Pulmonary Development and Disease in Down Syndrome : a Scientific Journey from the Bed to Bench Site

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Children's Hospital Colorado

Affiliated with University of Colorado Anschutz Medical Campus

Pediatric Heart Lung Center

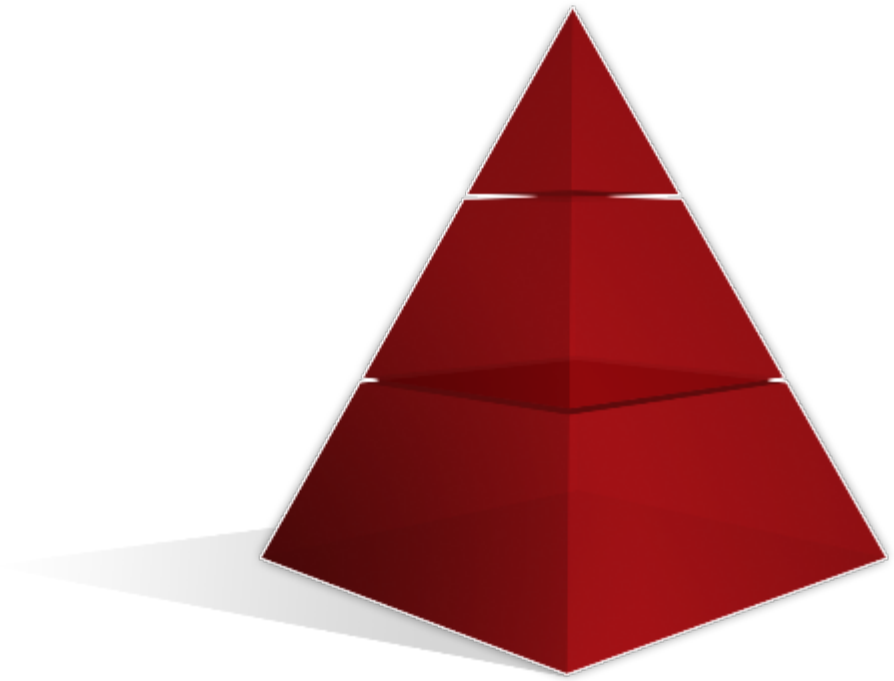
Research → Therapy → Laboratory → Education → Training

Innovation

PHLC

Collaboration

Pediatric Pulmonary Vascular Biology & Pathology



Diagnostic Pediatric Pathology (Lung)

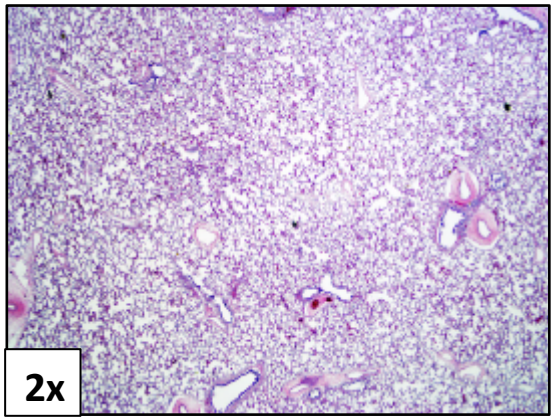
Bench Research on Lung Development & Disease

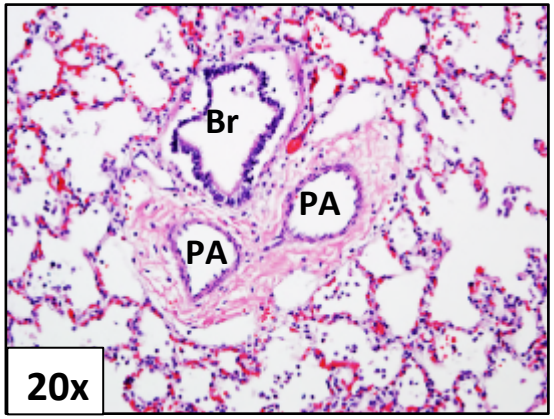
Case study

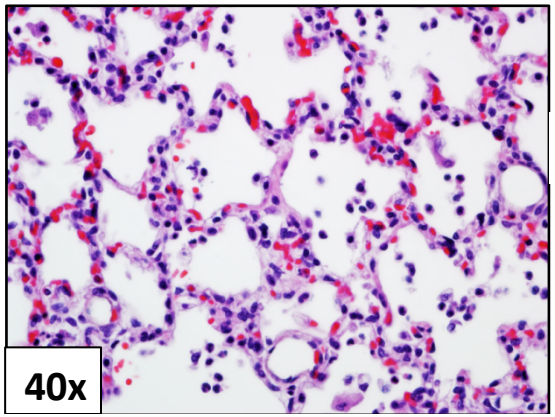
- 4 month old male with trisomy 21
- born at 36 4/7 weeks gestational age
- birth weight of 3080 grams
- delivered by Cesarean section to a 34 year old G2P1 mother
- multiple respiratory problems since birth including pulmonary hypertension, chronic respiratory failure, and tracheobronchomalacia requiring assisted ventilation
- No cardiac or other organ anomalies
- After a long hospital course without improvement in his cardiorespiratory status, his family decided to withdraw life support and an autopsy permit was granted

Histopathology

Control





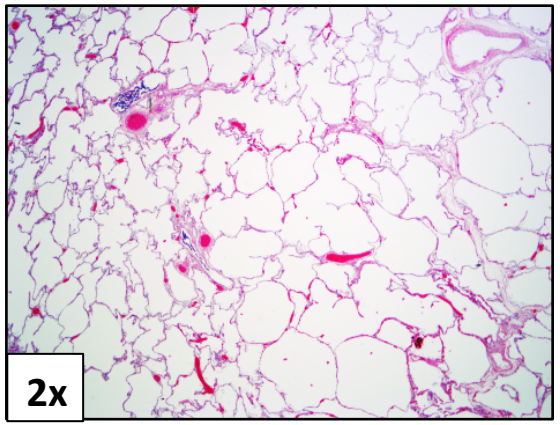


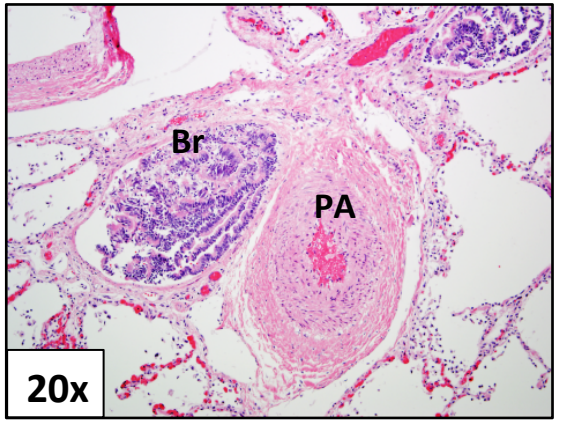
DIMINISHED ALVEOLARIZATION →

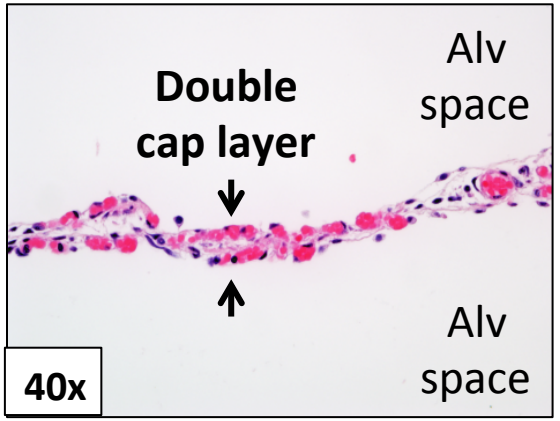
DEFECTIVE VASCULAR REMODELING →

IMPAIRED MICROVASCULAR GROWTH →

Down syndrome







Cause of Death

Respiratory*: severe chronic lung disease, pulmonary hypoplasia and pulmonary hypertension related to *Down syndrome

Babies with DS succumb to respiratory disease without any co-morbid condition

Pulmonary disease and Down syndrome

- **DS associated with increased risk for developing respiratory disorders including severe pulmonary hypertension (PHT) and persistent pulmonary hypertension of the newborn (PPHN)**
- **Prevalence study at Sie Center for Down Syndrome (ongoing):**
  - 1,252 children with DS, 27.6% (n = 346) with PHT
  - PPHN incidence: 9.9% (vs 0.1 in general population)
- **Children with DS and co-morbidities, such as congenital heart disease or obstructive sleep apnea, are more susceptible for developing accelerated PHT than children without DS**
- **One of the leading causes of death in people with DS is respiratory related**

The genetic and molecular mechanisms responsible for pulmonary hypoplasia and PHT in DS are unknown

Down syndrome associated lung disorders are understudied

Molecular Syndromology

Syndrome Conferences, Symposia or Workshops

Mol Syndromol 2016;7:251–261
DOI: 10.1159/000449049

Accepted: July 21, 2016
by M. Schmid
Published online: September 16, 2016

Changing Paradigms in Down Syndrome: The First International Conference of the Trisomy 21 Research Society

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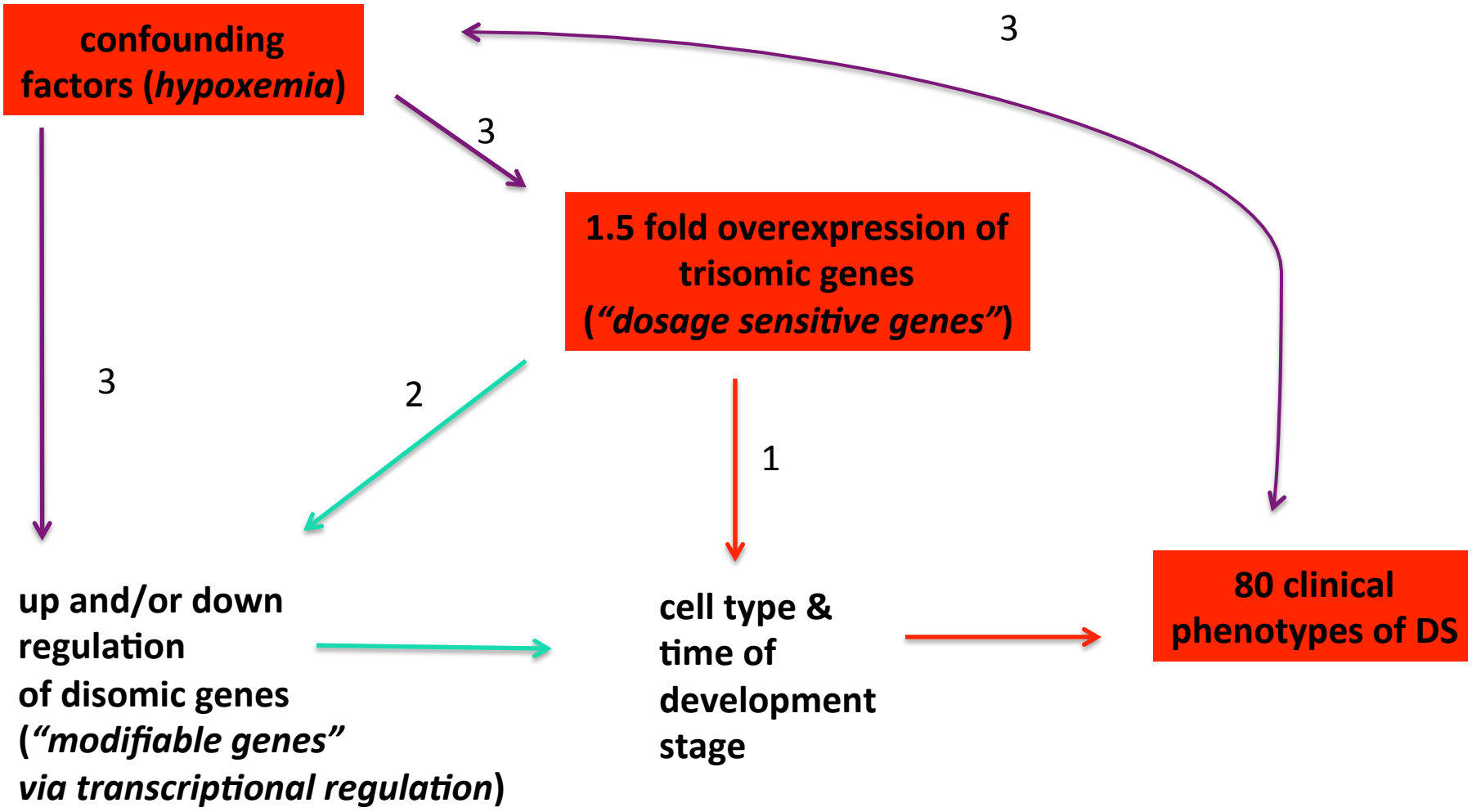
Challenges of Down syndrome research

- **many genes (300+): *one gene dysfunction-one pathway-one disease phenotype* paradigm does not apply**
- **genes are overexpressed**
- **~80 clinical phenotypes in various combinations**

GENETIC PATHWAYS AND CONFOUNDING FACTORS LEAD TO VARIABLE DS PHENOTYPES

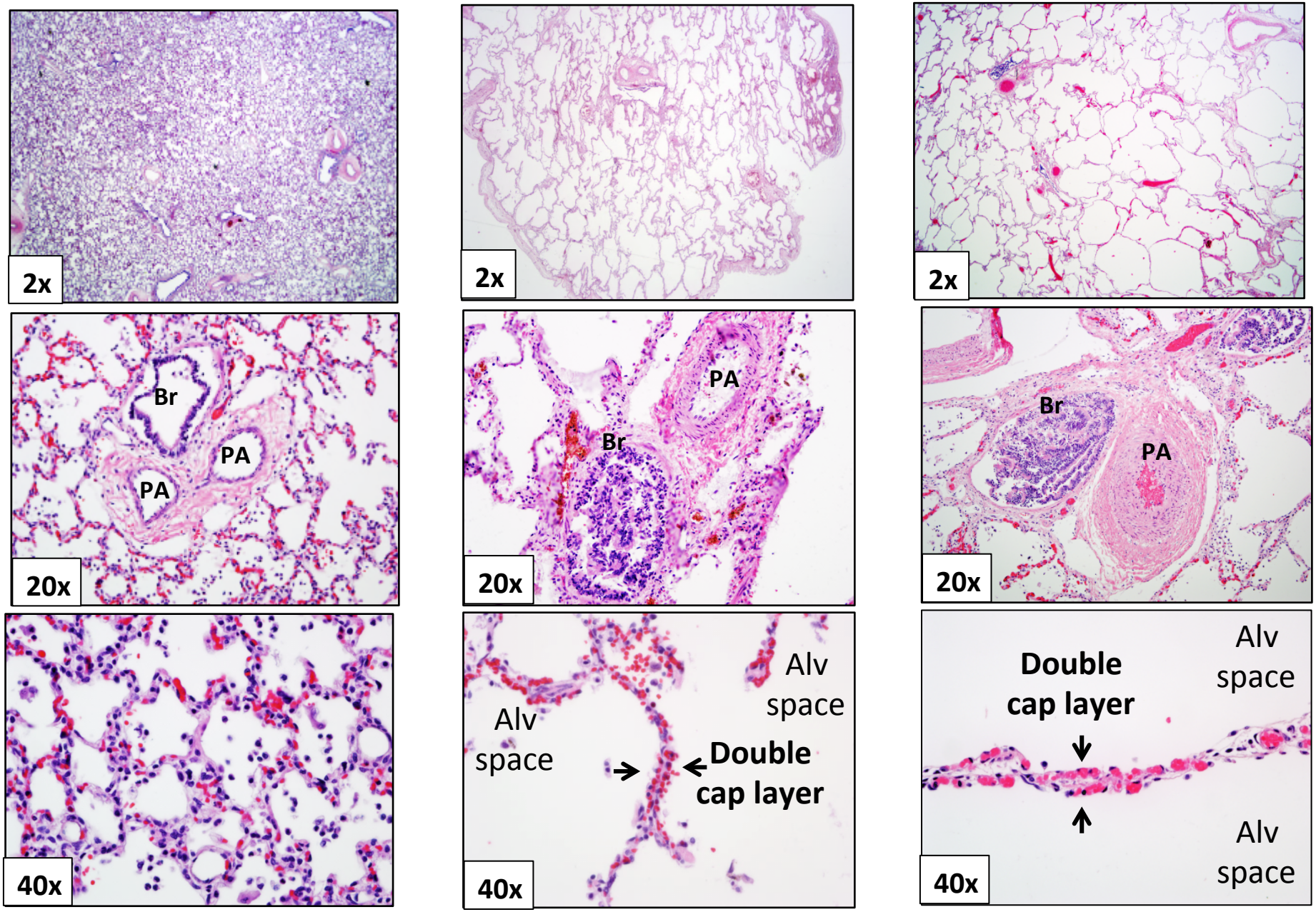


Control

Bronchopulmonary Dysplasia

Anti-angiogenic Disorder

Down syndrome



Are DS-related lung hypoplasia and PHT *anti-angiogenic* disorders?

Judah Folkman, MD
Surgeon-Scientist
Founder of angiogenesis research

- Patients with DS have a decreased incidence of pro-angiogenesis related diseases including solid tumors, atherosclerosis, diabetic retinopathy, and vascular anomalies
- Genes for potent anti-angiogenic factors are present on the triplicated chromosome 21 and overexpressed in patients with DS and in DS animal models

Endostatin (ES), B-amyloid peptide (BAP) and Calcineurin Regulator-1 (RCAN-1/DSCR-1)

- All prominent endogenous anti-angiogenic factors
- All located on Chromosome 21 and all individuals with DS have an extra copy
- Patients with DS have increased serum and/or tissue levels
- They specifically inhibit the proliferation and migration of vascular endothelial cells
- They suppress VEGF-VEGFR2 induced signaling causing marked angiogenesis inhibition

It is unknown whether ES/BAP/RCAN1-related antiangiogenic mechanisms contribute to abnormal lung development and PHT in DS

OVERRIDING HYPOTHESIS

Overexpression of chromosome 21-related anti-angiogenic factors play a critical role in the development of lung hypoplasia and pulmonary hypertension in infants and children with Down syndrome

Specific study questions

1. Do lungs in people with DS show impaired alveolar and vascular growth/remodeling?
2. Do lungs of a fetus with Down syndrome overexpress ES, BAP, RCAN-1 and other anti-angiogenic genes (disomic) and show vascular growth impairment?
3. Are angiogenic functions of endothelial cells and progenitors isolated from individuals with DS impaired?

Study question -1

Do lungs in people with DS show impaired alveolar and vascular growth/remodeling?

Study 1- Design

Retrospective Autopsy Review
Children 0-8 years
With DS or typical with CHD
Excluded disorders of lung development
Study Population
Patients with DS (n=13)
Typical patients age and CHD matched controls (n=4)
Clinical data obtained from autopsy reports
Routine H&E lung histology reviewed
Serial sectioning and 3D image reconstruction to clarify microvascular anatomy

Study 1-Results

Clinical Features	DS (n=13)	non-DS (n=4)
Congenital Heart Defect (%)	77	100
Clinical Diagnosis Pulmonary Hypertension (%)	46	25
Histologic Features (%)		
Pulmonary Arterial Hypertensive Remodeling	85	25
Pulmonary Vein Hypertensive Remodeling	15	0
Prominent Bronchial Vessels	100	25
Prominent Intrapulmonary Anastomotic Vessels	100	25
Quantification of Histologic Findings (0-3)		
Pulmonary Arterial Hypertensive Remodeling	1.69	0.5
Pulmonary Vein Hypertensive Remodeling	0.23	0
Prominent Bronchial Vessels	2.54	1.5

Study 1-Summary

Infants and Children with DS who died of severe cardiopulmonary disease have:

Histologic evidence of abnormal lung development
- Alveolar simplification

Histologic evidence of abnormal pulmonary vascular development
- Prominent arterial hypertensive remodeling
- Prominent bronchial vessels
- Persistence of double capillary network
- Prominent intrapulmonary anastomotic vessels

Findings present in those with DS with and without CHD are more prevalent than typical patients who were age and CHD matched controls

Study 1 supports angiogenic hypothesis

D Bush et al. J Peds 2017

Study question-2

Do lungs of a fetus with DS overexpress ES, BAP, RCAN-1 and other anti-angiogenic genes (disomic) and show vascular growth impairment?

Study 2 - Design

Human fetal lung tissue* from
- Down syndrome (n=4)
- Typical controls (n=4)

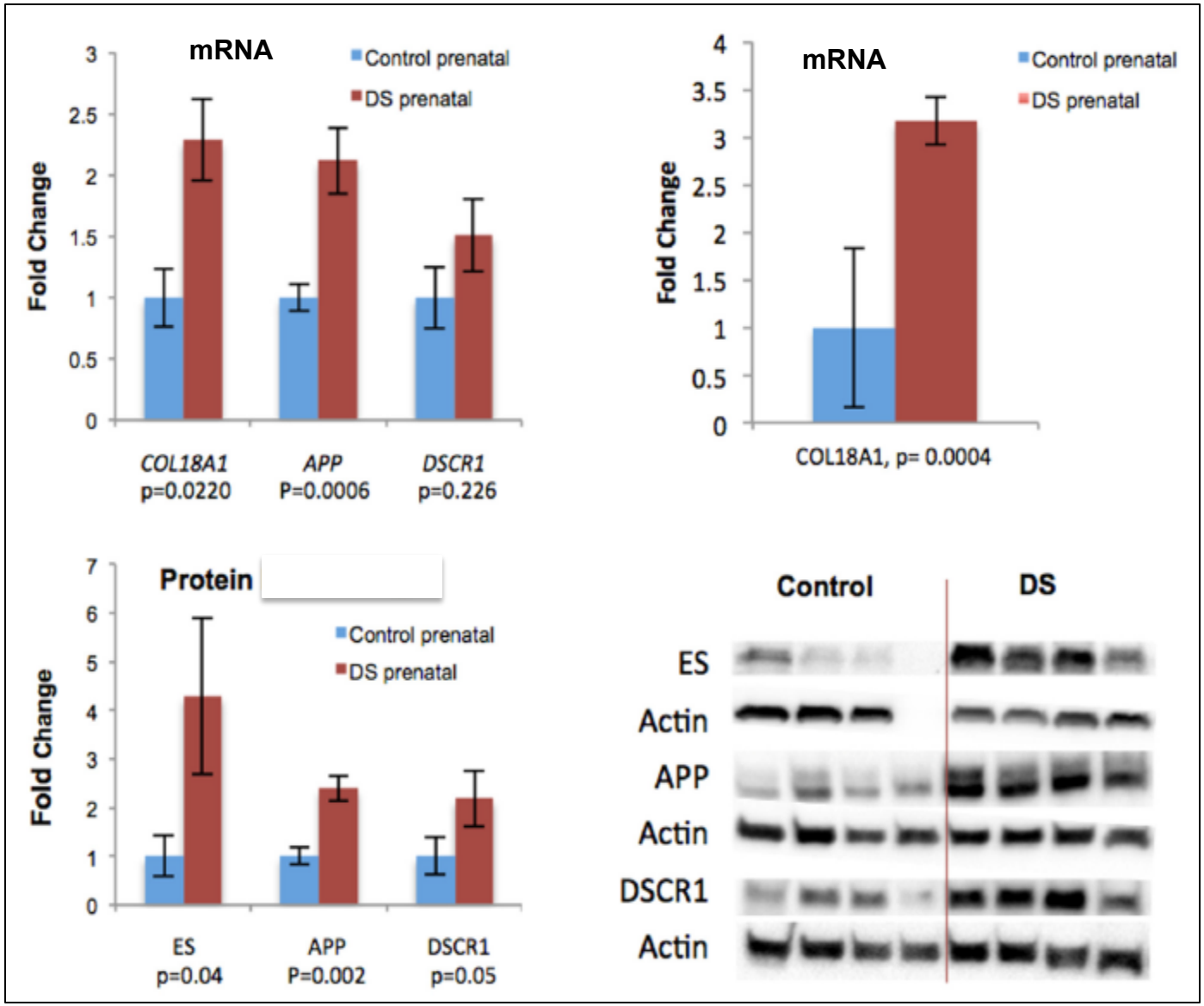
Individual qPCR (mRNA) and Western Blot Analysis (Protein)
- Endostatin
- B-Amyloid Protein (BAP)
- Regulator of Calcineurin-1 (DSCR1)

Angiogenesis-associated mRNA microarray (84 angiogenic genes)

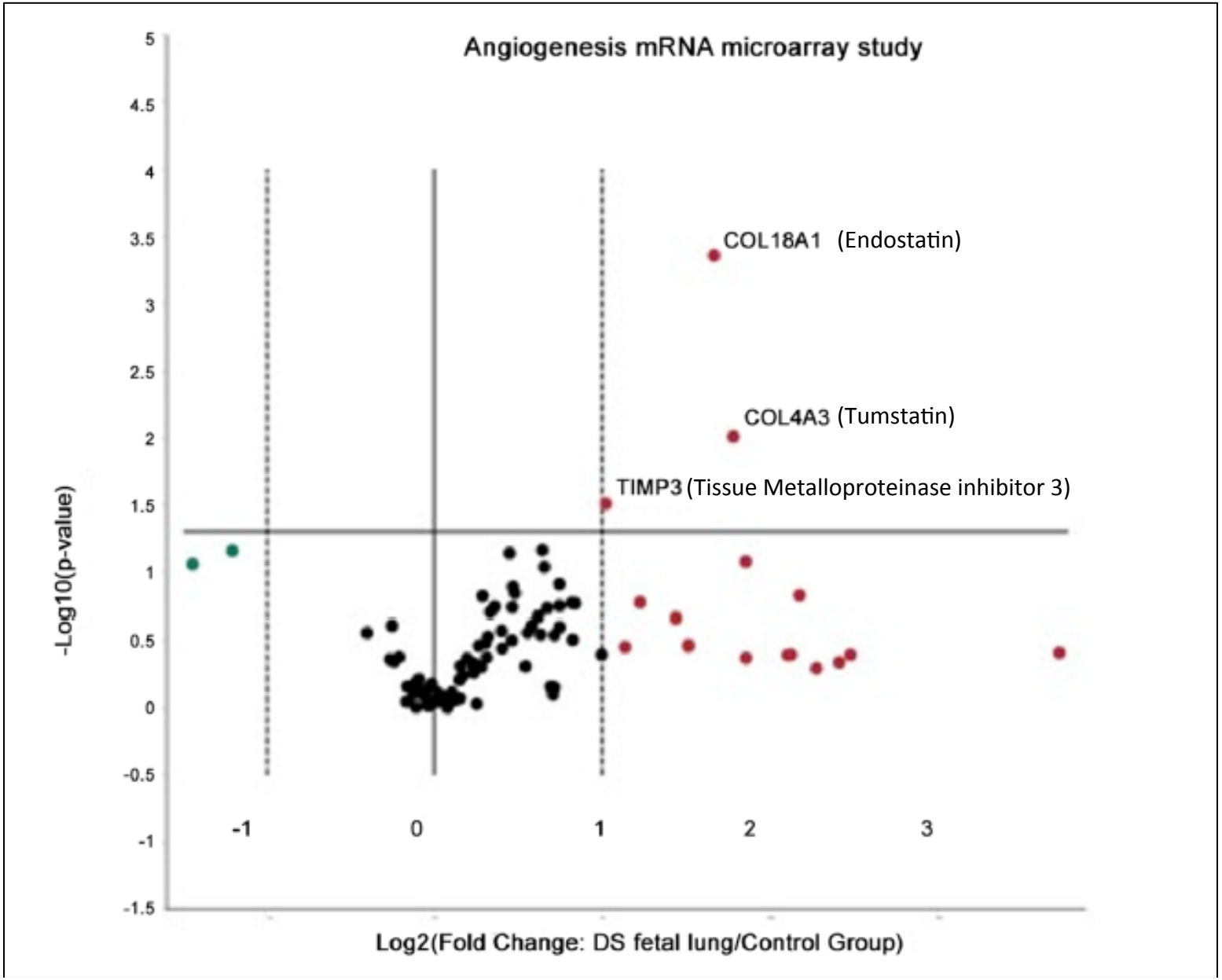
IHC evaluation for impaired angiogenesis:
- Vessel Density (CD31)
- Vessel Thickness (SMA)

**U. Maryland, Baltimore/NICHD Brain &Tissue Bank for Developmental Disorders*

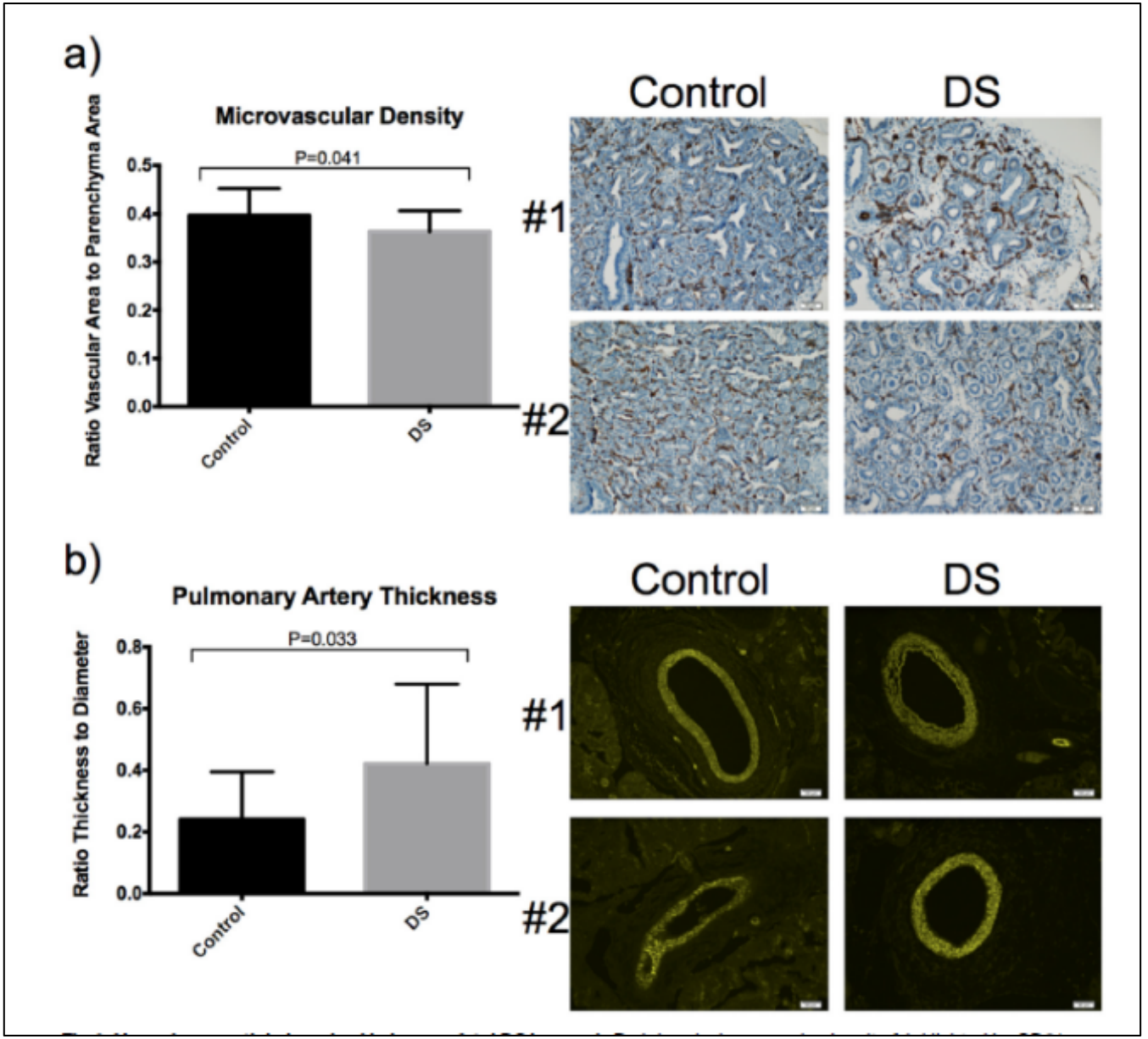
Study 2 - Results



Study 2 - Results



Study 2 - Results



Study 2 - Summary

Anti-angiogenic factors are over-expressed in the lungs of fetuses with DS

- Chromosome 21-related Genes (trisomic) and Proteins
  - *ENDOSTATIN*
  - *BAP*
  - *RCAN1/DSCR1*
- Non Chromosome 21-related Genes (disomic)
  - *TIMP3*
  - *COL4A3*

Early abnormal vascular growth in the lungs of fetuses with DS

- Reduced vessel density
- Increased vessel wall thickness

Study 2 supports angiogenic hypothesis

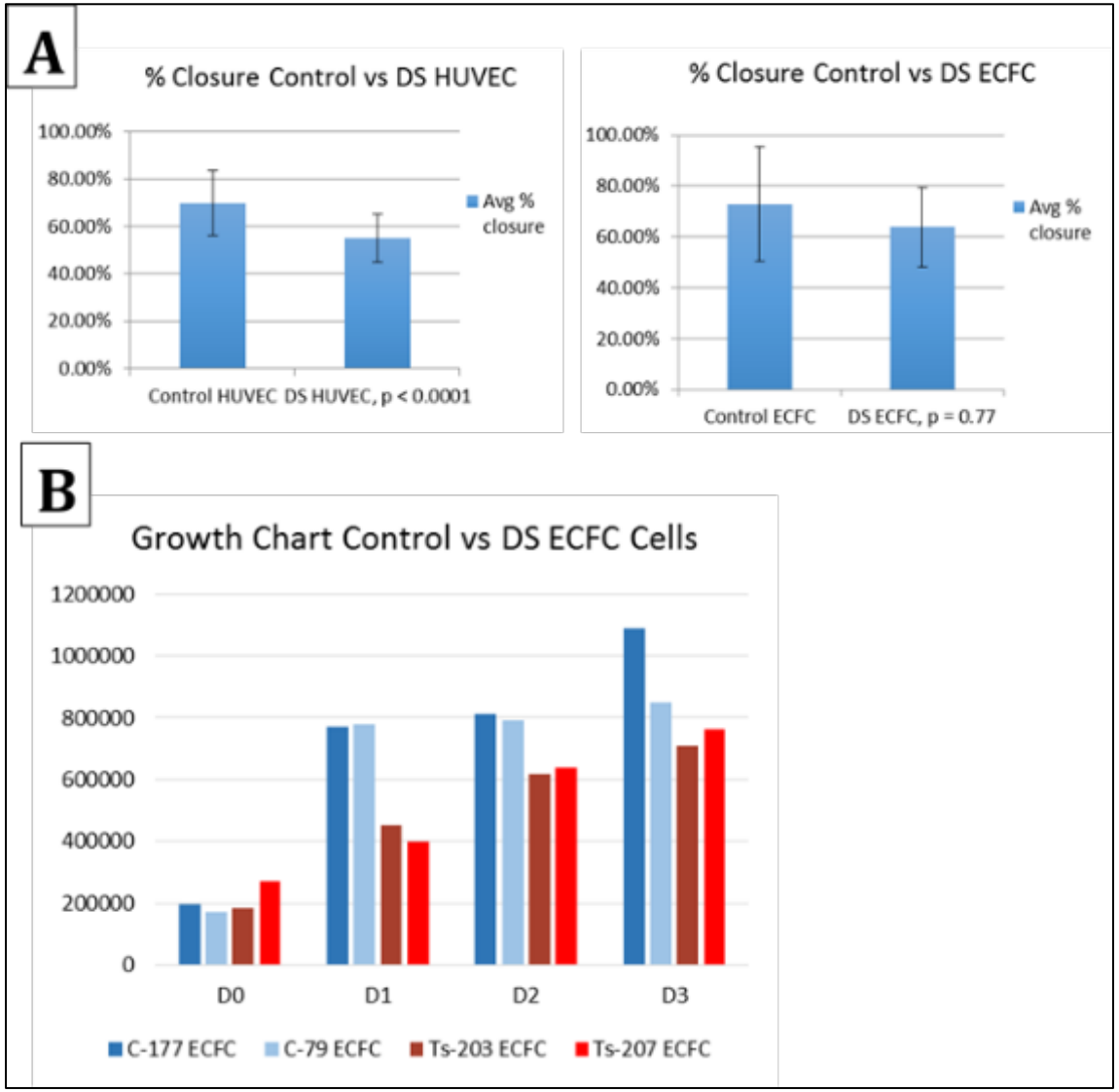
C Galambos et al. PLOS-1 2016

Study question-3

Are angiogenic functions of endothelial cells and progenitors isolated from individuals with DS impaired?

Study 3-Results (ongoing)

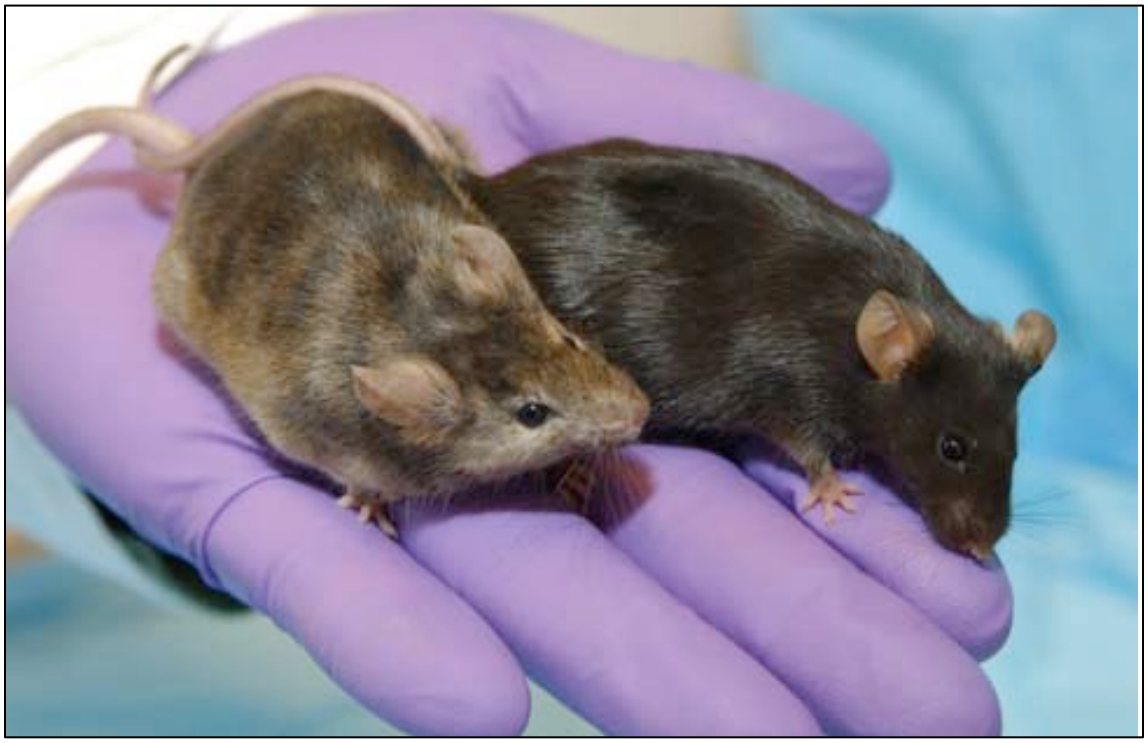
Endothelial cells (HUVEC) and precursors (endothelial colony forming cells) isolated from individuals with DS show impaired angiogenic functions



Dr. C Baker, PHLC

Disease specific DS mouse models

- to further test our angiogenic hypothesis
- study specific molecular mechanisms
- explore a variety of interventions/treatment options

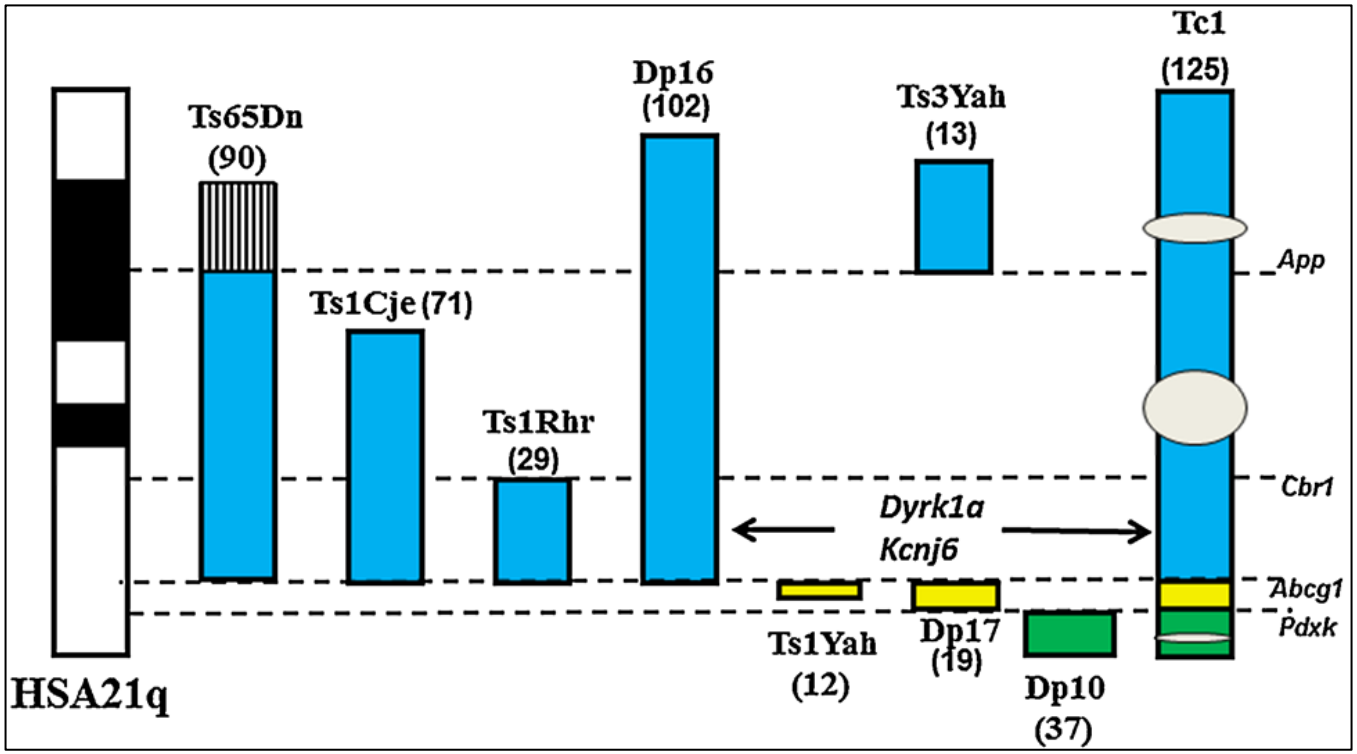


www.down-syndrome.org/research-practice

Mouse models of Down syndrome

No characterization/research of lung disorders has been done in any of DS mouse models

- Mouse orthologues of human Chr21 on 3 separate mouse chromosomes
  - **Mmu 10 –** ***ENDOSTATIN (37 genes)***
  - **Mmu 16 –** ***DSCR1/RCAN1 & BAP* (102 genes)***
  - **Mmu 17 –** ***NO ANTIANGIOGENIC GENE (17 genes)***



Gupta et al, Mamm Genome, 2016

Summary

- Lung disease (PHT/PPHN) is a significant contributor to morbidity and mortality of infants and children with DS
- DS Lung histology is characterized by vascular and alveolar growth abnormalities
- Lung disease in DS is understudied

- Lung pathology of DS is driven by anti-angiogenic mechanisms
- Early intervention with angiogenic stimulators or blockers of anti-angiogenic pathways may prevent lung disease in those with DS
- Angiogenic biomarkers will help predict lung disease in those with DS

Members of Pediatric Heart Lung Center

Angela Minic, Doug Bush, Gregory Seedorf, Blair Dodson, Chris Baker
Dr. Steve Abman—Scientific Director

Members of Dept. of Pathology

Drs. Ann Thor and Mark Lovell

Collaboration

Dr. Charles Hoeffer Lab at University of Colorado Boulder
The Linda Crnic Institute for Down Syndrome-Drs. Fran Hickey, Joaquin Espinosa
Univ. Maryland, Baltimore/NICHD Brain &Tissue Bank for Developmental Disorders

Funds

Foundation Jérôme Lejeune for Down Syndrome
Children's Hospital Colorado Research Institute
The Linda Crnic Institute for Down Syndrome Challenge Grant
Jayden de Luca Foundation (Dr. Dunbar Ivy) for Pulmonary Hypertension
Dean's Bridge Fund, University of Colorado Denver

“Down Syndrome Takes The Breath Away”

“People with DS are gift. By studying their biology can help them and the rest of mankind.”

----Dr Tom Blumenthal, Former Executive Director of LCI for DS

Self-Advocates, Families, & Researcher celebrate World Down Syndrome Day in the CO Capitol together with Governor John W. Hickenlooper (3/22/17)