



# YC-XLH NEWS



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## Introduction

Here's a quick look back at the accomplishments of the YC-XLH during 2008. The Center remains heavily committed to our sponsored research projects (p. 3-4). In October 2008 our Administrative Core hosted the second annual meeting of the YC-XLH External Advisory Board Meeting. Our updated website (<http://www.ycxlh.yale.edu>) complements the material in this issue of the YC-XLH newsletter. In addition to these activities, a major focus of the Administrative Core in 2008 was the establishment of a Pilot and Feasibility Program, which, after a rigorous review process has funded three exciting projects described below (p. 5-6).

This edition of the newsletter also features highlights from recent scientific meetings (p.2) and an introduction to the hard working members of our Research Core.

Finally, the Administrative Core is working with other centers around the country to establish a consortium of investigators interested in XLH. Currently we are spearheading (together with Indiana and Duke Universities), a Phase 1 clinical trial evaluating an important new therapeutic for XLH, developed by Kirin Pharmaceuticals.

## XLH CLINICAL STUDIES!

The YC-XLH is currently carrying out several clinical studies. We invite interested potential participants to contact us.

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## YC-XLH: Who We Are

Director: Dr. Thomas Carpenter

Associate Director: Dr. Karl Insogna

Principle Investigator Project 1: Dr. Carpenter

Principle Investigator Project 2: Dr. Marie Demay

Principle Investigator Project 3: Dr. J. Schlessinger

Clinical Research Coordinator: Elizabeth Olear

Administrative Coordinator: Danielle Frank

## Advisory Board Members

Dr. Joseph Craft

Dr. Francis Glorieux

Dr. Ralph A. Meyer, Jr.

Mrs. Joan K. Reed

Dr. Larry Winger

## Highlights from Recent Meetings

### ***American Society for Bone and Mineral Research (ASBMR), September 11-15, 2008. Montreal***

The field of phosphate homeostasis was again a “hot topic” at the 2008 ASBMR meeting in Montreal. Studies presented included investigations of XLH, phosphate transport, FGF23, MEPE and DMP1. Abstracts from YC-XLH members, including work from Drs. Demay, Carpenter and Insogna were presented. Two members of YC-XLH laboratories received Young Investigator Awards; Dr. Catherine Mendenhall-Brownstein in Dr. Carpenter’s laboratory and Dr. Tsutomu Kawano in Dr. Insogna’s laboratory.

Business was brisk at the XLH-Network booth where volunteers manned a display and served as an important resource for patients, clinicians, and researchers. The XLH-Network distributed an attractively updated information sheet on XLH to visitors at the booth throughout the meeting.

### ***American Academy of Pediatrics, October 11-14, 2008. Boston, MA***

The American Academy of Pediatrics sponsored a symposium on “Bone Health in Children” during its annual meeting. The AAP is the country’s largest organization of practicing pediatricians. Dr. Carpenter co-chaired (with Dr. Catherine Gordon from Boston Children’s Hospital) two sessions at the symposium. Dr. Carpenter delivered a lecture on XLH in which he discussed the problems encountered in making the correct diagnosis of the disorder.

### ***The YC-XLH Advisory Board Meeting, October 16, 2008. New Haven, CT***

On October 16, 2008 the YC-XLH hosted its 2<sup>nd</sup> annual Advisory Board meeting. Attendees included Dr. and Mrs. Ralph Meyer, Dr. Joseph Craft, Dr. Larry Winger and Mrs. Joan Reed. Dr. Michael Collins, from the NIH, was our featured scientific speaker. He spoke on “New insights into the action and regulation of FGF23.” The Advisory Board reviewed the Administrative and Research Cores in a closed session and also reviewed in detail the progress of each of the funded projects. They made a number of useful suggestions that the Administrative Core is currently implementing. The YC-XLH thanks the Board for their conscientious oversight of our Center.



### **2008 YC-XLH Advisory Board Meeting**

### ***The First Advances in Rare Bone Diseases, Scientific Conference, October 22-24, 2008. Bethesda, MD***

This “first of its kind” conference held at the National Institutes of Health was supported by the United States Bone and Joint Decade, the Rare Bone Diseases Patient Network, pharmaceutical industry representatives, and the NIH. The conference was chaired by Drs. Craig Langman and Michael Econs who have had career-long interests in phosphate disorders. The meeting assembled scientists, clinicians, pharmaceutical industry scientists and administrators, disease-related support groups, and patients affected with a variety of rare bone diseases in a single venue. The novel format of the meeting facilitated productive exchanges between the various groups attending the conference. In addition to state-of-the-art scientific lectures, disease-specific meetings were held to discuss clinical issues, scientific advancements and pharmaceutical advancements in the context of priorities voiced by those individuals affected with rare bone disorders. Joan Reed, President of the XLH-Network, and other members of the group participated. In addition to Drs. Econs and Carpenter other attendees with primary interest in phosphate disorders included Drs. Ken White and Michael Whyte, and representatives from Kirin Pharmaceuticals. Several proposals for immediate implementation were formulated during sessions focused on XLH. These include: 1) posting age-related reference ranges for serum phosphorus, TRP and TMP/GFR to help eliminate errors in interpretation of laboratory values (thereby reducing the number of missed diagnoses for XLH); and 2) Plans for a “treatment guidelines” document to be posted at our website. The document will be developed by the YC-XLH leadership over the coming year.

## Update on Center Projects

### Project 1: The Role of Parathyroid Hormone in the Pathogenesis of Skeletal Disease in XLH

This project seeks to test the hypothesis that parathyroid hormone (PTH) contributes to skeletal disease in XLH. A cross-sectional observational study is designed to evaluate the correlation of disease severity with circulating levels of PTH, FGF23, and phosphorus. Disease assessment includes quantifying skeletal symptoms, measurement of height and weight, fracture history, surgical interventions, and dental abscesses. Thus far, 37 affected subjects and 7 control subjects have been studied. Subjects are evaluated during an overnight stay in our Hospital Research Unit where serial blood and urine collections are made. A scintigraphic bone scan, a radiographic skeletal survey and an echocardiogram are performed. We expect to enroll 50 volunteers with XLH, and 20 normal controls. We are very grateful to the subjects who have participated thus far, and invite interested potential subjects to contact us.

Our second study employs a double-blind, placebo controlled design to determine if correction of secondary hyperparathyroidism in XLH is accompanied by a reduction in severity of skeletal disease. Secondary hyperparathyroidism is an all-too-common complication of XLH and we are using the vitamin D analog, paricalcitol, to suppress PTH in volunteers with this condition. The initial dose is 2 mcg per day, with upward titration based on the degree of suppression of PTH levels, to a maximum dose of 4 mcg per day. We have recruited 16 affected subjects; 5 have completed the study and 11 are currently receiving study drug (or placebo). We plan to enroll a total of 30 subjects in this interventional study.

### Project 2: Phosphate, PTH and FGF23 as mediators of the rachitic growth plate

The goal of project 2 is to determine how low ambient phosphate concentrations lead to the development of rickets. These studies use genetically modified mice, and employ cells isolated from mouse models of disease. Four mouse models are used to address these questions. The first model lacks the vitamin D receptor. Although it is known that vitamin D deficiency leads to rickets, previous studies have demonstrated that vitamin D action is not required when phosphate levels are normal. The second model lacks a renal phosphate transporter (Npt2a), which results in low blood phosphate levels. These mice develop rickets, however spontaneous reversal of the disease occurs with age. As these mice get higher levels of the active form of vitamin D, it is possible that 1,25 dihydroxyvitamin D levels compensate for the low phosphate in some manner. A newly developed third model employs mice that lack both the vitamin D receptor and Npt2a to determine if impaired vitamin D action will prevent time-dependent correction of rickets in Npt2a null mice.

In parallel, we are examining cultured cartilage cells from these animals to determine if vitamin D alters the susceptibility of these cells to undergo programmed cell death (apoptosis) in response to phosphate. Since impaired programmed cell death of the most mature cartilage cells in the growth plate leads to rickets, these studies aim to clarify the mechanism by which the mice lacking Npt2a cure their rickets. Additional investigations are being performed in Hyp mice, a well-characterized model of XLH. These mice have impaired activation of vitamin D, like their human counterparts. Thus, if our studies demonstrate that the active form of vitamin D can restore programmed cell death to the cartilage cells of these mice, it would suggest that higher doses of vitamin D, or modified vitamin D compounds, could help prevent rickets associated with low phosphate.

### Project 3: Mechanism of FGF signaling in phosphate homeostasis.

Recent experiments indicate that phosphate homeostasis is regulated by the Klotho-FGF23-FGFR signaling complex in kidney. Klotho is a type I membrane protein highly expressed in the distal convoluted tubules. It consists of an N-terminal signal sequence, an extracellular domain with two internal repeats that share sequence similarity with  $\beta$ -glucosidases, a single membrane spanning region, and a very short intracellular domain. Klotho converts the canonical FGF receptor to a specific receptor for FGF23. This FGF contains a canonical signal sequence followed by an FGF-homology domain and an OK C-terminal extension suggesting a specialized function of this growth factor. Unlike other FGFs, which act locally, FGF23 acts as a systemic endocrine factor. Specific missense mutations in FGF23 prevent proteolytic cleavage of FGF23, resulting in excessive renal phosphate wasting. Strikingly, mice deficient for FGF23 or Klotho are phenotypically similar (hyperphosphatemic and hypercalcemic) suggesting that FGF23 and Klotho may regulate a common signaling pathway involved in mineral homeostasis.

Presently, no direct link has been established between FGFRs and phosphate reabsorption in the kidney. Phosphate reabsorption occurs mainly in the proximal tubules of the kidney, which is also the site of 1,25 dihydroxy vitamin D synthesis. Both processes are influenced by Klotho. Yet Klotho is expressed in the distal tubules, and it is not known how Klotho signals to the proximal tubules.

To answer this fundamental question, we are performing biochemical and genetic studies that are required to examine the physiological function of Klotho. In that Klotho binds primarily to the c-isoforms of FGFRs, we are conducting experiments to determine the molecular basis of Klotho interaction with FGFR. The results show that the introduction of the c-isoform-specific sequences located at F and G strands of the Ig-like domain 3 convert the b-isoform to associate with Klotho. Next, we will perform an alanine scan mutational analysis, in which the residues in the F and G strands of the c-isoform will be individually replaced by alanine to determine the key residues that mediate the interaction of FGFR with Klotho. Moreover, Klotho is expressed as a membrane-bound form and soluble form. The distinct functions of these two forms are not known. Therefore, we are creating transgenic mice, in which Cre recombinase will be driven by the Klotho promoter, to delete FGFRs specifically where Klotho is co-expressed without affecting the expression of membrane bound and soluble Klotho. This will determine whether Klotho signals to the cells by autocrine or paracrine function to regulate phosphate homeostasis. Understanding of the molecular mechanism(s) of Klotho signaling is of great importance for therapeutic interventions in the phosphate wasting syndromes, abnormal calcium and vitamin D metabolism, osteopenia, and other potentially age-related disorders.

## Research Core

The overall goal of the Research Core is to facilitate CORT basic and translational research projects by supporting the detailed assessment of musculoskeletal homeostasis. Specifically, the Research Core 1) provides histological preparation and analysis of mouse tissue for CORT basic research projects; 2) performs mutational analysis of the PHEX gene in patients with XLH followed in Project 1; and 3) measures markers of bone turnover, FGF23, and calciotropic hormones in human and mouse samples using purchased kits and in-house assays for all three Projects of the CORT.



*Dr. Caren Gundberg*



*Nancy Troiano*



*Sherry Nieman*



*Bruce Ellis*

## New Directions: The Pilot and Feasibility Program

The Yale Center for X-Linked Hypophosphatemia launched its Pilot and Feasibility program in early 2008 with a request for applications. We received four applications deemed of sufficient merit to warrant external review. After peer review by 8 national experts, we selected the three proposals receiving the best scores, for funding. Awardees receive up to two years of funding. The awardees include two young investigators, one of whom is new to the field of phosphate metabolism and one international expert in intermediary metabolism who will bring unique technologies to his project. The funded studies all have immediate clinical relevance and fit the Center's mission of supporting translational science. They are briefly summarized below.

### ***Use of an animal model of Epidermal Nevus Syndrome (ENS) to understand the mechanism of FGFR signaling in renal phosphate wasting syndromes.***



Dr. Veraragavan Eswarakumar is a new Assistant Professor in the Department of Orthopaedics. His project, "Use of an animal model of Epidermal Nevus Syndrome (ENS) to understand the mechanism of FGFR signaling in renal phosphate wasting syndromes" will focus on mechanism of disease in a rare phosphate-wasting syndrome associated with FGF receptor dysfunction. Dr. Eswarakumar notes, "Because ENS is due to mosaicism resulting from a postzygotic mutation of the FGFR3 gene, we hypothesize that cells other than keratinocytes harbor the mutation and thus contribute to the skeletal abnormalities and phosphate wasting observed in ENS patients. We plan to create an animal model of ENS by introducing the lethal FGFR3-R248C mutation into the mouse germline using a loxP-flanked stop

sequence to overcome prenatal lethality and then selectively activate the mutation in specific tissues including skin, bone, and kidney. Identifying disease-causing sites of expression of the FGFR3-R248C mutant receptor will contribute to an improved understanding of the mechanism of FGFR signaling in phosphate homeostasis and ENS."

### ***A study of enthesopathy in X-Linked Hypophosphatemia***



Dr. Carolyn Macica is an Assistant Professor in the Department of Medicine. Her project, "A study of enthesopathy in X-Linked Hypophosphatemia," is focused on a particularly debilitating complication of XLH. She will explore the progression and pathogenesis underlying the paradoxical calcification of tendon and ligament insertion sites. Dr. Macica states, "The formation of enthesophytes will be our focus, with a major emphasis on characterizing the cellular changes that occur in enthesophyte formation using the murine model of XLH, Hyp mice. We have found that mineralization, while thought to originate from the bone, is actually due to both an expansion of fibrocartilage cells that express the FGFR3 receptor and an increase in alkaline phosphatase activity. Our goal is to study the role of elevated circulating levels of FGF23 in fibrocartilage expansion using FGFR3 knockout mice on the Hyp background and in Dmp1 knockout mice, another model of osteomalacia characterized by elevated FGF23 levels, independent of the PheX mutation. We have evidence that an increase in chondrocyte-derived alkaline phosphatase is likely involved in the mineralization of both entheses and in articular cartilage. We also aim to better characterize the dysregulation of alkaline phosphatase specific to cells of chondrocyte (vs. osteoblast) origin."

## New Directions: The Pilot and Feasibility Program (Cont.)

### ***In vivo <sup>31</sup>P NMR studies of Intracellular Phosphate, Mitochondrial and Whole Body Energy Metabolism in Normal and Hypophosphatemic Mice***



Our third Pilot awardee is Dr. Gerald Shulman, an internationally known clinical investigator in the field of carbohydrate metabolism and diabetes. His project "In vivo <sup>31</sup>P NMR studies of Intracellular Phosphate, Mitochondrial and Whole Body Energy Metabolism in Normal and Hypophosphatemic Mice" will examine the in vivo levels of intracellular phosphate in states of disordered phosphate homeostasis, something

that has eluded evaluation for decades. Dr. Shulman notes, "In preliminary studies we have found that dietary Pi-deprivation in normal mice results in severe hypophosphatemia and a 50% reduction in skeletal muscle ATP synthesis as measured by in vivo <sup>31</sup>P -MRS saturation transfer methods. In addition, these mice exhibit reduced whole body energy expenditure, activity, and oxygen consumption. We will examine intracellular phosphate metabolism, mitochondrial ATP synthesis and whole body energy metabolism in hypophosphatemic, euphosphatemic, and Hyp mice using these in vivo <sup>31</sup>P -MRS saturation transfer methods. It is anticipated that the results from these studies will provide important new insights into the role of intracellular phosphate metabolism in the regulation of mitochondrial and whole body energy metabolism under normal physiological conditions as well as provide new insights into intracellular phosphate metabolism in patients with X-linked hypophosphatemia."

The YC-XLH is delighted to have Drs. Eswarakumar, Macica and Shulman as pilot project awardees, and will meet with them regularly to monitor their progress and provide guidance as needed.

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*We're on the Web!*

*Visit us at:*

[www.ycxlh.yale.edu](http://www.ycxlh.yale.edu)

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