

Genetic and phenotypic characterization of Cystic Fibrosis airway disease in non-human primates

Principal Investigator: Jennifer L. Taylor-Cousar, MD

Co-Investigators: Nancy Lung, VMD, Joe Smith, DVM, Cindy Cossaboon, BS and Garry Cutting, MD

BACKGROUND AND SPECIFIC AIMS

Chronic sinus drainage, infection of the laryngeal air pouch (air sacculitis, see Figure 1), gram negative rod infection, and bronchiectasis characterize the respiratory syndrome that is present in approximately 20-40% of captive Sumatran (*Pongo abelii*) and Bornean (*P. pygmaeus*) orangutans.¹ Recently these respiratory signs and symptoms have also been described in orangutans that presented from the wild to rehabilitation centers.²⁻⁴ A mortality and population review of North American orangutans recently conducted by the Orangutan SSP showed that chronic respiratory disease negatively affects the sustainability of the captive population.⁵

Respiratory disease accounts for almost 16% of adult mortality, and is the most common cause of death in adolescents in the U.S. captive orangutan population.⁵ In the 2012 U.S. Orangutan Health Survey, 38% of zoos reported that they were managing orangutans with chronic respiratory infections.¹ Acute symptoms may also be associated with anorexia and weight loss.²

The etiology and pathogenesis of this syndrome in orangutans is incompletely understood.⁶ One widely accepted theory is that the chronic upper airway drainage contaminates the air sac and subsequently drains into the lower airway via openings in the laryngeal cavity.^{2,7} In the case series of juvenile Bornean orangutans with air sacculitis, 50% had evidence of upper respiratory tract infection in the 6 months prior to presentation.² However, air sac removal does not always lead to complete resolution of symptoms.^{8,9} Other possible predisposing factors have been thought to include exposure to human pathogens, overcrowding with fecal contamination of the environment, stress-related immunosuppression, and altered airway flora related to chronic antibiotic use.⁶

In an effort to evaluate predisposing factors for chronic respiratory disease, Zimmerman *et al* studied the medical records of 201 orangutans from 20 European zoos.⁶ Contrary to theories suggesting a primarily environmental association with chronic sinopulmonary disease, they demonstrated that diseased animals were more often genetically related to animals with respiratory disease (93%) than to healthy animals (54%).⁶

Based on the finding that genetic relatedness is associated with chronic respiratory symptoms in combination with the relatively small available orangutan breeding pool, it is highly possible that a genetic cause of respiratory disease enhanced by the founder effect may explain the frequency with which members of this critically endangered population¹⁰ are suffering increased morbidity and early mortality from respiratory disease.^{1-3,6,7,11,12}

In January of 2011, I was consulted for assistance with the medical management of a 30 year-old male Sumatran orangutan (*P. abelii*) who began to experience intermittent upper and lower respiratory infections at the age of 14 years. Further review of medical records and interview of the affected orangutan's keepers and veterinarians revealed that his symptoms have included deep rumbling cough, mucoid nasal discharge, and sluggish behavior. Worsening symptoms were treated intermittently with oral Ciprofloxacin. Cultures from bronchoscopies in 2009, 2012 and 2013 grew *P. aeruginosa*, *S. pneumonia*, and *M. morganiae* and methicillin sensitive *S. aureus*, respectively. CT scans of the sinuses and chest revealed chronic sinusitis and bronchiectasis, respectively. In addition to his chronic upper and lower respiratory tract symptoms, the affected orangutan requires an increased amount of oral intake compared to other orangutans to maintain his weight, suggesting that pancreatic insufficiency may be an associated sign of his disease. Therefore, stool was evaluated for fecal elastase. The affected orangutan's fecal elastase was 20 ug/g, compared to 47 ug/g in a healthy orangutan from the same zoo. (Normal fecal elastase ranges for the orangutan have not been established.) Since initiation of pancreatic enzyme replacement in June 2013, the affected orangutan has had substantially less flatus and bloating.

It has recently been reported that there is 97% genetic homology between humans and orangutans.¹³ In order to elucidate the cause of the affected orangutan's symptoms, evaluation for two genetic disorders with similar symptoms that are seen in humans [Cystic Fibrosis (CF) and Primary Ciliary Dyskinesia (PCD)] was undertaken. Cilia obtained from tracheal brush were structurally normal based on analysis by the Cell Biology/Ultrastructure Facility at the University of North Carolina, Chapel Hill (UNC-CH). No sweat was

produced during pilocarpine iontophoresis. Whole blood was sent for CFTR gene sequencing. Three variants were identified. Notably, *none* of the CFTR variants identified in the affected orangutan were found in the DNA of a healthy orangutan from the same zoo.

The **overall objective of this proposal** is to establish if mutations in the CFTR gene cause the phenotype of chronic sinopulmonary disease associated with increased morbidity and mortality in orangutans by establishing the presence of CFTR mutations in affected orangutans and its absence in unaffected orangutans.

Specific Aim 1. To rigorously phenotype all of the affected orangutan's living and deceased relatives based on orangutan genealogic data and through careful review of medical records.

Specific Aim 2. To determine if mutations found in the affected orangutan occur uniquely in orangutans affected with air sacculitis and bronchiectasis by analyzing the CFTR gene in affected and unaffected orangutans.

STUDY DESIGN

Specific Aim 1: To rigorously phenotype all of the affected orangutan's living and deceased relatives based on orangutan genealogic data (grandparents, parents, siblings/half siblings as well as their offspring) and through careful review of all available medical records (including necropsy reports and available imaging procedures).

Methods: I will review the medical and life history records of all of the affected orangutan's living and deceased relatives. The following information will be extracted for living relatives: 1) History of sinopulmonary symptoms with date of onset and all treatments received 2) Reports/images of all respiratory imaging 3) Bronchoscopy reports if available 4) History of gastrointestinal symptoms with date of onset and all treatments received and 5) All available weights. For deceased relatives, in addition to the information described for living relatives, I will extract the date/age of death and cause of death based on necropsy reports. Finally, for living relatives, we will obtain stool for fecal elastase.

Data Analysis: Descriptive statistics will be used to summarize demographic variables including range, mean, standard deviation and median for all continuous variables. Discrete demographic values will be summarized in tabular form. A detailed genealogical chart will be created. Fecal elastase will be compared in affected and unaffected orangutans.

Specific Aim 2: To determine if mutations found in the affected orangutan occur uniquely in orangutans affected with air sacculitis and bronchiectasis by analyzing the CFTR gene in affected and unaffected orangutans.

Methods: For identified study subjects, whole blood for DNA analysis will be obtained via venipuncture in sodium citrate tubes. For orangutans that have been trained for voluntary venipuncture, samples will be acquired immediately. For orangutans who have not been trained for voluntary venipuncture, blood will be obtained during routine scheduled anesthetized procedures.

Stool samples will be shipped frozen and stored at -20°C. Fecal elastase will be quantified using the monoclonal ELISA method (BIOSERV Diagnostics, Rostock Germany) in Dr. Nick's laboratory (Denver, CO).

We will extract DNA from the blood samples of all the living affected and unaffected members of the affected orangutan's family, as well as from other unrelated affected orangutans. Samples will be shipped to the Cutting Lab at Johns Hopkins University School of Medicine, Baltimore, MD. DNA will be extracted using the phenol chloroform procedure. DNA will be suspended in 10 mM TE buffer, pH 8.0 and stored at -20°C freezer.

In the next stage, all 27 exons and adjacent intron boundaries of CFTR will be amplified by PCR and sequenced. Sequencing data will be analyzed using the Sequencher program and compared with orangutan WT CFTR sequence downloaded from UCSC genome browser to search for variants in CFTR gene. Segregation analysis will be performed to determine if the DNA variants are inherited in affected family members, and not seen in healthy orangutans. Subsequently, any novel substitution variants identified in will be analyzed for conservation and, using predictive programs, for their disease causing potential. All substitution variants predicted to be disease causing or probably damaging will be subjected to further

experimental validation. Finally, the variants prioritized from the bioinformatics tools will be tested experimentally. Both CFTR processing and function will be assessed.

Data Analysis: Genetic data for the affected orangutan, that of the affected orangutan's affected and unaffected relatives, and that of other unrelated affected orangutans will be depicted in genealogical charts and summarized in tabular form.

Significance: This study **would be the first** to establish CF in non-human primates, and would potentially identify the cause of the increased morbidity and mortality of this critically endangered species. Such information could be used to guide the use of CF therapies and breeding decisions in the orangutan population.

Future Directions: Data from this study may be used to apply for a larger grant to rigorously phenotype and genotype all captive orangutans with chronic sinopulmonary disease in the U.S. This information could aid in not only in breeding and therapy decisions for orangutans, but also further understanding of appropriate therapeutic intervention in affected orangutans.

Specific Materials Requested

1. Medical Records
 - a. Pedigrees
 - b. Clinical Records
 - c. Imaging reports (and discs if available)
 - d. Necropsy reports
2. Specimens
 - a. Whole blood collected in heparinized (purple top) tubes in accordance with standard veterinary procedures.
 - b. Feces collected in an unpreserved stool transport vial
 - c. Banked pathologic samples on deceased animals

Commitment to Participating Zoos:

1. All participating zoos will receive an annual update on the progress of the research.
2. All identifying information on samples and records will be considered strictly confidential in the same way it is considered with human medical record numbers; no identifying information will be included in any public reporting of data.
3. Acknowledgement of the Orangutan SSP's support and participating zoos will be noted in all output materials and/or presentations.
4. Shipping and collection supplies can be provided upon request to Dr. Taylor-Cousar.

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SHIPPING INSTRUCTIONS

1. Blood

- a. Collect whole blood in a CaEDTA/purple top tube in accordance with standard veterinary procedures. A minimum of 3-5 ml in a purple-topped tube is needed.
- b. Label tubes with orangutan's stud book number, name and date of collection.
- c. Please include a copy of the identifier form with the sample.
- d. Ship blood immediately or stored overnight at 4°C and ship the following day at room temperature.
- e. Please alert Dr. Taylor-Cousar when a shipment is being sent: taylor-cousari@njhealth.org
- f. Ship blood at room temperature via overnight Federal Express (account number for shipping will be provided by Dr. Taylor-Cousar) to:

Dr. Neeraj Sharma
Johns Hopkins University | Institute of Genetic Medicine
733 Broadway, BRB 553
Baltimore, MD 21205

2. Stool

- a. Collect stool within 24 hours of it being excreted.
- b. Transfer 5 g stool to an unpreserved stool transport vial.
- c. Please include a copy of the identifier form with the sample.
- d. Please alert Dr. Taylor-Cousar when a shipment is being sent: taylor-cousari@njhealth.org
- e. **Freeze at -20°C** and ship on dry ice via overnight Federal Express (account number for shipping will be provided by Dr. Taylor-Cousar) to:

Silvia Caceres
National Jewish Health
1400 Jackson Street, A-531
Denver, CO 80206

3. Medical records

- a. Send copies of medical records, necropsy reports and available imaging discs to Dr. Taylor-Cousar (can be sent with stool or separately)
- b. Please include a copy of the identifier form with the sample.

Dr. Jennifer Taylor-Cousar
National Jewish Health
1400 Jackson Street, J-327
Denver, CO 80206