GENETIC AND PHENOTYPIC CHARACTERIZATION OF CHRONIC AIRWAY DISEASE IN NON-HUMAN PRIMATES

Orangutan Husbandry Conference
Houston, TX

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October 6, 2014
Epidemiology of Chronic Respiratory Disease

- Occurs in approximately 20%-40% of captive orangutans
  - Recently has also been described in orangutans that presented from the wild to rehab centers
- Accounts for ~16% of adult mortality
  - Most common cause of death in adolescents in the U.S. captive orangutan population

Photo courtesy of Nancy Lung, VMD
Etiology of Air Sacculitis

• Incompletely understood
• Chronic upper airway drainage contaminating air sac
  – Case series of juvenile Bornean orangutans with air sacculitis: 50% had evidence of upper respiratory tract infection in the 6 months prior to presentation
• Other hypothesized predisposing factors
  – Exposure to human pathogens
  – Overcrowding with fecal contamination of the environment
  – Stress-related immunosuppression
  – Altered airway flora related to chronic antibiotic use

Photo courtesy of Denver Zoo

Proc Am Assoc of Zoo Vet 2008:40;
J Med Primatol 2011;40:365-75
Factors Associated with Development of Respiratory Disease

• Zimmerman *et al* studied the medical records of 201 orangutans from 20 European zoos
  – Bornean orangutans had more chronic respiratory disease than Sumatran orangutans (13.6% vs. 3.6%)
  – Males were affected more often than females (15.8% vs. 3.9%)
  – Hand-reared orangutans developed disease more often than parent-reared orangutans (21% vs. 5%)
  – **No** environmental factors were associated with disease
  – Diseased animals more often genetically related to animals with respiratory disease (93%) than to healthy animals (54%)

J Med Primatol 2011;40:365-75
Symptoms of Airway Disease

• Cough
• Halitosis
• Nasal drainage
• Lethargy
• Anorexia
• Pyrexia
Signs of Air Sacculitis

- Induration
- Air sac distension
- Palpation of fluid collection
- Spontaneous abscess drainage

Photo courtesy of Nancy Lung, VMD
Signs of Upper Airway Disease

- Sinusitis
- Infection with gram negative bacteria
Signs of Lower Airway Disease

- Acute inflammation
- Infection with gram negative bacteria
- Bronchiectasis
Surgical Management of Airway Disease

- Drainage
- Marsupialization
- Closure of ostia
- Removal of air sac
- Sinus surgery

Medical management of Airway Disease

- Inhaled steroids
- Inhaled bronchodilators
- Inhaled saline
- Oral and/or inhaled antibiotics
Causes of Diffuse Bronchiectasis in Humans

- Cystic Fibrosis (CF)
- Primary Ciliary Dyskinesia (PCD)
- Immunoglobulin deficiency
- Young’s Syndrome
- Rheumatologic Disease
- Alpha-1 antitrypsin deficiency
- Idiopathic
Cystic Fibrosis Transmembrane Regulator (CFTR) Gene
Pathophysiology of CF

Genetic and Protein Defect

Abnormal Salt and Water Transport

Persistent Airway Infection

Accumulation of Leukocyte-Derived DNA and Elastase-Rich Secretions

Exacerbations of Infections

Airway Obstruction

Progressive Lung Destruction

Early Death

Murphy TM and Rosenstein BJ
Epidemiology of CF

• Most common autosomal recessive genetic disorder leading to decreased life span in Caucasians
  • Incidence ~ 1: 3,200
  • Carrier rate 1 in 29
• Approximately 70,000 patients worldwide
• Majority of patients diagnosed in childhood
  • Median age of diagnosis = 5 months

Data from CFF Registry 2012
Age Distribution of the CF Population

Median age 17.5 yrs
Survival 27.1 yrs
Projected Survival 37 yrs

Data from CFF Registry 2012
Diagnosis of CF

• Newborn screening
  • Immunoreactive trypsinogen/genetic screen

• Sweat chloride testing
  • Quantitative pilocarpine iontophoresis

• Nasal Potential Difference measurement

• DNA analysis
  • Buccal smear/blood
  • Analysis for 70 common alleles identifies 90% of patients with CF
Respiratory Manifestations

• Pulmonary infections
  – Cough
  – Sputum production
  – Chest pain
  – Airway reactivity

• Sinus disease
  – Nasal polyps
  – Chronic sinusitis
Prevalence of Respiratory Organisms

![Graph showing prevalence of respiratory organisms by age group. The graph indicates the percentage of patients infected with various organisms, including P. aeruginosa, H. influenzae, S. aureus, MRSA, S. maltophilia, Achromobacter xylosoxidans, and B. cepacia complex. The data is based on the CFF 2010 Annual Patient Registry Report.]
Gastrointestinal Manifestations

- Malabsorption caused by pancreatic insufficiency
  - Failure to thrive
  - Vitamin deficiencies
- MI/Distal Intestinal Obstruction Syndrome (DIOS)
- Chronic pancreatitis
- Bile sludging/cholelithiasis
Pillars of Therapy

• Replete/maintain adequate nutrition

Shwachman H. Pediatrics 1951;7;153-63.

• Promote mucous clearance


• Control respiratory tract infection
Anatomy of Respiratory Disease

• Main differences between apes and human occur in the upper respiratory tract
  – Sinuses
    • Large maxillary sinus
    • Small sphenoid sinus
    • Lack of frontal/ethmoid sinus
  – Extensive laryngeal air sac
• Lower tract virtually the same across species

Photos courtesy of Nancy Lung, VMD

Case presentation

• 28 yo male Sumatran orangutan with complaint of “deep rumbling” cough, mucoid nasal drainage and fatigue

• PMHx
  – Intermittent upper/lower respiratory infections since 1997
  – Dec 2009 bronchoscopy
    • *P. aeruginosa* from tracheal washes and sinuses
    • Received Cipro for 14d
  – Recurrent symptoms in April 2010
    • Received Cipro for 14d
Clinical Course

• NJ Pulmonary consult for worsening symptoms in February 2011
  – Bronchodilators, inhaled saline and QOM tobi started with improvement in symptoms

• Clinical course
  – Subsequent bronchoscopies have grown *S. pneumonia* (2012), and *M. morganiae* and methicillin sensitive *S. aureus* (2013)
  – *Sinus/Chest CT (2013) confirmed clinical suspicion of sinusitis and bronchiectasis*
  – *Pancreatic function assessed/ found to be low*
    • *Chronic flatus/bloating improved on pancreatic enzyme replacement therapy (PERT)*
Diagnostic Evaluation

• Sweat test performed

• Bronchial brushing sent to UNC/Dr. Michael Knowles for ciliary analysis

• Blood sent to Johns Hopkins/Dr. Gary Cutting for DNA Analysis
Summary of Case Presentation and Evaluation

<table>
<thead>
<tr>
<th>CF Diagnostic Criteria</th>
<th>Case signs and symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Features</strong></td>
<td></td>
</tr>
</tbody>
</table>
| Chronic Sinopulmonary Disease | 1. Chronic nasal drainage and chronic sinusitis  
|                          | 2. Chronic cough and bronchiectasis       |
|                         | 3. Bronchoscopic cultures demonstrating *S. aureus*, *P. aeruginosa* |
| Gastrointestinal abnormalities | Fecal elastase 20 ug/g, improvement in chronic flatus and abdominal distension with initiation of pancreatic enzyme replacement therapy |
| Evidence of CFTR dysfunction | Sweat test QNS |
| CFTR genetics           | + for 3 CFTR variants   |
| Ciliary biopsy          | Normal                  |
Summary of Variants

- Ten variants were identified in case’s CFTR gene.
  - Six variants did not change the encoded amino acid
  - One variant was a single nucleotide substitution in non-coding sequence that is not predicted to affect gene function
  - Three variants were predicted to cause amino acid substitutions

- Conservation of an amino acid across species can imply functional significance
  - One variant was well conserved across humans, non-human primates and other mammals
  - Other variant was also conserved across human, non-human primates and other mammals except mouse
Objective and Specific Aims

• **Objective**
  – Establish if mutations in the CFTR gene cause the phenotype of chronic respiratory disease in orangutans

• **Specific Aim 1.**
  – Rigorously phenotype all of case’s living and deceased relatives based on orangutan genealogic data and through careful review of medical records.

• **Specific Aim 2.**
  – Determine if mutations found in case occur uniquely in orangutans affected with air sacculitis and bronchiectasis by analyzing the CFTR gene in affected and unaffected orangutans
Significance and Future Directions

• This study will potentially identify the cause of the increased morbidity and mortality of this critically endangered species
  – Such information could be used to guide
    • Use of CF therapies
    • Breeding decisions in the orangutan population

• Data from this study may be used to apply for a larger grant
  – Rigorously phenotype and genotype all captive orangutans with chronic sinopulmonary disease in the U.S.
    • Provide further guidance breeding, and therapy decisions for affected orangutans
Progress

• Completed research paperwork/received approval from 13 zoos

• Gene sequencing completed on 14 orangutans

• Fecal elastase evaluations completed on 14 orangutans
Fecal Elastase Activity

- Female: P=0.09
- Male: P=0.08
Summary of the variants identified in *CFTR* gene of orangutans

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of orangutans genomic DNA tested</td>
<td>14</td>
</tr>
<tr>
<td>Total number of CFTR variants identified</td>
<td>29</td>
</tr>
<tr>
<td>Nonsense</td>
<td>1</td>
</tr>
<tr>
<td>Missense</td>
<td>3</td>
</tr>
<tr>
<td>Intronic</td>
<td>10</td>
</tr>
<tr>
<td>Synonymous</td>
<td>15</td>
</tr>
</tbody>
</table>

- Among 14 orangutans only four presented with CF related respiratory and/or GI symptoms
First report of nonsense mutation identified in the *CFTR* gene of orangutan

- Lys162X was found in a heterozygote state.

- This mutation is expected to cause nonsense mediated RNA decay and no CFTR protein production from the affected allele.
**CFTR** intron 10 variant identified in young orangutan presenting with respiratory and GI symptoms

- c.1393-22T>A was found in the homozygous state
- In humans c.1393-42G>A,
  - Reported in an 18 month old male with chronic lung disease, hypoxia and pulmonary hypertension.
  - Reported in a 9 year old with failure to thrive and a 6 month old child with clinical suspicion of CF.

(http://www.genet.sickkids.on.ca/)
Summary

• Fecal elastase
  – Results may be affected by gender/subspecies
  – No association with GI symptoms, but more samples from orangutans with GI symptoms are needed

• CFTR genetics
  – One stop mutation identified
  – One mutation identified that is close in proximity to known mutation in humans
    • Functional testing of mutation needed
  – More samples from orangutans with respiratory disease (and their family members) are needed
Thank you

- Participating Zoos
  - Atlanta
  - Cleveland
  - Columbus
  - Como
  - Denver
  - Houston
  - Louisville
  - Memphis
  - Miami
  - Philadelphia
  - Sedgwick

- NJ CF Research Team
  - Silvia Caceres, MS
  - Connie St. Claire, RN
  - Marion Jones, RN
  - Katie Poch, MS
  - Jerry Nick, MD

- UNC Collaborators
  - Kim Burns
  - Michael Knowles, MD
  - Syanne Olson

- Johns Hopkins Collaborators
  - Garry Cutting, MD
  - Neeraj Sharma, PhD

- Orangutan SSP
  - Cindy Cossaboon
  - Nancy Lung, VMD
  - Lori Perkins
  - Joe Smith, DVM

- Funding
  - Gilead Sciences Research Scholars Program in CF
I think you'll find the sofa's free.