the 2012 CHORI SUMMER STUDENT RESEARCH SYMPOSIUM

a showcase for young minds in research

August 10, 2012

C·H·O·R·I
Children's Hospital Oakland Research Institute

CHILDREN'S HOSPITAL
& RESEARCH CENTER OAKLAND

C · H · O · R · I

Children's Hospital Oakland Research Institute

August 10, 2012

Welcome to the 2012 CHORI Summer Student Research Symposium! Today's symposium presentations are a culmination of the 8-week long CHORI Summer Research Program that featured a rigorous research and education curriculum. The program provides training to students who are considering research careers in biomedical science and other health related fields.

This year we had a potpourri of high school, undergraduate, post-graduate and pre-medical students from across the country, coming from a broad range of backgrounds and experience. They were provided research education, training and awareness on a range of state-of-the-art topics including stem cells, vaccine therapy, nutritional genomics, obesity and diabetes in a mentored environment. This was made possible by funding from the National Institutes of Health (National Heart, Lung and Blood Institute) Short Term Research Education Program to Increase Diversity in Health Related Research.

New this year is the additional funding we received from Doris Duke Charitable Foundation to provide Clinical Research Experiences for High School Students and the Creativity Award from the California Institute for Regenerative Medicine for introducing high school students to the concept of stem cells and developmental biology. We are excited about these new research education opportunities and look forward to hosting a broad cross section of students and trainees.

We would like to take this opportunity to thank Deborah Ellen and Phillip Bollinger, Program coordinators who did an outstanding job in coordinating the program. We also thank all the mentors and supervisors who are the backbone of the Program: we appreciate their time, effort, and deep commitment to train the students. A note of appreciation also goes out to all the guest speakers for taking time out of their busy schedules to discuss their research, career challenges, issues related to ethics and integrity in academia and research with the students.

Last but not the least, as the program continues to grow in size and scope, and given our budget constraints, we urge you to consider supporting our program. Your philanthropic support will ensure continuation of this important scientific and educational experience for the interns as we remain committed to educating and fostering tomorrow's leaders.

We thank all members of the CHORI/CHRCO family for making the Program a huge success. We wish the students all the best in their future endeavors and hope that they will keep in touch with us, as we would like to know if the program had any impact on their academic and career decisions.

Sincerely,

Bertram H. Lubin, M.D.
President, Chief Executive Officer and Principal Investigator
Children's Hospital & Research Center Oakland

Alex Lucas, Ph.D.
Executive Director, Senior Vice President Research
Children's Hospital Oakland Research Institute

Vasanthy Narayanaswami, PhD
Associate Scientist,
Principal Investigator and Co-Director of Basic Research Program
Children's Hospital Oakland Research Institute

Barbara Staggers, MD, MPH, FAAP
Division Chief, Adolescent Medicine
Co-Director of Clinical Research Program
Children's Hospital & Research Center Oakland

5700 Martin Luther King Jr. Way / Oakland, California 94609-1673
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Support for the 2012 CHORI Summer Student Research Program is provided by:

The Short Term Research Education Program to Increase Diversity in HealthzRelated Research from the National Institutes of Health/National Heart, Lung and Blood Institute
#5 R25 HL096365
PI: Bertram Lubin, MD & Vasanthy Narayanaswami, PhD

NIH Grant #5 R37HL064159-12
PI: Robert Ryan, PhD

Doris Duke Charitable Foundation
Clinical Research Experiences for High School Students Program (CREHSS)
#2011114
PI: Vasanthy Narayanaswami, PhD and Bertram Lubin, MD

California Institute for Regenerative Medicine (CIRM) Creativity Award
TC1-05946
PI: Vasanthy Narayanaswami, PhD

Jennifer Leigh Wells Fellowship

Elizabeth Nash Foundation

Children's Hospital & Research Center Foundation

2012 Program Directors

Bertram H. Lubin, MD

President & Chief Executive Officer
Children's Hospital & Research Center Oakland

Barbara Staggers, MD

Director, Adolescent Medicine
Children's Hospital & Research Center Oakland

Vasanthy Narayanaswami, PhD

Associate Scientist at CHORI
Assistant Professor, Department of
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California State University Long Beach

2012 Program Coordinators

Phillip Bollinger

Deborah Ellen

Mentors

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Mindy Benson, PNP
Cassandra Calloway, PhD
Michael Conboy, PhD and Marc Cooljian, UCB
Deborah Dean, MD, MPH
Pieter de Jong, PhD
Stephanie Doniger, MD
Ervin Epstein, MD
Horst Fischer, PhD
Heidi Flori, MD
Ellen Fung, PhD
Rachel Gilgoff, MD
Ward Hagar, MD
Karen Hardy, MD
Caroline Hastings, MD
Jacqueline Hogan-Schlientz, RN
Beate Illek, PhD
Damini Jawaheer, MD
David Killilea
Janet King, PhD
Ashok Kumar, PhD
Rachel Kuperman, MD
Frans Kuypers, PhD
Desiree LaBeaud, MD
Edward Lammer, MD
Sandy Larkin
Greg Moe, PhD
Vasanthy Narayanaswami, PhD
Jacob Neufeld, MD, MSPH
Christopher Newton, MD
Janelle Noble, PhD
Michael Oda, PhD
Robert Ryan, PhD
Julie Saba, MD
Barbara Staggers, MD
Jodi Stookey, PhD
Jung Suh, PhD
Marsha Treadwell, PhD
Grace Wang, MD
Wen-Shu Wu, PhD

2012 CHORI Summer Student Research Program

Selection Committee for NIH Funded Program to Increase Diversity

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Selection Committee

Children's Hospital Oakland Research Institute's Summer Research Program for High School Students: Doris Duke Charitable Foundation Clinical Research Experiences for High School Students Program (CREHSS) and California Institute for Regenerative Medicine (CIRM) Creativity Award

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2012 Weekly Discussions/Lectures

Thursday, June 7, 2012
9:30AM to 3:30PM
Summer Student Research Program Orientation
Keynote Speaker: Troy Duster, PhD, Professor Emeritus, UC Berkeley, Professor Sociology, NYU

"Personalized Medicine vs. Racialized Medicine: The Tangled Fates of 'Big Pharma' and 'Big Science' in the Pursuit of Explanations Of Health Disparities"

Friday, June 8, 2012 9:30AM
Safety Training
Miriam Fang

Monday, June 11, 2012 9:30AM
Make up Orientation
Deborah Ellen and Phillip Bollinger

Tuesday, June 12, 2012 2:00PM
Make up Safety Training
Miriam Fang

Thursday, June 14, 2012 at 12:00PM
Julie Saba, MD, PhD,
CHORI Center for Cancer Research
CHORI Senior Scientist, Chair

"Cancer Research, Finding Your Passion, and Swimming for A Good Cause"

Friday, June 15, 2012, 10:00AM
Common Equipment Overview:
General Shared Room Philosophy - Lorelle Parker
Microscopy at CHORI – Horst Fischer
Centrifuges - Jennifer Beckstead
Tissue Culture Rooms - David Killilea and Jennifer Beckstead
Alpha Imager - David Killilea and Phillip Bollinger
Autoclave - All

Tuesday, June 19, 2012 at 4:00PM
Bo Zheng
PhD Candidate, Bioengineering
UC Berkeley, UC San Francisco

"Magnetic Particle Imaging for Stem Cell Imaging and Angiography"

Thursday, June 21, 2012 at 12:00PM*
Alex Wulff
Pulmonary Research Coordinator
Bay Area Pediatric Pulmonary Medical Corporation
CHORI Summer Student Alumnus
California Pacific Medical Center

"BAPP...Life After the Summer Student Program"

Tuesday, June 26, 2012 at 4:00PM
Vasanthy Narayanaswami, PhD
Basic Science Program, Co-Director
Associate Scientist, CHORI
Faculty, California State University, Long Beach

"Apolipoprotein E: A Tale of Two Diseases"

Thursday, June 28, 2012 at 12:00PM
Phillip Bollinger
Senior Systems Analyst, CHORI

Fear and Loathing of the Big Red X
"How to Correctly Assemble Your Microsoft PowerPoint Presentation Utilizing Sanctioned Procedures and Methodologies Within Accepted Microsoft Parameters"

Tuesday, July 3, 2012 at 4:00PM
Janet King, PhD
Scientist, CHORI

"Prenatal Nutrition: The Past, Present, and Future"

Thursday, July 5, 2012 at 12:00PM
Bertram Lubin, MD
President, Children's Hospital & Research Center Oakland

Film: "Unnatural Causes"

Friday, July 6, 2012 at 12:00PM
Tyrone L. McGraw,
BA with Honors, American Studies,
Stanford University Class of 2012

Tuesday, July 10, 2012 at 4:00PM
Paul Harmatz, MD
Physician, Gastroenterology and Nutrition
Children's Hospital & Research Center Oakland

"Mucopolysaccharidosis and Clinical Trials of Enzyme Replacement Therapy: Physician and Patient Perspective"

Thursday, July 12th at 12:00PM
Doris Duke and CIRM High School Students Only
Victor Urista, MA, Med-2
"Emotional Intellegence" & Survey
Ward Hagar, MD

"Designing Clinical Research"
Online Course

Tuesday, July 17th at 4:00PM
Carolyn Hoppe, MD
Hematologist/Oncologist, Children's Hospital & Research Center Oakland
Clinical Scientist, CHORI

"Do Statins Play a Role in Sickle Cell Disease?"

Thursday, July 19th at 12:00PM
Elizabeth Sanseau
Medical Student,CHORI Summer Student Alumnus

"Art History To Medicine: My Non-Traditional Path to Medical School"

Tuesday, July 24th at 4:00PM
Beate Illek, PhD
Staff Scientist, CHORI

"CFTR: A Novel Drug Target in Cystic Fibrosis and Diarrheal Disease"

Thursday, July 26th at 12:00PM
John Matsui, PhD
Director, Biology Scholars Program,
Department of Integrative Biology
University of California, Berkeley

"Your Place in Science"

Tuesday, July 31st at 4:00PM
Ben Haynes
Senior Systems Analyst
CHORI

"PowerPoint/InDesign Preparation for Posters and Talks"

Thursday, August 2nd at 12:00PM
Nghi Nguyen
Stanford University
CHORI Summer Student Alumnus

"Oh, The Places You'll Go: What's Next After CHORI"

Wednesday, August 8th at 1:00PM
Phillip Bollinger
Senior Systems Analyst, CHORI,
and Vasanthy Narayanaswami, PhD

"PowerPoint Oral Presentation Practice"

Friday, August 10th, 2012, 8:30AM to 5PM
Summer Student Research Symposium

2012 CHORI Summer Student Research Program Curriculum

ORIENTATION, JUNE 7, 2012

There will be an all-day orientation for summer interns on Thursday, June 7, 2012, from 9:30AM until 4:00PM. Continental Breakfast will be served at 9:00AM. Lunch will be served at 12:00PM.

Agenda to include:

- Introduction by Alex Lucas, PhD, Senior Vice President, Research, Executive Director, CHORI
- Introduction and overview by Vasanthy Narayanswami, PhD, Scientist, and Bertram Lubin, MD President & CEO, Children's Hospital & Research Center Oakland
- Keynote Lecture:
  Troy Duster, PhD, Professor Emeritus, UC Berkeley, Professor, Sociology, New York University
  "Personalized Medicine vs. Racialized Medicine: The Tangled fates of 'Big Pharma' and 'Big Science' in the pursuit of Explanations of Health Disparities"
- Explanation of curriculum:
  Vas Narayanaswami, PhD
- Lunch
- IT Orientation: Phillip Bollinger
- Administrative Review: Deborah Ellen
- CHORI Tour

SAFETY TRAINING, JUNE 8, 2012

The mandatory Safety Training with CHORI Safety Officer, Miriam Fang will be held on Friday, June 8 from 9:00AM until 12:30PM. The students will be required to complete this training BEFORE beginning their project.

CHORI TOUR

RESEARCH PROJECT: JUNE 11, 2012 TO AUGUST 10, 2012

The students will conduct research with assigned mentors. The details of project and research plan are left entirely to the individual mentor and each summer intern will follow the procedures and schedule, laid out by their respective labs.

SUBMIT WRITTEN RESEARCH PLAN: JUNE 25, 2012

Students must submit to the Program Coordinator a written Research Plan outlining their project. The Research Plan should be 3-4 pages long and include:
(a) Statement of hypothesis
(b) Specific aims
(c) Background
(d) Methods
(e) Anticipated outcome of project

Students will work closely with their mentor in the preparation of these reports, and mentors should review and approve the reports before submission. Figures, flow charts and schematics may be used to illustrate the research plan. The written report will be sent to: summerstudentprogram@chori.org, and must include student's name, mentor's name and the title of the project.

WEEKLY LECTURES: CURRENT TOPICS IN HEALTH AND DISEASE

Students are required to attend weekly lectures delivered by CHORI and Children's Hospital & Research Center Oakland faculty members. The lectures will cover various scientific topics, including women's and minority's issues, career decisions, teen health issues and Responsible Conduct of Research.

STUDENT PHOTO DAY: JULY 10, 1:00PM

All students must be present.

2012 CHORI SUMMER STUDENT SYMPOSIUM, AUGUST 10, 2012

A one-day symposium will be held on August 10, 2012 where all students are required to participate. The students will submit an abstract concisely summarizing their work, which will be considered for an oral or a poster session for the Symposium. Abstracts are due on July 23, 2012 by 5:00PM. A committee comprised of the Director, Co-Directors and other leading members of the CHORI scientific community will review the abstracts for the Symposium. Students will work closely with their mentors in the preparation of abstracts and presentations. Family members, teachers, lab members and friends are welcome to attend. Details of the Symposium and the scientific sessions will be available by August 1, 2012.

The Symposium will be comprised of oral presentations (10 minutes each, with 5 minute discussion), and a poster session during which the presenters will be on-hand to explain their research project. An abstract book, which will include the Symposium program, personal statements, and the research project abstracts, will be presented to each student. A catered breakfast and lunch will be provided for all attendees on the Symposium day.

A certificate of participation in the CHORI Summer Student Research Program will be awarded to those who successfully complete the program.

SUMMARY OF IMPORTANT DATES:

June 7, 2012
Orientation: 9:00AM – 4:00PM

June 8, 2012
Safety Training: 9:30AM - 12:30PM

June 11, 2012
Make-up Orientation: 9:30AM - 12:00PM
Badging: 1:00PM

June 12, 2012
Make-up Safety Training: 2:00PM – 5:00PM
Badging: 8:30AM

June 25, 2012
Written Research Plan due

July 2, 2012
Personal Statement for Program Guide due by 5:00PM

July 10, 2012
Student Photo Day: All Students must be present

July 23, 2012
Abstract for Program Guide due by 5:00PM

August 10, 2012
Summer Student Research Symposium

Please e-mail any additional questions and concerns to: **summerstudentprogram@chori.org**

Volunteer Recognition 2012

We would like to thank all the volunteers who took time out of their busy schedules to help this program succeed.

CHORI

Jennifer Beckstead
Tate Brazas
Horst Fischer
Ben Hanes
Damini Jawaheer
David Killilea
Kristine Munir
Lorelle Parker
Shirley Poy
Hector Sandoval
Kathy Schultz

Student Volunteers

Andrea Akabike
Neil Alameida
Kevin Chen
Ogochukwu Chukwu
Rigoberto Del Toro
Joan DeVoe
Adriana Gonzalez
Kimpreet Kaur
Nikitha Kosaraju
Lydia Ruesch
Manolis Sueuga

Symposium Schedule – Friday, August 10, 2012

8:30 – 9:00	**Check-in and Continental Breakfast** at the CHORI Library
9:00 – 9:15	Welcome by CHORI Executive Director, Senior Vice President of Research **Alex Lucas, PhD** Introduction and Welcome by Program Directors **Vasanthy Narayanaswami, PhD**, Basic Research Program Co-Director Scientist at CHORI, Faculty at California State University, Long Beach **Bertram H. Lubin, MD,** President, Chief Executive Officer and Principal Investigator, Children's Hospital & Research Center Oakland **Barbara Staggers, MD**, Clinical Co-Director, Director, Adolescent Medicine, Children's Hospital & Research Center Oakland, Executive Director, External Affairs and Community Relations
9:15 – 12:30	Oral Presentations in the CHORI Library

	ORAL PRESENTATIONS - SESSION 1	**PAGE**
Chairs:	**Jennifer Beckstead**, Senior Research Associate Children's Hospital Oakland Research Institute **Neil Almeida,** Senior, Washington High School	
9:15 – 9:30	**Napala Pratini**, Senior, Saint Mary's College of California Mentor: Jacob A. Neufeld, MD Title: **Early consequences of damage to the prefrontal cortex**	78
9:30 – 9:45	**Kevin Chen**, Senior, Alameda High School Mentor: Wen-Shu Wu, PhD Title: **Modified approaches for efficient assembly of TALEs**	28
9:45 – 10:00	**Joan DeVoe,** Senior, University of Alabama Huntsville Mentors: Beate Illek, PhD, Horst Fischer, PhD Title: **Proton Secretion by the HVCN1 channel and pH equilibrium in cystic fibrosis airway epithelial cells** **Kayla Horton**, Senior, California State University, Sacramento Mentors: Beate Illek, PhD, Horst Fischer, PhD Title: **Determining CFTR expression and HCO_3^- secretions in cystic fibrosis bronchial epithelium**	36 54
10:00 – 10:15	**Rocio Ochoa**, Junior, Holy Names High School Mentor: Robert Ryan, PhD Title: **Barth Syndrome associated neutropenia: effect of cardiolipin-nanodisks on myeloid progenitor cells**	72

	ORAL PRESENTATIONS - SESSION 2	**PAGE**
Chairs:	**Damini Jawaheer, PhD**, Assistant Scientist Children's Hospital Oakland Research Institute **Ogochukwu Chukwu**, Post Baccalaureate, UC Berkeley Extension	
10:15 – 10:30	**Rylan Rosario**, Post-Baccalaureate, California State University East Bay Mentor: Barbara Staggers, MD Title: S**creening practices of providers at Children's Hospital Oakland teen clinic for interpersonal violence**	82
10:30 – 10:45	**Issam Hamdallah**, Freshman, Kenyon College Mentor: Deborah Dean, MD Title: **Mapping the distribution of *omp*A genotypes among clinical samples from different geographical areas**	48
10:45 – 11:00	**BREAK**	
11:00 – 11:15	**Lydia Tesfamariam**, Freshman, Dominican University of California Mentor: Janelle Noble, PhD Title: **The key variables in differential diagnosis of pediatric type 1 and type 2 diabetes**	94
11:15 – 11:30	**Andrea Akabike**, Sophomore, University of California Berkeley Mentors: Janet King, PhD, Lisa Sawrey-Kubicek, M.S., RD Title: **The effects of ethnicity on the distribution and amount of body fat**	16
	ORAL PRESENTATIONS - SESSION 3	**PAGE**
Chairs:	**David Killilea, PhD**, Associate Staff Scientist Children's Hospital Oakland Research Institute **Nikitha Kosaraju**, Junior, Piedmont High School	
11:30 – 11:45	**Kelly Bauer**, Junior, University of California Berkeley Mentor: Greg Moe, PhD Title: **Finding pathway used for the uptake of exogenous NeuPSA**	22
11:45 – 12:00	**Yaqiao Li**, Senior, University of California Berkeley Mentor: Frans Kuypers, PhD Title: **Establishing novel red blood cell morphology data collecting and analyzing methodology**	62
12.00 – 12:15	**Tuyen Ngoc Tran**, Senior, California State University Long Beach Mentor: Vasanthy Narayanaswami, PhD Title: **Proteomics analysis of oxidative stress modification of apolipoprotein E by acrolein**	96
12:15 – 12:30	**Ogochukwu Chukwu**, Post Baccalaureate UC Berkeley Extension Mentors: Ashok Kumar, Postdoctoral Fellow, Julie Saba, MD Title: **To study the effect of S1P lyase deficiency on pluripotency in human embryonic stem cells**	32
12:30 – 1:30	Lunch in the Library Courtyards	
1:30 – 3:00	**POSTER SESSION IN THE SENIOR CENTER COURTYARD**	
	1:30 – 2:15: Even numbered Posters	
	2:15 - 3:00: Odd numbered Posters	

	POSTERS	PAGE
#24	**Brianna Pope**, Senior, Vista del Lago High School Mentors: David Killilea, PhD, Janet King, PhD Title: **Zinc absorption and measurement**	76
#25	**Maritza Rodriguez**, Senior, American Indian Public High School Mentor: Damini Jawaheer, PhD Title: **The influence of gender and age of onset on the severity of pediatric lupus**	80
#26	**Hira Safdar**, Sophomore, University of California Berkeley Mentors: Beate Illek, PhD, Horst Fischer, PhD Title: **Expression of dual NADPH oxidase and production of hydrogen peroxide in cystic fibrosis cell**	84
#27	**Kasey Siu**, Post-Baccalaureate, Mills College Mentor: Michael D. Bell, MD Title: **Comparison of nitrous oxide to intranasal midazolam for sedation in minor procedures in the pediatric emergency department**	86
#28	**Betty Su**, Post-Baccalaureate, University of California Berkeley Mentors: Trudy Forte, PhD, Robert Ryan, PhD Title: **Targeted-Nanodisk as a drug delivery vehicle for mantle cell lymphoma**	88
#29	**Manolis Sueuga**, Freshman, Stanford University Mentors: Marc Chooljian, Michael Conboy, PhD **Title: Investigation of molecular factors in muscle regeneration**	90
#30	**Siddhant Talwar,** Senior, Mountain Ridge High School Mentor: Ellen Fung, PhD Title: **A link between zinc and diabetes in patients with thalassemia**	92
#31	**Danielle Tucker**, Junior, University of California Berkeley Mentors: Grace Wang PhD, Ervin H. Epstein, Jr. MD Title: **Patched-1 genotyping analysis of microscopic basal cell carcinomas**	98
#32	**Autumn Turpin**, Freshman, Stanford University Mentor: Heidi R. Flori, MD Title: **Long-term follow-up of pediatric patients with acute respiratory distress syndrome (ARDS)**	100
#33	**Neha Verma**, Senior, Mission San Jose High School Mentor: Caroline Hastings, MD Title: **Yield of routine CT imaging of the pelvis in detection of relapse in pediatric patients with hepatoblastoma**	102
3:00 – 5:00	**CERTIFICATE PRESENTATION AND RECEPTION IN THE CHORI LIBRARY**	

Notes

the 2012 CHORI SUMMER STUDENTS

ANDREA AKABIKE

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley, Senior

Mentor: Janet King, PhD and Lisa Sawrey-Kubicek, M.S., RD

As a current undergrad at UC Berkeley, Andrea is majoring in American Studies. Andrea's interest in research sparked while interning at UCSF's Center for Health and Community last summer. While interning at UCSF, she worked on the MAMAS Study, which enrolls low-income, overweight pregnant women in a stress reduction program in hopes of assisting the women achieve and maintain healthy weight during their pregnancy.

This summer, Andrea has had the priviledge of working with Dr. Janet King. Over the course of the summer, she has worked on Dr. King's study on the postprandial response to almond consumption in pregnant women. In addition to working on Dr. King's project, Andrea spearheaded the design, execution, and qualitative data analysis of her own clinical research study on the effects of ethnicity on the amount and distribution of body fat.

Andrea's career goal is to advance to dental school, where she hopes to enroll in a dual degree program to obtain both her D.D.S and MPH degrees. By obtaining both degrees, she plans to develop projects to reduce oral health disparities in vulnerable populations. Andrea is very grateful and appreciative of her mentor, Dr. King and her lab partners for being so welcoming and helpful over the summer. The training she has received over the summer has not only strengthened her clinical research skills, but has helped her become a better independent worker and critical thinker. These skills will not only prove useful as Andrea moves forward in the field of research, but the skills she has gained will be essential to her success as a dentist.

The Effects of Ethnicity on the Distribution and Amount of Body Fat

Andrea Akabike, Lisa Sawrey-Kubicek and Janet King

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Studies have shown that a person's distribution of fat can put them at risk for metabolic disorders such as, type 2 diabetes and hypertension. People either store fat centrally (apple body shape) or peripherally (pear body shape). Scientists have proposed that people who have an apple-shaped fat distribution have an increased risk of metabolic disease when compared to people who have a pear-shaped fat distribution. We are studying African-American, Asian, Caucasian, and Hispanic young women to see if the amount and distribution of their fat is affected by their ethnicity. There has been a recent emergence of information suggesting that there may be ethnic differences in fat distribution at a given weight or waist circumference. However, there has been a lack of representation of minorities and younger people in clinical studies. In this study, we hope to recruit a diverse population of young women to see if in fact ethnicity influences fat distribution. We hypothesize that African Americans and Hispanics will store more fat centrally, i.e. have more visceral fat (apple-shaped fat distribution), and Whites and Asian will store more fat peripherally, i.e. have more subcutaneous fat (pear-shape fat distribution).

Objective: The specific aims of this project are to collect preliminary information about the relationship between BMI or ethnicity and the amount and distribution of body fat.

Methods: We will recruit African-American, Asian, Caucasian, and Hispanic young women, age 18-30, for our study. We plan to enroll a minimum of twenty-four women, six per group to participate in the study. Before entering the study, subjects will be screened to determine their eligibility for the study. All women who participate in the study must meet the following criteria: BMI between 20 and 35, not currently taking prescribed medicines to treat medical conditions such as, asthma, diabetes, heart disease, and/or hypertension, and willing to visit the Cholesterol Research Center (CRC) for one, one-hour afternoon assessment.

During the assessment at the CRC, we will measure the subjects' weight to the nearest 0.1 kg by using a standing digital scale (BWB-800, Tanita, Arlington Heights, IL.) These measurements will help us calculate the subject's Body Mass Index (BMI). Next, we will measure the subjects' waist and hip circumferences to the nearest 0.1 cm. These measurements will help us calculate their waist-to-hip ratio. Finally, we will measure the subjects' body fat by the following **Methods:** 1) measuring the density and volume of their body 2) measuring the thickness of a fold of fat under their skin. We will estimate the subjects' body density using the Bod Pod body composition system (Life Measurement Instruments, Concord, CA, USA). The subjects' fat fold thickness measurements will be made at four sites (biceps, triceps, subscapular, and suprailiac). At the conclusion of the subjects' visit, we will give them a print out of their body composition information.

Anticipated outcome: We predict that neither ethnicity nor BMI will be related to the amount of body fat, but that the distribution of body fat may vary by ethnicity.

NEIL ALMEIDA

Funded by the Jennifer Leigh Wells Fellowship

Washington High School

Mentor: Gregory Moe, PhD

I am Neil Almeida, and this fall I will be a senior at Washington High School in Fremont, California. This is the first time I have had the opportunity to participate in a research program. Here at CHORI, I am learning a great deal of new information that is not commonly found in high school course material. Unlike the school setting, we strive to find results instead of reading them from a book. At CHORI, I experienced many of today's cutting-edge technology and procedures in the biomedical field and I am confident that the research we conducted here will have a positive turnout and will help save many lives in the future.

First off, I would like to thank everyone in my lab group, especially Dr. Moe. He believed in my capabilities and gave me the chance to participate in such a prestigious program. This summer in the Moe Lab, our research focused on which ORF6 *genes are expressed in Nm strain H44/76 under different culture conditions. The Moe Lab is working on several projects that developed from studies of a sugar molecule, NeuPSA.*

Also, the frequent seminars on current issues related to bio-medical field and the importance of ethics in our research provided me with a great deal of insight. The information that I learned here is very important to me and will help lay a strong foundation for my work later on. The learning experience at CHORI was enjoyable and rewarding. All those brilliant individuals around me made this summer remarkable and I am proud to be part of this team. This program has given me a higher appreciation for the work of scientists and will help me pursue a career in biomedicine.

Expression of *ORF6* genes by *Neisseria meningitidis* under different culture conditions

Neil Almeida and Gregory Moe

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: *Neisseria meningitidis (*Nm) is a human pathogen that can cause life-threatening bacteremia and meningitis. Although carriage of Nm in the nasopharnyx is relatively common, meningococcal disease is rare. A recent epidemiological study by Bille et al, [1,2] showed that Nm strains causing invasive disease carried prophage DNA, particularly a gene called *ORF6*. There are three *ORF6* genes in strain H44/76. Our hypothesis is that expression of one or more *ORF6* genes is dependent on human serum.

Objective: The aim of this project is to find out how many and which *ORF6* genes are expressed in Nm strain H44/76 under different culture conditions.

Methods: First we isolated the mRNA from wild-type and *ORF6* knockout mutants using the GeneJet RNA purification kit (Fermentas). The number of *ORF6* transcripts will be determined by Northern blot analysis using DNA probes labeled with biotin by PCR amplification of a conserved segment of *ORF6* in the presence of Biotin-16 dUTP (Roche). RNA transcripts on the Northern blot will be detected with Strepavidin IRDye®800CW (LICOR). To determine which *ORF6* genes are expressed, we will use Nuclease Protection.

Results: So far, we have created the Biotin-16-dUTP-labeled probes by amplifying a 680bp, highly conserved segment of *ORF6* from genomic DNA prepared from wild-type H44/76 using Taq polymerase and biotin-16-dUTP. Results from detection of mRNA transcripts by Northern blot hybridization are still pending.

Conclusions: Though the study is still in progress, the anticipated outcome is that one or more of the *ORF6* genes are expressed and the nuclease protection assay will reveal the identity of the expressed genes.

1. Bille E, Ure R, Gray SJ, Kaczmarski EB, McCarthy ND, et al. (2008) Association of a bacteriophage with meningococcal disease in young adults. PLoS One 3: e3885.

2. Bille E, Zahar JR, Perrin A, Morelle S, Kriz P, et al. (2005) A chromosomally integrated bacteriophage in invasive meningococci. J Exp Med 201: 1905-1913.

ANAIS AMAYA

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley, Senior

Mentor: Mindy Benson

My name is Anais Amaya and I'm a senior at UC Berkeley. I am also a mother to an 8 year old boy and a military wife. Life has taken me in a different direction from most women my age. Finding a research program that understands different backgrounds is difficult to find. I was first introduced to CHORI by an advisor who believed this program was the perfect match for me. I am glad to admit she was correct and this is my second year as a CHORI summer intern with Mindy Benson as my mentor. This program has impacted my life in ways I never imagined. I will always be grateful for the lessons I've learned from the Asthma Team. I never imagined I would enjoy doing research as much as I have during the past year. I would like to thank my mentor Mindy Benson, Robert Mok, Lisa Caine, and everyone else at the Claremont Primary Clinic for all their support and guidance.

Outcome of Self-Management Education at Camp Breathe Easy

Anais Amaya, Christine Schudel and Mindy Benson

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: Asthma is the leading chronic illness and cause of school absences in children and adolescents. Low income and inner city children have the highest number of Emergency Department visits and hospitalizations than any other asthmatic group. Camp Breathe Easy targets inner city children with asthma who face health disparities. The goal for the children who attend the camp is for them to leave with a better understanding about asthma and therefore acquire better skills to manage it. The campers are able to participate in traditional camp activities in an environment suited for their needs. There are two educational sessions given during the four day camp that last forty-five minutes each. It is hypothesized that children who successfully complete the camp have a reduced number of Emergency Department visits and hospitalizations due to asthma symptoms one year after attending the camp.

Objective: The objective of this project is to evaluate whether the camp's educational sessions increases the attendee's skill to manage their asthma and therefore decrease Emergency Department visits and hospitalizations for asthma symptoms post camp. The purpose of this project is to build and strengthen the evidence base for future camp studies and ultimately increase the funding available for the camp.

Methods: This project is a one group pretest-posttest design. A list of campers who attended Camp Breathe Easy in 2011 was compiled**.** Due to limited resources, only CHRCO patient hospital records were reviewed. The number of times each individual attended Children's Hospital Oakland Emergency Department for asthma symptoms within one year prior to attending Camp Breathe Easy was recorded as well as the number of times the camper returned to Children's Hospital Oakland Emergency Department for asthma symptoms one year after attending the camp. Once the data collection was completed, the data was entered in statistical software and descriptive and analytical analyses were performed using SPSS. A t-test was used to determine if there was any significant difference between the means of Emergency Department visits and hospitalizations pre-camp vs post camp.

Results: Pending

Conclusions: Pending

KELLY BAUER

Funded by the Jennifer Leigh Wells Fellowship

Harvard University, Junior

Mentor: Gregory Moe

I have wanted to become involved with Oakland Children's Hospital, since the day I stepped through the doors to visit my younger sister who was being treated for Leukemia. My passion for medicine and biology was sparked by observing her treatment throughout. I cherished the moments when the doctors would take the time to explain to me exactly what tests they were performing, what measurements they were calculating, and how the various medications were eradicating my sister's cancer. After three years of treatment, her health was restored, and my interest for medicine was unwavering.
I made a promise to myself, that one day I would become a member of the medical community, and finally get the chance to become the actor, not the observer. Through working for CHORI this summer, I have started the journey of achieving this goal. Working in the lab with my mentor, Dr. Moe, has been an absolutely amazing experience, and has really opened my eyes to the countless opportunities there are to discover and solve the mysteries of disease. The exposure to the research staff, projects, and procedures at CHORI has helped develop my appreciation and skills in medical research, and has allowed me to the opportunity to explore and actively immerse myself in an element of human biology which I have been seeking. I am so thrilled and honored to have spent the summer surrounded by so many bright and incredible minds, and I hope to continue pursuing my passion for science, which CHORI has made so much stronger.

Finding Pathway used for the Uptake of Exogenous NeuPSA

Kelly Bauer and Gregory Moe

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Moe et al identified a derivative of Polysialic Acid (PSA), called de-N-acetylated polysialic acid (NeuPSA), which is highly expressed in human melanoma tumors and other cancer cell lines. Human SK-MEL-28 melanoma cells have been shown to exogenously take up NeuPSA from their environment, which results in an increased production of NeuPSA modified antigens. However, the mechanism for NeuPSA uptake is not known. We hypothesize that exogenous NeuPSA uptake is mediated by fluid phase pinocytosis. This prediction is based on the findings of Bardor et al, who showed that a sialic acid derivative acquired by humans from dietary sources, N-glycolylneuranimic acid (Neu5GC), is mediated by fluid phase pinocytosis. Because NeuPSA is also a sialic acid derivative, it may be scavenged by a similar mechanism. However, in preliminary studies, the Moe lab observed that survival of human Jurkat lymphoblastic leukemia cells incubated with lethal concentrations of NeuPSA and given drugs that blocked different uptake pathways, increased when pinocytosis mediated by clathrin was blocked. Therefore, an alternative possibility is that NeuPSA uptake is receptor-mediated through clathrin coated pits.

Objective: Because the way in which cells absorb exogenous NeuPSA is unknown, our objective is to block three potential pathways, including a clathrin-dependent pathway using Chlorpromazine, clathrin-independent pathways using Nystatin and Genistein, and fluid phase pinocytosis using Amiloride. We will measure the amount of PSA and NeuPSA expressed in the presence of these different drugs to determine which blocked pathway is most affected, and therefore used in the reuptake of NeuPSA.

Methods: We incubated SK-MEL-28 cells in media alone or in media supplemented with amiloride (3mM), chlorpromazine (6 μg/ml), genistein (200 μM), or nystatin (25 μg/ml) that contained no PSA derivative, or 1 mg/ml NeuPSA (57% Neu) or PSA. We then made the cell membranes permeble using Triton-X100 (0.25% vol/vol), and added the primary antibody, Seam 2 (10 μg/ml), 4to each of the wells, and then added the secondary antibody, goat anti-mouse IgG3 conjugated to AlexaFluor-488. We then used flow cytometry to measure the relative binding of Seam 2.

Results: We are still in the process of performing our experiments, however we anticipate that by blocking fluid phase pinocytosis, we will not see an increase in the production of NeuPSA when exogenous NeuPSA is added to the media. However, given the background of other studies, it is possible that by blocking the Clathrin pathway, we will not see an increase in the production of intracellular NeuPSA-modified antigens in the presence of exogenous NeuPSA.

Conclusions: If, in fact, we do not see an increase in the production of NeuPSA after blocking the fluid phase pinocytosis with amiloride, we can conclude that the pathway cells use to uptake exogenous NeuPSA is fluid phase pinocytosis.

SAMANEH BOLOURCHI

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley, Junior

Mentor: Edward Lammer, MD

The expression, "curiosity killed the cat", never applied in my family. As a child, my parents always encouraged me to ask questions and provoke discussions, constantly trying to motivate me to learn more and more everyday. On our annual trips to Lake Tahoe and Yosemite, I was always fascinated by the wildlife, giant trees and enormous mountains. I loved to examine things up close, questioning how they were made and contemplating the purpose of their existence. It was from that age that I knew I had a passion for science that only seemed to grow as I grew. Since then, not much has changed: my inquisitiveness about the world and our environment continues to push me towards broadening my knowledge about the things that surround me. This fall, I will be beginning my third year at Cal pursuing a Bachelors of Science in Molecular Environmental Biology and a minor in Ethnic Studies.

Through this internship at CHORI, I have strengthened my enthusiasm for science and learned so much through hands on lab work, lectures, and from the faculty around me. Because of CHORI, I have now had the opportunity to perform clinical lab research, as well as wet lab research. I have received invaluable guidance and knowledge from everyone in the Lammer lab. I must thank Dr. Edward Lammer, Kazutoyo Osoegawa, Nebil Mohammad, Kathleen Schultz and Christina Parodi for answering my numerous questions, taking time to explain concepts, and showing me proper lab techniques. Through their dedication, I have been able to expand my understanding of the theory and application of scientific research and I am forever thankful for such an opportunity.

Using genotyping to fine map candidate loci on chromosome 1 in an infant with Tetralogy of fallot

Samaneh Bolourchi, K. Schultz, C. Parodi, N. Mohammad and K. Osoegawa, E. Lammer

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Congenital heart defects are among the most common form of birth defect in infants, affecting roughly 4 to 8 per 1,000 births (Pierpont et al. '07). In this case, Dr. Lammer's lab is focusing on two specific conotruncal heart defects: tetralogy of Fallot (TOF) and dextro-transposition of the great arteries (d-TGA). Together, tetralogy of Fallot and d-TGA constitute 75% of conotruncal defects, presenting us with an alarmingly high percentage of infants with life-threatening cyanotic heart defects. Treatment of these infants requires palliative and/or major corrective surgery within the first year of life, often requiring further interventions throughout their lives. Research identifying the genetic factors involved in heart defects is crucial for improving individualized case management.

Objective: Fine mapping of candidate loci within the 2Mb at 1q44ter that has the greatest possibility of being associated with conotruncal heart defects.

Methods: This study examines the chromosomal microdeletions of case-C, an infant born with Tetralogy of fallot. We used the case-control research method to conduct SNP genotyping on possible candidate genes in the target loci within the 2Mb region at 1q44-qter. TagSNPs with the greatest minor allele frequency were selected across the segments, and the MALDI-TOF mass spec was used for genotyping for association analyses after designing multiplexed genotyping assays for the Sequenom MALDI-TOF Mass Array System. Samples were sorted into batches including controls, cases, and blank wells for data quality assurance.

Results: Results from this project are still pending. Data will be analyzed to test Hardy-Weinberg Equilibrium for the SNP genotypes among the controls and to examine associations between SNP genotypes/halpotypes and risk for conotruncal heart defects by calculating odd ratios.

Conclusion: If the results narrow the regions of risk, we can then implement a plan to sequence the narrowed regions of interest for cases as well as controls that will lead us to identify a gene or regulatory region important for heart development. Often times, due to the lack of genetic information regarding mutations and which pathways of gene expression they impact, it is unknown which organ systems will be most severely affected. Thus, this information would allow for pediatricians to optimize the quality of care they can provide for their patients.

KAREN BURTT

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley, Senior

Mentor: Pieter de Jong, PhD, Christine Jung and Alfred Tsang

As senior at UC Berkeley studying Bioengineering, the majority of my coursework focuses on mathematical derivation and computational modeling. Consequently, having the opportunity to learn essential lab techniques at CHORI has been incredibly beneficial in expanding my scientific abilities. With the skills I have learned this summer, I will face the forthcoming task of finding employment and establishing a career with aptitude, confidence, and hopefully success.

Returning to the CHORI Summer Student Program for my third year, I was placed into a completely different environment than I have become accustomed to at Children's Hospital Oakland. Transitioning from clinical research to a laboratory environment has been a humbling, yet vastly rewarding, transformation. Analyzing x-rays and optimizing cerebral palsy treatment has been replaced by midi-prepping cell cultures and running PCR. Having enjoyed working in both clinical and basic research environments, I have learned a wide range of research skills and have been involved in a variety of different fields of medicine and science. Based on these experiences, I look forward to completing my degree and doing research as a career.

My PI and mentor, Dr. Pieter de Jong, and my fellow lab members and supervisors, Dr. Christine Jung and Alfred Tsang, have made this summer memorable and unique. They have shown me how to be a scientist. I would like to thank them for their continued support and guidance.

Conversion of Human Pluripotent Stem Cells into Mouse Embryonic Stem Cell-like State Facilitates Genetic Engineering

Karen Burtt, Alfred Tsang, Christine Jung & Pieter de Jong

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Human embryonic stem cells (hESCs) hold great potential in regenerative medicine as an unlimited source of pluripotent cells for use in tissue replacement, as well as an efficient cellular model for studying various human diseases. However, in spite of the exciting potential engendered in these cells, several major obstacles have hindered the translational potential of hESCs toward clinical applications: 1) because hESCs cannot undergo clonal selection, genetic manipulation necessary for evaluating the differentiation potential through lineage tracking have been very difficult; and 2) hESCs are prone to frequent karyotypic instability, raising concerns regarding their safety in the transplanted tissue. Unlike hESCs, mouse ESCs are capable of undergoing clonal selection, and are karyotypically more stable than hESCs. During the past two decades, these characteristics have allowed for extensive studies delineating the nature of pluripotency and 'stemness' using mESCs as a model for mammalian development. Interestingly, an emerging body literature suggests that the differences between human and mouse ESCs may be attributed to the fact that hESCs are not *embryonic* in nature, but in fact, more close to *epiblast* stem cells. Indeed, several recent studies have shown that *epiblast* stem cells isolated from the developing mouse embryo appear very similar, both morphologically and behaviorally, to human ESCs. Hence, we sought to determine whether it is possible to convert hESCs into a mESC-like state, and whether this conversion would give rise to a cell type that is karyotypically more stable, and capable of undergoing clonal selection following genetic engineering.

Objective: The primary objective of our study is to convert hESCs into a mESC-like state. In so doing, we seek to generate a pluripotent human stem cell type that is karyotypically more stable, and capable of undergoing clonal selection following genetic manipulation.

Methods: In order to convert hESCs into a mESC-like state, we will utilize chemical inhibitors to suppress the activation of two major signaling pathways that are reported in the literature to play an important role during early differentiation. The conversion process from hESCs to mESC-like state will be determined by the expression level of a gene called *Dazl*, which is highly expressed mESCs, while the expression is severely attenuated in hESCs. Reverse transcriptase PCR, real-time qPCR, and western-blot assays will be utilized to evaluate the expression level of *Dazl*. Metaphse spread will be used to evaluate the stability of the karyotype following the conversion process. Fluorescent reporter will be introduced into the converted hESCs, followed by clonal selection, to determine whether these cells are capable of undergoing genetic alteration. Lastly, the converted hESCs will be differentiated along an endodermal cellular lineage in order to evaluate the differentiation potential.

Results: Pending.

Conclusion: Pending.

KEVIN CHEN

Volunteer

Alameda High School, Senior

Mentor: Wen-Shu Wu, PhD

The greatest challenge I face is my ceaseless hunger, my insatiable appetite. Not just for food, but also for knowledge, understanding, and solutions; for I firmly believe in the potential humanity has to overcome obstacles, impediments, and adversity through the utilization of the mind. This faith in humanity's capacity seemed to go hand in hand with my parents' work: science. Being the son of two scientists exposed my young, weldable, impressionistic, mind to the great scientific theories: Einstein's Special Theory of Relativity, the Bohr Atomic Model, Darwin's Theory of Evolution, and much more. Captivated by science, I often immersed myself in the readings my mother supplied to me. Yet, despite my passion for the sciences, school science was my least favorite class throughout middle school and high school.

Grade school science class fails to capture the excitement, vigor, and rigor of laboratory research work, and those aspects of science, I experience each day when I come to CHORI in the morning. The laboratory is the ultimate arena to put knowledge to applications, and results to solutions, unlike the dry, monotonous, pedestrian environment of a school science class. CHORI unlocks the gateway I yearn for, to enter into the forefront of the frontier that is research. To Dr. Wen-Shu Wu, Fransisca Heriyanto, and all the other wonderful people in the Wu Lab, thanks for the great help you have all given me and thanks for allowing me to experience that special quality of science that always draws me back!

Modified Approaches for Efficient Assembly of TALEs

Kevin Chen, Fransisca Heriyanto and Wen-Shu Wu

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Transcription Activator-Like Effector Nucleases (TALENs) are artificial restriction enzymes generated by fusing the DNA binding domain of Transcription Activator-Like Effector (TALE) to the non-specific DNA cleavage domain from the end of the FokI endonuclease. The central domain of the TALE consists of 15.5 to 19.5 tandem repeats, termed monomers; each monomer consists of 34 amino acids, which facilitate DNA recognition and binding.

TALEN technology offers potential application for the treatment of Sickle Cell Disease, caused by a single nucleotide mutation in the human hemoglobin (HBB) gene; recently, the Wu Lab demonstrated cleavage activity of an HBB-specific TALEN.

There are several ways to assemble TALEs, but all of the contemporary approaches involve stepwise cloning of TALE monomers, which require at least two to three weeks until completion of the TALEs. Efficient TALE assembly would facilitate growth in the field of genetic engineering.

Objective: This experiment is an endeavor to optimize the construction process of the first hexamer of HBB-TALEN3, left binding site. The steps include monomer amplification by polymerase chain reaction (PCR), T7 Exonuclease digestion, and ligation into hexamers. During amplification, the reagents under study are the modified primers for proper overhang during exonuclease treatment, Phusion GC Buffer, HF Buffer, dimethyl sulfoxide (DMSO), and Phusion HF Taq Polymerase. Those under study during ligation are RecA with T4 Ligase and Tth RecA with 9°N Ligase.

Methods: Improved amplification of the monomers by PCR will provide greater quantities of monomers with their respective primers. T7 Exonuclease digestion and subsequent ligation with and without RecA and Tth RecA test the efficacy of these two different enzymes and their respective ligases. The conditions: on ice, at 70°C, and clean-up are tested in between digestion and ligation to determine optimal conditions for exonuclease activity.

Results: PCR with 3% DMSO produces higher concentrations of each monomer, bearing 5 times greater concentrations than those from previous trials. Ligation with and without RecA in the T4 Ligase mix, for all three intermediate conditions, produced a smear, but no clear bands after gel electrophoresis. However, the 9°N Ligase mix produced clearer bands for both conditions: with and without Tth RecA; the clearest ligation bands occurred after clean up as an intermediate step between digestion and ligation.

Conclusions: Our results so far demonstrate which of the reagents under study are most efficient during hexamer construction. However, even at peak performance, these reagents still produce a majority of dimers rather than hexamers; for this reason, further study is necessary to improve our protocol.

LOIS CHEN

Funded by California Institute for Regenerative Medicine Creativity Award

American Indian Public High School, Senior

Mentor: Wen-Shu Wu, PhD

My passion in pursuing a career in health sciences encouraged me to apply for CHORI's summer program. Attending a small school with a senior class of 21, I was always sheltered in a small community where I knew everyone. Due to my school's lack of resources, I had never worked in a science lab or had any hands-on experience in my science classes. Being involved in a research group this summer, I want to break away from my comfort zone, seek a new adventure, and learn more about illness and pathology after witnessing the death of my father and illness of close family members. I will be studying nursing at New York University in the fall. This research on stem cell will help me to achieve my goal to improve people's lives by developing potential therapeutics for heart disease, Parkinson's disease, and diabetes.

Construction of a single lentiviral vector for efficient generation of iPS cells

Lois Chen, Fransisca Heriyanto, Di Xiang and Wen-Shu Wu

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Embryonic stem cells are pluripotent, which can differentiate into human somatic cells, and are self-replenished. However, the use of human embryonic stem cells has become an ethical issue among the science community and the general public. Serving as an alternative to embryonic stem cells, induced pluripotent stem (iPS) cells enable the possibility to obtain healthy renewable cells and tissue to treat numerous illnesses, including heart disease, Parkinson's disease, and diabetes. iPS cells are a type of pluripotent stem cell induced from a non-pluripotent cell through the introduction of transcription factors.

Shinya Yamanaka's team first produced iPS cells in 2006 from mouse cells and in 2007 from human cells at Kyoto University, Japan. This allowed researchers to obtain pluripotent stem cells, which are critical in research and possibly have therapeutic applications. Since iPS cells are created from a patient's own somatic cells, any cell forming the body of an organism, treatment of the iPS cells would prevent any immune responses of the body.

iPS cells are created by forced- expression of certain stem cell-associated genes (p65-Oct4, Sox2, KIF4, c-Myc) into non-pluripotent cells. However, the efficiency of iPS cell generation is very low. Here, we constructed a new lentiviral vector to enhance iPS cell generation. We used the two required genes (p65-Oct4, Sox2) for the reprogramming of somatic cells, gene miR302 to enhance the reprogramming efficiency, and gene IRES-GFPuro for selection purpose.

Objective: 1) Creating pLTiSFFV lentiviral vector with the SFFV promoter needed for gene expression of p65-Oct4, Sox2, miR302, IRES-GFPuro; 2) Creating pLTiSFFV-p65VP16-OS-miR302-IRES-GFPuro lentiviral by cloning of the four genes into pLTiSFFV vector.

Methods: 1) Construction of pLTiSFFV vector with SFFV promoter. Through PCR we obtained the SFFV-OS DNA fragment. We digested the pLM-vexGFP-Oct4 vector and the SFFV-OS DNA fragment using Xho1 and Sal1 restriction enzymes. We ligated these two digested DNA fragments together to generate the pLTiSFFV vector. 2) Construction of the lentiviral vector pLTiSFFV-p65VP16-OS-miR302-IRES-GFPuro. With PCR, we will construct the pLTiSFFV-p65VP16-OS-miR302-IRES-GFPuro lentiviral vector by the cold-fusion cloning of the four genes (p65-Oct4, Sox2, miR302, IRES-GFPuro) into the pLTiSFFV vector.

Results: We successfully constructed five PCR fragments (p65-Oct4, Sox2, miR302, IRES-GFPuro, PCR SFFV) and the pLTiSFFV vector by digesting both the plasmid pLM-vexGFP-Oct4 and PCR SFFV fragment with enzymes Xho1 and Sal1 and ligating the plasmid with PCR SFFV fragment.

Conclusions: Pending

OGOCHUKWU CHUKWU

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

Post-Baccalaureate UC Berkeley Extension, Certificate Program in Biomedical Sciences

Mentors: Ashok Kumar, PhD and Julie D Saba, MD, PhD

Losing several family members to Cancer has made me aware and passionate for the need for me to gain fundamental knowledge on the biology of Cancer. The past two years, I have launched a cancer awareness program at my local Nigerian Village Association: Amafor Village United- Bay Area Chapter. The program strives to make members of our village more proactive about managing their health. Although we are making great strides in educating our population, there are still several myths remaining on the minds of members of my community regarding cancer and other diseases that may plague us.

Through my experiences, I now know about health care disparities that plague inner city and underserved communities. Coming from an inner city community that has a high immigrant and minority population, a majority of the population lacks adequate knowledge of how to be proactive about their managing their health. I know firsthand the importance of proper health education, through cultural competency, and how it can noticeably increase quality of care.

I am so thankful for the opportunity to work in Dr. Saba's lab under the mentorship of Dr. Kumar. Through the CHORI Summer Research Program, I have gained a fundamental understanding into the world of cancer research. Having received such training from CHORI and acquiring a solid foundation, I will now have the proper skills and knowledgeable facts that I can educate my family and other members of my community with. Through CHORI, I also have regained confidence in my aspirations to pursue my medical degree. This in turn, allows me to play my part in helping to educate and support my community.

To study the effect of S1P lyase deficiency on pluripotency in human embryonic stem cells

Ogochukwu Chukwu, Ashok Kumar, Julie D Saba

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Embryonic stem cells (ESC) are pluripotent stem cells derived from the inner cell mass of an early developing embryo. Sphingosine-1-Phosphate, S1P, is a sphingolipid molecule that regulates several physiological functions including muscle regeneration, angiogenesis, lymphocyte trafficking, and pluripotency in stem cells. S1P's catabolizing enzyme, sphingosine-1-phosphate lyase (S1P lyase) irreversibly converts S1P into hexadecenal and ethanolamine phosphate and regulates S1P levels in tissues. From previous studies conducted in our lab, there has been evidence favoring the notion that S1P lyase regulates pluripotency in mouse ES cells through indirect inactivation of Signal Transducer and Activator of Transcription 3 (STAT3), an oncogenic protein. We hypothesized that S1P lyase deficiency in human ES cells would enhance the expression of pluripotency markers and would enhance cell proliferation.

To knockdown the S1P lyase gene, we infected human ES cells with lentivirus carrying short hairpin RNA against the human S1P lyase gene *(SGPL1)*. We selected stable clones of hES cell lines that had integrated shRNA into the genome by growing the cells on the media containing puromycin. We have verified the knockdown of S1P lyase gene expression by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and by western blotting. We are in the process of comparing the expression of pluripotency markers such as Oct-4, nanog and SSEA1 and oncogenic transcription factor STAT3 that regulates cell proliferation in control and S1P lyase knockdown cells.

RIGOBERTO DEL TORO

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley, Post-Baccalaureate

Mentor: Jodi Stookey, PhD

I would like to thank Dr. Jodi Stookey for her support and guidance and Dr. John Matsui for my academic and career accomplishments. *Exploration of my educational opportunities started when I met Dr. Stookey. Since becoming a volunteer in November 2009 at Children's Hospital of Oakland Research Institute (CHORI), I had the privilege of not only working with Dr. Stookey but with patients at the Outpatient Clinic. I was able to appreciate the different factors needed to provide the greatest quality of care, such as nutritional history, patient-doctor relationship, and personal and cultural sensitivity. I translated the concerns of patients, and my presence as a Latino helped them feel comfortable and confident in their nutritional care. Through the CHORI program, I have been able to take advantage of my opportunities to investigate the functions of a Physician Assistant (PA). My commitment to becoming a PA is strong. I have the ability and desire to help people through community effort. The PA pathway will help me find ways of helping people in need of health access and to work more directly with the underserved community. As a result, I will be attending Touro University California in the Fall 2012 to do a dual Masters in Public Health and Physician Assistant Studies.*

This summer 2012, I will be researching the food intake of fruits and vegetables among pregnant adults as well as overweight children with a low-income background throughout the bay area. The purpose of this study is to investigate if children who are given FREE fruits and vegetables like WIC or ready-to-eat like school lunch would increase their dietary intake of fruits and veggies. This is a pilot study to help inform agencies and the community that healthy eating habits are possible.

I am very delighted for taking part and thankful for the continued support and community at CHORI. Their appreciation for my support was extremely rewarding and my experience at CHORI further motivated me to pursue a career in the health sciences. This accomplishment empowered me to promote Latino and minority participation in medical research through not only the Biology Scholars Program but through my local community as well.

Free Fruits and Vegetables for Pediatric and Pregnant Patients

Rigoberto Del Toro and Jodi Stookey

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Reversing obesity is a national priority. Strategies to end excess pregnancy weight gain and childhood obesity are particularly needed for lower income groups, to reduce health disparities and prevent tracking of *overweight* from infancy through adolescence into adulthood. Tailoring a food assistance program to the needs of overweight participants holds great potential to reverse obesity, because half of the foods provided by existing food assistance programs (e.g. WIC approved foods) are *high glycemic foods that favor weight gain by depressing fat oxidation.* Many low income patients cannot change their dietary intake because they rely on food assistance programs. A program that primarily provides low glycemic of fruit and vegetables to overweight participants might better leverage the power of 'free' food for dietary change, and quickly shift large numbers of overweight lower income individuals towards normal weight. Unlike higher income individuals who do not have the time, resources, inclination, or transportation to shop and cook from scratch, low income individuals with the same constraints have a much more limited range of healthy food options. A program that addresses the income disparity in access to ready-to-eat salads, soups, bean/vegetable entrees, and fruit may facilitate compliance with clinician dietary change recommendations.

Objective: Our primary goal is to determine if a food assistance program that provides **ready-to-eat fruit** and vegetables **for free**, in amounts consistent with the value of other foods offered by WIC or school meals, can facilitate dietary change for low income children and pregnant women counseled to increase fruit and vegetable intake.

Methods: Overweight patients who are advised to increase intake of fruit and vegetables by a clinician at La Clinica de la Raza in Fruitvale Oakland are recruited for this study. Both children and pregnant women are included in this study. ALL patients seen at this clinic are under-resourced and underserved in the areas of nutrition and health access. By signing the consent materials, individuals indicate willingness to participate in three phone surveys, approximately two weeks apart, about factors that influence what people eat day-to-day. All participants receive one Safeway home delivery of ready-to-eat fruit and vegetable dishes valued at $50. Half of the participants are randomly selected to receive the food delivery after the first survey. The other half are surveyed twice before they receive the food delivery. This design will allow us to determine if the food delivery is associated with a change in fruit and vegetable intake compared to the group that has not yet received the delivery.

Expected Results: We expect delivery of ready-to-eat, free foods to be associated with significant increases in fruit and vegetable intake of low income, overweight pediatric and pregnant patients.

Conclusion: Data collection is still in progress.

JOAN DEVOE

Funded by the Elizabeth Nash FoundationI

University of Alabama in Huntsville, Senior

Mentors: Horst Fischer, PhD and Beate Illek, PhD

I am clearly the oldest of the summer students at CHORI this year. Some of them are the same age as my twenty-five year old daughter who is one of the main reasons I am working as a summer intern this year. In 1987 my daughter was diagnosed with cystic fibrosis (CF). Fortunately for all of us whose lives are affected by CF, the gene responsible for causing this genetic disease was discovered in 1989. Since that time we have anxiously awaited the moment for a cure to be found. Research and clinical trials take a lot of time, work and dedication, not to mention funding, but the progress that has been made since that time is nothing short of amazing. The life expectancy for a child born with CF in 1986 was approximately eighteen years; today the estimate has been extended to the late thirties. Recent drug discoveries bring renewed hope for a normal lifespan to young patients with cystic fibrosis.

My desire to be a part of research and my love for science motivated me to return to school, and I will complete my B.S. in Biological Sciences this December. Working as a summer intern in the Fischer-Illek Lab has been such an exciting and valuable experience for me. I feel privileged to have been able to gain laboratory research experience while participating in work that is important to so many people with Dr. Illek and Dr. Fischer. They have been patient and have taught me an enormous amount during the short time I have been here; their enthusiasm and determination has been an inspiration to me. They have provided me with renewed resolve to continue CF research, so I plan on pursuing a graduate degree in cell molecular biology.

I want to express my deepest appreciation to the Elizabeth Nash Foundation whose generosity and dedication to CF research has provided me with this opportunity. I am also grateful to Deborah Ellen whose support and guidance helped make it possible for me to participate in the CHORI internship. Adding to the list of things I have gained from CHORI are the friendships with my coworkers, who I will always cherish. They helped to make this a summer I will never forget. I look forward to seeing what goals they and the other CHORI students go on to achieve after this experience.

Proton Secretion by the HVCN1 channel and pH Equilibrium in Cystic Fibrosis Airway Epithelial Cells

Joan DeVoe , Kayla Horton, Beate Illek & Horst Fischer

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Cystic fibrosis (CF) is caused by a defect in the manufacture of the CF transmembrane protein conductance regulator (CFTR) responsible for anion transport and pH maintenance in airway epithelial cells. The voltage-dependent H+ channel (HVCN1) acts to regulate pH in the thin layer of fluid lining human airways called airway surface liquid (ASL). Evidence shows that pH regulation is crucial for the successful activity of the airway defense mechanism. Our hypothesis states that HVCN1 cells are localized in the apical membrane of airway epithelial cells and their function is to secrete H+ ions into the extracellular space, contributing to pH maintenance. Dual oxidase 1 (DUOX1) and dual oxidase 2 (DUOX2) are expressed in airway epithelium and generate the reactive oxygen species (ROS) used by lactoperoxidase to produce hypothiocyanate, an important bactericidal. The DUOX/lactoperoxidase system appears dependent on a neutral or slightly alkaline pH for proper function. Studies find CF ASL is one pH unit below normal and the lowered expression of the CFTR Cl- channel may be responsible for this difference.

Objective: The objectives of this experiment were to determine the ASL equilibrium pH of different types of airway epithelial cells, to localize HVCN1 in CF bronchial epithelial cells by confocal imaging and to determine the conductance activity of HVCN1.

Methods: CF bronchial epithelial cells homozygous for ΔF508 (CFBE41o-) were grown to confluence on Snapwell inserts (area = 1cm2) and used as a model for CF airway. Cells were stained for expression of HVCN1 with HVCN1 rabbit primary antibody R2 (kindly provided by Dr. Lishko, UC Berkeley) and secondary antibody Alexa 633 goat anti-rabbit IgG1. Cell orientation was determined by staining for zona occludens (ZO-1) tight junction protein with ZO-1 mouse primary antibody IgG and secondary antibody Alexa 488 goat anti-mouse IgG2. Cells were observed with a confocal microscope (LSM710, Zeiss, Inc.) using laser lines 488 nm and 633 nm. Rates of H+ and HCO_3^- secretion were measured with a TIM 856 Titration Manager using CFBE41o-, JME, CFBE41o-CFTR (stably transfected with a 6.2 kb cDNA construct containing the open reading frame of CFTR) and Calu-3 cells. pH equilibrium was used as a measure of ASL pH.

Results: Cells with normal CFTR have a higher equilibrium pH than cells with defective CFTR. pH equilibriums were JME/6.70±0.043, CFBE41o-/6.434±0.075 and CFBE41o-CFTR/7.179±0.145. Confocal imaging while exciting the stain at 633 indicated the presence of HVCN1 on apical cell surfaces and excitement at 488 localized ZO-1 in the tight junctions of CFBE cells. Current work is in progress on remaining objectives.

Conclusions: The more acidic pH in CF ASL inhibits effective airway defense. Airways require proper pH to support antibacterial activity of the DUOX/lactoperoxidase system. CFTR activity is compromised in CF and absence of regulation leads to lung inflammation and damage. Future studies to determine ways of altering ASL pH may offer new therapies for the CF treatment.

JASMINE EDELSTEIN

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

Princeton University, Junior

Mentor: Karen Ann Hardy, MD

I am entering my third year at Princeton University as a Biological and Chemical Engineering major, with the larger goal of conducting medical research from an engineering perspective. Working at Children's Hospital Oakland has been an excellent introduction to the clinical side of medical research. Under the supervision of Dr. Hardy, I investigated whether scheduling cystic fibrosis patients in a sequence based on their microbiologic flora categorization reduced exposure to antibiotic-resistant strains. In addition, I collaborated with a research team to assess the efficacy of aerosols and Airway Clearance Treatments in improving the outcome of patients with respiratory diseases. As part of my research, I had the opportunity to shadow physicians and respiratory therapists at the pulmonary ward, engaging in their discussions of unusual conditions. At the cystic fibrosis outpatient clinic, I caught a glimpse of what it feels like to be a patient diagnosed with a chronic disease and how families learn to thrive despite the diagnosis. I witnessed how the data collection sheet I helped design informed and empowered doctors to improve patient care, something I could have never done if I had just remained in a lab. With this new perspective, I proceed with a better of understanding of the impact my future research may have.

Outside of medicine, I have pursued my interests in renewable energy through research at UC Davis in crop sustainability and at Lawrence Berkeley National Labs in PEM fuel cells. However, medicine has remained my passion ever since reading books about the Black Death as a child. My time at the hospital has inspired a career in medical research. I would like to thank Dr. Hardy and Alex Wulff for integrating me into their team. Their passion was truly contagious.

Efficacy of Aerosols and ACTs in Patients with Respiratory Diseases

Jasmine Edelstein, Alex Wulff, Karen Hardy

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: ACTs move mucus from the periphery to the central airways, allowing the patient to cough to clear the airways. Aerosols thin mucus and moisten airways to make coughing more productive. Although these techniques have proven effective in CF patients, their efficacy in non-CF patients remains unclear except for those with cerebral palsy, neuromuscular disease, and atelectasis as found by a Cochrane systematic review.

Objective: To investigate the use of aerosols and ACTs in non-CF patients with respiratory problems by correlating diagnosis with outcome and cost. To assess whether an evidence-based approach facilitated communication among respiratory therapists (RTs) and ordering physicians.

Methods: Consecutive patients admitted to the pulmonary ward became subjects if ACT/aerosol was ordered. CF patients were excluded from study. A tool was devised for RTs to record indications, treatment plan, and daily outcome for each patient. A second tool was developed to summarize RT findings to the ordering physicians. Every 48 hours, a required outcome reassessment was made. Based on the sheets, investigators determined whether a subject's indications were appropriate, if his condition improved, and what cost was incurred.

Results: Seven patients have been enrolled in the study thus far.

Table: Acceptance (A) or rejection (R) of RT request to physician for change in treatment. Conditions improved (+), stabilized (0), or worsened (-) following treatment. For example, A,+ means the RT's request was accepted and that the patient's condition improved.

Pt	Indication for ACT	Timeline	Tx after Discharge
1	atelectasis with recurrent aspiratory pneumonias, DD	6/13: Admitted — 6/28: Discharged	able to return to home therapy
2	CPDD, abnormal gag	7/11: Admitted — 7/18: Discharged	able to return to home therapy
3	pulmonary dysphagia, ineffective cough; secondary to transverse myelitis	6/19: Admitted — 6/29: A,+ — 7/3: A,+ — 7/13: R,0 — 7/22: Discharged	returned home on new therapy
4	chronic lung disease	7/10: Admitted — 7/17: unknown — 7/20: Discharged	able to return to home therapy but new meds
5	ineffective cough; secondary to stroke from vasculitis	3/21: Admitted — 7/16: Discharged	able to return to home therapy
6	absent gag, absent cough; secondary to necrotizing encephalopathy	5/8: Admitted — 7/12: R,0 — 7/16: Discharged	transferred to different part of hospital for rehabilitation
7	congenital hypotonia, ineffective cough, sleep hypoventilation	7/11: Admitted — 7/11: R,- — 7/12: R,0 — 7/12: Discharged	able to return to home therapy

Conclusions: Use of ACTs and aerosols may help stabilize a patient's condition, but a larger sample size is needed to determine which conditions benefit most from treatment. Tracking of RT-physician interactions showed that RTs tend to reevaluate patient progress more often and favor less frequent treatment or less invasive techniques, whereas physicians are hesitant to change treatments. Further study regarding physician decision-making still required.

MICHELE FLETCHER

Volunteer

Saint Mary's College of California, B.S. Biochemistry

Mentor: Stephanie Doniger, MD

I've always loved a challenge, especially when it comes to answering difficult questions, and as a double major in Biological Basis of Behavior and Philosophy, I definitely encounter a lot of them. It definitely wasn't hard for me to decide on a career in medicine: I love helping people, and the opportunity to use my critical thinking in an ever-changing field to that end was an obvious choice.

This summer, I wanted to find a program that was serious about teaching its students about medicine and professional research. CHORI's summer program was just that and beyond. To me, scientific research is the perfect blend of analysis and creativity, and my project at CHO—using ultrasound to diagnose constipation in the Emergency Department—really exemplified both. Though ultrasound is a fairly common imaging technique, its use in pediatrics has yet to be fully understood was exciting to work on a study that was the first of its kind. My mentors, Dr. Stephanie Doniger and Dr. Nel Latronica, despite their busy schedules, were amazingly supportive as I learned how to conduct research with human subjects.

I loved working with the children who participated in the study, and learned a lot about the human aspect of research from both through the guidance of my mentors and my own experiences. Within only a week in the Emergency Department, I realized good patient care was more than just protocol. Every nurse and doctor I encountered treated the patients and families with such genuine compassion; I couldn't help being inspired. Before this summer, I had never considered working in the emergency room, but being a part of the collaborative, high energy atmosphere showed me a whole different side of medicine—one that I definitely enjoyed.

I'd like to thank my mentors Dr. Doniger and Dr. Latronica, for helping me so much with this project, as well as Dr. Bertram Lubin for introducing me to the program and for his guidance throughout the process..

Emergency Department Bedside Ultrasound Diagnosis of Constipation in Children

Michele Fletcher, Eva Delgado, Nel Latronica, Augie Saulys, Kevin Whitelaw, Stephanie Doniger

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Introduction: Constipation affects between 4% and 36% of children. A purely symptomatic diagnosis is not always accurate, especially when distinguishing functional constipation from other pathologies such as irritable bowel syndrome. Plain radiographs can visualize large fecal masses in the rectum, yet this imaging method is expensive and exposes patients to dangerous radiation. Ultrasonography can also image fecal masses, yet there is no literature on its use for the diagnosis of constipation in the emergency department. This study would be the first of its kind and a potentially valuable adjunct to confirming a clinical diagnosis.

Objective: The objective of this study was to determine the reliability of an abdominal ultrasound examination in assisting a diagnosis of functional constipation. As a result, constipation could be more quickly and effectively diagnosed, as ultrasonography could replace plain radiographs in imaging the diameter of the rectum and determining the presence of a fecal mass. Ultrasound could provide insight into the physiology of the patient's illness in an attempt to distinguish constipation from other gastrointestinal diseases with similar symptoms.

Methods: This observational study took place in the Emergency Department of CHO. XX children ages 4-18, whose chief complaint upon ED entry was abdominal pain, were enrolled in the study. Patients who had a suppository in the preceding 24 hours or had an anatomical abnormality were not enrolled. A Rome III Diagnostic for gastrointestinal disorders was administered to each patient, and the results scored according to Rome III guidelines. Additional patient history, including recent procedures, medications, and history of urinary tract infection, was also noted. An abdominal ultrasound was then performed to measure the transverse diameter of the rectum, using the bladder as an acoustic window. The volume of the bladder was calculated using measurements of its transverse width, transverse height, and sagittal width, with correction factor 0.75. Data were analyzed to determine a correlation between results of the Rome III diagnostic and the ultrasound examination.

Results: Pending

Conclusions: Thus far, the usefulness of ultrasonography remains unclear in diagnosing constipation. While the rectum is well visualized in the ultrasound exam, there is not enough data to establish a correlation between the results of the Rome III diagnostic and the ultrasound examination.

EIA GARDNER

Volunteer

Agnes Scott College, Senior

Mentor: Dr. Rachel Kuperman

I am Eia Gardner, a senior at Agnes Scott College in Atlanta Georgia, majoring in Public Health-Laboratory Track with a minor in Biology. This summer has been both a privilege and an honor participating in the Children's Hospital of Oakland Research Institute. The weekly lectures and seminars have grasps my attention and increased my knowledge on various subjects such as Angiography and Stem Cells along with improving my presentation skills. Being in a learning environment with the rest of the students all with different educational backgrounds was another good way to meet new long-term friends and learn from each other.

The highlight of my summer was conducting research with my mentor Dr. Rachel Kuperman. I could not thank her enough for giving up her time around such a busy schedule working in the Neurology Department at Children's Hospital. She has guided me through the process of how to properly research a subject, review charts properly and collecting data using software such as excel to create spreadsheets. My use of medical terminology has expanded along with my ability to be an independent worker.

Last year, participating in CHORI helped me decide that the medical field was right for me but not necessarily as a physician. This year, I have become more and more interested in Pediatric Nurse Practitioners; they have a wide range of opportunities in the medical field. As a Practitioner I feel as though I could combine my interest in clinical work with my interest in public health and welfare working with the community. With out the amazing team at CHORI, I would have never been able to make that decision. I thank all of CHORI's marvelous staff, especially Deborah Ellen because of her committed work and contact that she has with each student, she goes over and beyond what a coordinator is and I thank her for that. I would like to thank my mentor Dr. Kuperman for the opportunity to assist her on her research, along with Rhonda Burton, PNP and Rosalia Macias for always being available to assist me if needed and to all the staff currently in the Neurology Department. I thank Students Rising Above (Lauren Brener and Susan Troung), the program that supported me through this process and gave me the information about CHORI. Lastly, my family for their support through out the summer, my amazing grandmother, and God for placing the opportunity in my life.

Frequency of Hemiconvulsive Status in Dravet Syndrome

Eia Gardner and Rachel Kuperman

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: Dravet is a serve form of epilepsy. Children with Dravet Syndrome (DS), also known as severe myoclonic epilepsy of infancy (SMEI), have frequent episodes of prolonged seizure activity produced by fever. Known as pharmacoresistant febrile status epilepticus (FSE), where classic seizure medication fails to sufficiently control seizures typically seen during the first year of life or early childhood. DS can be caused by a defect in a gene that is required for signal propagation. DS is usually diagnosed after sodium testing occurs, where a positive mutation is found in the Sodium Ion Channel, alphatype sub-unit 1a gene (SCN1a). Children with DS typically experience poor development of language and motor skills, hyperactivity and difficulty relating to others. Currently there is no cure, but seizures can be reduced by anti-convulsant drugs and a Ketogenic diet, which is high in fats and low in carbohydrates. Most children with DS will be dependent on caregivers for life.

Objective: Approximately 50% or more of patient's with DS are seen in the Emergency Department or have contact with EMS after failed anticonvulsant abortive medication for status epilepticus annually. There have been no randomized trials looking at the effectiveness of acute anticonvulsant treatment in this population. The goal of this retrospective chart review is to determine baseline frequency of convulsive seizures requiring emergent anticonvulsant medications and /or presentation to the Emergency room. Therefore, using a retrospective chart review of children with DS we aim to determine the frequency of administration of emergency anticonvulsants, types of anticonvulsants administrated, and the duration of seizures.

Methods: Our experimental study design is a general study design. All patients with DS in the neurology department will have their charts retrospectively reviewed to look for frequency convulsive seizures, frequency of acute anticonvulsants management, frequency of emergency room visits and hospitalizations for status epilepticus. The data will be placed in an excel spreadsheet. No patient identifiers will be kept in the database. There are no inclusion and exclusion criteria for the study, all patients with DS, clinical or gene confirmed diagnosis will be included in the study. For the biostatistical design and analysis the sample size is approximately 10-15 patients in clinic at CHRCO. In analyzing data, likely raw data will be used- such as frequency. All subject population includes all gender's and ethnicity. The source of research material obtained from individually identifiable living human subjects includes a chart review. There are no recruitment plans or potential risks for the patients.

Results: Data are still being collected and analyzed using the retrospective chart review of children with DS. Data show that seizures have occurred as early as 3 months to 12 years. It was analyzed for each patient from 6 months to 3 years. With current data we determined that EMS was called on average 9.3 times. Patients suffering from a seizure were seen in the Emergency Department on average 11.5 times, and patients were hospitalized or admitted on average 3.6 times. Out of the 12 patients observed in the retrospective chart review, 9 of them have been tested positive for the SCN1a gene. There was an average of 21 seizures by each patient, and the average time for the duration of seizures was 9.9 minutes.

Conclusion: These preliminary results indicate that by creating a retrospective chart review of children with DS, we will observe a frequency status epilepticus. Knowing more about DS can improve the overall approach, treatment and education given to patients seen in the Emergency department annually.

ALICE GIANG

Funded by the Doris Duke Charitable Foundation

University of California, Los Angeles, Freshman

Mentor: Rachel Gilgoff, MD

My name is Alice Giang and I recently graduated from American Indian Public High School. I will be attending University of California, Los Angeles, in the fall and I will be majoring in psychobiology with a tentative double minor in sociology and dance. As of now, my plans for the future have not been set in stone and my wide array of interests continues to guide me in many directions. However, I aspire to be a criminal or forensic psychologist in the future because my ultimate goal is to lower recidivism rates in cities where homicide rates and crime rates are high, such as here in Oakland. I want to take a psychological perspective, noting the little factors involved people's lives before drawing conclusions.

This summer, I have the wonderful opportunity to be a part of CHORI and to work with a magnificent mentor from whom I am already learning so much. Even though our research project just began, I have already become immersed in this project because it has already captured my attention in an entirely positive manner. Everything involved in this project reminds me of why I aspire to be a criminal psychologist and why I love working with children. I hope that by pursing that career, I can help ensure the safety of children, and keep that young population educated and motivated to do what they aspire to do. This research project will help reinforce my understanding of what goes on with young children currently.

Causes of Subcutaneous Emphysema of the Neck and Pneumomediastinum

Alice Giang and Rachel Gilgoff

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: The finding of air within the skin, or subcutaneous emphysema, is a very uncommon pediatric finding. From a mechanistic standpoint, subcutaneous emphysema occurs when air tracks into the skin from external or internal perforation. Several case reports have suggested that unexplained subcutaneous emphysema of the neck is extremely concerning for child physical abuse.

Objective: The goal of this study was to expand on the current body of literature, to identify patients at CHRCO with pharyngeal subcutaneous emphysema to evaluate the mechanisms of injuries, and to determine if patients with spontaneous or unknown pharyngeal perforations are more likely to have other unexplained injuries such as bruising or broken bones than patients with known accidental perforations to suggest child physical abuse.

Methods: This study was a descriptive analysis involving a retrospective chart review of patients 18 and under who were admitted to CHRCO between Jan 1, 2009 and Dec 31, 2010 with a finding of subcutaneous emphysema of the neck and/or pneumomediastinum. Location of the emphysema, patient age, patient history, review of associated symptoms, diagnoses, studies done, suspected child abuse, and other related medical findings were collected and placed into a spreadsheet. Statistical analysis was done with the help of Clinical Research Services offered through CHORI's Clinical and Transitional Science Institute (CTSI) program.

Results: Twenty cases of subcutaneous emphysema and/or pnemomediastinum were found during our study period**.** The average age for the patients was 7.7±5.5. The causes of emphysema were 15% (3/20) iatrogenic, 20% (4/20) due to asthma, 35% (7/20) related to a lung infection (i.e. H1N1 or rhinovirus), 15% (3/20) caused by trauma (internal and external) and 15% (3/20) of unknown cause. Of the 20 cases, only one case was suspected of child abuse and reported to Child Protective Services (CPS). There were 3 cases in which the mechanism for the emphysema was unknown: one was the child abuse case, one was a child with cancer and multi-organ failure, and one was a "possible" pneumomediastinum that was "spontaneous" with no other injuries to suggest child abuse. Of the cases with only subcutaneous emphysema of the neck without pneumomediastinum, 50% (1/2) were of unknown cause and 50% (1/2) were from trauma. Of the cases with pneumomediastinum without tracking into the neck, 25% (1/4) were of unknown cause ("possible"), 25% (1/4) were from trauma (chugged Gatorade), 25% (1/4) were iatrogenic, and 25% (1/4) were related to a lung infection. Of the patients with both neck and mediastinal emphysema, 7.14% (1/14) were of unknown cause, 7.14% (1/14) were from trauma, 14.29% (2/14) were iatrogenic, 42.86% (6/14) were related to a lung infection, and 28.57% (4/14) were from asthma.

Conclusions: In our sample we found five different mechanisms for subcutaneous emphysema or pneumomediastinum. The mechanisms found included iatrogenic, asthma, infection, trauma, and unknown. It was interesting to note that over half of our subjects had a form of lung disease, either asthma or viral pneumonia as the cause. There were only three cases in which a cause of the emphysema was not discovered on initial evaluation. One of these was ultimately discovered to be a case of suspected child physical abuse. While the exact mechanism for the emphysema was not known in the child abuse case, the child was ultimately found to have numerous unexplained broken bones and bruising in addition to the subcutaneous emphysema. The other 2 cases of unknown cause did not have any signs or symptoms to suggest missed cases of child physical abuse. Only 3 cases total involved trauma: child abuse, swallowing a chicken bone, and a gunshot wound. Overall, the sample size was small and more research will be needed to determine the mechanistic link between subcutaneous emphysema of the neck and child physical abuse.

ADRIANA GONZALEZ

Funded by the Doris Duke Charitable Foundation

Holy Names High School, Senior

Mentor: Dr. Ward Hagar, MD

Hello! My name is Adriana Gonzalez and I just graduated from Holy Names High School. In the fall I will be a freshman at Denison University in Granville, Ohio. I plan on double majoring in Biology and Spanish. I want to attend Medical School to become a Pediatric Hematologist-Oncologist. Ever since I was a little girl I had always wanted to be a doctor because I knew it would be a perfect way for me to go out to impoverished communities to help those who are in need. After my experience from last summer I decided that I wanted to help kids with cancer, after meeting a little friend with stage 4 Neuroblastoma. Just seeing how happy he was when people were around him made me realize that kids with cancer need a lot of support, therefore I want to be a hematologist-oncologist.

This is my second year being a part the CHORI Summer Student Program and it has been a great way for me to get closer to my career goals. Having this wonderful experience helped me get into thirteen colleges because I wrote about my experience conducting clinical research. All the college admissions counselors were surprised with what I had accomplished and recognized me for that in my acceptance letters. I have learned so much about myself and about the work that I want to pursue in the future.

I have had the privilege of working with Dr. Hagar for the second year in a row. He has been such a great mentor and he has taught so much. He is a great mentor and I would like to thank him for taking time out of his busy days to help me continue my project. I would also like to thank Christy Hoehner for all of her help these last two years, and lastly I would like to thank the CHORI Program Directors for allowing me to be a part of the summer program.

Discordance between DXA Scans and Bone Health in Adult Sickle Cell Patients

Adriana Gonzalez, Christy Hoehner, Ellen Fung and Ward Hagar

Children's Hospital Oakland Research Institute, Oakland, and Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: Sickle cell disease (SCD) has been linked to low bone mineralization, avascular necrosis, and microfractures. SCD patients are routinely transfused with red cells in order to keep a high hemoglobin concentration to suppress sickle cell formation. My pilot study from last year showed the unexpected inverse relations of serum vitamin D levels and Bone Mineral Density (BMD) by DXA in hips and spine bone. The lower the vitamin D levels, the higher the bone density. Furthermore, the higher the liver iron concentrations were, the lower were the vitamin D levels. Possible explanations were, too small a data set, effect-cause with the lowest vitamin D level patients having spine compressions that would lead to higher DXA BMD values, or other unknown interactions. The clinical question raised by last year's work is whether normal DXA scans in sickle cell disease patients indicate healthy bones.

Objectives: 1. To see if the relationship of low vitamin D and high bone mineral density holds up in a larger dataset. 2. To examine the DXA scans of those patients with high BMD to see if there is evidence of vertebral compressions. 3. To see if subgroup analysis identifies a group where BMD results are misleading in sickle cell patients.

Methods: The adult sickle cell program has longitudinal records on patients with sickle cell disease. The database constructed last summer was expanded this year to include more recent and complete information on patients' spine and hip bone mineral densities. Relevant patient information was recorded in an ACCESS relational database as a nested cohort study. SQUID (Superconducting Quantum Inference Device) values are available on many patients to estimate liver iron storage. Images of the lumbar spine and thoracic spine will be evaluated to determine if there is bony vertebral compression. Co-variates such as Body Mass Index (BMI), spine bone mineral density, gender, and age will be collected. Data will be analyzed by STATA 12.1 software using appropriate statistical tests for continuous and categorical variables.

Results: Detailed results, including multivariate regressions, are pending.

Limitations: Cross-sectional studies have inherent limitations of patient selection, have no time component, and have possible unrecognized confounding variables. No clear biologic model is available to guide possible statistical analysis. Although the larger database is important and helps refine the statistical estimations, it will still be unclear how robust our results may be.

Conclusions: DXA scans are an unreliable measure of bone health in sickle cell patients. Low vitamin D levels associate with compression factors. Hip bone density is the most sensitive to vitamin D levels and possibly liver iron levels.

ISSAM HAMDALLAH

Funded by the Doris Duke Charitable Foundation

Kenyon College, Freshman

Mentor: Deborah Dean, MD

My name is Issam Hamdallah. I have just graduated from Riordan High school on a scholarship funded by Barbra Bass Bakar, and I will be attending Kenyon College in the fall. From an early age, I knew that I wanted to become a doctor. Initially, that goal was inspired by a belief that doctors were somehow immortal but as I grew up, a strong interest in the sciences and the chance to make a significant impact on the lives of others became my inspiration.

Growing up, I spent most of my time working with my father in a convenience store, as most boys in my family do. Although I enjoyed the company of the people I encountered on a daily basis, I realized that working in a store is not something I could see myself doing for the rest of my life. I am genuinely grateful to be working in the Dean lab where I will be researching Chlamydia under Dr. Dean and Thomas Pham. I am lucky to be working in a field that is both interesting, and will give me some skills and knowledge to help me reach my goal of eventually becoming a doctor. Most importantly, I am honored to be working with a team committed to mitigating the impact of Chlamydia on the world.

Mapping the distribution of *omp*A genotypes among clinical samples from different geographical areas

Issam Hamdallah, Aishwarya Shukla and Deborah Dean

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Chlamydia *trachomatis* is the most common bacterial sexually transmitted infection in the world. It is transmitted through intercourse or childbirth. According to the WHO, over 100 million *C. trachomatis* urogenital infections occur each year. The number of infections is estimated to be greater because most infections are asymptomatic. People often pass the infection onto their partner without even knowing that they are doing so. As a result, *C. trachomatis* is becoming an epidemic. Systematic screening, especially in developing countries, is not in place to mitigate the rapid spread. The lack of a vaccine, effective methods of prevention, and a rapid point of care diagnostic for screening, and test-of-cure are some reasons why *C. trachomatis* is spreading so rapidly. Symptoms of *C. trachomatis* include an abnormal discharge from the penis or vagina, urethral itch and burning upon urination. It is also known to cause infertility, abnormal bleeding and abdominal pain. Infants born with the infection are more susceptible to lung and eye infections. Men and women with the disease are more likely to contract other STD's such as HIV. *C. trachomatis* also causes Trachoma, which leads to the roughening of the eyelids and blindness. The *omp*A gene encodes the major outer member protein of *C. trachomatis*. The *omp*A gene can sometimes distinguish between ocular and genital isolates that represent the same serovar. That suggests that *omp*A genotyping can identify more *C. trachomatis* subtypes or strains than other methods such as serotyping. That is why our research focuses specifically on the *omp*A genotype.

Objectives: 1. Extract genomic DNA from clinical samples (urine and cervical swabs)**.** 2. *omp*A genotype each sample**.** 3. Identify mutations in *omp*A compared to reference *omp*A sequences**.** We hope to map the distribution of *C. trachomatis* *omp*A genotypes from the Boston and Amsterdam urine samples and cervical swabs positive for *C. trachomatis*. We also hope to discover patient samples that may be interesting to further investigate. For example, some samples may include a mutation that would need to be sent for further sequencing because little is known about it.

Methods: The ultimate goal is to genotype the ompA gene. In order to do that, we must first purify the genomic DNA from the urine, or cervical swab. Then we run PCR (Polymerase Chain Reaction) using primers specific for *omp*A, which amplifies the target DNA so that further analysis can be done. We then take the amplified DNA, place it into an Agarose gel and run Gel Electrophoresis, which isolates the amplified product. Next, we take a picture of the gel and extract the desired portion of DNA. Lastly, we send the DNA to a sequencing lab for sequencing.

Results: We are still working to modify our genomic DNA purification from urine so that we can extract genomic DNA from our urine samples that is pure enough to be successfully sequenced. The way to see whether the DNA obtained after genomic purification of DNA is pure enough for sequencing, is to measure it in a nanospectometer. If we obtain a 260/280 ratio between 1.7 and 2.0, that means that the machine absorbed mostly genomic DNA, but if we obtain a ratio greater or less than that range, that means that our sample does not contain pure genomic DNA. So far, the best 260/280 ratio we have gotten is 1.64. Also, we have recently begun practicing how to purify genomic DNA from cervical swabs. We have successfully purified genomic DNA from cultured samples with a ratio of 1.98 which is ideal. We have also successfully amplified *omp*A and purified the DNA from the amplified product so that it can be sequenced.

Conclusion: We have made significant progress in modifying our genomic DNA purification from urine protocol, but we still have to modify our protocol so that we can consistently extract pure genomic DNA from our urine samples. We cannot begin working on our clinical samples until this is achieved because we do not want to waste precious samples.

CHELSEA HEIMBAUGH

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

Cal Poly San Luis Obispo, 5th year

Mentor: LaBeaud, Desiree, MD, MS

I am a biology major with a minor in anthropology/geography. As such, I am passionate about both the health field and learning about different cultures. These interests recently compelled me to volunteer at a health clinic in Lima, Peru where I learned the impact of limited access to health care (including a high rate of infectious diseases) and the devastation that poverty brings, especially for children. I feel truly privileged for all the opportunities I've been given and for my parent's consistent love and support. I am also grateful for the opportunity to participate in Children's Hospital Oakland Research Institute's (CHORI) summer program.

My experience at CHORI so far has been eye opening. I have my mentor, Dr. LaBeaud, a pediatric infectious diseases physician scientist who does global health research, to thank for that. She showed me the utmost patience when answering all my questions, having me shadow her around Children's Hospital, and aiding me in the lab. I can only hope to share her enthusiasm and passion for my career one day. From working in the infectious diseases lab at CHORI, I now understand the value of medical research and how participating in research allows you to make a large impact in people's lives. More than ever, I want to go into pediatrics. Now, however, I am also strongly considering research as well.

Dengue Virus Seroprevalence in Rural Kenya

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Introduction: Dengue virus (DENV) infections cause incapacitating fever syndromes world-wide, yet they are often "overlooked" by public health and research programs, particularly in African countries where DENV has not been considered a major problem (1-3).

Objective: Serosurveys are needed to better understand DENV prevalence in Kenya where limited surveillance and likely misdiagnosis may have led to underestimates of DENV infection rates (4). We aimed to measure the seroprevalence of DENV in Milalani, a rural village on the Kenyan coast, and link seropositivity to age, gender, socioeconomic status, anthropometrics, parasitic infections, and other exposures at the household and individual levels.

Methods: Participants in a household-based cluster study of Milalani village were administered demographic, household inventory, and exposure questionnaires along with anthropometric, parasitic and hemoglobin testing. Sera were tested for exposure to DENV using the InBios DENV DetectTM IgG ELISA test kit, consisting of a "two-step" sandwich-type immunoassay. Plaque reduction neutralization testing (PRNT) will be performed to confirm seropositivity. Bivariate relationships for each potential predictor of DENV seropositivity were assessed based on x2 testing. Multivariable logistic regression was used to further test predictor variables for association with DENV seropositivity. Statistical analysis was performed using SAS version 9.3.

Results: Of the 560 Milalani study participants, 33 had equivocal ELISA results and were excluded from analysis. Of the remaining 527 participants, 286 (54.3%, 95% C.I. 49.5%-58.4) were DENV seropositive, aged 4 to 90 years. 222 (42.1%) of the 527 samples were from children (≤15 years of age) and of these, 38 (17.1%, 95% C.I. 12.1%-22.9%) were seropositive. 248 (81.3%, 95% C.I. 75.0%-84.4%) of the 305 adults in the study tested positive for DENV. Children were less likely to be seropositive than adults (17.1% vs. 81.3%; $p<0.0001$). Women were more likely to be seropositive than men, however, this difference was not significant (56.7% vs. 50.7%; $p=0.183$). PRNT and predictor variable results are pending.

Conclusions: Dengue is common in rural coastal Kenya. Cross-reactivity with other related viruses in the Flaviviridae family may have led to false positive DENV results, but it is clear that flavivirus exposure is widespread in this area of Kenya. Although analysis of DENV seroprevalence and epidemiologic data is pending, we expect DENV seropositivity to vary significantly according to socioeconomic status, exposure variables and parasitic co-infections. Further studies are needed to determine if there is large-scale variability in exposure and DENV seropositivity among other Kenyan villages.

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ROY HERNANDEZ

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

California State University, Long Beach, Post-Baccalaureate

Mentor: Vasanthy Narayanaswami, PhD

My interest in the inner workings of cell biology has changed my outlook on the world. With so many unanswered questions in biochemistry that need to be explored, my curiosity has driven me forward. I knew that I would dedicate myself to pursue a path of science. I come from a first generation working class background; most of my family has been relegated to service of manual labor work. Despite their strong work ethic, education to them has not always been a priority. I had a very different path in mind. Over the years, during my college career my interest in biochemistry has been developing and has concentrated on protein biochemistry and cell biology. With many interesting topics in biochemistry today and the unfilled gaps in science, my education thus far will not suffice for what I truly want to become. Thus the most logical path for me is the research setting where I can truly show my desire for enhancing the knowledge that exists today.

My mentor Dr. Narayanaswami and I have been working on the molecular structural differences of apolipoprotein E3 and aplioprotein E4 using hydrogen-deuterium exchange coupled with mass spectrometry. The utilization of fluorescence techniques to understand order of helix unfolding is also important to understand mechanistic differences between the two isoforms. ApoE4 is an isoform of the protein, which displays an increase development of atherosclerosis and Alzheimer's. Apolipoproteins play a huge role in maintaining proper levels of cholesterol, lipid, and fatty acid contents in human blood. ApoE3 deficient mice are perfect models for atherosclerosis, stressing the importance of functional apoE3. Being part of this summer research at CHORI has given me the wonderful experience to work with fellow researchers that love and enjoy their work. I would love to thank all the people that worked hard to put this together because without them, this wonderful research opportunity would not be possible.

Structural Analysis of Human Apolipoprotein E3 by Fluorescence Spectroscopy and Hydrogen/Deuterium Exchange Coupled to Mass Spectrometry

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Introduction: Apolipoprotein E3 (apoE3) is an important anti-atherogenic protein that helps maintain cholesterol levels in the brain and plasma. It is responsible for binding and cellular uptake of plasma lipoproteins via the low-density lipoprotein receptor family of proteins. It is a highly helical protein, which can exist in lipid-free and lipid-bound states. ApoE3 is composed of two domains in lipid-free state: an N-terminal (NT) domain folded into a 4-helix bundle and a C-terminal (CT) domain that mediates apoE3 oligomerization via inter-molecular helix-helix interactions.

Objective: The objective of this study is to understand the conformational organization of lipid-free apoE3 in its oligomeric state and to examine its order of unfolding.

Methods: We employed chemical-induced denaturation coupled to fluorescence spectroscopy to obtain information regarding helical mobility of apoE. Environmentally sensitive fluorescent probes monitored the mobility of different helices in the two domains. In a complementary approach, we used hydrogen/deuterium exchange coupled to mass spectrometry (HDX-MS) to understand amide-backbone structural dynamics, solvent accessibility, and helical contours of apoE3.

Results: Fluorescence intensity and polarization studies indicate that: (i) the unfolding is likely initiated at the C-terminal end of the protein, (ii) the CT domain unfolds prior to NT domain, and (iii) the NT domain forms a highly stable helix bundle. Fluorescence polarization data also suggest that helices 1 and 4 unfold prior to helices 3 and 2 in the NT domain 4-helix bundle. This allows for opening via a two-hinge mechanism. HDX-MS studies confirm data from fluorescence studies showing that: (i) the CT domain is relatively more accessible to exchange compared to NT domain, and (ii) the NT domain forms a tight helix bundle with lower accessibility to exchange with the exception of a loop segment between H2 and H3.

Conclusions: Our studies suggest that the two domains of apoE may undergo independent conformational reorganization, a concept that bears significant relevance in terms of apoE interaction with lipids and lipoproteins.

KAYLA HORTON

Funded by the Elizabeth Nash Foundation

California State University, Sacramento, Senior

Mentors: Beate Illek, PhD and Horst Fischer, PhD

My name is Kayla Horton and I am a senior at California State University of Sacramento. In Spring 2013 I will graduate with a B.S. in Cell and Molecular Biology and in Chemistry. Following graduation, I will be applying to graduate school and eventually striving for an MD/PhD I have always found science to be compelling, and the further I get in my education the more passionate and curious I have become for research.

My brother, Ben, has shaped my life in more ways than I can even begin to describe. He was born with Cystic Fibrosis (CF) and has battled with the disease for twenty years. His relentless passion for life, despite all of the struggles he faces, inspires me everyday. I have chosen to pursue a future in biomedical research with a focus on lung biology and pulmonary disease and I hope to take part in improving the successful treatment of CF.

This summer at CHORI has been the most amazing experience. Although it has been difficult to be away from my tight-knit family, the rewards from participation in this program are endless. It has provided me the opportunity to perform basic medical research inside of the laboratory. The Fischer-Illek lab has given me the chance to do hands-on bench science, where the exposure ranged from cell culturing to confocal microscopy. Dr. Illek's and Dr. Fischer's focus on CF and inflammatory airway disease was incredibly satisfying to be a part of. They are passionate and dedicated to their work, and for those of us with loved ones that can benefit from their research it is heartwarming. I thank them for their patience and for the knowledge that I gained working closely with them this summer. I also want to thank the Elizabeth Nash Foundation for providing me with this opportunity. I feel so fortunate to have been chosen by the foundation, and I know without Elizabeth Nash, my time at CHORI wouldn't have been made possible. Throughout this summer I gained friendships that will last a lifetime. Joni and Hira, the summer wouldn't have been the same without you and I can't wait to see what your futures hold. Lastly, I want to thank my family and friends for their infinite support and interest in what I am passionate about. They provide me the love that I use as fuel on this journey to pursue my dreams.

Determining CFTR Expression and HCO_3^- Secretions in Cystic Fibrosis Bronchial Epithelium

Kayla Horton, Joan DeVoe, Beate Illek & Horst Fischer

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Introduction: Cystic Fibrosis transmembrane conductance regulator (CFTR) is an apical anion channel, that recent studies suggest operates in parallel with H^+ channels (HVCN1) to determine airway surface liquid (ASL) pH. Proper functioning CFTR secretes bicarbonate (HCO_3) into the ASL, which drives the proton flux of HVCN1 into the ASL, neutralizing the alkaline environment. The airway defense mechanism of epithelial cells, DUOX/lactoperoxidase, generates the bactericidal compound hypothiocyanate, and is dependent on pH to function properly. In Cystic Fibrosis (CF), the CFTR is defected and the ASL tends to be more acidic than normal airways. This difference in pH is expected to cause inhibition of DUOX/lactoperoxidase, and the airways will be more susceptible to infection.

Objectives: (1) Localize CFTR protein in Cystic Fibrosis bronchial epithelial (CFBE) cells. (2) Determine the secretions of HCO_3^- across the apical membrane and the equilibrium pH of the CFBE, Calu-3, and JME cells. (3) To determine the efficacy of clinical trial compounds VX-809 (Kalydeco) and VX-770 (Ivacaftor) for restoration of HCO_3^- secretion in the CFBE cells.

Methods: CFBE41o-, homozygous ΔF508 CFTR, were complemented with a 6.2kb wildtype CFTR plasmid (CFTR-CFBE) or a 4.7kb DF508-CFTR plasmid (DF508-CFBE) and were grown as confluent monolayers. Calu-3 and JME p38 were used for further cell models. An immunostaining protocol was designed in order to stain for CFTR, Zo-1 (a tight junction protein), and nuclei. Images were taken using confocal microscopy method (Zeiss LSM 710). Equilibrium pH for the CFBE and JME cells was recorded using Ussing chambers with unbuffered solution (N_2) mucosally and a 25mM HCO_3^- solution (CO_2) serosally. The pH electrode detected mucosal pH after 30-35 minutes of equilibration, as a measure of ASL pH. The system was then titrated using TIM 856 with 0.5mM HCl and a target pH of 6.0.

Results: Calu-3 and CFBE cells were successfully stained for CFTR, Zo-1 and nuclei when using a primary antibody of 1:400 monoclonal CFTR 450 mouse IgG1 and 1:300 monoclonal Zo-1 rabbit, incubated 1-2 hours at room temperature. The secondary antibody was 1:500 of both polyclonal AlexaFluor 488 goat anti-mouse IgG2 and AlexaFluro 633 goat anti-rabbit, incubated, protected from light, for 1 hour. The nuclei were stained using Hoechst dye 33342. Calu-3 stains had the most abundant CFTR expression, while CFTR-CFBE had some areas of high expression and CFBE 41o- showed none. When other methods were used there was non-specific staining of the antibodies and comparisons couldn't be made. The equilibrium pH (n=3) averages were: CFTR-CFBE/7.179(+/-0.145), CFBE41o-/6.434(+/-0.075), JME/6.700(+/-0.043). The HCO_3^- secretion average rates (baseline, Forskolin, and CFTR-inhibitor) in nanomole HCO_3^-/hour: Calu-3/325, 285, 0.01, CFTR-CFBE/294, 270, 147, CFBE41o-/159, 80, 50 respectively. Results pending for VX-770 and VX-809 objectives.

Conclusions: Both of the CF cell models had more acidic equilibrium pH of the ASL than those with normal CFTR, which is an important factor that hinders the airways ability to fight off bacterial infection. There was an overall high expression of CFTR in all Calu-3 cells, while the CFTR-CFBE only had high expression in a low percentage of the cells. This difference of expression did not affect the CFTR-CFBE cells ability to secrete HCO_3^-; in fact, the two cell lines had similar secretion rates. It was seen that the proper pH of ASL could be maintained even when the bronchial epithelium CFTR expression was low. Therefore, studies with compounds, such as VX-770/VX-809, which could rescue small percentages of CFTR, could prove to have a significant impact in future CF treatment.

KIMPREET KAUR

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley; BA Integrative Biology

Mentor: Marsha Treadwell, PhD

Hello! My name is Kimpreet Kaur and I am a recent graduate from the University of California Berkeley, and also a returning CHORI summer student. Through out my educational career I knew I wanted to pursue becoming a physician, but it was not until last summer that I found my hidden passion for public health research. At that time, I began working with Dr. Marsha Treadwell at Children's Hospital Oakland, who mentored me on a health services research project evaluating mental health symptoms, barriers to accessing health care, and health related quality of life (HRQOL) among underserved minority patients with sickle cell disease (SCD). We found that models combining mental health symptoms and barriers to care predicted HRQOL. This work was chosen for oral presentation at the American Society of Hematology Annual Meeting, giving us a chance to educate providers on the importance of mental health screening and the potential impact on HRQOL and access to care for patients with SCD. Overall, my experience with my mentor has influenced my view of research and my career goal. I still want to become a physician, but also obtain a masters degree in public health so I can continue contributing to bettering the health of communities as well as individuals.

The CHORI program has been a big influence in my life. I could not have asked for a better educator, intellectual, and person as my mentor. She is one of my biggest supporters and always receptive to new research ideas. I truly appreciate Dr. Marsha Treadwell for exposing me to public health issues within my community that I would have been oblivious to otherwise. I am gaining knowledge about the disparities that exist within our community and I am becoming better able to appreciate the different factors that are essential to providing the greatest quality of care for people with chronic illnesses. I would like to thank the Sickle Cell Team for welcoming me and allowing me to contribute to their overall project. I am grateful and thankful to the wonderful people at CHORI for this summer internship experience.

Differential Reactivity in Children with Sickle Cell Disease

Kimpreet Kaur and Marsha Treadwell

Children's Hospital & Research Center Oakland, Oakland, CA 94609

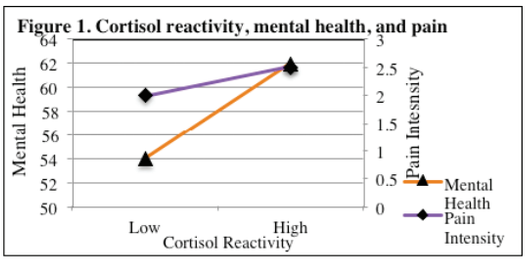
Introduction: Sickle cell disease (SCD) is a genetic condition characterized by multiple clinical complications, including acute and chronic pain and co-morbid mental health symptoms. Expression of complications is variable and not easily linked with genotype. Stress reactivity, measured by autonomic nervous system (ANS) and hypothalamic pituitary axis (HPA) responses has been associated with mental health symptoms and physical illness for healthy children. Demonstrating a relation between stress reactivity and clinical complications in SCD, including daily pain, could aid in predicting which children will express severe disease and therefore benefit the most from high-risk disease-modifying therapies.

Objective: 1. To investigate the relation between stress reactivity, pain episodes and frequency and intensity of daily sickle cell related pain; 2.To investigate the relation between stress reactivity, mental health symptoms. We hypothesized that higher levels of ANS and HPA axis reactivity would be associated with higher rates of pain episodes and greater frequency and intensity of sickle cell related daily pain. We also hypothesized that increased stress reactivity would be associated with increased prevalence and severity of mental health symptoms.

Methods: We examined ANS and HPA reactivity to challenging everyday tasks in relation to pain and mental health symptoms in a prospective cohort study of children with SCD. Our measures of ANS reactivity include cardiac measures - respiratory sinus arrhythmia and pre-ejection period. HPA reactivity was measured using assays of salivary cortisol, from saliva collected immediately before and following the autonomic reactivity protocol. We evaluated the children's reactivity in relation to pain episode rates from medical record review; pain frequency and intensity from daily diaries; and mental health symptoms from parent report. Parents rated family stress and child mental health symptoms.

Results: Participants were 60 children (*M* age 8.1, 5-12 years). 52% were girls; 55% were diagnosed with Hemoglobin SS disease. We found significant associations between greater cortisol reactivity and increased mental health symptoms (Figure 1, $p<.05$); higher resting cortisol and greater daily pain intensity ($p<.05$); and increased mental health symptoms and frequent daily pain ($p<.05$). Increased ANS reactivity predicted poor pain coping, which in turn was associated with greater pain intensity.

Figure 1. Cortisol reactivity, mental health, and pain



Conclusions: Our findings suggest that HPA reactivity may directly, and ANS reactivity indirectly, offer complementary approaches for characterizing children vulnerable to expressing severe SCD. Understanding the stress reactivity profile for children with SCD may allow for patient-specific clinical interventions that focus on regulation of biological processes and physiological reactivity. Our future research attends to developing interventions that reduce vulnerability to mental and physical health problems for children with SCD by focusing on coaching children to improve in their stress management and active pain coping at an early age.

NIKITHA KOSARAJU

Funded by the California Institute for Regenerative Medicine Creativity Award

Piedmont High School, Junior

Mentor: Ward Hagar, MD

Throughout my life, I have always wondered how things work. I would ask my parents, teachers, and friends hundreds of questions about the things around me. They always nurtured my curiosity. Eventually, I got some answers, but there was still so much to learn. In school, science classes helped quench my endless quest for answers. By far, my most favorite class was the biology class I took as a freshmen because we got to learn about the human body. I learned about all the different systems that work together in harmony to make up the amazing human body, but while I gained so much knowledge, there was still so many things unanswered. So I turned to my grandfather who has worked in the medical field and has shared many stories and experiences with me. He along with my parents inspired me to find the answers myself and have instilled in me a strong sense of helping other people and now, I picture myself doing this by becoming a doctor. I have known that I have wanted to go to medical school since I was in middle school, and this program has helped to cement that choice.

This summer, I worked closely with my mentor, Dr. Hagar, to study the relationship between hypertransfused sickle cell patients, metabolic syndrome, and mesenchymal stem cells. This project was both interesting and educational, providing more information about adult patients with sickle cell disease. I would like to thank Dr. Hagar, Christy Hoehner, Dr. Narayanaswami and CHORI for a rewarding summer full of learning and new experiences.

Possible Clinical Effects of Altered Mesenchymal Stem Cell Functioning in Iron Overloaded, Hypertransfused Sickle Cell Patients

Nikitha Kosaraju, Christine Hoehner and Ward Hagar

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Introduction: Sickle cell disease causes severe pains and loss of joint function. Only three treatments exist: bone marrow transplant (which is limited by donor availability), hydroxyurea, and red cell transfusions. Monthly red cell transfusions are increasingly used to suppress the production of sickle cells. An unavoidable effect from red blood transfusions is iron overload, which leads to oxidative stress. Mesenchymal stem cells are multipotent cells derived from bone marrow that differentiate into a variety of cell types such as osteoblasts, adipocytes, chondrocytes, endothelial cells, and smooth muscle cells. Oxidative stress increases the production of adipocytic cells from mesenchymal stem cells, thereby, increasing the risk of type 2 diabetes and cardiovascular disease. Metabolic syndrome, defined as the occurrence of metabolic risk factors from both cardiovascular disease and type 2 diabetes, has also been associated with insulin resistance, low insulin-like growth factor 1 levels, high sensitivity C-reactive protein levels, fatty liver disease, chronic kidney disease, and sleep apnea. Iron-related oxidative stress has been observed in patients with sickle cell disease. Additionally, most patients have low bone mass, bony infarcts, and clinical and biochemical changes suggestive of metabolic syndrome. The effects of transfusional iron overload have been studied mostly for its liver, endocrine, and heart effects. It is unknown whether transfusional iron overload may lead to metabolic syndrome and low bone mineralization in patients with sickle cell disease. This could be mediated by a shift of mesenchymal stem cell differentiation from osteoblasts to adipocytes.

Objective: This retrospective cohort study examined associations between iron overload, vitamin D, insulin-like growth factor 1, and C-reactive protein, as markers of metabolic syndrome in adults with sickle cell disease.

Methods: The Adult Sickle Cell Program has 390 patients. We looked at patients with and without DXA scans for vitamin D, insulin-like growth factor 1, high sensitivity C-reactive protein, diabetes, sleep apnea, SQUID, triglycerides, glucose, blood pressure, and HDL cholesterol. Data was collected using Meditech and analyzed using STATA 12.1 software.

Results: DXA scans were used as surrogate markers for possible iron overload as their serum iron levels are higher than those not sent for DXA scans. This study also shows that these patients have low insulin-like growth factor 1 levels and high sensitivity C-reactive protein. Further regression analysis is pending.

Limitations: For this pilot study, we used a convenient sample of subjects, which may have bias. Additionally, we assessed markers for metabolic syndrome. Ideally, we would want to be able to directly measure mesenchymal stem cells in sickle cell subjects with and without iron overload.

Conclusions: This suggests an association between hypertransfused sickle cell patients and metabolic syndrome. This may be an effect of mesenchymal stem cell differentiation from osteoblasts to adipocytes induced by iron loading. This study provides preliminary data to design a prospective cohort study that further examines the relationship between transfusional iron overload and mesenchymal stem cell differentiations.

NANCY LI

Volunteer

Albany High School, Senior

Mentor: Donald Reason, PhD

This fall, I will enter my senior year at Albany High School. Whenever people ask me what I want to do when I grow up, my first thought is "How old exactly is 'grown up' supposed to be, anyway?" My second thought is "When am I going to know how to answer this question?"

Up until recently, I didn't even know what I wanted to major in in college. I was, and am still, interested in a wide variety of things that I couldn't seem to reconcile. I wanted to learn more about politics; I wanted to write for a big newspaper; I wanted to join the Peace Corps and help the less privileged in Africa. More recently, I wanted to learn the ins and outs of the human body.

In my junior year, I took an AP Biology course that really sparked my interest in the biological sciences field. My teacher was genuinely enthusiastic and knowledgeable about the subject, and I found that her interest in the subject influenced my views of the course. My parents would literally have to drag me away from my biology textbook, much to their exasperation. Over the dinner table, I would regale my parents with explanations of mitosis, the Krebs cycle, and the importance of maintaining calcium levels in the body while they nodded in the right places. However, despite how amazing this past year of biology has been, I realized that the field of biology wasn't all about textbooks and learning facts.

I want to thank Dr. Donald Reason and Dr. Jinying Sun for taking the time to provide guidance to me this summer. This summer program has allowed me to experience hands-on scientific research in a lab environment and has afforded me with invaluable insight in current biological studies.

Epitope Mapping of Toxin-Specific Antibodies using Yeast Surface Display

Nancy Li, Jinying Sun and Donald Reason

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Anthrax is a lethal infectious disease caused by the spore-producing bacterium *Bacillus anthracis*. Toxins secreted by the bacterium are important in the pathogenesis of the disease. The primary anthrax toxin system is composed of three proteins: edema factor (EF), lethal factor (LF), and protective antigen (PA). PA binds the entire toxin to mammalian cells and allows EF and LF to enter the cell. PA is also the immunogenic component of the licensed anthrax vaccine. Vaccination produces antibodies that bind to PA, but few are actually capable of neutralizing the toxin. PA is folded into four functional domains; domain 4 (D4) is required for binding to the cell receptor, and a subset of the antibodies that bind to domain 4 neutralize the toxin. Our hypothesis is that neutralizing antibodies recognize epitopes that impede cell surface binding of the toxin, while the epitopes recognized by non-neutralizing antibodies do not obstruct toxin binding to the cell.

Objective: Our objective in this study is to identify the antigenic epitopes recognized by a neutralizing and a non-neutralizing PA-specific human monoclonal antibody.

Methods: Yeast surface display was used to map the epitopes bound by different D4-specific human monoclonal antibodies. A yeast expression library was constructed that expresses randomly mutated D4 peptides on the yeast surface, and binding loss mutants for each of the antibodies identified. Comparison of the mutated sequences to the sequence of wild type PA indicated those amino acids important for antibody binding. This information was used to guide modeling and docking studies.

Results: The toxin neutralizing human monoclonal antibody 4A12 and the non-neutralizing antibody 11F12 were used to probe a yeast library displaying mutated versions of the PA toxin. Sequence analysis of 4A12 binding loss mutants identified a region of domain 4 at residue Asp671 as being important for binding. Analysis of 11F12 binding loss mutants identified two regions centered around Glu625 and Tyr688 as being involved in antibody binding. Although separated in the primary sequence, these two regions are in proximity in the three dimensional structure of the toxin molecule. Modeling and docking studies indicate that the 4A12 neutralizing antibody occludes residues in both domain 4 and domain 2 that are known to interact with the cellular receptor for PA.

Conclusions: Our results indicate that the toxin neutralizing and non-neutralizing antibodies we examined bind to different antigenic epitopes on the toxin molecule. Although the epitope for the non-neutralizing antibody 11F12 is closer to the cellular binding region of the toxin than that of the neutralizing antibody 4A12, docking studies demonstrate that 4A12 efficiently blocks both region of the toxin known to interact with the cellular receptor. Our results suggest that steric hindrance by 4A12 of receptor binding is the mechanism by which this antibody neutralizes the PA toxin molecule.

YAQIAO LI

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley, Senior

Mentors: Frans Kuypers, PhD, Sandra Larkin, Marcel Fens

Since I was younger, I have found happiness by focusing on the good things in life. Lived away from my parents and constantly moved among my relatives, school was inconsistent. After coming to the United States from rural China, I started to feel the joy of learning, especially in science. It was the most interesting and enjoyable to understand the logic behind how things work in this very biological and physical world. I realized I had the ability and the motivation to further pursue knowledge. Time became a treasure when I had to work part time to support my own living. Spend a whole summer doing research study was such a luxury thought.

Lucky for me, I am in the land of opportunity. After getting in CHORI this summer, I have gained so much valuable experience and mentorship that I could never have in a large university campus like UC Berkeley. The weekly seminars exposed us to all the possibilities for a career in the medical field. I was encouraged by my mentors to design my own experiment and put in critical thinking on each procedure. They introduced me to a lot of technical equipment and procedures that I could use to acquire and process my data with. They are extremely patient with me. They are giving me all the freedom and time I need to put out my ideas and expand them, instead of throwing instructions. My experience here changed the way I tackle a scientific question and trained me to be a critical thinker.

I want a career where I can improve patient health, especially researching diseases that are more likely to affect low-income populations. I see a need to advocate for those who lack the financial ability to maintain physical well being. My CHORI experience really guided me in the right direction to pursue a career in clinical research. I gained valuable knowledge regarding research processes that I will utilize for the rest of my career.

Establishing Novel Red Blood Cell Morphology Data Collecting and Analyzing Methodology

Yaqiao Li, Sandra Larkin, Marcel Fens and Frans Kuypers

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: The hemoglobin molecule within the red blood cell (RBC) is the iron-containing biomolecule that binds oxygen and responsible for oxygen transferring. Mature RBCs have and flexible biconcave disk shape. This special shape gives RBC the ability to deform; allowing them to pass through small capillaries thinner than the diameters of RBC. This allows highly efficient oxygen exchange in the lungs and peripheral body tissues. Sickle Cell Disease (SCD) is an inherited chronic hemolytic anemia caused by a single nucleotide mutation. Under low oxygen conditions, the mutated hemoglobin polymerizes, altering the shape of the RBC to the peculiar "sickle shape. This altered shape and increased rigidity due to long hemoglobin polymers disable RBC from deforming and proper flow through small capillaries. This in turn leads to a syndrome called vaso occlusive crisis in sickle cell patients. Failure to delivery of oxygen to tissues leads to extreme pain and can cause tissue and organs damage.

Objective: This project aims to develop a novel, objective, and systematic method to identify, categorize, and characterize sickled RBC from normal RBC using light microscopy and computer software. The newly developed protocol will assist in studies that evaluate the ability of drugs to reduce the sickling process. The established standard protocol for RBC image data collection from a blood smear will be used in the second part of this project to test the antisickling capacity of RRx-001, a novel hemoglobin modifying drug currently used in an anti-cancer treatment trial.

Methods: SCD patient's blood samples were incubated in a tonometer at 37°C for different times under mixtures of air and nitrogen gas, under conditions that mimic the blood environment in the body. The SCD blood samples were fixed with formaldehyde after 1, 3, 5, 10, 20, and 40 minutes. Blood smear were obtained from these samples on microscope slides. Images were taken by light microscope under various light conditions including DIC (Differential interference contrast) to find the most optimal image generation for the pixel analysis software (ImageJ). The Image analysis parameters were optimized to render parameters that allowed automated assessment of the sickling process.

Results: The establishment of a novel image collection and software analysis protocol has resulted in a new approach that allows rapid and automated assessment of the shape of cells. Data on different sickle cell samples exposed to various de-oxynation regimen is currently being collected and analyzed. The results will be presented at the symposium.

Conclusions: Analysis of sickle cell morphology can be greatly assisted with the method developed in this project. This approach will allow rapid evaluation of potential anti-sickling drugs. The effectiveness RRx-001, to inhibit sickling will be established once the data has been fully collected and analyzed.

YUN LIANG

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley, College of Chemistry, Senior

Mentor: Wen-Shu Wu PhD

I was born in Haikou, China. I am a first generation college student in my family. My father did not finish high school and my mother only graduated from elementary school; both had to work in order to support their families. However, they knew the importance of higher education, so my parents supported and encouraged me to go to college. I attended Rio Hondo College, a community college near Los Angeles, California. Then, I transferred to the University of California - Berkeley with a major in chemistry in the fall of 2011. After I earn my Bachelor's degree, I will pursue an MD/PhD My goal is to become a physician scientist – a career that will allow me to provide medical care for patients and find new solutions for curing human diseases that affect millions. Not only am I enthusiastic to cure patients, but I also dream of becoming a leader in the field of new medicine invention and discovery.

I am very glad that I am given the opportunity to work as a summer intern at CHORI. CHORI is a fantastic institute to work and learn about research. I learned everything from scratch with my little biology background. Every person here is friendly and encouraging. I want to thank my mentor Wen-Shu Wu. Dr. Wu helped me set up my project – Construction of a New PiggyBac Transposon Vector for Induced Pluripotent Stem (iPS) Cell Generation and assigns me books and papers to read that provide the background information for the stem cell research I also want to give my gratitude to Dr. Chunping Zhang. Dr. Zhang provides me guidance in my research activities on a daily basis. He explains and shows me how to use the equipment in our lab and gives me many opportunities to do the real lab work. He encourages me to perform the experiment procedure even though I made all kinds of mistakes in the beginning. He also figures out the solution for my mistakes and recovers the experiment. From him, I learned from my mistakes and engaged in stem cell research. CHORI summer internship is going to be a milestone on the way to my career goal.

Construction of a new PiggyBac (PB) *transposon* vector for Induced pluripotent stem (iPS) cell generation

Yun Liang, Chunping Zhang and Wen-Shu Wu

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: A non-pluripotent cell, usually an adult somatic cell, is induced artificially to a type of pluripotent stem cell by a "forced" expression of specific genes. These cells are called induced pluripotent stem (iPS) cells. iPS cells have same functions as natural pluripotent stem cells, for example embryonic stem (ES) cells. They have the similar stem cell genes, chromatin, proteins, embryoid body formation, methylation patterns, teratoma formation, doubling time, potency and differentiability.

The PiggyBac transposon vectors "cut and paste" genes efficiently at chromosomes without footprint. During transposition, the Transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector were recognized by PB transposase. PB transposase efficiently transfers and integrates the sequences from the original sites into TTAA chromosomal sites. The PB transposon vector is a highly useful tool to move and integrate the gene into target genomes, because there is no cargo limit and gene integration is reversible when PB transposase is re-introduced into cells. PiggyBac is also unique because it does not associate with a superfamily of transposable elements, however, its TTAA insertion site is shared by other elements specificity.

Objective: Our first goal is to generate a new PB transposon vector (pBacII-SFFVgfpuro) with GFP and puromycin selection markers, and spleen focus-forming virus (SFFV) promoter that will drive high gene expression in hematopoietic cells. The second goal is to clone the two reprogramming factors (Oct4 and Sox2) in pBacII-SFFVgfpuro vector simultaneously. This new PB transposon vector will stably express reprogramming factors in mammalian cells, including hematopoietic cells.

Methods: 1) Construction of a PB transposon vectors – pBacII-SFFVgfpuro with five DNA fragments (WPRE, SV40PA, EF1-promoter, GFpuro and SFFV). 2) Enzyme-BglII was used to digest the insert site on pBacII-SFFVgfpuro vector by Restriction enzyme digestion technique. 3) Cold-fusion cloning, the two factors (Oct4 and Sox2) were inserted into pBacII-SFFVgfpuro vector simultaneously, 4) transfection, the new PB transposon vector will be introduced into target cells. 5) The pBacII-SFFVgfpuro vector will be verified by Western-blot analysis.

Results: 1) The pBacII-SFFVgfpuro has been generated, 2) The two reprogramming factors (Oct4 and Sox2) have been inserted into the pBacII-SFFVgfpuro vector simultaneously. The other goal is pending.

Conclusions: It is doable that multiple DNA fragments, including two reprogramming factors, are inserted into the PB transposon vector simultaneously by the cold-fusion cloning. This new technique will facilitate construction of various reprogramming vectors for iPS cell generation.

ZHE JERRY CHRISTOPHER LIN

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

Saint Mary's College of California, Post-Baccalaureate

Mentors: Mark Borja and Michael Oda, PhD

I was born in Kunming, China, where I spent the first eight years of my life before my family immigrated to the United States. Kunming is a city, but when I was a child it was not nearly as sophisticated as Shanghai or Beijing, or any of the other urban centers that China advertises as examples of its incredible growth. We were a moderate family, and both of my parents worked hard every day, so I spent much of my childhood with my grandmother, a nurse in Kunming City Hospital. I was a rambunctious child, and I would play in the halls while the nurses chased me. Aside from the joy of using the hospital as my personal playground, I still remember the stoic doctors who quietly walked the halls, checking on patients. I remember watching these men in white coats from around corners, and my delight when one would let me walk next to him when visiting patients. To me, these men were heroes, and I saw the good they did every time a patient would smile, or would leave the hospital better than when they came in. At school, kids would talk about how they wanted to grow up to be firemen or soldiers, but I always knew that I would become a doctor.

This passion for medical science has followed me throughout my life. In high school I excelled in science, and I carried that focus through five years at Saint Mary's College of California where I majored in both Biochemistry and Dance. While at Saint Mary's, I participated in several projects and college symposiums, presented research at the Experimental Biology conference in Washington, D.C., and worked as a scribe at Saint Rose Hospital in Hayward. Although my schedule was rigorous, and at times even a little too much so, I found that the vitality and excitement of medical science kept me endlessly interested.

I am excited to be working at CHORI this summer, which I know will further my love and passion for medical science. I have much to learn before I embark on a long journey as a physician scientist. Past research done at CHORI has provided immeasurable good in the fight against disease, and though I realize that I will only be a small piece of the puzzle in this wide world of medical research, I am grateful to this program for allowing me to contribute to medical science and for fostering my ultimate goal of helping humanity. I am deeply honored to be a part of the great work that has been, and will be, done at Children's Hospital Oakland Research Institute.

Analysis of the Loop Regions of the Lipid-free apoA-I Structure by FRET

Zhe Jerry Christopher Lin, Kalistyn Lemke, Mark Borja and Michael Oda

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: There are five different types of lipoproteins in the human body and high-density lipoprotein, commonly known as HDL, is one of them. These lipoproteins help move lipids, such as cholesterol and triglycerides, through the water-based bloodstream. High-density lipoprotein consists of several different proteins, and the major protein in HDL is apolipoprotein A-I, which comprises about 70% of the protein in a HDL particle[1]. High levels of HDL have been shown to correlate with decreased coronary artery disease[2], making the quantification of HDL important for the prediction of a patient's risk of coronary heart disease. One of the best-characterized functions of apoA-I is reverse cholesterol transport, which promotes the removal of cholesterol from peripheral tissues back to the liver or to steroidogenic organs, such as the adrenals, ovaries, or testes[3]. This process is promoted by the lipid-poor apoA-I conformation. The reverse cholesterol transport pathway is what gives HDL its antiatherogenic characteristics. Understanding the structure of ApoA-I is important for understanding the function and interaction of apoA-I. Thus, this project is to help ensure that the structure provided by EPR analysis[4] is correct.

Objective: The objective of this project is to use fluorescence resonance energy transfer (FRET) to verify the presence of several loop regions of apoA-I. Our goal is to confirm that the current structural model derived from electron paramagnetic resonance spectroscopy (EPR) studies is correct.

Methods: We generated four sets of double mutations: Q98W and Q84C; M148W and A130C; A124W and A154C; K208W and L189C. The production of recombinant apoA-I and purification protocols was followed as described in Oda et. al[5]. Recombinant plasmid DNA was verified for correct mutations by sequencing, and was transformed into an E. coli cell line grown in NZCYM medium with ampicillin. AEDANS labeling still needs to be done as described by Cavigiolio et. al[6] to the cysteine mutants. After fluorescent labeling fluorescent spectroscopy will be done.

Results: We have thus far mutated and purified Q98W, Q84C, M148W, K208W, L189C, K208W/L189C, and wild type trp-null.

Anticipated Conclusions: The anticipated conclusion of this project is that the FRET assay will show fluorescence of the acceptor molecule in the proposed loop regions of ApoA-I. This is an indication that the structure deduced by EPR[4] is indeed the correct structure. This will give secondary confirmation about the lipid free structure of apoA-I.

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SANAM MOBIN

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Post-Baccalaureate

Mentor: Barbara Staggers, MD

I recently graduated from UC Berkeley as pre-med with a B.A in Developmental Studies. At Berkeley I played an active role in working on improving the health of my local community. I was funded by the Berkeley Blueprint Leadership Program to conduct my own own study on the cause of obesity in low-income community. During this time I visited various elementary school in order to integrate new curriculum in public school standard lesson plans that revolved around the students nutrition. Beyond Blueprint, I also worked as a house cook and health worker in the Berkeley Co-op, where I not only cooked and taught 50 plus students how to make healthy meals once a week but also worked on improving the health of the students living in the house by publishing weekly health tips and holding weekly office hour for students to share health concerns and questions. I plan to continue to expand my passion for community health by applying for a duel MN/MPH program in 2014. My career goal is to combine both clinical experience as a nurse practitioner which public health practices and theory. I believe that the key to truly understanding chronic health problems like cardiovascular disease, diabetes, cancer and obesity is by integrating social, behavioral and environmental research with clinical practice in order to gain greater insight on the foundation of these leading disease. I plan to work in underdeveloped communities throughout my hometown, San Francisco, with the hopes to not only focus on individual health but community health by finding root causes of medical problems through analyzing community behavior and creating long term health solution that creates change from bottom up.

Through CHORI and the help of Dr. Staggers, I was able to explore the steps and methods to proper clinical survey. I expanded my prior knowledge of the obesity epidemic and narrowed my focus on the health concern to a specific audience that is typically overlooked. The experience and skills gained from this program will help me guide my future goals in community health development.

The Effects of Stress and Depression Among Ethnic Disadvantaged Teen Mothers

Sanam Mobin and Barbara Staggers

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: Obesity is todays newest epidemic and it is currently on the rise. It is a major concern because it has a high potential in leading into many other medical complications like: arthritis, diabetes, heart disease, high blood pressure, sleep apnea, cancers (specific forms) and stroke[1]. It is a disease that affects the lives of all ages, race, gender and socioeconomic class. However certain populations are heavily targeted over others depending on specific environmental, educational, social and biological conditions. The 2011 YRBS report by the CDC not only illuminates that Latino and Black females are prone to have unhealthy weight gain and loss, but they are also most likely to become pregnant at an early age. This is why it is important to focus on Latino and Black teens as well as other females from disadvantaged backgrounds and provide them with the resources to prevent unhealthy weight gain after pregnancy. It is critical that we provide new teen ethnic mothers (esp. Blacks and Latinos) with the proper support and education to alleviate stress and common depression that are a consistent trend among these specific population that leads to the upward spiraling rate of adult obesity.

Objective: The Objective is to see if nutritional education and consistent weekly meetings to monitor postpartum stress and depression will significantly help reduce the excess (unhealthy) postpartum weight among the ethnic teen community.

Methods: A literature review was done to better understand what specific community was most at risk. After narrowing down to a specific target group (Black/Latina teen mothers). Another set of literature review was done to understand the root cause for excessive pregnancy weight gain and unhealthy postpartum weight retention. Two factors that were projected in literature review showed that stress and depression were key factors. A patient chart review was conducted to find common trends among patients that had a BMI over 30 (1 year after giving birth).

Results: Four of the six patients reviewed were single mothers who had to take on more responsibility then an average new mother, placing greater stress upon their daily life. Also all patients reviewed did not have a balanced diet or healthy practice of exercise even though they had stated that they would like too. Each of the 6 patients reviewed showed several common trends in their daily life. Beyond the instability, stress and lifestyle change that occurs after pregnancy many of the teen mothers also had to juggle work while finishing H.S/ taking community college courses. Even with this new extended responsibility these teen mothers spend most of their time with children, leaving little or no time to themselves.

Conclusion: The study is still in progress. Providers at Children Oakland Teen Clinic will be given a list of common trends that increases the daily stress and depression among their patients that have recently given birth (1 year) and have a BMI over 30. Among the list of trends providers will also be given information of free/low-cost resources within the local Oakland community. Patients will be given a weekly packet to complete, which will consist of: nutrition log, activity log, progress log and stress management tips. Every week the provider and clinic health worker will view these packets with the patient and document progress and goals for the next week. The results will hopefully illuminate that the weekly meeting with their providers will reduce some of the stress and depression that leads to unhealthy weight gain.

ADRIENNE NICHOLAS

Volunteer

Reed College, Junior

Mentor: Janelle Noble, PhD

Working with non-profit healthcare providers in developing countries has been a longtime ambition of mine, as a way to treat women with limited access to health resources while at the same time taking part in the training of local caregivers.

Returning for my second year in the Noble Lab at CHORI to analyze the most effective means of data collection in the field has been a valuable experience. Being able to study a certain disease in different regions across the globe is imperative, particularly when considering the differing effects it has on specific populations. Perhaps most importantly, this project has made me aware of the extent to which one must predict all possible setbacks in collecting data remotely. In addition, it has been wonderful to have the opportunity to work and compare products not yet widely available.

I would like thank Janelle Noble, Julie Lane, Shana McDevitt, and Jonah Hemphill for their guidance and contributions to the project. Also, a special thanks to Kevin Williamson, Nancy Keller, Kevin and Lydia for their encouragement.

Evaluation of the Performance of Blood and Saliva Kits For Field Use

Adrienne Nicholas, Julie Lane, Jonah Hemphill, Shana McDevitt and Janelle Noble

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Type I diabetes (T1D) is a disease that occurs when the insulin-producing beta cells of the pancreas are mistaken as foreign and attacked by the body's immune system. Prior research suggests that T1D stems from a combination of genetic and environmental factors,1 with HLA (Human Leukocyte Antigen) genes accounting for nearly 50% of the inherited risk.2 HLA alleles and haplotype frequencies differ across ethnic groups, which contributes to differences in disease prevalence among populations. In an ongoing effort to include worldwide populations in their genomic studies, the Noble Lab will collaborate with the International Diabetes Federation's "Life For A Child" project, an organization providing T1D treatment for children in developing countries.4 As a part of their proposed epidemiological study, Life For A Child will collect samples for HLA genotyping from up to 100 diabetes patients and 200 unaffected individuals, from 5 sites in Asia, Africa, and Eastern Europe. While blood samples may be collected from diabetes patients in conjunction with treatment administration, noninvasive techniques may facilitate sample collection from unaffected controls. We hypothesize that either collection of blood or saliva samples will allow for extraction of adequate DNA for HLA genotyping by sequencing; however, we expect that blood will give more consistent yields.

Objectives: 1) To collect blood and saliva samples from two individuals using the saliva and blood collection kits from three different manufacturers (DNA Genotek, Biomatrica®, IntegenX), and to compare DNA yields and performance in downstream processes (Polymerase Chain Reaction, and, ultimately, DNA sequencing). 2) To test DNA stability provided by kits through exposure to high temperature over a period of several days. 3) To compare the weight, volume, cost, and convenience of each of the DNA collection kits. 4) To evaluate natural variation in DNA yield between individuals and sample aliquots of the same individual.

Methods: Sample Collection and Stabilization: Blood from one individual was drawn by a hospital physician into an EDTA Vacutainer. In addition, "old blood" (drawn in 2005) from 2 random individuals was tested for DNA stability. Saliva was collected by spitting into the appropriate collection unit. Samples were stabilized either by addition of proprietary reagents, or by dessication onto IntegenX Matrix-Chaperones. Aliquots of stabilized blood and saliva were either processed immediately or incubated at 45°C for 5-6 days.

DNA Extraction: DNA was extracted according to the manufacturer's protocols. Most of the kits recommend processing 200 ul stabilized blood or saliva mixture on a Qiagen DNA Blood Mini spin-membrane. The DNA Genotek "Oragene" Kit-stabilized saliva was precipitated (500 ul) twice, and the resulting pellet resuspended in TE. In addition, we tested 200 ul of the Oragene Kit stabilized saliva on the Qiagen DNA Blood Mini spin-membrane. The IntegenX Matrix-Chaperones require a larger elution volume; the Qiagen DNA Blood Mini protocol was adapted to accommodate the larger volume.

Quantification: DNA concentrations were measured by Quant-iT™ PicoGreen® dsDNA reagent (Invitrogen™).

Evaluation of DNA quality: All DNA extracts were first run on the Fragment Analyzer™ capillary gel system from Advanced Analytical to view size distribution of purified DNA. To test utility of extracted DNA for HLA genotyping, we amplified 20 ng template DNA (where possible) with FastStart HiFidelity Polymerase and HLA*DRB specific PCR primers, purified the product with AMPure beads, quantitated the PCR products, and sequenced them using the Roche 454 GS Junior platform.

Results: Pending.

Conclusions: Pending.

ROCIO OCHOA

Funded by the California Institute for Regenerative Medicine Creativity Award

Holy Names High School, Junior

Mentor: Robert Ryan, PhD

My name is Rocio Ochoa and I am a returning senior at Holy Names High School. I work in the Ryan Lab alongside Dr. Ryan and Betty Su. I am a daring individual who enjoys taking positive risks. This year was my first year in CHORI and before beginning my summer project, the closest I've been to a science lab was the chemistry lab in my school. With my little knowledge of biochemistry, I was literally thrown into the deep end, but luckily with the help of Dr. Ryan and the people working in my lab, I've been brought up to speed and managed to learn everything there is to know about my summer project.

I've always known that I wanted to work in the medical field; I could never imagine myself working in an office filing papers all day. I've had various volunteer opportunities and internships at hospitals and care centers before which only gave me motivation to do whatever I could to get a job in medicine. Given that, one can assume how excited I was when I found out that I was accepted into CHORI. I knew that being part of this wonderful program would give me a taste of how things work in the medical field.

My goal in life is to become a Neo-Natal Physician or go into research and study pediatric diseases. I've gotten one step closer to this goal thanks to the CHORI Summer Program. At this time, I would like to thank Dr. Ryan and Betty Su for taking me under their wing and welcoming me into their lab. They've both had incredible patience with me and have dedicated their time to teach me lab techniques and catch me up to speed. I could not have had a successful summer without their guidance and I am grateful for all that they have done for me. I would also like to thank my parents for pushing me past my limits. They are my strength and the reason why I am where I am today. Thanks to CHORI I can honestly say that I have had the real lab experience. It was nothing like how I've imagined it but by far was the best summer experience I've had thus far.

Barth Syndrome associated neutropenia: effect of cardiolipin-nanodisks on myeloid progenitor cells

Rocio Ochoa, Betty Su, Jennifer Beckstead, Jens Simonsen and Robert Ryan

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Barth Syndrome (BTHS) is an X-linked genetic disease caused by mutation(s) in tafazzin (*TAZ*). There is no cure for this disease, which is characterized by neutropenia and cardiomyopathy. The tafazzin gene product is a phospholipid transacylase that localizes to the mitochondria and functions in cardiolipin remodeling. Patients with BTHS show characteristic defects in cardiolipin metabolism that result in mitochondrial malfunction including decreased production of ATP. While the cellular and molecular mechanism underlying neutropenia in BTHS patients is unknown, loss of *TAZ* function in myeloid progenitor cells is known to induce apoptosis, possibly due to defective mitochondrial function.

Objective: The objective in this project is to use shRNA to knockdown the *TAZ* gene in HL60 myeloid progenitor cells. Subsequently, the ability of cardiolipin therapy to reverse the BTHS phenotype of these cells will be assessed. Cardiolipin will be formulated into water soluble nanoscale delivery particles, termed nanodisks. The effect of cardiolipin nanodisk incubation on the apoptotic response of control and *TAZ* shRNA treated HL60 cells will be determined. If the BTHS model cells display less apoptosis upon treatment with cardiolipin nanodisks, these self assembled lipid-protein complexes may offer a therapeutic strategy for treatment of BTHS patients.

Methods: Cultured HL60 myeloid progenitor cells will be transfected with *TAZ* specific shRNA. The effect of TAZ knockdown on annexin V binding will be assessed by flow cytometry while cadiolipin nanodisks will be formulated using bacterial expressed recombinant apolipoprotein A-I and cardiolipin. Cardiolipin nanodisks will be characterized by gel permeation chromatography prior to incubation with cells.

Results: Cardiolipid nanodisks have been prepared and characterized. HL60 cells are in culture shRNA experiments are pending.

Conclusions: Currently, insufficient data is available for us to make a solid conclusion.

MICHAEL PAN

Volunteer

Graduated from University of California in Spring of 2012 – Applying to medical school

Mentor: Cassandra Calloway, PhD

I've recently graduated from UC Berkeley with a degree in Neurobiology this Spring of 2012. Sciences, especially those relating to biology and medicine, have always been a passion of mine. I started volunteering at CHORI during the 2011-2012 academic year and joining this summer program has allowed me to continue my research at CHORI with a more defined routine. I plan to go to medical school in the fall of 2013 and am currently in the process of application.

My interest in medicine began at a young age. I was intrigued by the efficacy of drugs, such as ibuprofen, in helping me manage any symptoms of illness that I had at the time. I was a pretty sick child and took a variety of different medications, each which I tried my best to memorize. Since then I have always taken an interest in pharmacology and all things related to medicine.

More recently, I have volunteered or worked for institutions related to the medical sciences. The summer after my Freshman year at Cal, I interned at Biogen Idec, a pharmaceutical company, and worked closely with monoclonal antibodies. Since then I found a position at CHORI under my mentor, Cassandra Calloway, PhD, and have followed her dedication in the analysis of mitochondrial DNA.

I'd like to thank Cassandra Calloway, PhD, for taking the time to help guide me through all my research, and I would also like to thank Esteban Gomez, MD, for his contributions to my current research.

Thalassemia and Iron Overload: The Effects on Mitchondrial DNA Copy Number and Deletion Frequency

Michael Pan, Esteban Gomez and Cassandra Calloway

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Thalassemia major is an inherited genetic disorder that affects an individual's ability to synthesize specific proteins constituting the hemoglobin of red blood cells. The resulting lack of functional hemoglobin in patients afflicted with thalassemia can cause severe anemia, which is usually treated with blood transfusions. However, frequent blood transfusions can lead to systemic iron overload, which in turn can cause multiple organ failure.

A previous pilot study in the Calloway lab has yielded data suggesting that iron overload is associated with a decrease in the ratio between mitochondrial and nuclear copy numbers and also an increase in the deletion frequency of ΔmtDNA4977, a common 4977 bp deletion. These changes have both been associated with oxidative damage in the mitochondria. Using either information as a potential biomarker for iron overload in thalassemia patients receiving blood transfusions may help diagnoses of iron overload before systemic organ failure occurs.

Objective: We will determine whether having thalassemia is associated with a decrease mtDNA to nuclear DNA ratio and also an increase in mtDNA damage. We are interested in determining if mitochondrial to nuclear DNA ratio and/or the deletion frequency correlates with iron burden and toxicity.

Methods: For the project, a total of 30 blood samples were studied. There were 10 control and 20 thalassemia samples. These samples were extracted using 200 ul of whole blood and the Qiagen DNeasy Blood Extraction Kit. The copy numbers of the mtDNA and nuclear DNA of the extracted samples were measured using qPCR assay. A standard curve was generated using standards with known copy numbers of both mtDNA and nDNA that the samples were compared with to quantify the samples' respective copy numbers. There was a unique probe for both the nuclear and mitochondrial DNA so that both could be detected in tandem. Using the mtDNA copy number determined from the mito-nuclear qPCR, we will normalize the amount of mtDNA to 10,000,000 copies per reaction to perform the deletion assay. Again, standards with known amounts of deleted mtDNA fragments are used to generate a standard curve that will allow us to quantify the deletion frequency in the samples. The deletion assay will yield the extent of ΔmtDNA4977 deletion per 10,000,000 copies of mtDNA in the samples.

Results and Conclusion: We are in the process of collecting data from the mito-nuclear qPCR assay. The extractions for the 30 samples have been completed and we will move on to perform the mito-nuclear qPCR. We expect the data to yield a decrease in mtDNA:nDNA ratio and an increase in the deletion frequency of the ΔmtDNA4977 deletion in the samples from thalassemia patients.

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BRIANNA POPE

Volunteer

Vista del Lago High School, Senior

Mentors: David Killilea, PhD, Janet King, PhD, Sarah Burke and Tai Holland

I am beginning my senior year at Vista del Lago High School. My primary schooling has taught me many things. More than anything else, I have learned that I am passionate about medicine. I have decided that after high school I would like to get my bachelor's degree in science and then attend medical school. I am particularly interested in forensic and pediatric medicine. I want to use the knowledge and privileges I would gain as a doctor to help less privileged people around the world. I now see how much doing research can help those people as well. I had never realized how interesting research could be. I learned so much working on Dr. King's clinical study and now have a strong interest in medical research. I was lucky to have four mentors for my summer at CHORI, Sarah Burke, Tai Holland, Doctor David Killilea, and Doctor Janet King. I appreciate the time and knowledge they used to teach me so much. Research on zinc biomarkers did not sound like something that would increase my love for medicine but learning about nutrition and the many ways we can improve it globally has been so fascinating for me and I would not trade this experience for anything. The benefits I have gained will help me throughout my schooling and my medical career. I would also like to thank the administrators who make this program possible and run it so well. I hope that CHORI will be a part of my life as I leave the Summer Student Program, whether I rejoin the program or work in one of the labs.

Zinc Absorption and Measurement

Brianna Pope, Sarah Burke, Tai Holland, David Killilea and Janet King

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: One third of the world's population is at risk of deficiency for the essential mineral zinc. While there are no distinct symptoms of minor to moderate zinc deficiency, there is evidence of a decline in metabolism and other bodily functions. Severe zinc deficiency symptoms include diarrhea, skin lesions, and dysfunction of the immune system, leading to higher infection and mortality rates. Children with severe zinc deficiency are likely to experience stunted growth and development. The only clinically validated target for determining zinc status in individuals is plasma. However, changes in plasma zinc are notoriously insensitive to changes in zinc intake. Our goal is to find a more sensitive target for determining zinc status.

Objective: By analyzing the sensitivity to whole body zinc in plasma, RBCs, WBCs, fingernails, and urine, we hope to identify a more sensitive target for zinc status than the clinically validated plasma target.

Methods: A 74-day nutritional study with 3 metabolic phases will be conducted using healthy adult males as the study participants. The three phases will include depletion with 4 mg of zinc (and 1.5 mg phytic acid) per day, a gradual repletion with 8 mg of zinc per day, and rapid recovery where participants consume at least 25 mg per day of zinc. Throughout the study plasma, WBCs, RBCs, urine, and fingernails of the study participants will be analyzed for a range of different metabolites, to determine how the changes in whole body zinc affect the separate physiological targets. My part in the study consisted of measuring the levels of zinc and specific protein in the WBCs, RBCs, and plasma.

Results: Two of twenty participants have completed the trial. In one participant, plasma zinc was sensitive to moderate changes in dietary zinc. Total RBC zinc was similar in these participants through the whole study. Surprisingly, WBC zinc showed an elevation during depletion and normalization during repletion and recovery. Levels of proteins that are known to be regulated by zinc, including albumin and hemoglobin, were also altered.

Conclusions: The identification of biomarkers that are sensitive to zinc status is in progress. The identification of sensitive targets to changes in zinc status will have a significant impact on future nutritional studies.

NAPALA PRATINI

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

Saint Mary's College of California, Senior

Mentor: Jacob A. Neufeld, MD

The more I learn about becoming a doctor, the more I understand that I'll never know it all. There is no perfect medical school applicant, no doctor that has an answer to every malady, no scientist with 100% successful experiments, or single drug that cures every ailment. While modern medicine is arguably light years ahead of medicine as recent as a century ago, there is still so much to investigate and so many discoveries to be made. Personally, the more I learn, the less I claim to know, and the more I want *to know. I owe the CHORI Summer Student Program and Dr. Neufeld a big 'thank you' for granting me this priceless opportunity to learn, now for two summers in a row. In addition to my research project last summer I shadowed doctors, assisted with publishing an article, and was introduced to the inner workings of a peer-reviewed medical journal. At the same time, my mentor and others I met through the CHORI summer program assisted me in preparing applications for the Rhodes and Fulbright scholarships for the year following my graduation from Saint Mary's. This summer, I am excited to be gaining experience with different clinical research projects and learning more both about how such projects begin, and how they are carried out. I am honored to also be preparing for a nine month Fulbright grant to Spain starting this September, during which time I will be working in the Melanoma lab of the Spanish National Cancer Research Center and perfecting my Spanish, as I wish to become multilingual. While my ultimate aspiration is to become a physician, I have lots of experiencing, exploring, and, most importantly, learning to do before realizing this goal. Going forth, I will always be grateful to this program for allowing me to do a little of all three.*

Early consequences of damage to the prefrontal cortex

Napala R. Pratini[1], Robert Knight[2], Sylvia Bunge[2], Chloe Green[2], Kenneth Martin[1], Peter Sun[1], Kurtis Auguste[1], Rachel Kuperman[1], Jacob Neufeld[1]

[1]Childrens Hospital Research Center Oakland, CA 94609;
[2]Helen Wills Neuroscience Institute and the Department of Psychology, University of California, Berkeley.

Introduction: The prefrontal cortex (PFC), or the front third of the human brain, contributes to the establishment of meaningful, goal-directed thought and behavior in typically developing children and adolescents. Unfortunately, traumatic brain injuries that affect the PFC are quite common, often seen in car accidents, sports injuries, falls, child abuse, strokes, epileptic foci, and brain tumors. Although PFC damage incurred later in life has been studied intensively, many barriers to research have prevented prospective or retrospective studies that investigate the effects of PFC damage incurred early in life. In the summer of 2011, researchers at Children's Hospital and Research Center Oakland were awarded a CHORI Clinical Research Grant to collaborate with the Cognitive Control and Development Laboratory of the Helen Wills Neuroscience Institute and the Psychology Department at UC Berkeley to administer cognitive assessments for children with PFC damage, following them over time with the final goal of determining and comparing their cognitive developmental trajectories to those of typically developing children.

Objective: Patient recruitment and testing for this study has occurred over the past year, but a larger sample was needed in order to include site of lesion, age at injury, and current age as covariates of interest in analysis. The purpose of this project was to assist with patient identification, recruitment, and testing.

Methods: Potential participants were identified by searching the hospital's imaging database for patients with the appropriate brain lesions. Recruitment included contacting the patient's primary care physician to verify eligibility, and also calling the family to explain the study and to enquire as to if they would like to participate. Patient testing involved various neuropsychological exams administered by a graduate student, and subsequent scoring of the exams.

Results: To date, over 128 patients have been identified as potential participants for the study. Of these, over 75 have been reviewed and accepted as appropriate by the research neurologist. Testing has begun on 10 patients. Results from testing are pending.

Conclusions: We have successfully identified the appropriate number of patients. Once 25 have been tested, preparation of a larger scale NIH grant proposal will begin.

MARITZA RODRIGUEZ

Funded by the Doris Duke Charitable Foundation

American Indian Public High School, Senior

Mentor: Damini Jawaheer, PhD

Hello, my name is Maritza Rodriguez, and I am from Oakland, California. I became interested in biology and medicine when I was a sophomore in high school. That year, I was taking AP Biology, but more importantly, that was the year my family began experiencing numerous medical emergencies. These events sparked in me a desire to help others as a doctor. In my senior year, I heard about the CHORI summer program, and I applied because I thought that CHORI could provide me experience and knowledge that would later become advantageous for me. After completing the program, I will attend University of California, Santa Cruz and major in human biology. After college, I plan on going to medical school in order to become a pediatrician.

The Influence of Gender and Age of Onset on the Severity of Pediatric Lupus

Maritza Rodriguez and Damini Jawaheer

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Systemic lupus erythematosus (SLE) is a life-threatening, autoimmune disease that affects about 1.5 million people in the United States. About 20% of all lupus patients develop the illness in childhood. Pediatric lupus, like adult lupus, mostly affects females and is more severe than adult lupus. However, it is not known whether gender or age of onset affects the severity of the disease. Since female hormones have been associated with adult lupus flares, we hypothesize that females and children older than 12 (post-pubescent) have more severe lupus.

Objective: To determine if gender and age of onset influence the severity of pediatric lupus

Methods: The severity outcomes examined were serositis, pericarditis, and renal symptoms. Gender and age of onset were the exposure variables with age of onset being categorized as ≤12 (before puberty) and >12 (post-puberty). For each exposure variable, differences in frequencies of each outcome were determined using chi-squared tests, and logistic regression was used to calculate an odds ratio with 95% confidence intervals (CI). In addition, we performed multivariate logistic regressions to determine whether gender and age of onset have independent influences on the severity of pediatric lupus.

Results: The study population was comprised of 1267 patients with pediatric lupus. The majority of the population was female (87.1%); a total of 46.4% of patients were older than 12 (post-puberty). Also, 28.6% of all patients had serositis, 16.9% had pericarditis, and 57.2% had renal symptoms. In our data set, the logistic regression results showed that females were significantly less likely to have renal symptoms, but that gender was not associated with any other severity outcomes (Table 1). Compared to onset before puberty, pediatric lupus onset after puberty was significantly associated with the severity outcomes serositis and pericarditis, but not with renal symptoms (Table 1). Our multiple logistic regression results suggested that this effect of age of onset on serositis and pericarditis was independent of gender.

Table 1

	Gender		Age of Onset	
Outcome	**Odds Ratio (95% CI)**	**p Value**	**Odds Ratio (95% CI)**	**p Value**
Serositis	1.38 (0.92, 2.06)	0.12	3.03 (2.33, 3.95)	<0.0005
Pericarditis	0.99 (0.60, 1.64)	0.98	2.05 (1.42, 2.94)	<0.0005
Renal Symptoms	0.65 (0.46, 0.92)	0.02	1.19 (0.94, 1.49)	0.14

Referent Categories for (a) gender: male, (b) age of onset: pre-pubescent (>12 years)

Conclusions: In our dataset, children with pediatric lupus who developed the disease after puberty experienced a more severe form of the disease, independently of gender. Although more males had renal symptoms, this result should be interpreted with caution because the number of males in the dataset was very small.

RYLAN ROSARIO

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

Californa State University East Bay, Post-Baccalaureate

Mentor: Barbara Staggers, MD

My name is Rylan Rosario a recent graduate from San Jose State University. I majored in Health Science with a minor in Biological Sciences. I was recently accepted to Cal State East Bay Post-Baccalaureate Program, which I will begin in the Fall. In the future I want to attend a joint MD-MPH program.

In my late teens I was diagnosed with epilepsy, my health was a continuous concern but it was than I realized a serious need. I remember feeling that my autonomy was taken away from me and I had to endure many obstacles because I could not afford healthcare insurance. During that time I decided that I would go into the health field and become advocate for those that felt powerless. It is my belief that patients and providers could work together as a team to improve health outcomes.

I have a vision for incorporating public health in everyday medical practice and the art of medicine is more than tertiary care. I knew it was critical that I gain experience in the different facets of public health. I had the opportunity to intern at Faces for the Future. This was the first time I would meet Dr Barbara Staggers and learn about the Teen Clinic. I left that summer with a since of urgency and confidence that I never had before.

The teen clinic inspired me it was and still is a safe haven for young people. I asked myself what would my life have been like if I had somewhere like this to go? The end of the summer I would become a co-founder of Global Medical Brigades and travel to Honduras to provide healthcare to hundreds. I was a part of a team of doctors, nurses, pharmacists, and health educators that worked together to provide the best medical experience to each patient. We did not have the advanced technologies or state of the art buildings but there was passion, commitment, and humility. My mentors like Dr Staggers and learning opportunities are providing me with the support and vision that someday I will have the privilege of serving in such a challenging but rewarding career.

Screening Practices of Providers at Children's Hospital Oakland Teen Clinic For Interpersonal Violence

Rylan Rosario, Jasolyn Harris and Barbara Staggers

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: Recently in San Leandro, California, a teenage boy took the life of his 15-year-old girlfriend and then took his own life. The adolescent was stabbed multiple times; she was a victim of interpersonal violence IPV. Her tragic story shows the imperativeness of educating providers, parents, and teens about IPV. "One in three adolescents reports experiencing physical violence by a romantic partner" (CDC, 2012). IPV can consist of physical, sexual, or psychological violence within a dating relationship.

Objective: The exploration of current screening protocols for Interpersonal Violence among adolescents seen at Children's Hospital Oakland CHO Teen Clinic. As a result develop efficient universal IPV screening methods for all teen clinics for CHO and provide educational materials for patients.

Methods: To comprehend IPV various literatures were reviewed. There was a further investigation of IPV by examining patient charts of IPV victims with the assistance CHO medical social worker and adolescent specialist. In person sessions were conducted with identified IPV victims to hear personal testimonials of their accounts of IPV. Following the sessions, a questionnaire was developed to assess the current IPV screening methods of current CHO Teen Clinic providers. The questionnaire was distributed in person. The questionnaire consisted of n= 7 multiple-choice questions, n=1 open ended question, and a section for additional feedback and comments.

Results: The questionnaire was administered to n= 10 Cho Teen Clinic providers. The providers consisted of n= 5 attendings, n= 4 residents, and n= 1 medical student. Sixty percent stated they always did continual screening and 40% said only sometimes. When providers were asked if they needed more IPV education 80% said yes, regardless of their credentials. More than half of the providers wanted to learn about local resources, and 40% wanted to know good questions to ask teens for IPV screening. They were also asked if they wanted computer-based screening and 80% answered yes.

Conclusions: The concluding results could not be generalized for all the CHO Teen Clinics because of the small sample size. The results have determined the need to administer the survey to all the providers of the three clinics. In addition to accompany the initial survey a second survey has been developed for patients. The patient's perspective will be valuable when developing educational and resource materials.

HIRA SAFDAR

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

University of California, Berkeley, Sophomore

Mentors: Beate Illek, PhD and Horst Fischer, PhD

Over the past few years, I have grown to realize that science is anything but perfect, just like life. As a Pakistani and Muslim female born and raised in San Francisco, I have constantly faced the struggle of trying to maintain a balance between honoring my culture's traditions and pursuing opportunities that will stretch me beyond my limits. Although my family has always supported me in my studies, the gender inequalities and misogynistic double standards deep-rooted in my culture daunt me till this day as they may limit my possibilities of working in the science and research field. However, these are the same aspects that motivate me to aim for success.

I want to invest my intelligence and perseverance into a field where the goal is to give back to the community; being a part of this summer program is the first of many steps that will get me closer to where I want to be. As a student and researcher at CHORI, I envision myself on a pathway that leads to success in a field in which women—especially of my background—are underrepresented. I aspire to become a role model for young Muslim females by becoming a devoted professional who deftly balances the positive aspects of respect, faith and courage from my culture with the boundless opportunities offered by life in the United States.

The weeks I have spent in the Fischer-Illek Lab have made this summer one to remember. I have learned a great deal about cystic fibrosis and have gained hands-on experience in basic medical research with a great group of people; for that I am very thankful. From learning about cell culture and confocal microscopy with Dr. Illek and Dr. Fischer to having deep conversations with my colleagues Kayla and Joni over lunch, I have grown so much and have become quite fond of working in the research environment. I offer my sincere thanks to the program directors/coordinators, seminar speakers, NHLBI/NIH foundations, and the amazing people at UC Berkeley's Biology Scholars Program for making this privileged experience possible. Thank you!

Expression of Dual NADPH Oxidase and Production of Hydrogen Peroxide in Cystic Fibrosis Cell

Hira Safdar, Beate Illek and Horst Fischer

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Initially, phagocytic white blood cells were considered as the major source of hydrogen peroxide (H_2O_2) production in the lung; however, with the identification of the NADPH oxidase gene family, epithelial cells were identified as additional producers of H_2O_2. The human lung epithelium produces a significant amount of H_2O_2, and there are two types of H_2O_2-producing isoforms of the NADPH oxidase family: dual oxidase 1 and 2 (DUOX1 and 2). Both localize to the apical membrane of epithelial cells and generate H_2O_2, which is required for the formation of the bactericidal molecule hypothiocyanate by the enzyme lactoperoxidase (LPO). Recent studies show that the DUOX/LPO defense system depends on the function of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel, which provides the airway surface liquid with thiocyanate (for LPO function) and bicarbonate (for pH adjustment).

Objectives: To determine the expression of DUOX in the apical cell membrane of CF bronchial epithelial (CFBE) cells and CF nasal (JME) cells; to determine the role of normal and mutant CFTR on H_2O_2 production across the apical cell membrane at various pH values.

Methods: *DUOX expression:* Monolayers of CFBE and JME cells were fixed, permeabilized, and blocked overnight. Cells were stained with polyclonal DUOX rabbit primary antibody, which was raised against the Arg618–His1044 fragment of DUOX1 and kindly provided by Francoise Miot (Brussels, Belgium). The AlexaFluor®633 antirabbit and AlexaFluor®488 antimouse secondary antibodies were applied, nuclei were counterstained with Hoechst 33342 dye, and the apical cell membrane was stained using the monoclonal mouse zona occludens (ZO-1) antibody. Cell filters were then mounted onto microscope slides. *H_2O_2production:* The Amplex® Red Hydrogen Peroxide Assay Kit was used to detect H2O2 production in CFBE and CFBE+CFTR cells.

Results: *DUOX expression:* Slides were viewed using a Zeiss LSM710 confocal microscope; laser lines at 405 nm and 633 nm were used to excite the nuclear stain and DUOX, respectively. Overview images were collected at 20x magnification, and a 63x oil objective was used to generate detailed Z stacks. The Z stack images of the CFBE cells stained with DUOX and Alexa633 and JME cells stained with DUOX/ZO-1 and Alexa633/Alexa488 both showed low signal for DUOX and nonspecific staining across the apical cell membrane. In addition, the ZO-1 counterstains for the tight junctions in the apical cell membrane stained in a nonspecific, perinuclear manner. To test if the ZO-1 mouse antibody was properly staining, a control experiment was performed: CFBE and JME cells were stained for ZO-1 and Alexa488 only. The images from these slides showed nonspecific, perinuclear staining as well and not the expected ringlike structures for ZO-1. *H_2O_2 production:* Fluorescence was measured with a microplate reader using excitation at 530nm and detection at 590nm. Bar charts and standard curves indicate that ~50% of H_2O_2 production was blocked by diphenyleneiodonium chloride, indicating DUOX activity. CFBE+CFTR cells produced 18% more H_2O_2 than CFBE cells, and H_2O_2 production for cells at pH 7 appeared higher than cells at pH 6 or 8.

Conclusions: *DUOX expression:* Staining for DUOX remained inconclusive as the ZO-1 control resulted in non-specific staining. The perinuclear staining does not provide enough information to identify expression of DUOX. *H_2O_2 production:* CFTR-treated cells showed higher production of H_2O_2; this suggests that the presence/function of DUOX may be greater in CFBE+CFTR cells. Since cells at pH 7 produced the most H_2O_2, this implies that the function of DUOX in both CFBE and CFBE+CFTR cells is optimal at a neutral pH.

References:

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2. "Background" from DUOX2 (Y-15): sc-49939 Santa Cruz Biotechnology, Inc. http://datasheets.scbt.com/sc-49939.pdf
3. "Abstract" from Mechanism and Function of DUOX in Epithelia of the Lung by Dr. Horst Fischer
4. "Cloning of Two Human Thyroid CDNAs Encoding New Members of the NADPH Oxidase Family" from The Journal of Biological Chemistry
5. "Amplex® Red Hydrogen Peroxide and Peroxidase Assay Kit" from Invitrogen, Molecular Probes®

KASEY SIU

Volunteer

Mills College, Post-Baccalaureate

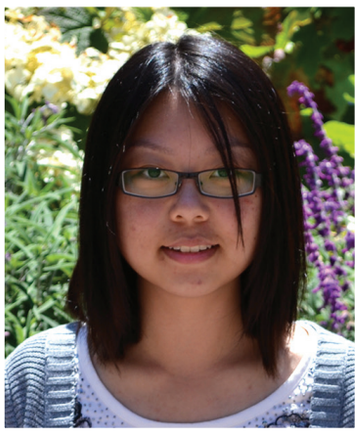
Mentor: Michael D. Bell, MD

My journey towards applied science and eventually medicine began nine years ago when Severe Acute Respiratory Syndrome (SARS) was first identified in Hong Kong. Within a short period of time of its discovery, a large number of people were infected and hospitalized. Some infected patients were treated with high doses of steroids to reduce swelling in the lungs; however, the high dosage of the drug had caused other side effects and complications. Therefore, clinicians and scientists diligently investigated the mutated virus in order to, hopefully, find an effective treatment for this new disease to keep it under control.

Because the disease was highly contagious, SARS patients were isolated in the intensive care unit (ICU). My secondary school's alumna, Dr. Ha-Yan Cheng, who voluntarily joined the medical team in the ICU in order to accommodate an increased demand in patient care, was unfortunately infected with SARS by a patient. She died at the age of 30, and was the youngest doctor to die from SARS. I learned of her death on television news and was astonished that she died at such a young age. While everyone was frightened about the disease, this young lady had the courage to fight against it in the front line, and it totally changed my life goal.

From the SARS outbreak, not only have I inspired by Dr. Ha-Yan Cheng's dedication and commitment to caring for patients, I have also learned that scientific research and technology are the keys to the doors of remedies in the medical field. Since then, my interest in applied science and medicine has developed, and it continues to grow the more new medical discoveries and technology I learn. After immigrating to the United States, I am very grateful for the volunteering experiences in the hospitals and laboratory settings. They have given me opportunities to observe procedures, to be familiar with medical terminology and healthcare equipment, and, more importantly, to help people by positively impacting their welfare and happiness in a clinical setting and through scientific investigation and discovery.

Unlike many of my peers, I do not come from a family of doctors or even college graduates. Because of this, I am so very thankful to Deborah Ellen for the opportunity to participate in the Summer Student Program at CHORI. It means so much to me and has a positive impact on my future endeavors. I also want to express my sincere appreciation and gratitude to my mentor, Dr. Michael D. Bell, for teaching, inspiring, and believing in me. Last but not least, I would like to thank the staff at CHORI and in the emergency department for making the project possible.



Comparison of Nitrous Oxide to Intranasal Midazolam for Sedation in Minor Procedures in Our Pediatric Emergency Department

Kasey Siu and Michael D. Bell

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: Nitrous Oxide (N_2O) is a colorless, tasteless gas. Its primary purpose is to reduce anxiety and distress in patients during minor procedures. It provides a rapid onset of 30 to 60 seconds. Upon discontinuation, N_2O provides a short recovery time after 3 to 5 minutes of 100% O_2 administration. Midazolam is a drug in the class of benzodiazepine that binds to gamma aminobutyric acid (GABA) receptors resulting in depression in the central nervous system. Intranasal midazolam (Versed) has an onset ranging from 10 to 15 minutes and a duration of effect of 60 minutes. Its reversal agent, Flumazenil, can be given to patients to treat an overdose of midazolam.

Objective: We are attempting to show that (1) high-concentration of up to 70% N_2O is safe to use in pediatric procedural sedation, and (2) N_2O is a superior agent compared to intranasal midazolam, the most commonly used drug for minor procedures in our pediatric emergency department (ED), that is (3) more effective, has less adverse effects and variability, and (4) allows shorter ED visits which in turn increases ED productivity.

Methods: We plan to use a randomized non-blinded study. To determine the number of participants needed and length of study, we will do a chart review of previous 6 months intranasal midazolam sedations. Nitrous oxide sedations will be reintroduced shortly before the study begins so there will be no baseline information. All patients who need sedation will be evaluated by using the exclusion criteria for N_2O and intranasal midazolam. N_2O is contraindicated in patients under 1 year of age, sickle cell disease, pneumothoraces or bullous lung disease, decompression sickness, air embolism, otitis media, cardiac failure, early pregnancy, impaired level of consciousness, and intoxication with drugs or alcohol. The exclusion criteria for intranasal midazolam include patients with a blocked or traumatized nose, respiratory depression, head injury, and patients who are taking narcotic or benzodiazepine medication. Eligible patients and parents will be given an information sheet about the study. If interested, parents will be asked to sign a consent form for enrollment in our study. Enrolled patients will be randomly assigned to N_2O or intranasal midazolam for sedation. Patients in the N_2O arm of the study will begin with 50% N_2O/50% O_2 and an increasing concentration of up to 70% N_2O will be administered if needed. Patients in the intranasal midazolam arm of the study will be administered a dose of 0.4mg/kg with a maximum of 10mg/kg intranasally with an atomizer. All patients will be monitored for their vital signs and O_2 saturation. Adverse effects, level of pain, level of sedation, and time of beginning of procedure to discharge will be recorded. A visual analog scale (VAS) will be used for the self-reporting of pain in children 7 years of age and older and for parental scoring of children under 5 years of age. Children between 5 and 7 years of age will use the Faces Pain Scale-Revised (FPS-R). The level of sedation will be rated by researchers or trained medical staff using Children's Hospital of Wisconsin Sedation Scale. A satisfaction survey will be given to the examining physician to rate on the efficacy and helpfulness of the sedation in allowing the procedure to be done. Parents will be contacted by telephone within 72 hours after sedation for a satisfaction survey.

Results: All data will be entered into a software database. κ^2 tests will be used for dichotomous variables and t tests for parametric variables.

Conclusions: We anticipate that 70% N2O is safe and is a better form of sedation than intranasal midazolam in our ED.

BETTY SU

Funded by NIH Grant #5 R37HL064159-12

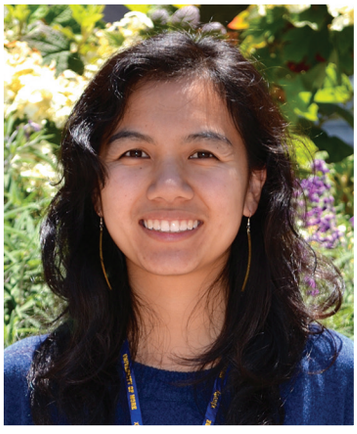
University of California, Berkeley, Post-Baccalaureate

Mentor: Trudy Forte, PhD, Robert Ryan, PhD

My name is Betty Su and I graduated from University of California, Berkeley, last spring with a degree in Molecular Cell Biology. My interest in cell biology and anatomy developed through the courses I took. In my third year, these interests led me to volunteering in Dr. Ryan's lab, CHORI, studying apolipoproteins. That was two years ago but this is my first opportunity to participate in the summer student program. I am very excited for this opportunity as it allows me to have my own project. Since graduating from UCB, I have been spending my time volunteering between CHORI and Children's Hospital. My goals are to explore and have enriching experiences before I go back for an advanced degree.

I am very grateful for the opportunities CHORI and Children's Hospital have provided me as they allowed me to grow as a budding scientist. As I move forward towards my career, I hope to become a medical professional involved in research.

I would like to thank, in particular, Drs. Forte and Ryan for their mentorship and patience and everyone else in the Ryan Lab for their support. I would not be able to accomplish so much without them.



Targeted-Nanodisk as a Drug Delivery Vehicle for Mantle Cell Lymphoma

Betty Su, Natasha Crosby, Jennifer Beckstead, Mistuni Ghosh, Rocio Ochoa, Robert Ryan, Trudy Forte

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Introduction: Mantle Cell Lymphoma (MCL) is a subtype of non-Hodgkin's lymphoma. This lymphoma has poor prognosis since current treatments are inadequate. All trans retinoic acid (ATRA) is one of many potential drugs currently being explored to treat MCL. ATRA is a nuclear hormone receptor ligand that effects gene expression. However, administration of free ATRA has not been effective thus far and has non-specific toxicity. A vehicle to transport the drug specifically to MCL cells is needed. Nanodisk technology has emerged as an ideal option. Nanodisk (ND) is a bioactive agent delivery vehicle composed of an apolipoprotein (apo), phospholipid, and the bioactive agent (drug). The complex itself is hydrophilic on the outside, and hydrophobic on the inside. This characteristic is useful in that it is able to transport hydrophobic drugs. CD20 is a cell surface marker unique to lypmphocytes and lymphomas; it is present on MCL cells. To improve upon drug delivery to MCL cells, a targeted ND was developed. A CD20-specific single chain variable antibody (scFV) was used to engineer a fusion protein consisting of anti-CD20 and apo AI. Incorporation of this fusion protein into ND (targeted-ND), will allow the ND to target specifically to those cells containing CD20 on the cell surface.

Objective: I aim to formulate a consistent and effective targeted-Nanodisk that will successfully deliver ATRA into mantle cell lymphoma cells which will result in concentration dependent apoptosis.

Methods: Formulate targeted-Nanodisks adapting from the protocol described in Ryan et al, 2008; Culture the MCL cell line, Granta, as a test system for ATRA cell killing; Use Non-Radioactive Cell Proliferation (MTT) Assay to determine cell viability.

Results: I was successful in making targeted-ND with and without ATRA. My initial studies showed that 48 hr exposure of Granta cells to 20μM targeted ATRA-ND resulted in only 4% cell survival. Non-targeted ATRA-ND at 20μM had an average of 24% cell viability after 48 hr compared to Free ATRA at 61%. Cell killing was dependent on ATRA concentration, which was less effective at 10 μM.

Conclusion: This study shows that the targeted nanodisk formulation is more efficacious in cell killing than either non-targeted ND or free ATRA. This observation is important since it infers that toxicity to normal cells avoided.

MANOLIS SUEUGA

Funded by the California Institute for Regenerative Medicine Creativity Award

Stanford University, Freshman

Mentors: Marc Chooljian, Michael Conboy, PhD and Irina Conboy, PhD

Throughout most of my experience at Berkeley High School, I found myself moving through class after class uninterested, passively completing assignments because I had to in order to move on to eventually study things that did interest me. This began to change in my junior year when I took my first biology class. My teacher was engaging and passionate about the study of biology, and soon I too became intrigued with the intricacies of life at a molecular level. From then on, I began developing a passion for biology, and a deep appreciation for science. Currently, I am on my way into my freshman year of college at Stanford and although I am undecided on a major, I will certainly be studying molecular biology and neurobiology, and continuing to expand my field of knowledge in the natural sciences.

When a close friend told me about the opportunity to actually partake in scientific research via the CHORI summer program, I was thrilled to apply, and even more so to get in. During my time in the program I have been able to gain a much deeper understanding of what it means to do research in a lab setting and pursue my interest in molecular biology through the study of a particular agonist, and its affect on muscle regeneration. I have been able to interact with other majors and doctorate students in the laboratory who are highly knowledgeable in the research process, and learn important skills in performing bench research. I look forward to continuing doing research in future years, and I am grateful to participate in such a stimulating summer program. I would like to thank Deborah Ellen, Christian Elabd, and Mike Conboy for supporting me in throughout the program, and my amazing mentor Marc Chooljian for his hard work and patience every step of the way.

Investigation of molecular factors in muscle regeneration

Manolis Sueuga, Christian Elabd, Wendy Cousin, Robert Chen, Andrea Tham, Sunny Kung, Marc Chooljian, Irina Conboy

Berkeley Stem Cell Center, Department of Bioengineering, University of California, Berkeley

Introduction: Muscle exhibits one of the greatest abilities to regenerate of adult tissue in humans. Regeneration occurs in a few stages, beginning with myotrauma and the subsequent inflammatory response. This stage is followed by activation and proliferation of satellite cells, a population of quiescent muscle progenitor cells that surround the myofiber, which then fuse with the damaged myofibers and fuse with other satellite cells to produce new myofibers. Finally, the nuclei migrate to the outer edges of the cell and the myofiber is re-inneravated. Impaired ability to regenerate muscles is well documented in older individuals due to a defect in satellite cell activation, and the search for the molecular basis of this phenotype is an important area of research.

Objective: We are investigating the impact of a new signaling pathway on muscle regeneration and aging.

Methods: We are evaluating muscle regeneration of young and old mice upon treatment with an agonist and antagonist of this pathway. Similar experiments will be performed using genetically modified mice that are deficient for the natural ligand of this pathway (knockout mice). Muscle progenitor cells cultured in vitro will also be used. The markers highly expressed in proliferating satellite cells include the sarcomere component desmin. Newly formed regenerating fibers express the embryonic form of Myosin heavy chain (MyHC-neonatal). The lab uses a BrdU incorporation assay and fluorescent immunostaining of these markers to identify proliferative muscle progenitor cells. Muscle regeneration assays will be performed using muscle sections from experimentally injured mice stained with hematoxylin and eosin.

Results: The exposure of old mice to the agonist of our pathway of interest has resulted in improved muscle regeneration of these mice, while the exposure of young mice to the antagonist of this pathway has caused reduced muscle regeneration (an “old mouse” phenotype) in these mice.

Conclusions: The next step in this process is the identification of downstream effectors in this pathway, and a further assessment of the pathway in knockout mice, for which results thus far have been unclear. However, we have identified that this pathway plays a role in the regulation of muscle regeneration.

SIDDHANT TALWAR

Funded by the Doris Duke Charitable Foundation

Mountain Ridge High School, Senior

Mentor: Ellen Fung, PhD

For years, I have been closely attached to the medical world due to my special medical situation. I was diagnosed with Thalassemia at the age of one and since have been receiving blood transfusions every three weeks. Being a patient of a chronic illness and being closely associated with the medical side of things, I feel that I have a different perspective of the adversities faced by patients just like me. I have built a personal connection with CHRCO as I myself have been a patient here at Children's Hospital in Oakland. Knowing how closely associated I have been to the medical world over the years, I gained a strong interest in the field of medicine and research. Within my schooling career thus far, I have always enjoyed science courses especially biology. I hope to use my interests to guide me into a future where I am able to work productively towards finding answers to improve life for patients.

I have just graduated from high school and am looking forward to my next year at college. I have been greatly thankful that I was told about the great summer internship program offered at CHORI and so far have enjoyed the experience immensely. I hope to gain knowledge and improve my abilities regarding medical research. This summer I am working on the aspect of diabetes in thalassemia. I am really optimistic that I will be able to understand a different matter regarding the disorder that I have not really known as much about. Through this process, I will gain understanding of the various aspects related to research and hope to advance my interest in the field. Up until now, I have gained some understanding of the initial processes related in starting new studies and the steps involved. I have gotten to experience interviews with patients who participate in clinical studies. Additionally, I was able experience discussions among doctors during the planning stages of different studies.

Ultimately, my aim is to end up the sector of medical research and I am very grateful that I have gotten such a great opportunities at CHORI to experience the field up close. Although it is not sufficient in words, I would like to give special thanks to my mentor Dr. Ellen Fung and the rest of the HEDCO staff for all the great support they have provided. Dr. Fung gave me countless opportunities to really get a great feel for research, which I am very fortunate for.

A Link between Zinc and Diabetes in Patients with Thalassemia

Siddhant Talwar and Ellen Fung

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Zinc is an essential mineral required for growth, pubertal development, immune function, bone health and glucose homeostasis. Preliminary data from our group suggests that patients with thalassemia (Thal) have a marginal zinc deficiency which is associated with poor bone health. Zinc deficiency maybe due in part to aggressive oral chelation therapy that is required for thalassemic patients to eliminate iron from the body. It is hypothesized that marginal zinc deficiency may also affect glucose and insulin regulation in Thal.

Objectives: 1) to explore the relationship between zinc status, impaired glucose tolerance and insulin sensitivity in patients with Thal, and 2) to assess the effect of a 25 mg/day zinc supplement on glucose homeostasis and insulin secretion in patients with Thal.

Methods: *Aim 1:* A retrospective chart review of patients with Thal currently enrolled in the CHRCO hematology clinic was conducted to explore the relationship between zinc status and response to a glucose challenge. Abstracted variables included fasting zinc and oral glucose tolerance tests. *Aim 2:* Frozen plasma samples previously drawn as part of a randomized placebo-controlled trial (ThinkZn study) were analyzed. Half of the subjects in this study received 25 mg zinc/day the other a placebo capsule daily for 18 months. Blood samples for fasting glucose and insulin were assessed at 2 time points, 12 and 18 months. C-peptide, a marker of insulin secretion, and fructosamine, an indicator of long term circulating glucose concentrations, were analyzed at a single time point; 12 months. All samples were sent to ARUP national laboratory for analysis. Other variables assessed included urinary zinc, plasma zinc and insulin-like growth factor 1. All data were analyzed using STATA v9.1 statistical software and a p-value <0.05 was considered significant.

Results: Aim 1: 16 subjects (27.1± 9.9 yr, 10 Male) with transfusion dependent Thal had serum zinc values drawn clinically within 6 months of an oral glucose tolerance test. Of these, 4 (25%) had low serum zinc. Insulin response to the 75g glucose load trended to be lower in those with low serum zinc ($p=0.08$), compared to those with normal zinc. None of the patients with abnormal fasting glucose values (n=3) had low serum zinc. In fact, glucose concentration at 30 and 60 minutes was significantly lower in those with low ($p<0.03$), compared to those with normal serum zinc. Aim 2: 38 subjects (17.2 ± 5.1 yr, 19 Male) with Thal were enrolled in the ThinkZn study, of which 20 received the zinc supplement. Ten subjects (26%) had low zinc at baseline and 6 of the 10 were prescribed an oral chelator medication. Seven others were diagnosed with diabetes. Those with diabetes had significantly greater urinary zinc excretion ($p=0.024$). Plasma zinc increased significantly with supplementation, and there was a trend towards and increase in insulin like growth factor-1 for those supplemented. Fasting glucose ($p=0.023$) and fasting insulin ($p=0.048$) both decreased with time of study in the zinc group compared to placebo. Results for C-peptide, which is an insulin marker and fructosamine are pending.

Conclusions: Poor zinc status is common in Thal, roughly 25% of subjects in this study had low circulating zinc concentration. These low levels appear related to oral chelator use. It appears that those with marginal zinc status may have an altered insulin response to an oral glucose load. Additionally, these results suggest that zinc supplementation decreases fasting insulin and glucose concentrations. These findings need to be confirmed in a larger study aimed at exploring this relationship more directly.

LYDIA TESFAMARIAM

Funded by the Doris Duke Charitable Foundation

American Indian Public High School, Senior

Mentor: Janelle Noble, PhD

Hello, my name is Lydia Tesfamariam, a high school graduate from American Indian Public High School. I'm the oldest sibling in my family, and the first to go to college: in the fall, I plan to attend Dominican University of California as a Biology major. Because I have much responsibility of taking care of my younger siblings, I was not involved in many researching activities during my high school career even though I dreamed of working in a lab. When I first heard about this internship from my college planning teacher, I was instantly hooked. I knew I had to apply for an internship at Children's Hospital Oakland Research Institute (CHORI) because I would finally be able to experience actual research and learn more about the life of a medical scientist.

I'm spending nine wonderful weeks at CHORI learning more about type II diabetes in children. Before this program, diabetes was only a word I associated with "eating too much candy," but after reading many articles and having frequent stimulating talks with the CHORI scientists around me, I discovered that diabetes was much more complicated. Learning about diabetes through my research is an exciting experience that makes me more interested about researching in the future. The lessons I take from this internship program will benefit me extremely as I apply my knowledge of patient confidentiality and the complexity of diseases to my work with patients from places of widespread poverty and large outbreaks. My CHORI experience greatly heightened my interests in the medical field and will be useful in the future.

The Key Variables in Differential Diagnosis of Pediatric Type 1 and Type 2 Diabetes

Lydia Tesfamariam, Nancy Keller and Janelle Noble

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Insulin is a hormone produced and secreted by pancreatic islet beta cells. This hormone is responsible for allowing glucose into cells. Type I diabetes (T1D) patients produce insufficient insulin to allow glucose to enter body cells because their pancreatic beta cells have been damaged by an autoimmune reaction which sees the beta cells as a threat. However, in type II diabetes (T2D), insulin levels are abnormally high, causing hyperinsulinemia. The insulin resistance prevents body cells from responding to insulin. The diabetes differential diagnosis model, or DDDx (Keller, et al., PLoS March 2012) is intended to aid doctors in making an initial diagnosis using variables such as age, gender, height, weight, and race/ethnicity before lab test results are available, which can be up to two weeks after presentation. The model will give doctors an idea of which type of diabetes they are dealing with at presentation, and this can immediately determine whether a patient must be hospitalized for 1 to 2 days (T2D patients) or 3 to 4 days (T1D patients).

Objective: To make these predictive models, we first must find the observational or other variables that could help distinguish T2D from T1D at presentation (when the patient first comes to the doctor). New data was gathered to extend and replicate the DDDx model cited above.

Methods: Observational and clinical laboratory data recorded at presentation (first visit) were abstracted from 37 charts of African American (AA), and 31 charts of Hispanic American (HA) diabetes patients, age 2 – 18, who presented in 2009 – 2010. Clinical laboratory data recorded at presentation will be collected for future comparisons that will determine whether or not the clinical laboratory data will improve or not improve the predictability of the models. Observational data recorded at presentation were used to find correlations more prevalent in T1D or in T2D patients of African American and Hispanic American ancestry.

Results: Observational variables measured at presentation included age, BMI, hyperpigmentation, gender, DKA, and family history. Statistically significant differences between T2D and T1D are seen for age with the p-value of .0001 for AA patients and .0003 for HA patients and BMI with the p-value of .0006 for AA patients and less than .0001 for HA. There is insufficient data to detect any significant differences between T2D and T1D in gender distribution, hyperpigmentation, DKA, and family history. The autoantibodies associated with beta-cell damage measured at presentation are GAD65, IA-2 and IAA. T1D presented with positive autoantibody tests GAD65 77.8% for AA patients and 81.0% for HA patients, IA-2 64.3% for AA patients and 81.8% for HA patients, and IAA 59.3% for AA patients and 47.6% for HA patients compared to T2D patients with positive autoantibody tests GAD65 0% for AA and HA patients, IA-2 0% for AA and HA patients, and IAA 0% for AA and HA patients.

Conclusions: Significant differences between T2D and T1D were found in both AA and HA populations for age and BMI. With more data for statistical analysis, we can determine whether there are significant differences between T2D and T1D for hyperpigmentation, gender, family history, and DKA.

TUYEN NGOC TRAN

Funded by The Short Term Research Education Program to Increase Diversity in Health Related Research from the NIH/NHLBI

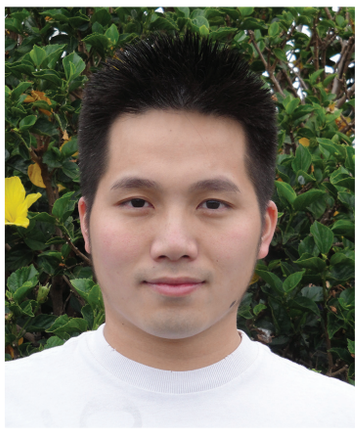
California State University, Long Beach

Mentor: Vasanthy Narayanaswami, PhD

Ever since cancer stole my father's life, it is my aspiration to become a researcher and medical practitioner to join the fight against diseases. Although it is too late to save my father, I take solace in knowing that I may someday have the chance to save the lives of parents, children, siblings, and friends. I have the determination and the confidence to achieve my dreams, and CHORI Summer Research Program is a part of these dreams. CHORI Summer Research Program offers an opportunity to sharpen my skills and gain experience in research. Also, as a program participant I will develop knowledge to prepare me for graduate study.

Thanks to the generous support of the program I am able to continue to conduct research under the direction of my mentor, Dr. Vasanthy "Vas" Narayanaswami. Dr. Vas' enthusiasm for encouraging her students to pursue biomedical research has dramatically increased my engagement in both my academic studies and undergraduate scientific exploration. Dr. Vas not only taught me how to conduct the research independently, but she also helped to hone my leadership skills. These skills will be extremely helpful for me as a future researcher.

Being a part of the CHORI Summer Research Program would bring me one step closer to achieving my goals, and I greatly appreciate the opportunity to be one of the recipients of this award. Especially, I would like to thank Dr. Vas for her mentorship, patience, and time during the summer.



Proteomics Analysis of Oxidative Stress Modification of Apolipoprotein E by Acrolein

Tuyen Ngoc Tran[1], Yuan Yu Lee[2], Koji Uchida[3] and Vasanthy Narayanaswami1[4]

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Introduction: Apolipoprotein E (apoE), an anti-atherogenic apolipoprotein, plays a significant role in the metabolism of lipoproteins. It lowers plasma lipid levels by acting as a ligand for low-density lipoprotein receptor (LDLr) family of proteins. ApoE mediates this function via essential lysine residues that interact with the LDLr. Preliminary studies from our lab showed that rats exposed to environmental tobacco smoke displayed oxidative modification of apoE and dissociation of lipoprotein-bound apoE; however, the specific sites of modification or the effect of modification on the LDLr binding function of apoE are not known.

Objective: The objective of this project is to study the effect of oxidative stress (specifically acrolein) mediated *in vitro* modification on recombinant rat apoE and its effect on the LDLr binding ability of apoE.

Methods: Recombinant rat apoE was obtained using a bacterial expression system. The purified protein was incubated with acrolein (10-fold molar excess over apoE) at 37 °C for 4 h. The modification was verified by probing with an acrolein-lysine specific antibody by Western blot analysis. LDLr binding ability was evaluated by conducting co-immunoprecipitation assay. Matrix Assisted Laser Desorption Ionization-Time of Flight/Time of Flight Mass Spectrometry (MALDI-TOF/TOF MS) was carried out to study the modification sites at the molecular level.

Results: SDS-PAGE confirmed that the protein was purified to homogeneity with no signs of degradation. Acrolein modification was confirmed by Western blot analysis. Functional assay demonstrated that the LDLr binding ability of acrolein-modified apoE was significantly impaired. Lastly, MALDI-TOF/TOF MS analysis identified Lys82, 85, 86, 156 and 252 as likely modification sites by acrolein.

Conclusions: Loss of LDLr binding ability is attributed to acrolein modification of Lys156, an essential residue located on helix 4, that is directly involved in interaction with the LDLr. Overall, we conclude that acrolein disrupts functional integrity of apoE, which is likely to affect its role in maintaining plasma cholesterol homeostasis.

DANIELLE TUCKER

Volunteer

University of California, Berkeley, Junior

Mentors: Grace Wang PhD and Ervin Epstein Jr., MD

Currently, I am at junior at UC Berkeley majoring in Molecular and Cell Biology with an emphasis in immunology. My earliest memory of the laboratory is looking up at the red, green, blue, and black squiggles on my dad's computer screen (DNA sequencing). This was back in the day when the lab coat my dad put on me touched the floor and size extra small latex gloves were too big. My dad's boss would ask if I was going to work for them when I got older and I, of course, said yes. When I was seven, my mom was diagnosed with Rheumatoid Arthritis. Soon afterwards I began saying that I was going to become a Rheumatologist because I wanted to cure my mom. Once I got older, my ambitions became more realistic. I wouldn't be able to cure my mom, but I still wanted to be a doctor. My mom's disease progressed quickly. She began receiving infusions of the strongest drug on the market, and even that was not a cure-all. That was when I realized that doctors have their limits for how much they can help their patients. A doctor's ability to treat his/her patients is dependent on the advancement of knowledge about the disease and the development of treatments. My hope is that like Dr. Epstein I will be able to not only contribute to healthcare as a physician but a researcher as well. I have enjoyed volunteering in the Epstein lab at CHORI and being a part of the CHORI Summer Research Program. The seminars have been great exposure to current research and issues in healthcare. Also, having my own project has pushed me to delve deeper into and to think more critically about the research done in the Epstein lab. I would like to thank Grace, Joy, and Lynn for mentoring me, sharpening my basic lab skills, teaching me new techniques, and introducing me to working in an animal facility.

Patched-1 genotyping analysis of microscopic basal cell carcinomas

Danielle Tucker, Grace Wang and Ervin Epstein Jr.

Children's Hospital Oakland Research Institute, Oakland, CA 94609

Introduction: Basal cell carcinomas (BCCs) are the most commonly diagnosed human cancer. The majority of BCCs occur sporadically, but patients with the heritable disorder called basal cell nevus syndrome (BCNS) have a high susceptibility to BCCs. Using family-based linkage studies of families with BCNS, the locus carrying the mutant gene was mapped to chromosome 9q22 and then to the PATCHED1 (PTCH1) gene (Epstein, E. 2008). All BCCs, whether sporadic or familial, have Hedgehog signaling activation likely due to PTCH1 gene abnormalities in at least 80% of these tumors. BCNS patients' susceptibility is similar to that of the transgenic Ptch1 +/- mice that we use in the laboratory. When Ptch1+/- transgenic mice are treated with IR at age 2 months they develop μBCCs first detectable generally from age 4 to 5 months. A small percentage of these progress into palpable/visible BCCs. The molecular underpinnings of the deletion/inactivation of the wild type allele of Ptch1 are not completely understood.

A previous study, Basal cell carcinoma and its development: insights from radiation-induced tumors in Ptch1-deficient mice (Mancuso, M., et al, 2004), looked at the stages of growth of BCCs, and genotyped macroBCCs and μBCCs. It found that macroBCCs had the mutant allele and μBCCs only carried the wild type allele. Considering the method used to isolate the μBCCs, we believe that the samples contained a large amount of stroma, which could have caused the samples to be genotyped as Ptch +/-. In the present study, laser-capture microdissection (LCM), a more precise method for obtaining tumor cells, will be used. With this method we will be able to determine the Ptch1 genotype in tumor cells.

Objective: It is of importance to characterize this stage of BCC development because not all μBCCs turn into visible, malignant BCCs. Also, the reason not to look earlier is that μBCCs are too small or too few to allow assessment. If we can determine when the genotype mutates to Ptch1 -/-, then it is possible to take steps towards the prevention of malignant tumors by targeting that stage of development. The goals of this study are: 1) to optimize LCM for genotype analysis of μBCCs 2) to determine the Ptch genotype in μBCC tumor cells.

Methods: LCM was used to isolate μBCC tissue. The DNA was extracted using FFPE extraction solution. Standard PCR for Ptch was then followed.

Results: The isolation of μBCCs from skin tissue using LCM and extraction of the DNA from the dissected tissue was optimized. The minimal amount of laser punches used in order to get DNA was 500 punches. Also, the FFPE extraction solution gave more intense bands on the agarose gel than the PicoPure extraction solution. The first μBCC sample was Ptch +/-. Additional results are pending.

Conclusion: Pending while more samples are being analyzed

AUTUMN TURPIN

Volunteer

Stanford University, Freshman

Mentor: Heidi Flori, MD

My name is Autumn Turpin, and this is my second year in the CHORI Summer Student Program. I graduated this year from St. Patrick St. Vincent High School, and will be attending Stanford University in the fall. I plan to major in biomedical engineering.

I became interested in engineering after spending last summer's CHORI program studying the effects of helium and Heliox on acute respiratory distress syndrome patients. I saw many types of technologies used for patient support, such as high frequency oscillatory ventilators, cardiopulmonary bypass used in heart surgery, and ECMO (external corporeal membrane oxygenation). During the internship, I was also able to see cardiac surgery, which sparked my interest in studying to develop cardiac devices. At the hospital, I also saw how I could combine my interest in research and patient care through clinical research. I enjoy the research and technology aspect, yet I don't want to be far away from seeing the people affected by medical discoveries.

This summer, the project I am working on is a long-term follow-up study focusing on quality of life of patients who have sustained an acute lung injury. I have learned more about different technologies used to assess patients, such as pulmonary function tests and the IOS machine.

My current education plans are to study biomedical engineering in undergraduate school, and apply for a coterminal master's program to graduate with a bachelor and master's degree after five years. After that, I am unsure whether I work towards a PhD or an MD/PhD program. I would love to someday work in a hospital or, if possible, in both a hospital and a lab. So far, I have not experienced lab research. After these two summers as a summer student, I know I really enjoy clinical research.

The CHORI Summer Student Program has given me amazing opportunities to see first-hand what clinical research is like and how a hospital runs and cares for its patients. It has helped me to refine my education and career goals, and I am extremely happy I had the chance to participate.

Long-Term Follow-Up of Pediatric Patients with Acute Respiratory Distress Syndrome (ARDS)

Autumn Turpin, Marsha Treadwell, Gwynne Church, Isabelo Elisan , Ginny Gildengorin and Heidi Flori

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: There are far more long-term follow-up studies for adult survivors of ARDS than for children. One particular study conducted on 109 adult survivors of ARDS one year after discharge evaluated functionality after their stay. Many adults had persistent limitations after discharge (including diminished motor functionality and problems with cognition) concluding that patients have trouble recovering from ARDS and the ICU stay.

Objectives: Aim One: To identify clinical predictors of 3 major outcomes in 23 pediatric patients with ARDS: 1) re-hospitalization rate, 2) risk of persistent abnormal pulmonary function, and 3) long term quality of life (QOL).

Aim Two: Analyze for possible differences in patients that: did not consent, consented and failed to return for follow up, and consented and did return.

Methods: From 2002 to 2005, all patients in the PICU were prospectively screened for study eligibility: intubation and meeting American European Consensus Conference definition of ARDS. After patient discharge from the PICU, follow-up visits were arranged. At the visits, both parent and child (if developmentally capable) completed the Child Health Questionnaire (CHQ) to assess QOL.

The CHQ results were graded according to the manual. Scores are on a 1-100 scale; 100 as optimal. CHQ results were used from the date of testing closest to 6 months post discharge.

PFTs were completed on 21 of the patients. Standard CHO procedures for both infant and child PFT testing were applied. Patients completed infant PFTs, full PFTs, or impulse oscillometry (IOS).

Data consisted of patient demographics, consent forms, PFT results, CHQ, and medical history questionnaire. Data from the patient files were added to an Access database with CHQ and PFT data. Descriptive statistics of demographic and clinical data, t tests for CHQ results, and Cox modeling for PFTs are currently underway.

Results: 23 of 50 consented patients returned for follow-up. Diagnoses associated with ARDS included pneumonia, aspiration, sepsis/meningitis, drowning, cardiac disease, trauma, sickle cell disease, Kawasaki's disease, and post-spinal fusion surgery.

The parent QOL data showed: 7 parents in the 41-60 category, 9 in the 61-80 category, and 9 in the 81-100 category (n=25). The child QOL data showed: no patients between 41-60, 1 patient in 61-80, and 7 patients in 81-100 (n=9). Analysis of PFT data is still in progress.

Conclusions: Interestingly, the children seem to have a more positive opinion of their QOL than parents. Likely, parents are concerned and worried about their child's well-being while the children are more resilient. Conclusions about pulmonary function will be made after analysis of PFT data is complete.

NEHA VERMA

Volunteer

Mission San Jose High School, Senior

Mentor: Caroline Hastings, MD

For my sweet 16, last year, I decided that I wanted to start making a difference in my community. I realized that there is no age cut-off for when anyone can make a difference in the world. I organized a charity fashion show for my birthday and raised $9000 for the Oncology Department at CHRCO.

It was through this fashion show that I met my mentor, Dr. Caroline Hastings, director of the fellowship program in the Oncology/ Hematology department at the hospital. After I told her about my interests in math and science, she encouraged me to apply for the CHORI Summer Program.

I was very excited to be accepted into CHORI, as it gave me an opportunity to get a glimpse of not only what life as a pediatric oncologist is, but also a thorough understanding of how research projects are conducted. It gave me the opportunity to try to make a difference using my knowledge of AP Biology, AP Chemistry and AP Statistics classes. With the help of the material in these classes, as well as endless support and guidance by Dr. Hastings and Dr. James Feusner, I carried out a retrospective review project. The aim of my project was to determine if the current practice of CT imaging after treatment is indicated to monitor for relapse in children with Wilms tumor and Hepatoblastoma.

I am so grateful to have been given the opportunity to be part of this program, and meet all the inspirational people this program allowed me to meet. I am sure that I will take the lessons I learned from this program wherever I go.

Yield of routine CT imaging of the pelvis in detection of Relapse in Pediatric Patients with Hepatoblastoma

Neha Verma, Caroline Hastings and James Feusner

Children's Hospital & Research Center Oakland, Oakland, CA 94609

Introduction: Hepatoblastoma is the most common type of pediatric liver cancer, occurring primarily in children under the age of 3. Survival is related to initial stage. Relapse, even in lower stage disease, is still a problem, creating a need for accurate surveillance off- therapy. Following completion of therapy, surveillance for potential relapse includes physical examination, imaging, and monitoring of tumor markers (AFP). Traditionally, CT imaging of the lungs, abdomen and pelvis have been utilized for diagnostic and surveillance purposes. The utility of routine pelvic CT imaging is questioned given the probability of finding a relapse in this area that my not be detected by other means such as an elevated AFP or on examination.

Objective: The aim for this project is to determine if the current practice of CT imaging of the pelvis after treatment is indicated to monitor for relapse in children with hepatoblastoma.

Methods: We performed a cohort retrospective study of all patients diagnosed with hepatoblastoma at the Children's Hospital & Research Center Oakland between January 1978 and June 2012. This study was approved by the Institutional Review Board. We reviewed written and electronic medical records. Data collected included gender, age at diagnosis, date of birth, stage, presence of pelvic extension, presence of metastatic disease, location of metastatic disease, histology, presence and level of positive tumor markers, chemotherapy treatment protocol, dates of surgical biopsy and/or resection, date off therapy, length of follow-up, occurrence of relapse, relapse location, and survival. We reviewed CT imaging data including date and locations of initial scans, other imaging done, date of baseline CT after surgery, and dates of all surveillance CT imaging by location.

Results: Thirty patients were diagnosed with hepatoblastoma at CHRCO between 1978 and June 2012. Two patients were not eligible for review due to inadequate medical records; therefore our cohort consisted of 28 patients. (20 males, 8 Females; Age Range 0- 3 yrs). CT scans performed included the pelvis, chest, and abdomen. Overall, 311 CT scans were performed for the 28 patients (average of 11.1 CT scans total per patient). There were 152 surveillance CT scans (average- 5.4 surveillance CT scans per patient). We focused on pelvic CT scans, and discovered that in total, there were 206 pelvic CT scans (average- 7.4 total pelvic CT scans per patient). Of these 109 were surveillance CT Scans (average- 3.9 pelvic surveillance CT scans per patient). Twenty-four patients survived after treatment (85.7% survival) and 4 passed away (14.3% death). Two patients relapsed off-therapy; one at 2 years and 2 months, and the other at 2 years and 4 months off therapy. Both patients experienced pulmonary metastasis and were successfully re-treated. In both cases, the AFP levels became significantly elevated at the time of the relapse. (4875ng/mL and 180ng/mL, respectively).

Conclusions: In our study, 85.7% of the children diagnosed with hepatoblastoma were cured with initial treatment. In our patients that experienced relapse, disease was confined to the lungs found on CT imaging and associated with an increase in AFP protein levels. None of our patients experienced relapse in the abdomen or pelvis. Therefore, the utility of off-therapy surveillance pelvic imaging is questionable, especially if AFP levels can be tracked routinely post- treatment. Not only does surveillance imaging pose a financial issue to some patients' families, but there also are concerns that exposure to radiation by CT imaging may increase risk of cancer. Therefore we should be prudent in how we determine when and how frequently to utilize CT imaging for surveillance of recurrence of hepatoblastoma.

Notes

the 2012 CHORI SUMMER STUDENT RESEARCH SYMPOSIUM

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