

in airway resistance than non-CF mice, with heightened response to methacholine. Importantly, this increase in airway obstruction in CF mice was reversible with albuterol treatment, indicating airway smooth muscle dysfunction as a principal driver of lung function abnormalities. Furthermore, TGFbeta induced an increased ASM burden on lung histology in both CF and non-CF mice ($p < 0.05$). IL-6 levels in the BAL of CF mice showed greater increases after TGFbeta treatment compared to non-CF mice ($p < 0.05$). Empty vector control treatment did not cause lung pathology. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Young children with CF have a unique pattern of pulmonary inflammation compared to inflamed non-CF control patients. In CF, TGFbeta pulmonary levels are uniquely associated with IL-6, a driver of ASM dysfunction in other pulmonary diseases. We followed up this clinical observation study by investigating the effect of TGFbeta on pulmonary disease in a mouse model. CF mice demonstrate increased pulmonary IL-6, airway obstruction, and ASM dysfunction after TGFbeta exposure. This study provides evidence that TGFbeta is associated with a distinct cytokine pattern that may promote ASM dysfunction in early CF lung disease. Understanding the mechanism of early CF pathophysiology will be critical in developing targeted therapeutics that can prevent early lung damage.

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The influence of early life experience on telomere length, health, and behavior of domestic cats

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OBJECTIVES/SPECIFIC AIMS: The primary objective of this research is to determine whether being hand-reared, and deprived of early maternal interaction, will affect telomere length in orphaned kittens. The secondary goal is to examine how early maternal separation impacts the health, growth and behavior of orphaned kittens. **METHODS/STUDY POPULATION:** Kittens were fostered through local rescue groups and shelters. We collected blood samples from 42 orphaned kittens during the first week of their lives. Due to high mortality of this population, we obtained a second blood sample at eight weeks of age from only 30 of these kittens. We collected blood samples from 12 control kittens raised with mothers at during the first and eighth weeks of life. Blood samples are currently being processed with real time quantitative PCR (qPCR) by the Real-time PCR Research and Diagnostics Core Facility at the UC Davis School of Veterinary Medicine (SVM). This includes RNA extraction, cDNA synthesis, Reference Gene Validation, and qPCR analysis. Relative telomere length (RTL) will be calculated by comparing the average telomere abundance across three samples cells with that of a reference gene (single copy number) for each sample. The resulting T/S ratio (telomere to single copy) is proportional to the average telomere length. If T/S = 1, then telomere length in the sample and the reference are the same. **RESULTS/ANTICIPATED RESULTS:** Because telomeres show the fastest rate of shortening early in life, we predict that maternal separation will increase the rate of telomere shortening in kittens. We also predict that the telomeres of orphaned kittens will be shorter at both one week and eight weeks of age, compared to controls. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This study will increase our understanding of early life adversity, a finding that can translate to other mammals. It will inform the practice of fostering neonatal kittens, and illuminate whether these kittens

might be at higher risk than mother-reared kittens for health problems (which could be investigated in future studies). If significant telomere shortening occurs between collection periods, then future studies can take more frequent blood samples to determine what stages of early development are potentially most sensitive. If differences between groups are found, this will establish a protocol for several future research projects, such as testing whether these detrimental effects can be mitigated by environmental enrichment via activation of telomerase. Telomerase is an enzyme that appears to counteract some shortening of telomeres, and is activated by several external factors, including exercise. Thus, a logical follow up study would be developing and testing age-specific and appropriate enrichments that may activate telomerase and reduce telomere loss. Physical contact, whether human, mother, or siblings, is another possible source of telomerase activation in young kittens. Future studies also could quantify the effects of different sources of physical contact on telomere shortening. Finally, a positive finding would establish a need for longitudinal studies of the effects of early weaning on feline health and behavior and whether differences in early-life telomere lengths predict health and longevity of cats.

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The Power of Phenotypic Extremes in Detecting Novel Genetic Modifiers of Hemophilia

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OBJECTIVES/SPECIFIC AIMS: 1. To identify novel genetic modifiers that result in a mild bleeding phenotype in patients with FVIII <1%. 2. To examine the feasibility of a practice model that incorporates the principles and methods for both obtaining consent for NGS and returning individual research results from the sources described above. **METHODS/STUDY POPULATION:** 1. We plan a 3-step approach for identifying novel genetic modifiers of hemophilia: a. Obtain samples from individuals with an extremely mild bleeding phenotype: The study will be narrowed to patients with confirmed FVIII <1%, a null mutation in the gene for FVIII, and a mild bleeding phenotype according to a detailed bleeding history. b. Identify variants that modify phenotype: Whole exome sequencing will be performed, followed by a focused analysis of genes known or suspected to be involved in thrombosis and hemostasis and prediction of variant impact using algorithms that account for conservation and deleteriousness of all variants. c. Verify the impact of novel variants in independent samples: In silico (analyze genetic databases for suspected variants), in vivo (assess bleeding in animal models of hemophilia after introducing presumed modifier variants). 2. We will employ a model for obtaining informed consent and communicating individual genetic research results and results with potential clinical impact to research participants: a. The informed consent process will be performed after potential participants read a pamphlet entitled "Genetic Research at The Rockefeller University Hospital and Center for Clinical and Translational Science." The pamphlet includes 16 questions that the potential participants are urged to ask the investigator, including, "What will you look for in my genetic information?", "Will I receive results from this study?". Potential participants will also be informed of the meaning of clinically actionable variants, either pathogenic variants related to phenotype or secondary ("incidental") findings (i.e. variants unrelated to phenotype, the knowledge of which could lead to actions that may improve health). Participants who do not want to receive