associated with a new type of lung histology referred to as “**new BPD**”
-

New BPD

- Characterized by impaired alveolar growth
 - Occurs primarily in very low birth weight and very early gestational age infants
-

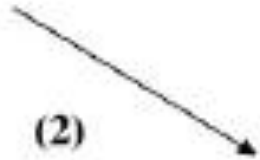
Poor alveolar growth characterizes chronic lung disease of prematurity

Fewer alveoli
Airspace enlargement





Normal alveolar sac

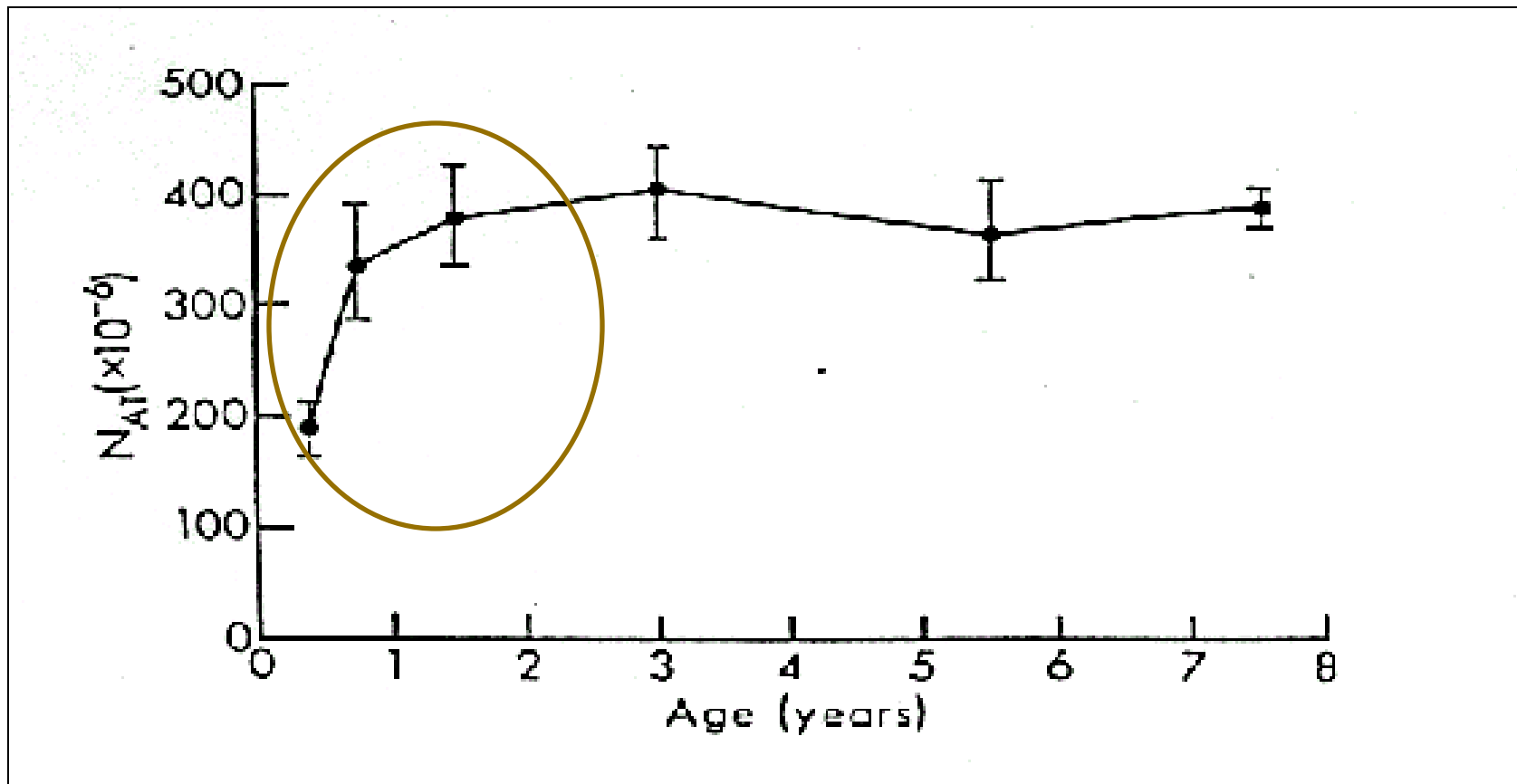


Alveolar sac in BPD

**Thebaud and Abman,
AJRCCM, 2007**

Why do we care about alveolar growth in the premature infant and what impact does that have on lung function?

Compensatory alveolar growth is limited- majority of alveolar lung growth occurs by age 2yrs.



Natural decline in lung function with age

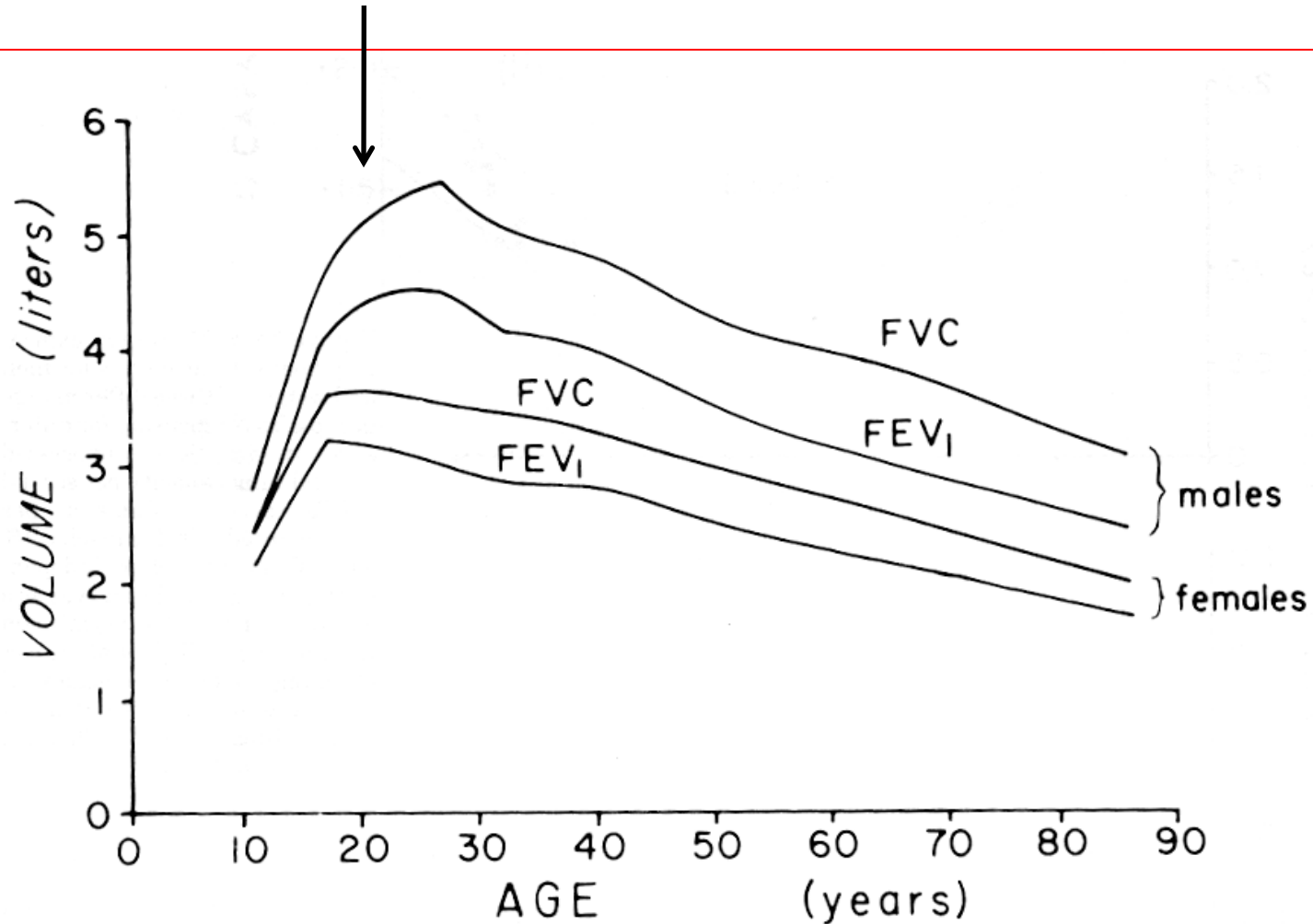


Figure 14-5. Mean values showing changes in 1 second forced expiratory volume (FEV₁) and forced vital capacity (FVC) with age in normal men and women. (Reprinted by permission from Knudson, R. J., et al.: The maximal expiratory flow-volume curve. Normal standards, variability and the effects of age. *Am. Rev. Respir. Dis.*, 113:587-600, 1976.)

In later life

- Approximately 25% of infants diagnosed with chronic lung disease of prematurity will have long term respiratory issues as young adults
 - Airflow obstruction, increased rates of wheezing
 - Exercise intolerance
 - Use of chronic respiratory medications
 - Respiratory difficulties with viral illnesses

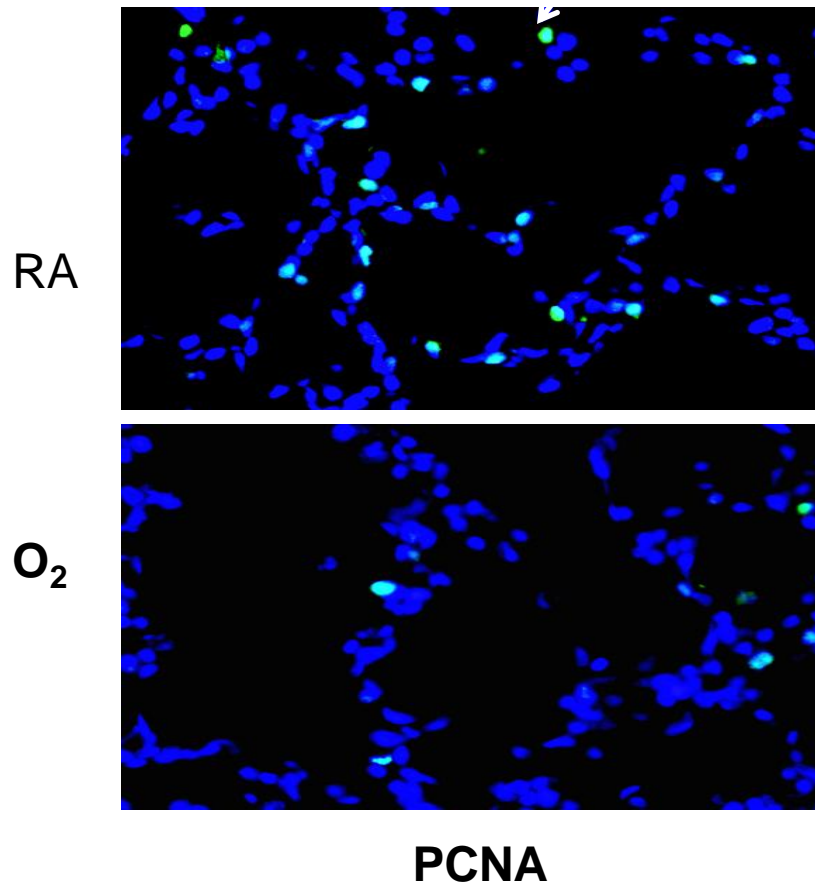
Doyle and Anderson. Long-term outcomes of bronchopulmonary dysplasia, Sem. in Fetal & Neo. Med., 2009

What factors limit alveolar lung growth in the neonatal lung?

Study design

- Neonatal mice like humans exhibit postnatal lung growth
 - Neonatal mice were placed in oxygen chamber
 - Oxygen concentration kept between 88-92% for the first 4 days of life
-

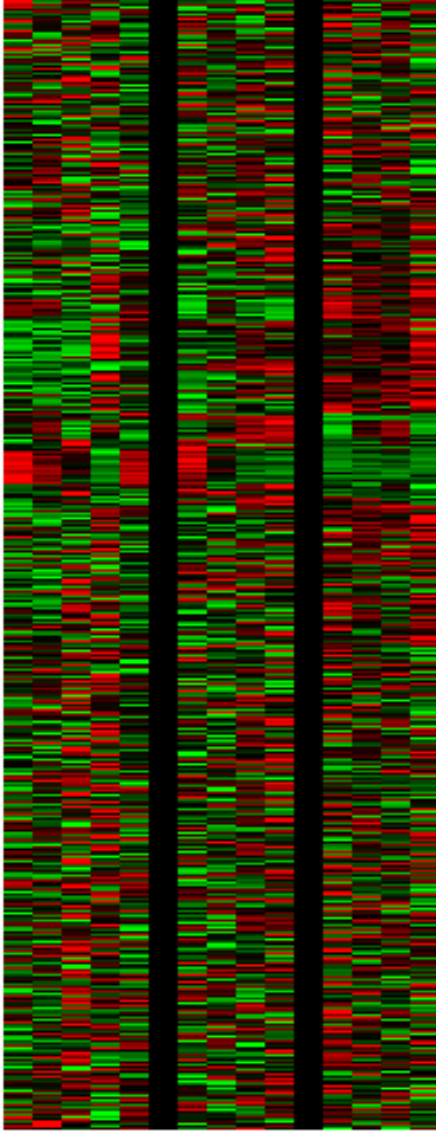
Neonatal lung exposed to hyperoxia has decreased alveolar cell proliferation



**What genes are induced in
in neonatal lung exposed to hyperoxia?**

Microarrays

-differences in gene expression between room air and hyperoxia neonatal lung



```
zzSample #250 (Control)
zzSample #251 (Control)
zzSample #252 (Control)
zzSample #254 (Control)
zzSample #255 (Control)
X
zzSample #256 (Neonatal + Ad?Lt CS)
zzSample #258 (Neonatal + Ad?Lt CS)
zzSample #259 (Neonatal + Ad?Lt CS)
zzSample #260 (Neonatal + Ad?Lt CS)
X
zzSample #282 (Ad?Lt CS)
zzSample #283 (Ad?Lt CS)
zzSample #284 (Ad?Lt CS)
zzSample #286 (Ad?Lt CS)
```

p53 regulated genes

Fold-change
compared to room air lung

Growth Arrest

p21	10.3
cyclin G1	4.8
Zmat3	4.8
Psrc1	4.4
Phlda	3.0
Sulf2	2.3
Sesn2	3.0
Gpnmb	2.1
Btg2	1.6
TRP53inp1	2.1
GDF15	2.5
RPS27L	2.6

Genes induced in hyperoxia
neonatal lung

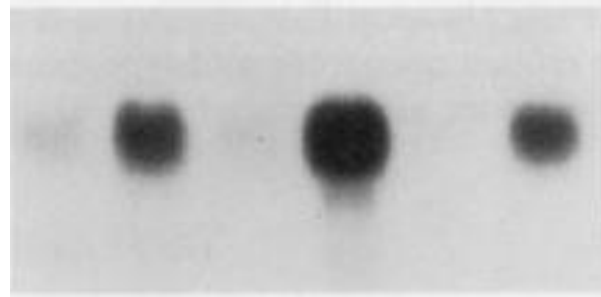
Apoptosis

Aen	2.9
Bax	2.6
Ptgs2	2.3
Nupr1	2.7

p21, a G1 cell cycle checkpoint regulator, is induced in neonatal lung exposed to hyperoxia

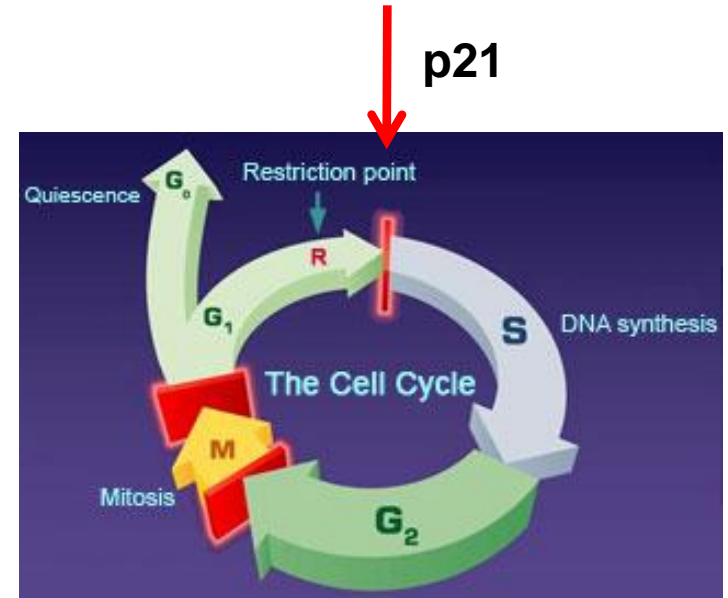
Neonatal lung exposed to hyperoxia

1.5 d 3.5d 6.5d



RA O₂ RA O₂ RA O₂

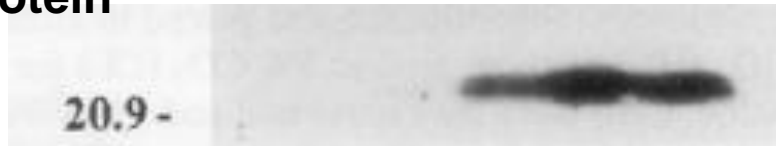
← p21



www.usyd.edu

Neonatal lung exposed to hyperoxia

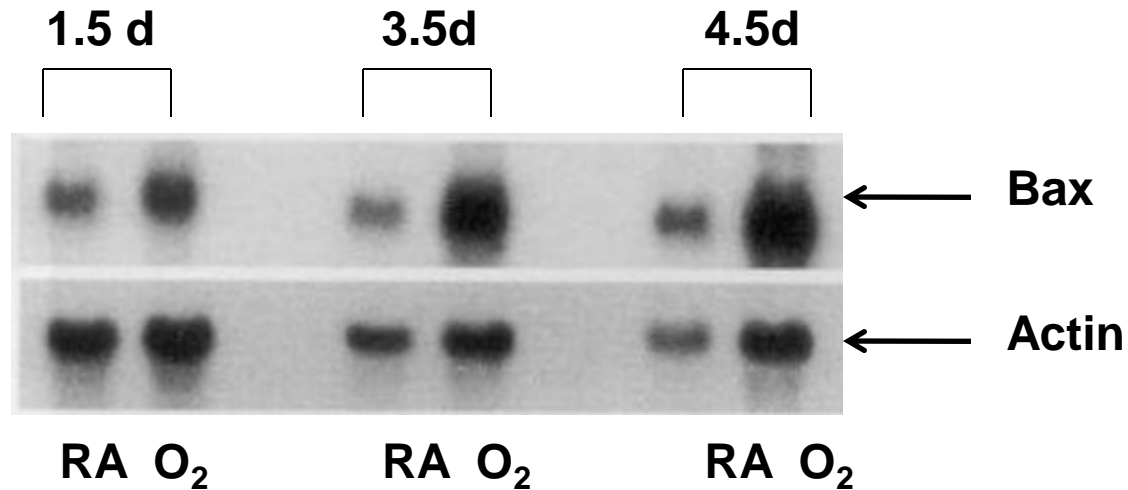
2d 3d 4d



RA

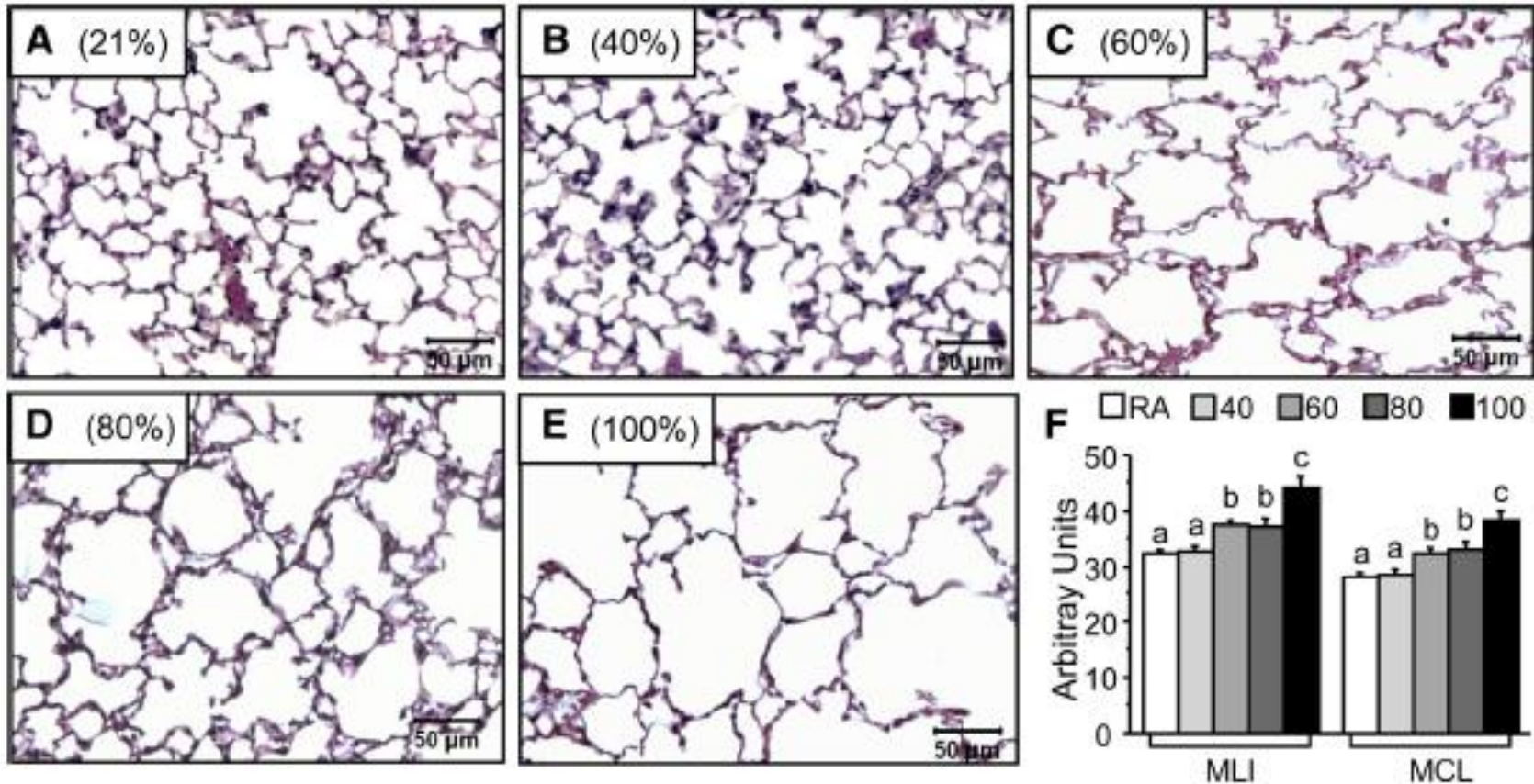
O₂

Bax an apoptotic (programmed cell death) gene is induced in neonatal lung exposed to hyperoxia



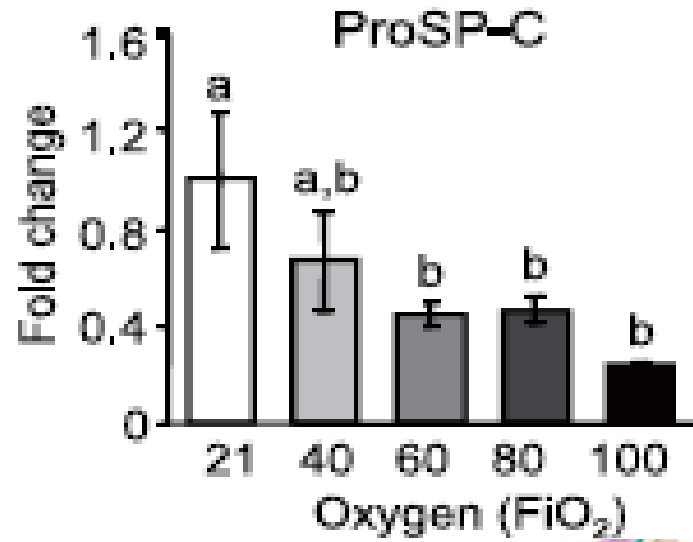
Does exposure to neonatal hyperoxia lead to structural changes in the adult lung?

High oxygen exposure in the neonate alters adult lung structure



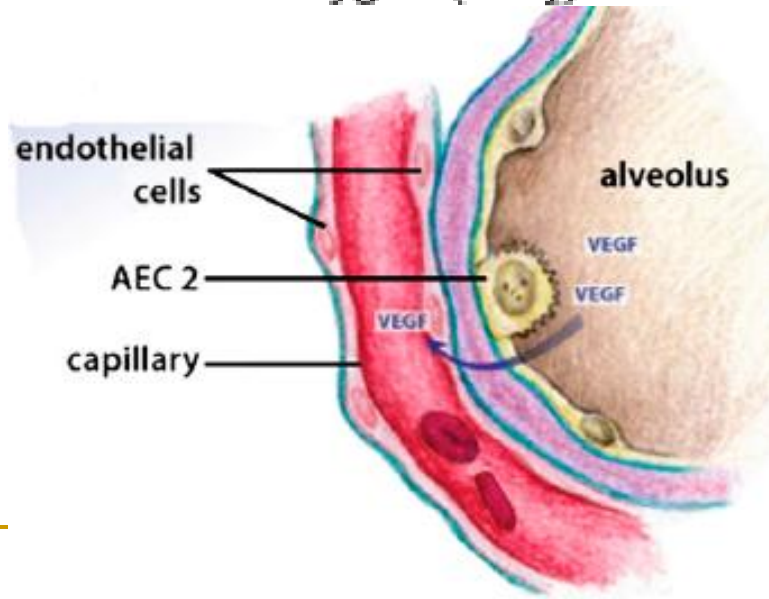
Hyperoxia causes loss of type 2 epithelial cells

Hyperoxia causes loss of type 2 epithelial cells



Yee et.al., AJP, 2009

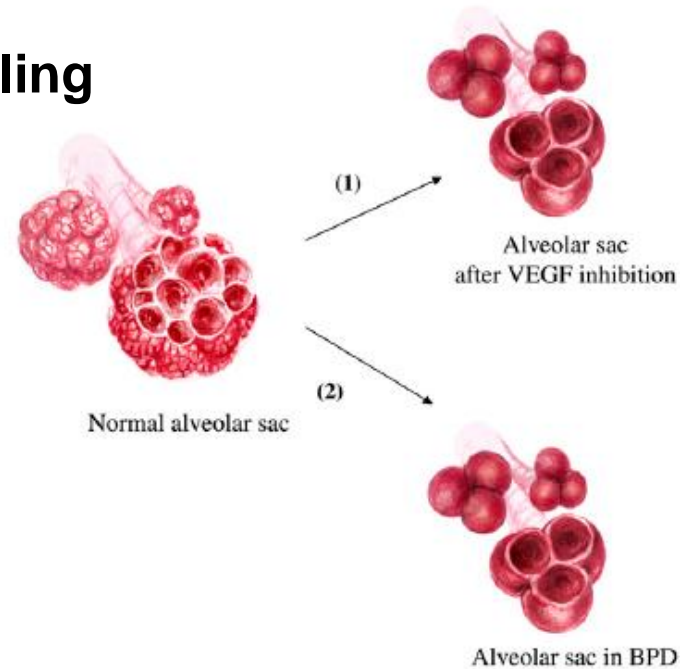
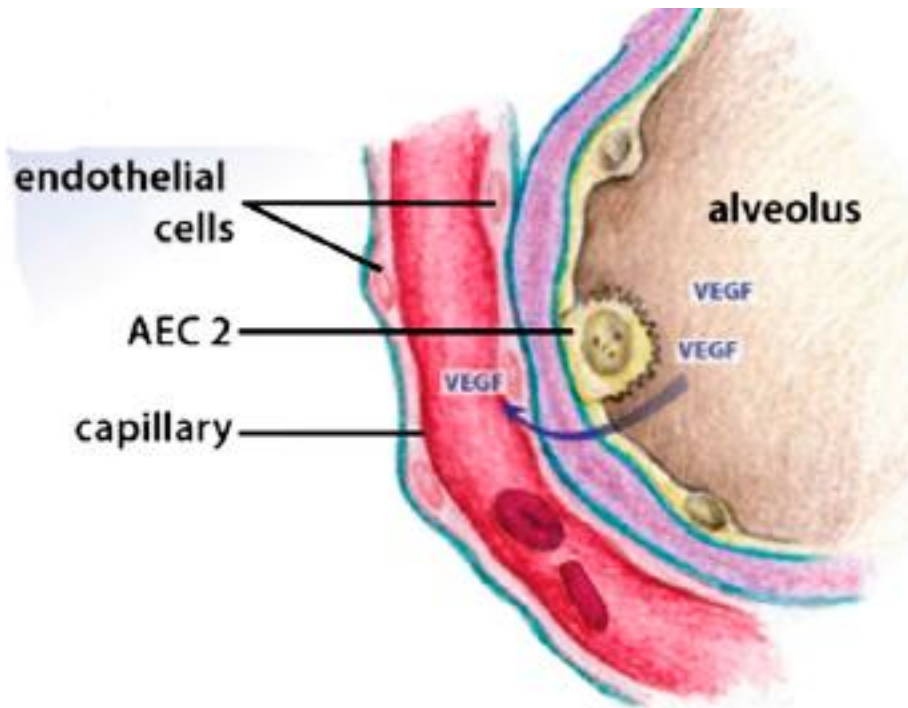
Type 2 Epi cells
secrete VEGF



Thebaud and Abman,
AJRCCM, 2007

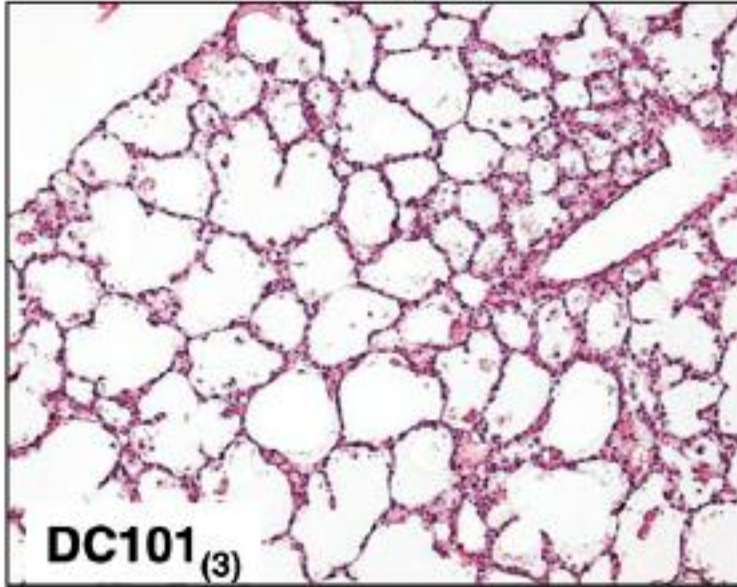
Vascular hypothesis

Hyperoxia interferes with VEGF signaling

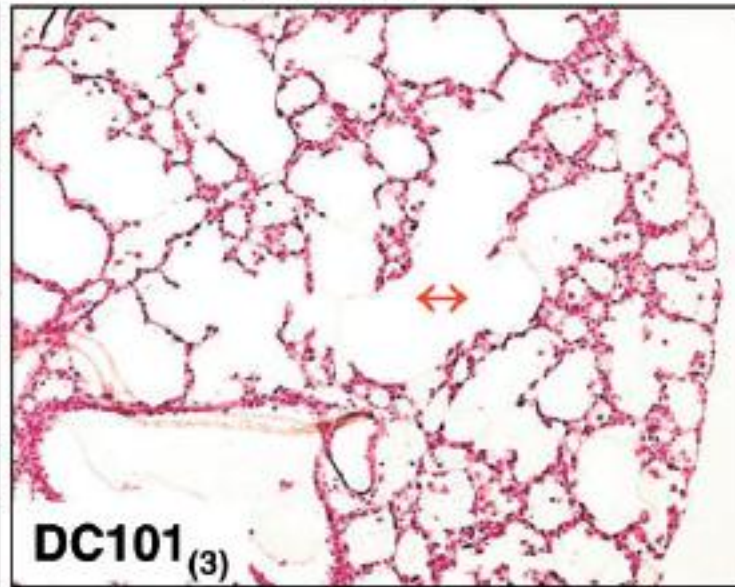


VEGF signaling critical for post-natal lung growth in neonatal mice

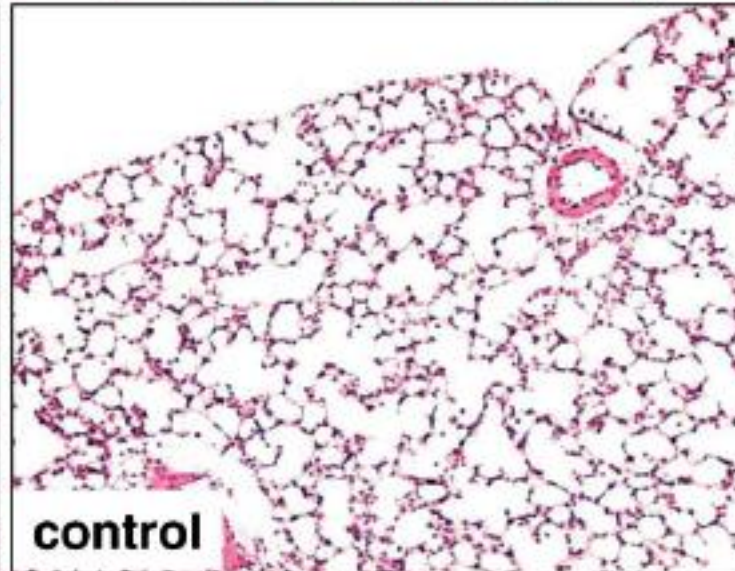
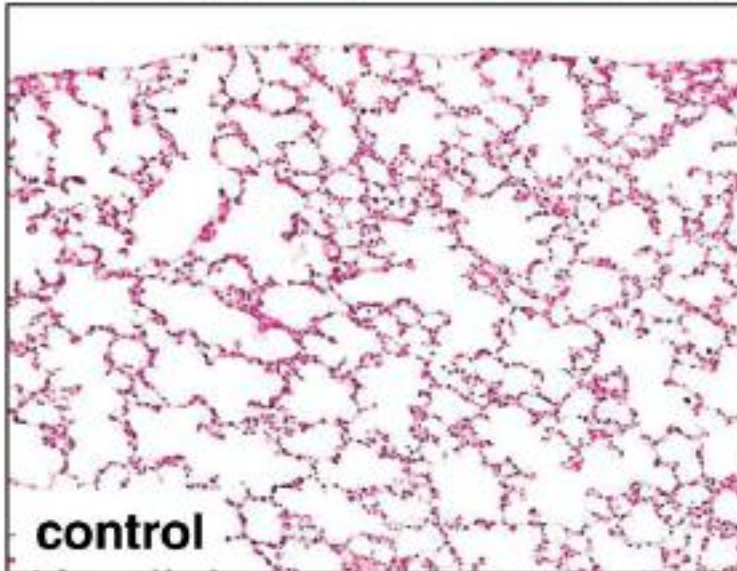
one week



two weeks



DC101 is a
VEGFR-2
antibody



Summary of Findings

- Hyperoxia induces cell cycle growth arrest by increasing expression of checkpoint and apoptotic genes regulated by p53
 - Hyperoxia impairs type 2 epithelial cell growth in the alveoli thus decreasing VEGF expression from type 2ECs
 - Blockade of VEGF signaling disrupts capillary growth inhibiting alveolar growth
-

What is the relationship between hyperoxia, alveolar growth arrest and oxidative stress in the neonatal lung?

Prenatal events

Chorioamnionitis
- Cytokine
exposure of the
fetus-

Postnatal events

- + Resuscitation
- + Oxygen toxicity
- + Mechanical ventilation
- + Pulmonary and / or systemic infection

Oxidative stress

Sequential
lung injury

Pulmonary inflammatory response

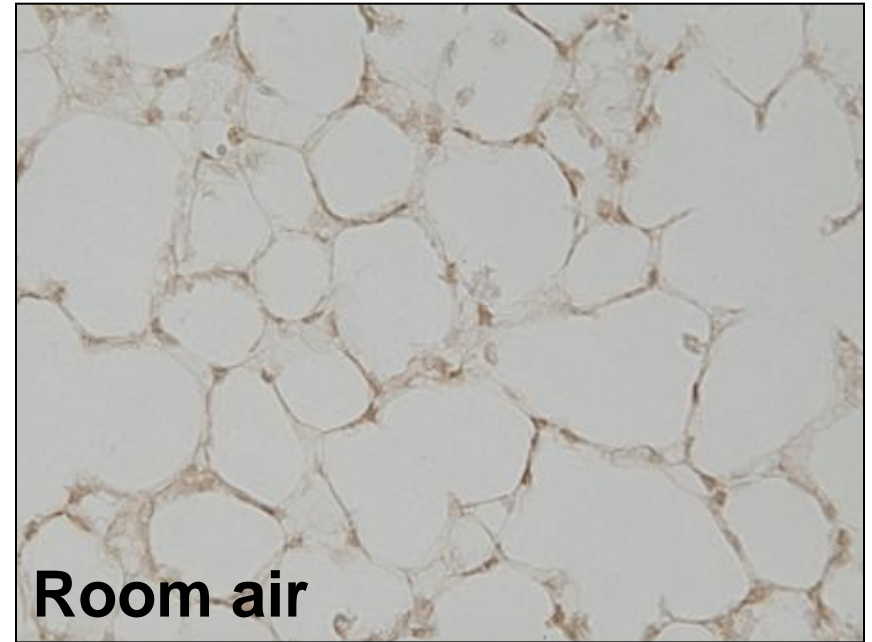
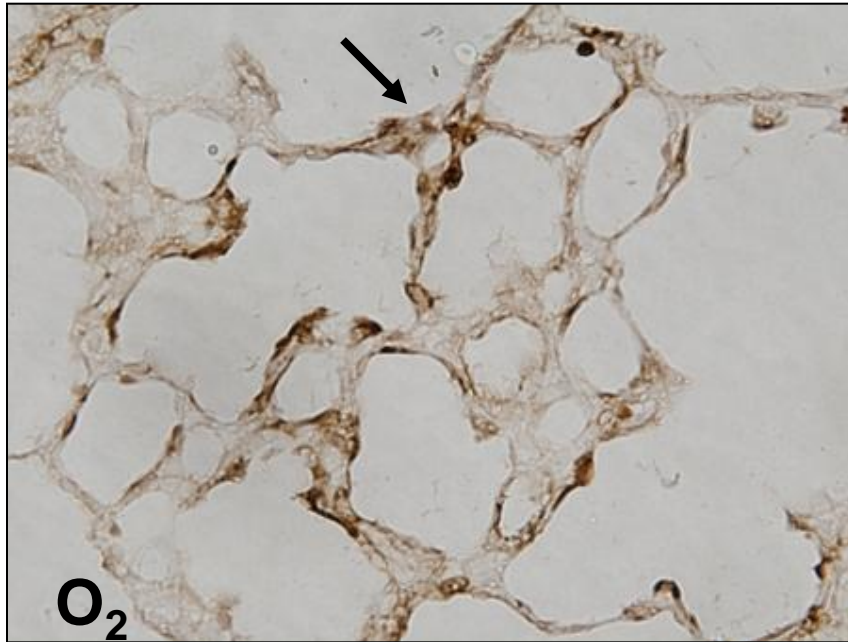
Aberrant wound healing

Inhibition of alveolarization and vascular development

"New" BPD

More severe BPD

Oxidative stress increased in 2-week old lung exposed to neonatal O_2



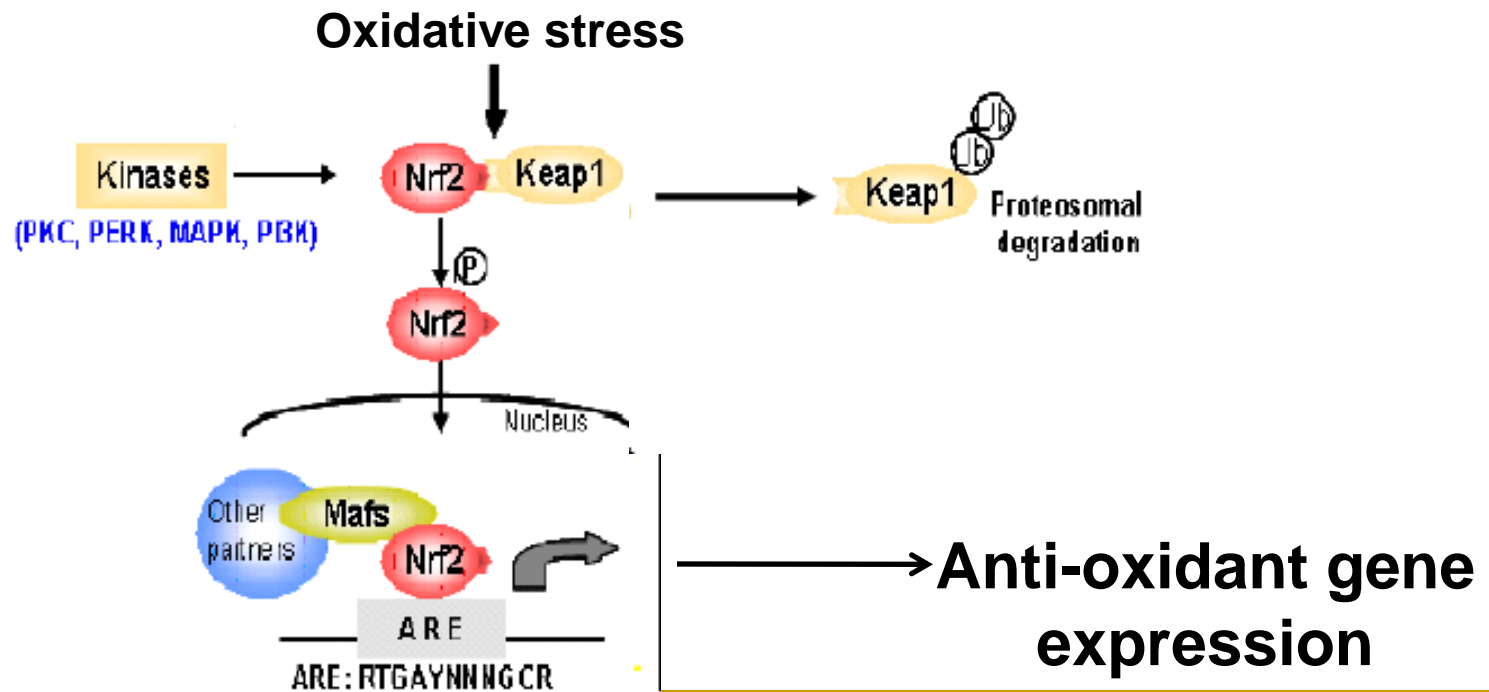
N-tyrosine staining

How does the cell respond to increased oxidative stress and is this important?

Induction of Nrf2

Transcriptional factor

-binds to the **antioxidant response element** on the promoter region of genes



Nrf2



Stress Response

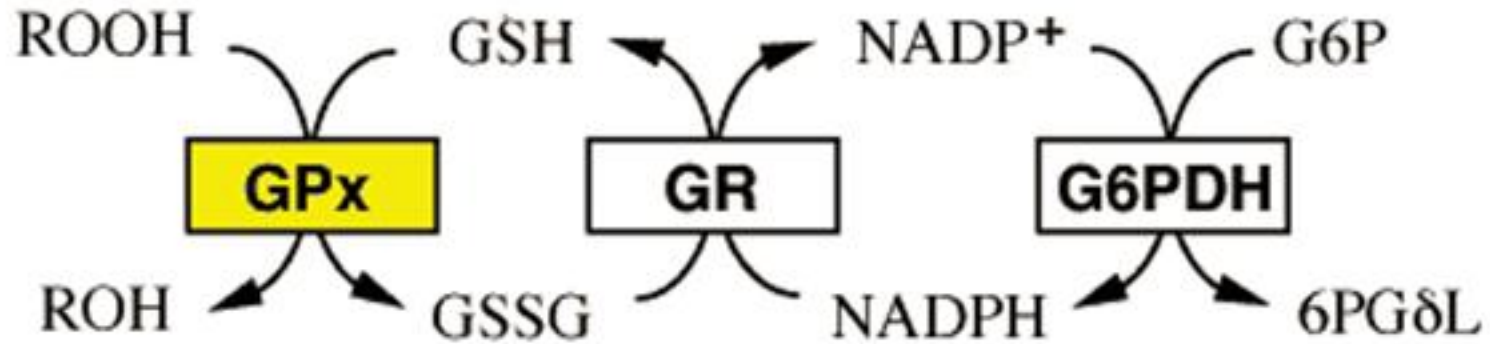
Antioxidant enzymes – HO-1, GST, NQO1

Glutathione/Thioredoxin pathway

Proteosomal pathway

Chaperones

GPx2 is induced by Nrf2 and the recycling of GSH in the cell



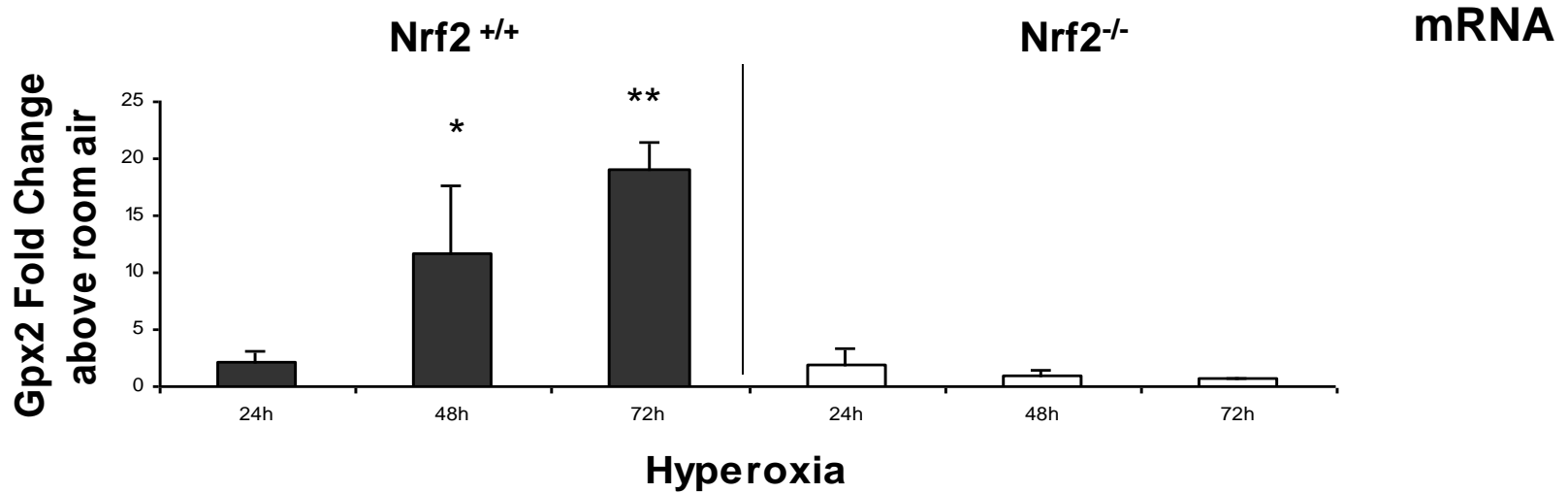
Nrf2 knockout mice

- Normal development
- Exposed Nrf2 knockout pups to hyperoxia

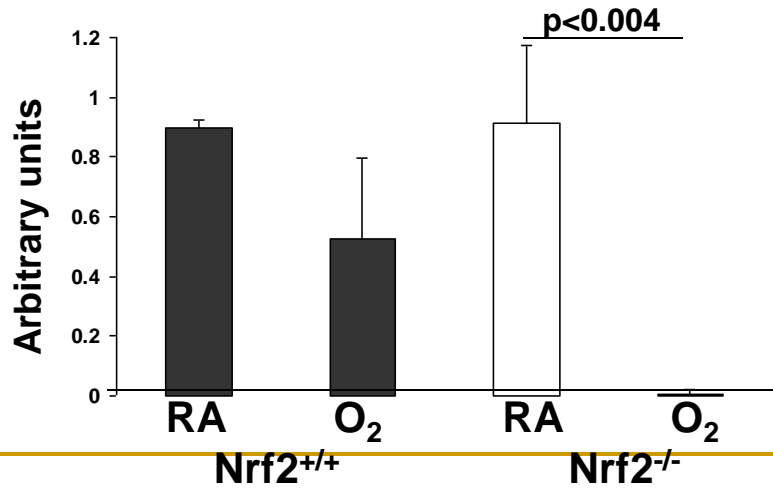


Gpx2 is not induced by hyperoxia in lung of Nrf2^{-/-} mice exposed to hyperoxia

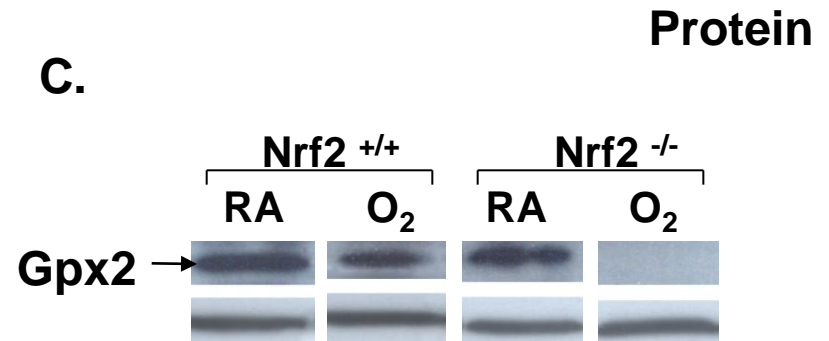
A.



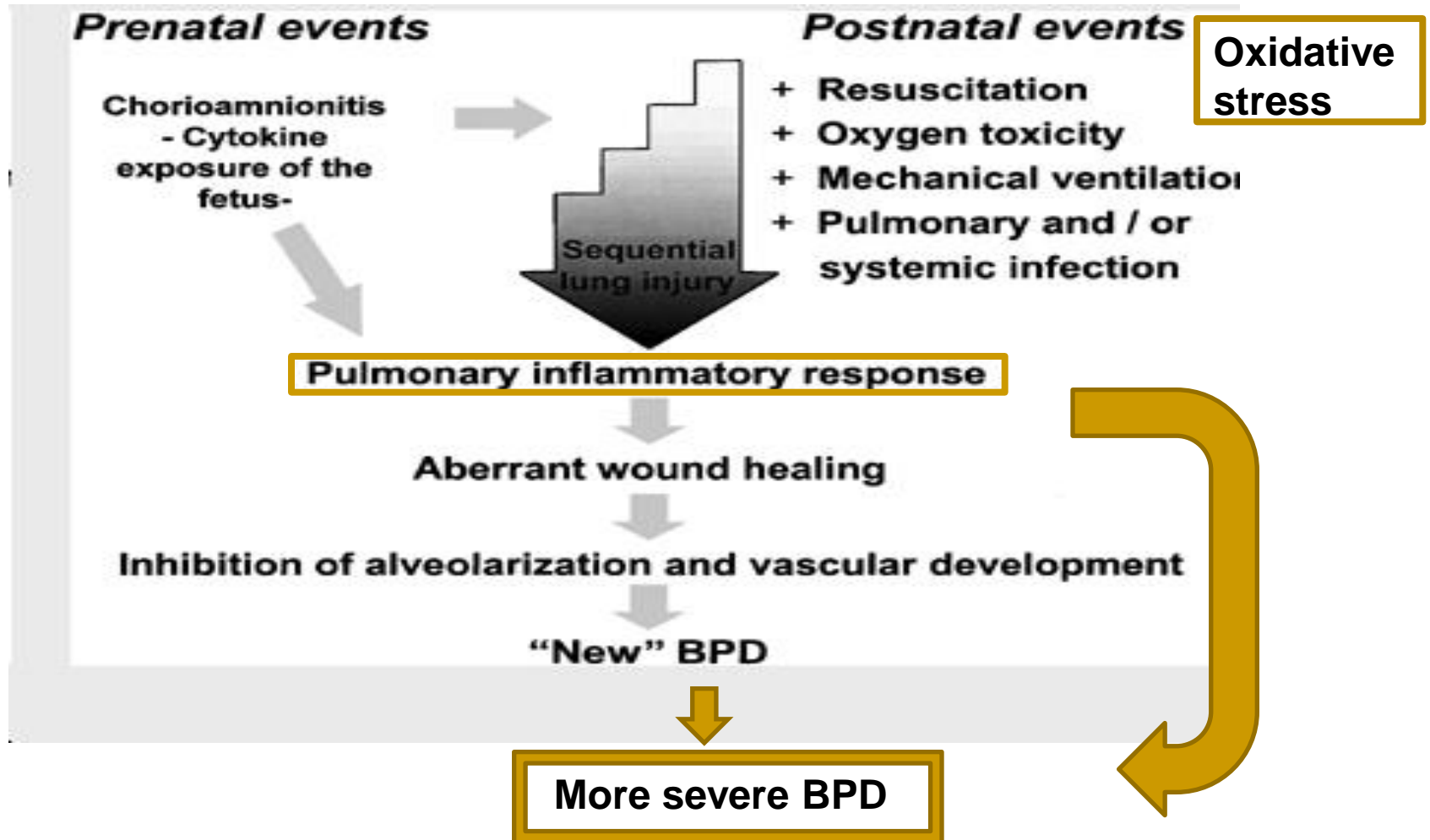
B.



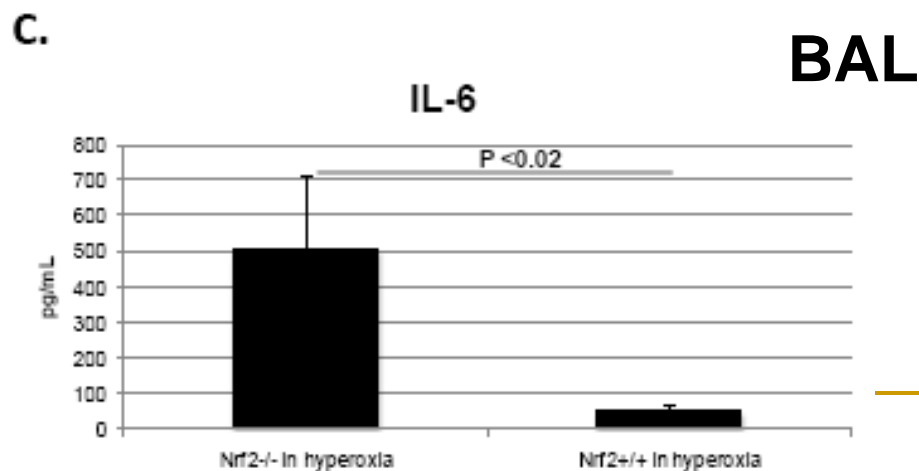
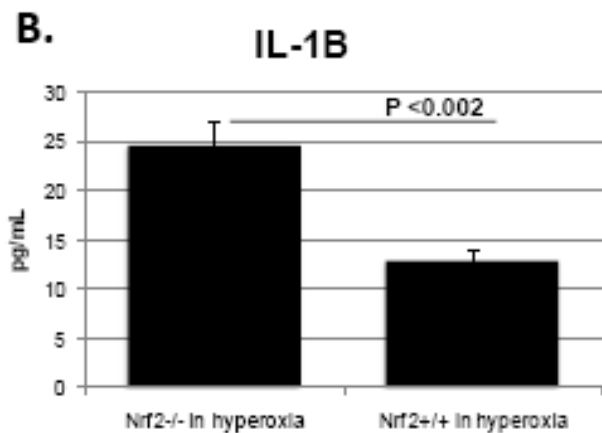
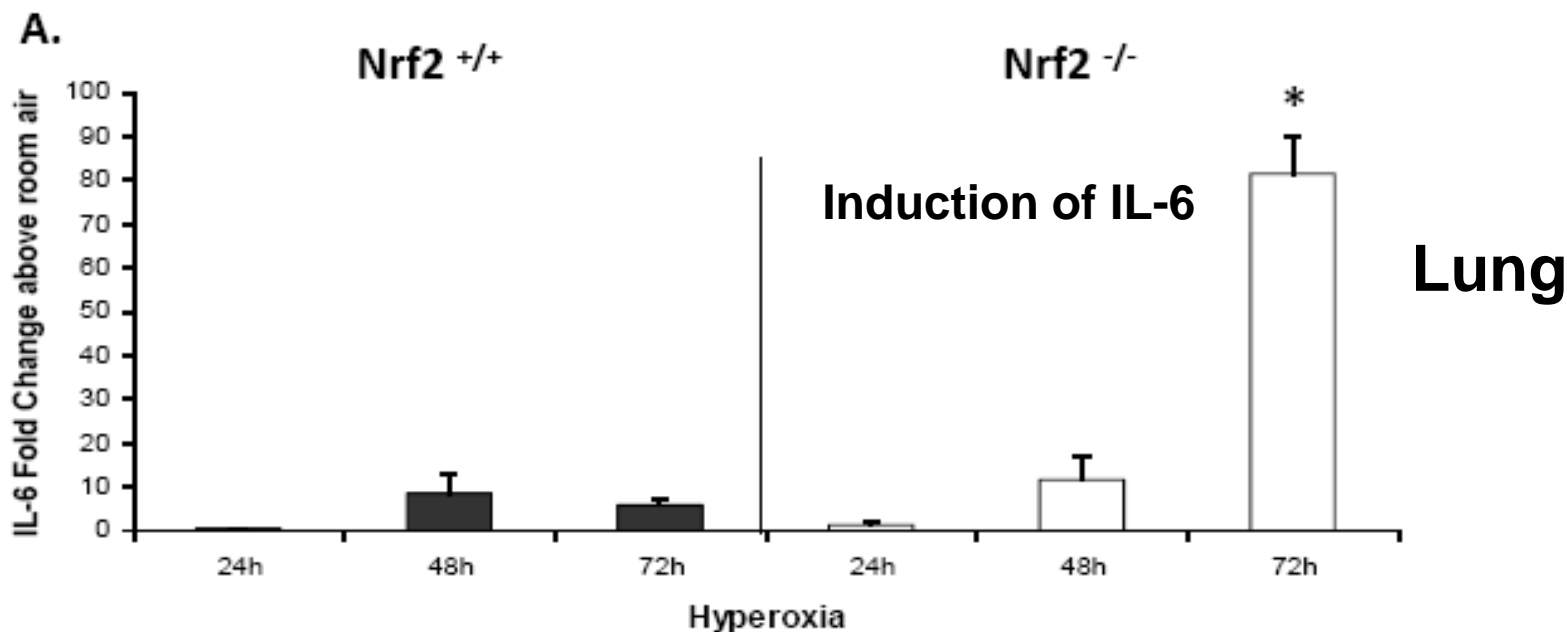
C.



Is lung inflammation increased in neonatal Nrf2 knockout mice exposed to hyperoxia?



Lungs of $Nrf2^{-/-}$ mice have high levels of the pro-inflammatory cytokine IL-6



Summary

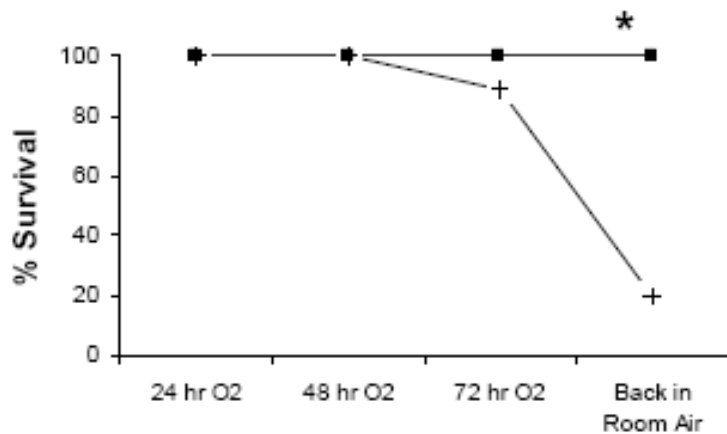
- Nrf2 induces anti-oxidant pathway genes in the neonatal lung exposed to hyperoxia
 - In the absence of Nrf2 induction, hyperoxia exposure leads to a significant increase in inflammatory gene expression in neonatal lung
-

Hyperoxia and Nrf2 knockout mice

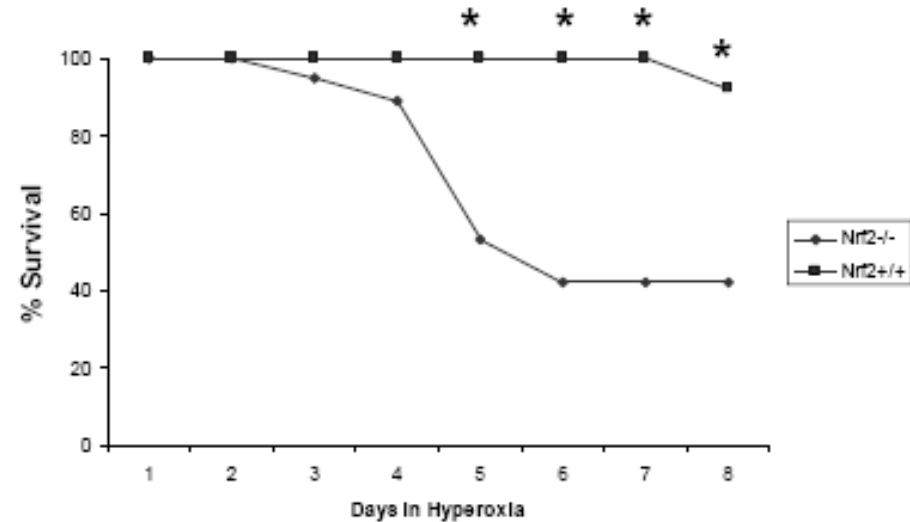
- Is mortality increased in neonatal Nrf2 knockout mice exposed to hyperoxia?

Mortality in hyperoxia increased in Nrf2 knockout mice

Survival curves of newborn Nrf2^{+/+} and Nrf2^{-/-} mice in hyperoxia



Hyperoxia/Hypoxic injury



Hyperoxic injury

**What is the impact of environmental factors
on lung phenotype of premature infants
after discharge from the hospital?**

Are there environmental insults that can worsen lung structure and function abnormalities?

- Airborne pollutants
 - Second hand smoke
 - Lower respiratory tract infections
-

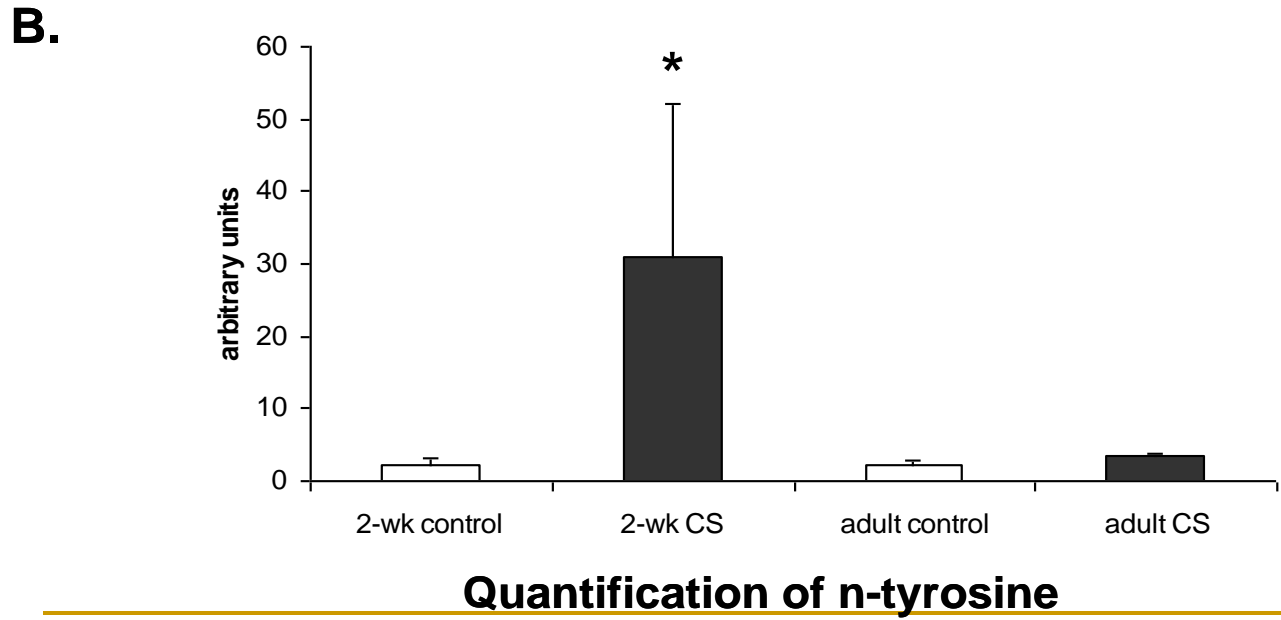
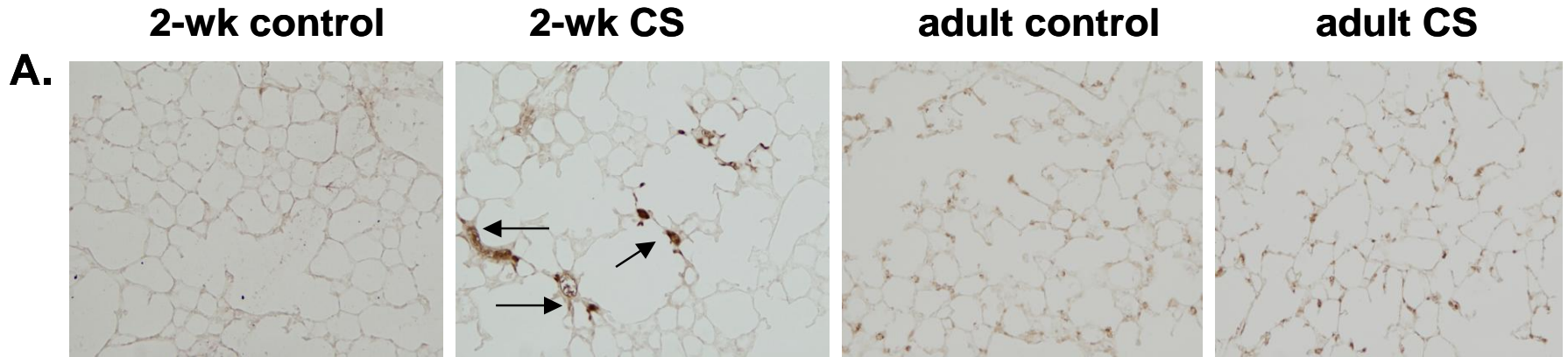
Rationale

- Children and adolescents exposed to high level of pollutants are at increased risk for suboptimal lung growth as adults (Gauderman et. al **Lancet 2007**)
 - Exposure to parental smoking during childhood is associated with poorer lung function in adulthood (Lebowitz et. al., **ARRD, 1987**)
 - Childhood respiratory illnesses and environmental smoke exposure increases the risk of developing COPD in adulthood (Berglund et. al., **Chest, 1999**)
-

Study Design

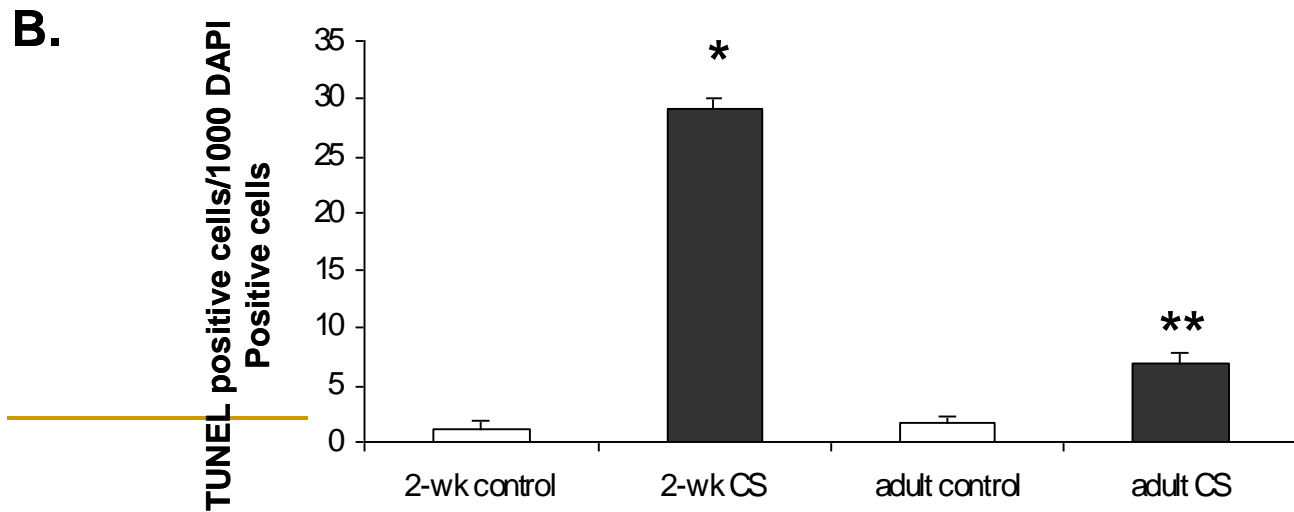
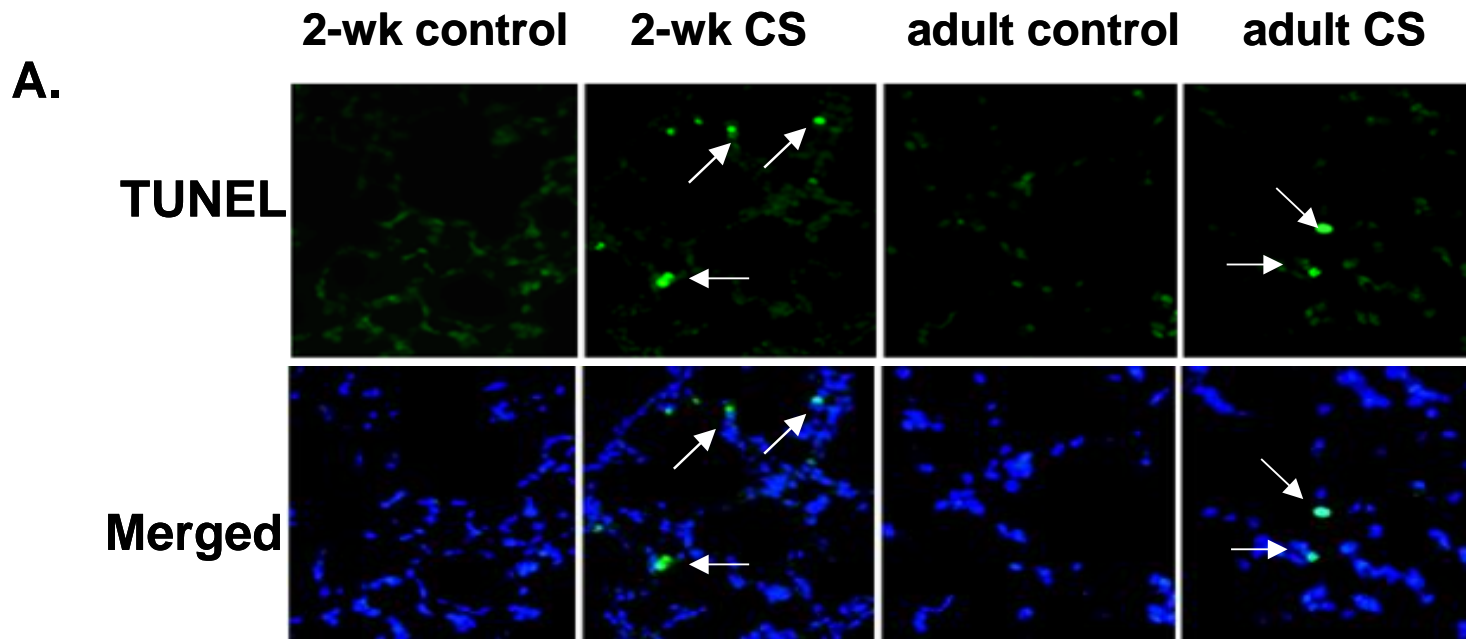
- Neonatal mice were placed in smoke chamber for the first 2 weeks of life (1hr/day for first 7 days, and 2hr/day for the last 7 days)
-

Increased oxidative stress in neonatal lung exposed to CS Figure 2



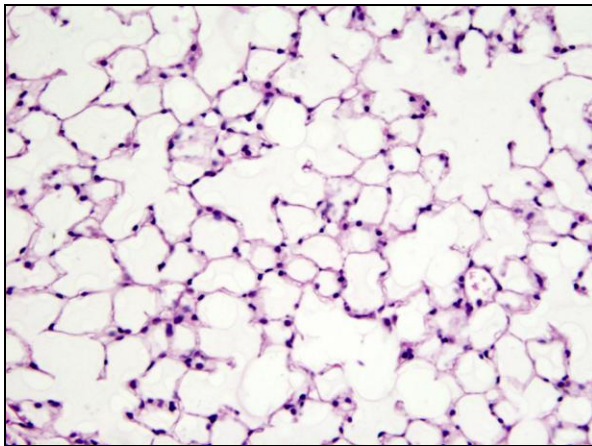
Increased cell death in neonatal lung exposed to CS

Figure 3

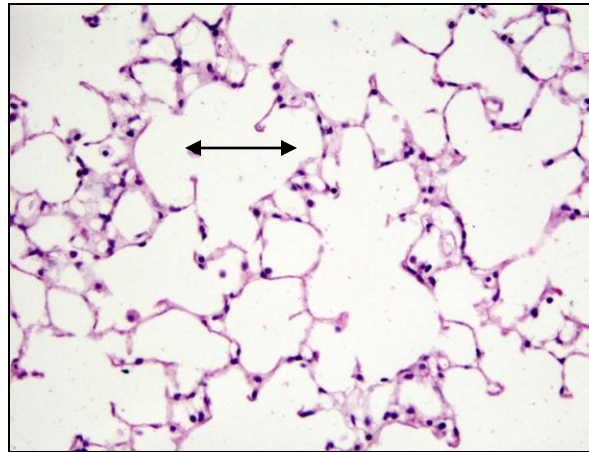


Impaired alveolar growth in adult mice exposed cigarette smoke in the neonatal period

8-wk control



8-wk neonatal CS



**Does cigarette smoke alter immune
gene expression in the developing
lung?**

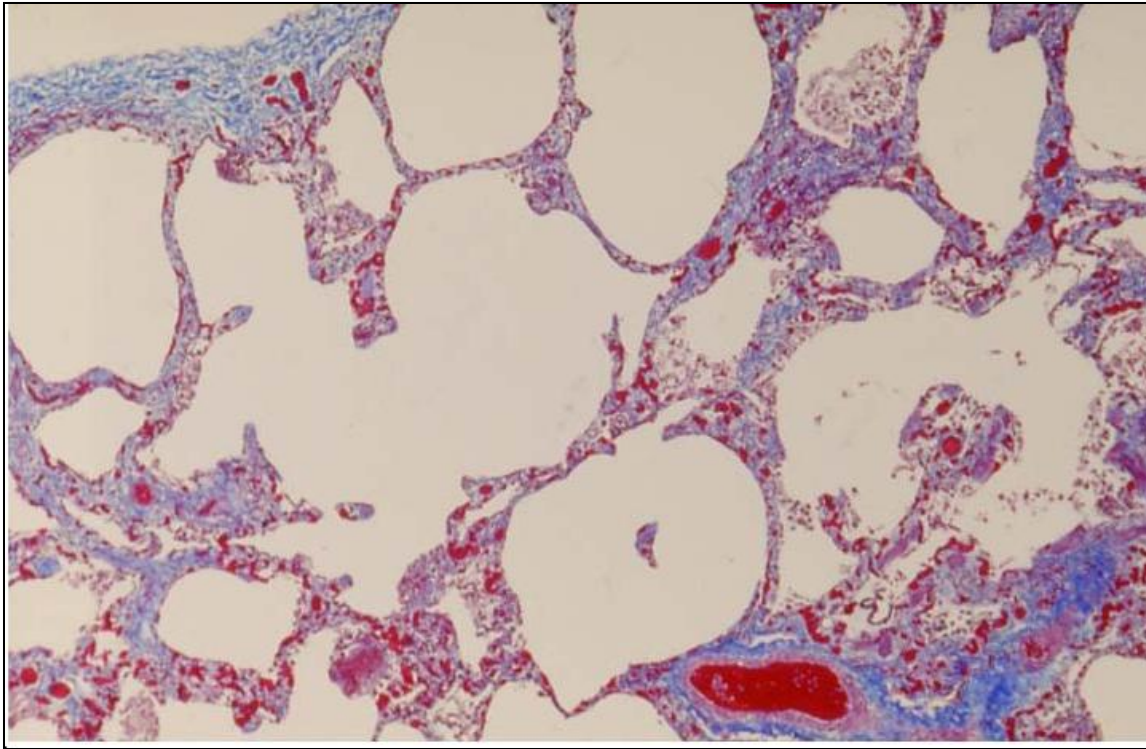
p47 GTPases	Gene symbol	Fold-change	P value
Interferon-inducible GTPase-1	IIGP1	-9.71	.005
Interferon-inducible GTPase-2	IIGP2	-6.77	.005
Interferon- γ induced GTPase	IGTP	-7.52	.005
Interferon inducible protein 1	LRG-47/ IFI1	-5.94	.005
Interferon gamma inducible protein 47	IRG-47/ IFI47	-5.74	.005
T-cell specific GTPase	TGTP	-6.68	.005
VLIG GTPases-secondary response			
GTPase, very large interferon inducible 1	GVIN1	-5.03	.005
p65 GBP GTPase			
Guanylate nucleotide binding protein 2	GBP2	-5.17	.005
Guanylate nucleotide binding protein 4	GBP4	-5.9	.005
Mx GTPases			
Myxovirus resistance 1	MX1	-5.5	.005
Myxovirus resistance 2	MX2	-19.56	.005

Activated by viral dsRNA	Gene symbol	Fold-change	P
Toll-like receptor 3	TLR-3	-3.4	.005
Retinoic-acid-inducible protein 1	RIG-1	-2.43	.005
Fas death domain-associated protein	DAXX	-1.88	.005
Melanoma differentiation associated gene 5	MDA5	-4.08	.005
DNA segment, Chr 11, Lothar Hennighausen 2, expressed	LGP2e	-11.63	.005
Interferon regulatory factor 7	IRF-7	-2.66	.005
Inhibits initiation of translation			
IFN- type I-induced and dsRNA-activated kinase	PKR	-6.54	.005
Interferon-induced protein with tetratricopeptide repeats 1	IFIT1/p56	-19.84	.005
Interferon-induced protein with tetratricopeptide repeats 2	IFIT2	-4.69	.005
Interferon-induced protein with tetratricopeptide repeats 3	IFIT3	-13.74	.005
2'-5' oligoadenylate synthetase-like 2	OASL2	-6.15	.005
2'-5' oligoadenylate synthetase-like 1	OASL1	-3.16	.005
2'-5' oligoadenylate synthetase-1A	OASLA	-4.79	.005
Adenosine deaminase RNA specific	ADAR	-5.5	.005
Z-DNA binding protein 1	ZBP1	-12.47	.005

Can lung insults in early life, influence the lung's ability to handle infections in later life ?

-
- “Disruptive effect of hyperoxia on neonatal lung development reprograms key innate immunoregulatory pathways in the lung,
 - -which may contribute to exacerbated pathology and poorer resistance to respiratory viral infections typically seen in people who had BPD”

18 year old with a history of severe BPD



What genetic and environmental factors influenced her lung phenotype?

Clinical Observations

- Wide variation in lung phenotype exists among children born prematurely
 - Infants of similar gestational ages may have no respiratory symptoms or may require long-term mechanical support
 - **Besides gestational age- what influences lung phenotype in infants with chronic lung disease of prematurity?**
-

**Clinic and patient registry established for
infants and children with a history of
chronic lung disease of prematurity**

Criteria for chronic lung disease of prematurity clinic

- Infants discharged from the NICU who are ≤ 34 weeks gestation
 - On respiratory medications and/or supplemental oxygen/mechanical ventilation
-

Data	Visit 1	Visit 2	Visit 3	Visit 4
BPD assessment surveys (BPD-CCI, BPD QOL)	X	X	X	X
QOL (Peds QL)	X	X	X	X
Vitals (RR, sats, weight, length)	X	X	X	X
Clinician Assessment	X	X	X	X
Demographics	X			
Environmental exposures	X			
Past Medical History	X			
Medications (diuretics, ICS, GER)	X	X	X	X
Developmental Status (Peds Response)	X			
Consent status	X			
Missed appointment	X	X	X	X
Health Insurance status	X			

Lung Disease and the Environment

- Do environmental factors play a role in lung disease in the premature infant with CLDP?
 - Can we alter lung disease progression or decrease exacerbations through non-medical interventions?
-

Observation

- Lower respiratory illnesses before 2 yrs of age associated with lower lung function in adulthood (Shaheen et.al., Thorax, 1998)

Hypothesis

- Attendance at daycare is a risk factor for seeking acute medical care in infants and children with chronic lung disease of prematurity
-

Study design and methods

- IRB approval for patient registry
 - Prospective collection of data from 1/16/2008 to 10/28/2009
 - Caregivers filled out questionnaires using a standardized collection instrument (BPDCCI) at initial visit and each subsequent visit
 - Age at time of form completion < 3 years of age
-

CLD of Prematurity Demographics

(Mean SD: n = 110)

Gestational age (weeks)	26.1	2.0	[Range: 23 – 32]
Estimated household income (\$)	45,655	14,910	[Range: 23,616 – 101,266]
# children in the home (n = 102)	2.9	1.0	[Range: 1 – 6]
# completed forms per subject	1.9	1.1	[Range: 1 – 6]

Environmental Factor: Ever Attended Daycare?

		YES (20%: n = 22)	NO (80%: n = 88)	P value
Caregiver educational level (%): (n = 87)	< High school	5.6	4.4	0.07
	H.S. grad	16.7	26.5	
	Some college	27.8	41.2	
	College grad	11.1	17.7	
	Any post-grad	38.9	10.3	
# completed forms per subject		2.5 ± 1.3	1.8 ± 1.0	0.01

*Groups did not significantly differ by other demographic factors

Daycare exposure is associated with increased acute care usage in CLDP

Attends daycare
(n = 211)

	Odds Ratio	<i>P</i> value
ED Visit	4.50 [1.70 – 11.96]	0.003
Admission	3.11 [1.03 – 9.36]	0.04
Prednisone	2.67 [1.32 – 5.42]	0.01
Antibiotics	2.93 [1.35 – 6.35]	0.01

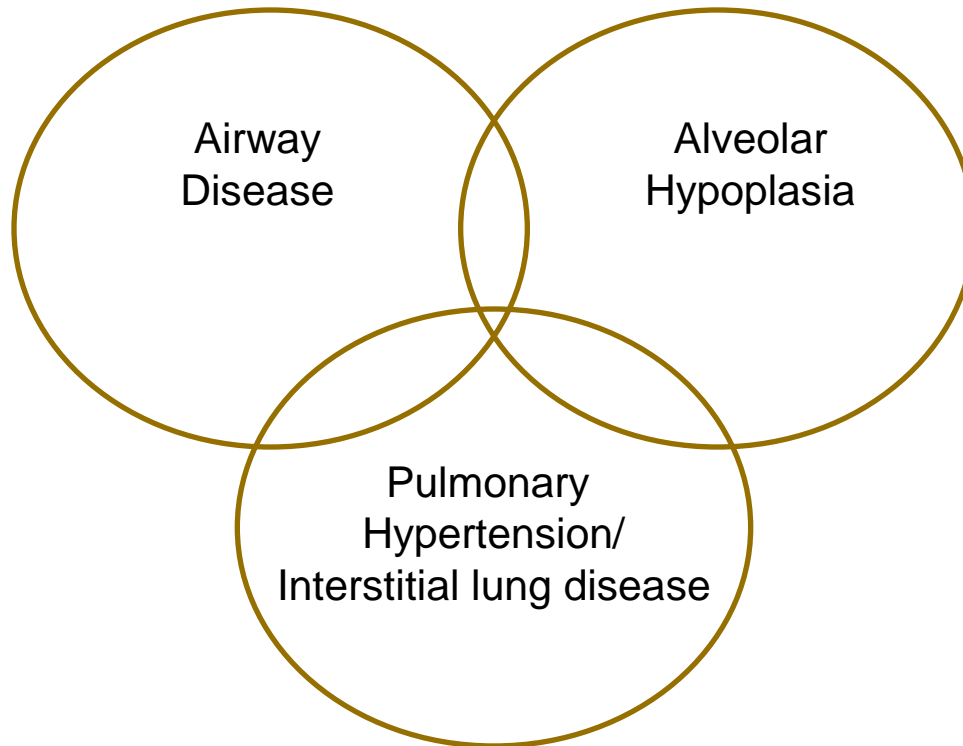
Odds ratios were generated through logistic regression with clustering by subject and adjustment for significant demographic differences

Summary of Findings

- Daycare increases acute care usage in infants and children with CLDP/BPD
 - Physicians should consider screening for daycare attendance to identify patients that may be at increased risk for poor respiratory outcomes
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Characterizing Phenotypic variability

- Characterize lung phenotype following discharge from the NICU
 - Can characterization of lung phenotype, predict outcomes, and allow for more standardized treatment plan?



Phenotypic Criteria

Phenotype	VARIABLE	DEFINITION	DISEASE
Airway Disease	Wheezing/Cough	History of wheezing or cough (Y/N)	Present if any two variables are positive
	Corticosteroids	Use of inhaled corticosteroids (Y/N)	
	β-agonists	Use of inhaled β-agonists (Y/N)	
	Dyspnea	History of shortness of breath with exertion (Y/N)	
Alveolar Hypoplasia	Assisted Ventilation	Use of any type of assisted ventilation from nighttime CPAP to continuous mechanical ventilation at home (Y/N)	Present if assisted ventilation <u>or</u> hypercarbia <u>or</u> both oxygen and diuretic use
	Hypercarbia	pCO ₂ > 40 on any blood gas or polysomnography obtained after 28 days of life	
	Oxygen Use	Use of any supplemental oxygen at home (Y/N)	
	Diuretics	Use of any diuretics in the home setting (Y/N)	
Interstitial Lung Disease	Crackles	Crackles on lung auscultation (Y/N)	Present if crackles <u>or</u> any 2 of the other variables are positive
	Tachypnea	Respiratory rate > 60 breaths per minutes after discharge from NICU (Y/N)	
	Diuretics	Use of any diuretics in the home setting (Y/N)	
	Systemic Steroids	Use of oral steroids for non-wheezing symptoms	
	Cough	History of cough (Y/N)	
Pulmonary Hypertension	Pulmonary Hypertension	Present on any echocardiogram (Y/N)	Present if either variable is positive
	Sildenafil	History of any sildenafil (or similar agent) use (Y/N)	

Future directions

- Determine association between socio- economic factors and lung disease
 - Determine if child's disease state impact quality of life of the primary caregiver
 - Determine if any exposure to cigarette smoke impacts child's lung disease
 - To establish a cohort of very low birthweight children to help understand the natural history of lung disease in this population
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