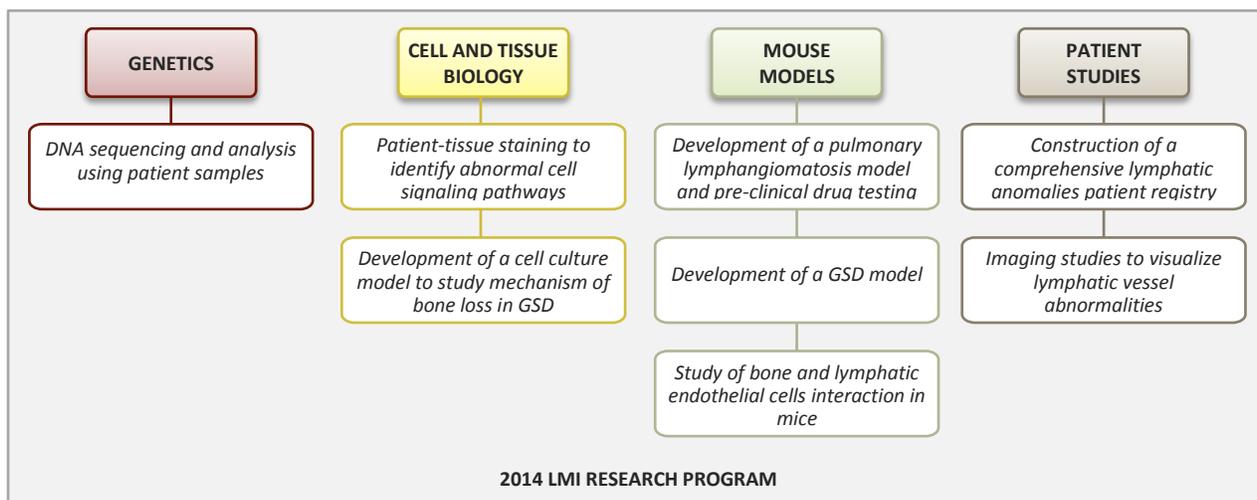


Lymphatic Malformation Institute
2014 ANNUAL REPORT

INTRODUCTION

The LMI funding program entered its fourth year in 2014, and spanned across several areas of research fundamental to advancing the understanding and treatment of generalized lymphatic anomaly (GLA) and Gorham-Stout Disease (GSD).



Overall, the program comprised of eight active projects in 2014. Out of these, a total of \$340,912.00 was awarded in grants to three continuing projects and one new project. The remaining were covered under funds awarded in 2013 and/or did not require additional funding in 2014.

CONTINUING PROJECTS

1. Genetic and genomic analysis in patients affected by Gorham-Stout Disease and General Lymphatic Anomalies

Victor Martinez-Glez, MD, PhD, Juan Carlos Lopez Gutierrez, MD, PhD and Pablo Lapunzina, MD, PhD; Institute of Medical and Molecular Genetics (INGEMM), Madrid, Spain

The project was approved for a second round of funding in mid-2014 to continue genetic sequencing of blood and affected tissue from GLA and GSD patients, and to develop a bioinformatic tool for integration and analysis of all the collected data. Using different approaches for DNA sequencing, the team has identified several candidate regions/genes that are being tested further to determine their involvement in disease. Once completed, this will be the single largest DNA sequencing study of GLA and GSD samples ever conducted.

2. Immunohistochemical characterization of the extraskelatal changes in generalized lymphatic anomaly (GLA) and Gorham Stout disease (GSD)

Erik Eklund, MD, PhD; Lund University, Lund, Sweden

This project has been focused on the collection and immunohistochemical-characterization of GLA and GSD patient samples. Using antibodies to visualize different signaling proteins, Dr. Eklund has been studying tissue-sections to determine if differences in their levels and/or cellular localization can be picked up between normal and diseased samples. Through his efforts, a large collection of patient samples has been established at Lund University, and Dr. Eklund remains very open to collaboration with researchers around the world to further utilize these samples; a collaboration with Dr. Lianpin Xing has already been initiated (see pg.3) for expanding her mouse studies to human samples. Results from the project are being compiled into a manuscript for publication. Though the LMI's funding for Dr. Eklund's project ended in mid-2014, the lab is continuing the work through another grant received from a Swedish foundation. The LMI remains in touch with Dr. Eklund to keep abreast of his research.

3. Molecular crosstalk and matrix metalloproteinases in Generalized Lymphatic Anomaly (GLA) and Gorham-Stout Syndrome (GSS) patients

Ramani Ramchandran, PhD; Medical College of Wisconsin, WI

This project was initiated in 2012, and has been focused on studying the interaction between bone cells and lymphatic endothelial cells using a cell-culture based model. So far, Dr. Ramchandran's studies have been successful at establishing culturing and differentiation conditions for cells. The project was renewed for a second round of funding December.. In the first phase (December 2014-May 2015), the work will focus on establishing a quantifiable assay for culturing osteoclasts (bone-resorbing cells) in the presence of normal and patient-derived lymph fluid. Once a robust assay has been established and osteoclast function can be reliably measured, the second phase of the grant (June - November 2015) will be awarded that will aim at identifying cellular pathways that affect osteoclast function in response to lymphatic fluid.

4. Transgenic mouse model to determine the mechanism and treatment of congenital pulmonary lymphangiectasia and lymphangiomatosis

Donald M. McDonald, MD, PhD; University of California, San Francisco, CA

The project was renewed for a third round of funding in mid-2014. The mouse model originally proposed has been well characterized and published (see pg. 4). In addition, studies so far have shown that rapamycin can significantly reverse the abnormal overgrowth of lymphatics, whereas treatment with other agents like dexamethasone, propranolol, DAPT and chloroquine does not have this effect. During the third year, the project will focus on determining the mechanism behind rapamycin's reversal of lymphatic growth, as well as testing agents that can augment the effect of rapamycin.

5. Development of a mouse model of Gorham-Stout Syndrome

Michael T. Dellinger, PhD; University of Texas Southwestern Medical Center, TX

This project was transferred from Dr. Rolf Brekken's lab (where it was initiated in 2013) to Dr. Dellinger's new lab. Dr. Dellinger tested different approaches for creating transgenic mice that overexpress VEGF-C in the bone. Mouse colonies have now been established for investigation and will be used to study if stimulating VEGF-C levels in bone leads to skeletal anomalies, and whether

these can be prevented or reversed through intervention. Application for renewal to continue the project has been approved and funding for the second phase will begin in 2015.

6. Lymphatic anomalies registry

Cameron C. Trenor III, MD; Boston Children's Hospital, MA

The first phase of the project, initiated in early 2012, was completed in mid-2014. During this phase, the patient registry was successfully built, implemented and launched: www.lymphaticregistry.org. As of December 2014, the registry comprises of a total of 387 patients with lymphatic anomalies, out of which there are 47 Gorham-Stout disease patients and 104 GLA patients. During the second phase, patients will continue to be enrolled in the registry and the data collected will be analyzed to study disease features such as presentation, outcomes, etc. In addition, expansion of the registry to additional sites is being explored, as well as initiation of patient-sample collection for establishing a Biorepository. Funding for the second phase has been approved and will begin in early 2015.

NEW PROJECTS

1. Bone cells and lymphatic endothelium interface

Lianping Xing, PhD; University of Rochester, NY

This project is aimed at exploring the potential interaction between bone cells and lymphatic endothelial cells to gain a better understanding of the bone loss that occurs in GSD. Using mice, Dr. Xing has identified a key factor secreted by LECs that could be promoting osteolysis, and is in the process of conducting further experiments to confirm these results. She has also begun a collaboration with Dr. Erik Eklund (see project description on pg. 1) to obtain patient samples to check if this factor is upregulated in GSD and verify the results she has obtained in mice. The project commenced in January 2014, and a renewal application for continuing the studies for another year is pending.

2. Imaging lymphatic function in normal subjects and in persons with lymphatic disorders

Eva M. Sevick-Muraca, PhD; University of Texas Health Science Center at Houston, TX

The purpose of this study was to visualize lymphatics in a GLA patient using Near Infra Red Fluorescence (NIRF) imaging, and explore whether abnormalities in lymphatic vasculature and/or lymphatic flow can be picked up. The idea for the study arose at the 2013 LMI-LGDA conference where Dr. Sevick-Muraca gave a presentation on the NIRF imaging technology. The patient who took part in the study was identified by the LMI's partner organization, the Lymphangiomatosis and Gorham's Disease Alliance (LGDA). Results from the study will be compiled into a manuscript for publication. The grant award for this project is pending.

CONFERENCE ATTENDANCE AND PARTICIPATION

The LMI staff attended and participated in several scientific and patient conferences throughout the year.

1. 20th International Workshop on Vascular Anomalies (ISSVA), Melbourne, Australia, April 1-4, 2014.

Attended by Dr. Mike Dellinger.

2. 27th Annual American Society of Pediatric Hematology & Oncology Meeting (ASPHO), Chicago, IL, May 14-17, 2014.

Attended by Tiffany Ferry and Dr. Mike Dellinger. The LMI provided \$1000 for a travel grant/young investigator award to ASPHO towards recognition of Drs. Denise Adam's, Leonardo Brandao's and Cameron C. Trenor's work for the Vascular Anomalies Special Interest Group. Dr. Dellinger was a presenter for a special symposium titled, "Updates in lymphatic anomalies for the hematologist/oncologist", and gave a presentation on the basics of lymphangiogenesis and animal models of lymphatic diseases.

3. Inaugural International Lymphangiomatosis & Gorham's Disease Alliance Patient & Family Conference, Dallas, TX, June 13-14, 2014.

Attended by Tiffany Ferry and Dr. Mike Dellinger. The LMI provided \$1500 sponsorship toward the banquet dinner. Dr. Dellinger gave a presentation in which he discussed several of the research projects funded by the LMI and future research projects on generalized lymphatic anomaly and Gorham-Stout disease.

4. American Society for Bone and Mineral Research 2014 Annual Meeting (ASBMR), Houston, TX, September 12-15, 2014.

Attended by Dr. Mike Dellinger.

5. ASPHO Vascular Anomalies Special Interest Group Meeting, Boston, MA, November 9, 2014.

Attended by Tiffany Ferry and Dr. Mike Dellinger.

6. 2014 LAM International Research Conference, Chicago, IL, March 28-30, 2014.

Attended by Tiffany Ferry. The LMI provided \$500 sponsorship for this meeting.

PUBLICATIONS

Two LMI-supported research projects resulted in peer-reviewed journal articles in 2014:

1. Pulmonary lymphangiectasia resulting from VEGF-C overexpression during a critical period

Yao LC, et al., 2014, Circulation Research 114, 806-822.

This paper from Dr. Donald McDonald's lab reports studies conducted using the VEGF-C transgenic mice.

2. Kaposiform lymphangiomatosis: a distinct aggressive lymphatic anomaly

Croteau SE, et al., 2014, Journal of Pediatrics 164, 383-388.

This paper from Dr. Cameron Trenor's lab discusses the clinical and imaging features of Kaposiform lymphangiomatosis (KLA) using analysis of data contained in the patient registry at Boston Children's Hospital.

In addition, the review article on Gorham-Stout disease co-authored by the LMI team with Dr. Bjorn Olsen (Harvard Medical School) following the 2013 LMI-LGDA research conference was published in 2014:

Viewpoints on vessels and vanishing bones in Gorham-Stout disease

Dellinger MT, et al., 2014, Bone 63, 47-52.

CONCLUSION

The LMI research program has grown significantly in the last four years, and has established itself as the largest global source of funding for research on generalized lymphatic anomaly and Gorham-Stout disease. During this time, we have also strived towards building close relationships with the labs we fund and positioning ourselves as their collaborators. We are deeply aware that biological research is seldom a straight path, and requires constant and real time assessment. We hope that by promoting an interactive and open approach to research-funding, we will be able to work hand-in-hand with the research community to address roadblocks and forge ahead.