

Lymphatic Malformation Institute
2013 ANNUAL REPORT

This report summarizes the proceedings of the Lymphatic Malformation Institute's research funding program for the year 2013.

RESEARCH PROJECTS

The LMI's research program comprised of six active projects in 2013, of which three were initiated this year (total awarded: \$487,730.30). In addition two new projects were approved for funding in 2014 (total approved: \$93,116.25).

1. Lymphatic anomalies database

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

Work on this project continued from early 2012, and aims to build a searchable database of patient data for the large number of vascular anomalies cases seen at BCH to facilitate data organization, analysis and accessibility. The pilot phase of the project to test database design and setup was completed and compiled into a manuscript for publication. Consent documents, and patient questionnaires for data-collection and entry into the database were finalized, and are being actively sent to patients for recruitment. As of September, around 60 patients had been enrolled for prospective data collection.

2. 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)

The project was renewed for second year of funding in August. Studies conducted in the first year were helpful in characterizing the mouse model, and establishing a critical role for aberrant VEGF-C signaling in lymphatic overgrowth in the lungs. Studies in the second year will build upon these results, and test whether different therapeutic agents can be used to reverse the growth of the abnormal lymphatic vessels, including dexamethasone, rapamycin, and propranolol.

3. Molecular crosstalk and matrix metalloproteinases in generalized lymphatic anomaly (GLA) and Gorham-Stout syndrome (GSS) patients

(Ramani Ramchandran, PhD and Kelly Duffy, PhD; Medical College of Wisconsin)

The project is aimed at investigating the signaling between lymphatic endothelial cells (LECs) and osteoclasts to learn if and how the deregulation of this communication causes disease. During the year, osteoclasts were successfully cultured from normal blood monocytes, and preliminary studies using lymph from a Gorham's patient show increased osteoclastic activity. The project was funded in November, 2012 but studies commenced in March 2013. The project has been extended until March 2014 to accommodate the delayed start.

4. Genetic and genomic analysis in patients affected by Gorham-Stout disease and general lymphatic anomalies

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

Work on the project began in January 2013. Several GLA and GSS patient samples including blood and affected tissue were, and continue to be analyzed using genomic tools such as CGH arrays, SNP arrays and next generation sequencing (NGS) to uncover potential genetic cause(s) behind the disorders. CGH studies have revealed copy number variations (CNV) in the genome of a handful of patients that are being further investigated. Results from SNP arrays and the sequencing work are anticipated in the near future.

5. Immunohistochemical features of lymphangiomatosis and Gorham's disease

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

The project began in March and is focused focus on staining patient tissue samples to visualize and quantify the levels of signaling molecules, cell-surface receptors, and several other proteins.

Through collaboration with other labs, including Dr. Gutierrez, Dr. Eklund has procured a large number of biopsy samples for conducting his studies.

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

(Rolf Brekken, PhD; UT Southwestern Medical Center, Texas)

The project began in March and is aimed at establishing a mouse model for GSS. Transgenic mice will be used to test if over-expression of VEGF-C in bones leads to their resorption.

7. Bone cells and lymphatic endothelium interface

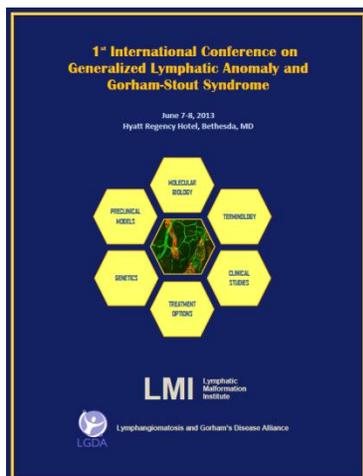
(Lianping Xing, PhD, University of Rochester, New York)

The idea for this project was formulated during the LMI-LGDA conference in June. The proposal was approved for funding in October, and work will commence in January 2014. TNF-alpha transgenic mice, a model for studying arthritis, will be utilized to investigate the interaction between lymphatic endothelial cells and osteoclasts, and to also test if osteoblast inhibition by lymphatic endothelial cells is a contributing factor for bone loss seen in GSS.

8. The mTOR and RANKL pathways in lymphangiomatosis and Gorham-Stout syndrome

(Alison Boyce, MD; Children's National Medical Center, Washington, DC)

The project t aims to explore the involvement of mTOR and RANKL signaling pathways in the promotion of lymphatic and bone pathologies respectively. Though the proposal has been approved for funding , the grant is yet to be awarded due to the lack of availability of clinical samples to carry out the experiments. Dr. Boyce is currently working with LGDA to procure patient samples through NDRI, and through CNMC.



SCIENTIFIC CONFERENCE

In the past, lymphatic anomalies have been the focus of a number of international conferences. However, GLA and GSS are very rarely, if at all, discussed at these gatherings. The LMI and LGDA sought to fill this critical gap by organizing and sponsoring the 1st International Conference on Generalized Lymphatic Anomaly and Gorham-Stout Syndrome held at the Hyatt Regency in Bethesda, Maryland on June 7-8. The meeting was successful in bringing together a very broad group of experts including not only clinicians and scientists who are actively involved with these disorders, but also introducing new scientists to the field whose work could be relevant to furthering our understanding of GLA and GSS. Following are some key elements of the meeting. For a detailed description, please see the addendum to this report: *Proceedings of the 1st International Conference on GLA and GSS*.

Management and leadership

The LMI and LGDA spearheaded the overall management of the conference including designing the conference program, shaping the attendee list and overseeing the logistical aspects. Dr. Bjorn Olsen (Professor, Harvard Medical School) was invited to co-chair the meeting along with LMI's Director of Research, Dr. Michael Dellinger. We are very grateful to Dr. Olsen for his valuable input and guidance throughout the planning process, and for stimulating scientific dialogue at the meeting by raising thought-provoking questions.

Opening address for the meeting was given by LMI's Program Director Dr. Nupur Garg, and LGDA's President Mr. Jack Kelly spoke to the gathering on day two. Each session was pre-assigned a discussion leader from amongst the attendees who was responsible for leading the Q&A after each presentation. Dr. Stephen Groft (Director, Office of Rare Diseases, NIH) addressed the meeting at the dinner reception on the first day. Concluding remarks on day two were made by Drs. Bjorn Olsen and Michael Dellinger.

Scientific agenda

Drafting of the meeting's scientific program, including assembling the oral sessions, planning the talks and speakers for each session and designing the breakout sessions was led by Dr. Dellinger, in close collaboration with Dr. Olsen. There were a total of five oral sessions, each featuring four talks, which comprehensively covered different topics including basic science research, preclinical models, clinical features, treatments and genetics. Speakers were encouraged to present unpublished data, and talk about ideas and hypotheses to generate novel areas for investigation. The program also featured breakout sessions where the attendees, as a part of one of four groups, brainstormed on ideas for addressing topics such as terminology and classification, etiology, therapies and biomarkers. For details of each session, please see addendum.

Attendees

There were a total of 62 attendees at the conferences, including 20 invited speakers from 7 different countries, additional scientists and clinicians, and members of the LMI and LGDA. Several representatives from the different institutes of the NIH, including NHLBI, NIDCR and NIAMS were invited to the meeting to promote future collaboration and support from the NIH for GLA and GSS.

Meeting book and handouts

All the attendees were provided with a conference bag containing the meeting book, and other printed material such as the LMI's 2012 annual report and a brochure for LGDA's global patient registry. The meeting book contained the speaker-abstracts, description and discussion items for each breakout session, background information on the LMI and LGDA, and a pictorial list of all the attendees. The book was designed and compiled by Dr. Nupur Garg.

Organization and sponsorship

The LMI and LGDA are grateful to Charlene Waldman, former Executive Director of The Paget Foundation, for helping with the conference organization.

All conference-related costs, including travel and hotel expenses for all the attendees, were covered by sponsorship provided by the LMI (\$42,748.39) and the LGDA (\$20,000.00). Half of LGDA's sponsorship was contributed by The Alfie Milne Lymphangiomas Trust, UK.

PUBLICATIONS

Two articles were compiled for publication following the June conference:

1. Conference report

1st International Conference on Generalized Lymphatic Anomaly and Gorham-Stout Syndrome
Michael T. Dellinger, Nupur Garg, Tiffany Ferry, Jack Kelly and Bjorn R. Olsen

Accepted for publication in Bone Key (November)

The article summarizes the proceedings of the conference and provides an overview of the current status of GLA-GSS research. It includes a session by session overview of the speaker presentations, as well as the key discussion items from the breakout sessions.

2. Review article on GSS

Viewpoints on Vessels and Vanishing Bones in Gorham-Stout Disease

Michael T. Dellinger, Nupur Garg and Bjorn R. Olsen

Submitted to: Bone (November)

In addition to reviewing the current status of basic and clinical research on GSS, the article goes beyond previous reviews on GSS by delving deeper into the possible molecular mechanisms behind the disease. Contribution of potential signaling pathways and the interplay between various cell types is discussed.

CONCLUDING REMARKS

The highlight of the LMI program this year was the organization of the 1st international conference focused solely on GLA and GSS. The meeting spurred lively discussions on the many questions facing the field, and created a platform for interaction amongst a broad group of experts. Building on the momentum gained at the meeting by transforming ideas into concrete projects, supporting newly formed partnerships and addressing the gaps identified, will be critical in the coming year.

Lymphatic Malformation Institute

**Proceedings of the 1st International Conference on
Generalized Lymphatic Anomaly and Gorham-Stout
Syndrome**

Bethesda, MD, USA, 7-8 June 2013

Michael T. Dellinger, Nupur Garg, Tiffany Ferry, Jack Kelly and Bjorn R. Olsen

Introduction

Gorham-Stout Syndrome (GSS) is a rare disease of unknown etiology characterized by intraosseous lymphatic vessels and massive osteolysis. Generalized Lymphatic Anomaly (GLA, also known as lymphangiomatosis) is a related disease that features an increase in the number of lymphatic vessels in affected tissues and commonly affects bones. Unfortunately, patients with these disabling, deforming, and sometimes life threatening diseases have limited therapeutic options. To address this urgent medical need, the Lymphatic Malformation Institute (LMI) and Lymphangiomatosis & Gorham's Disease Alliance (LGDA) sponsored the 1st International Conference on Generalized Lymphatic Anomaly and Gorham-Stout Syndrome. This inaugural conference was held from June 7-8, 2013 in Bethesda, MD, and was chaired by Drs. Bjorn Olsen and Michael Dellinger. Participants of the meeting included 20 invited speakers from 7 different countries, 34 additional scientists and clinicians, and several representatives from the LMI and LGDA. The **objectives** of the conference were: **1)** to bring together leaders in bone and endothelial cell biology to discuss the latest advances in basic science and clinical research relating to GLA and GSS; **2)** to identify and develop new avenues of research; and **3)** to foster collaboration among investigators studying these rare diseases. The highlights of this exciting conference are presented in this meeting report.

Session I: The VEGF family - a driver of lymphangiogenesis and osteogenesis

A better understanding of the molecular pathways regulating lymphangiogenesis and osteogenesis could shed light on the underlying pathology of GLA and GSS. There is mounting evidence that the vascular endothelial growth factor (VEGF) family regulates the growth of the lymphatic vessels as well as osteoblast and osteoclast differentiation and activity. Talks in this session focused on the effect of VEGF family members on lymphatic endothelial cells (LECs), osteoblasts and osteoclasts and the potential relevance of this family to GLA and GSS. **Dr. Marc Achen** (Australia) started the session by discussing the proteolytic activation of VEGF-D and the role this factor serves in regulating the size and function of lymphatic vessels. Dr. Achen showed that the diameter of initial lymphatic vessels was smaller in *Vegfd*^{-/-} mice than wild-type mice and that *Vegfd*^{-/-} mice displayed defects in lymph transport and wound healing (1). He also showed that tumors overexpressing VEGF-D specifically induced the enlargement of tumor draining collecting lymphatic vessels (2). Together, these findings point to a novel role of this growth factor in regulating the diameter of lymphatic vessels. Next, **Dr. Bjorn Olsen** (USA) discussed the regulation of osteoblast differentiation by VEGF-A. Conditional inactivation of VEGF-A in osteoblastic precursors in mice resulted in an osteoporosis-like phenotype characterized by reduced bone mass and increased bone marrow fat (3). Importantly, bone marrow-derived mesenchymal stem cells lacking VEGF-A displayed a reduced capacity to differentiate into osteoblasts (3). Surprisingly, it was found that osteoblast differentiation was controlled by intracellular rather than extracellular VEGF-A (3). This is the first report to show that intracrine VEGF-A controls bone development. **Dr. Lianping Xing** (USA) then gave a presentation that linked the lymphatic growth factor VEGF-C to osteoclast

function. Dr. Xing showed that RANKL induces VEGF-C expression by osteoclast precursor cells and mature osteoclasts (4). Importantly, exogenous and endogenous VEGF-C stimulated osteoclast-mediated bone resorption (4). This effect was blocked by a soluble VEGFR3 decoy receptor (4). Together, these findings show that VEGF-C produced by osteoclasts in the bone marrow cavity can stimulate lymphangiogenesis and osteolysis, two key features of GLA and GSS.

VEGF-D has emerged as a central figure in the biology of lymphangiomyomatosis (LAM), a progressive lung disease characterized by smooth muscle cell infiltration of lungs. VEGF-D serves as a diagnostic and prognostic biomarker for LAM. **Dr. Frank McCormack** (USA) gave a presentation on the concerted efforts by several groups to study and treat LAM. These efforts have been greatly facilitated by the LAM Foundation (www.thelamfoundation.org). This presentation showed how a foundation could help the scientific community make discoveries that significantly impact the lives of patients with a rare disease.

Session II: Development of preclinical models to study GLA and GSS

Patient-derived cell lines and animal models will greatly enhance the ability of researchers to investigate the underlying cause(s) of GLA and GSS and to identify effective therapies for treating these disorders. Talks in this session focused on the generation of *in vitro* and *in vivo* tools to study GLA and GSS. The session started with **Dr. Ramani Ramchandran** (USA) describing a novel approach to isolate and culture LECs from GLA and GSS patients. In this method, lymph fluid is collected from patients prior to sclerotherapy and LECs are isolated from this fluid by FACs sorting with antibodies against CD31 and podoplanin. This technique has also been used to purify LECs from a pleural effusion. Dr. Ramchandran is currently using LECs and osteoclasts from GLA and GSS patients to determine whether crosstalk between these two cell types contributes to the pathology of GLA and GSS. The focus of the session then switched to animal models. **Dr. Stefan Schulte-Merker** (Netherlands) discussed the usefulness of zebrafish to study lymphangiogenesis and osteogenesis and reviewed several of the lymphatic and skeletal mutants discovered in his lab. He also discussed the advantages of zebrafish, which include the ability to perform *in vivo* imaging as well as forward and reverse genetics with ease. Additionally, the recent implementation of Transcription Activator-like Effector Nucleases (TALENs) has made generating loss-of-function alleles in zebrafish trivial. Genetic manipulation of zebrafish could someday lead to a model of GLA and GSS. Presentations on animal models continued with **Dr. Donald McDonald** (USA) describing a mouse model of pulmonary lymphangiectasia and lymphangiomatosis. This mouse model relies on the Tet-On genetic system to drive VEGF-C expression in the lung. He showed that *CCSP-rtTA;TetO-Vegfc* transgenic mice exhibit a profound growth of pulmonary lymphatic vessels and develop chylothorax when given doxycycline shortly after birth. Importantly, *CCSP-rtTA;TetO-Vegfc* mice display phenotypic similarities to GLA patients with pulmonary involvement. In the future, this mouse model could potentially be used to test therapies for treating pulmonary involvement in

GLA. **Dr. Michael Dellinger** (USA) was the last speaker of this session and he presented a hypothesis for a mouse model of GSS. He is in the process of developing a mouse that over-expresses VEGF-C in osteoclasts and believes that this mouse will display a phenotype that mimics GSS.

Session III: Clinical and histological features of GLA and GSS

Talks in this session focused on the history, clinical features and histological characteristics of GLA and GSS. The session began with **Dr. Marlys Witte** (USA) reviewing the major milestones in GLA and GSS research. This included the successful culture of LECs from a female patient with GSS seen by Dr. Witte over 20 years ago (5). She raised the point that our knowledge about the lymphatic abnormalities in GLA and GSS patients is far from complete. Dr. Witte suggested that a better understanding of the lymphatic abnormality in GLA and GSS could be achieved by imaging the lymphatic system through lymphoscintigraphy or other techniques. The next presentation was by **Dr. Gulraiz Chaudry** (USA) and focused on the skeletal features of GLA and GSS. He performed a retrospective review of 32 GLA and 19 GSS patients in the Vascular Anomalies Center database at Boston Children's Hospital (BCH) to determine whether GLA and GSS patients display differences in bone disease (6). He discovered that the pattern of bone loss was dramatically different between GLA and GSS patients. Cortical bone was preserved in GLA patients but was lost in GSS patients (6). Additionally, GLA patients typically had more bones affected than GSS patients and involvement of the appendicular skeleton (6). Macrocytic lymphatic malformations were also more frequently observed in GLA patients (6). Next, **Dr. Paula North** (USA) described the histopathology of GLA and GSS. She presented several images of affected tissues stained with antibodies against either podoplanin or LYVE-1 (commonly used markers of LECs). These images showed lymphatic vessels in marrow spaces and in cortical regions of affected bones in both GLA and GSS patients. Soft tissues affected in GLA and GSS also showed evidence of abnormal lymphatic vessels. Surprisingly, the LECs in GLA and GSS sections displayed a low mitotic index. Thus the mechanisms driving the expansion of the lymphatic network in GLA and GSS remain unclear. Additionally, there was no evidence of osteoblast activity in areas of bone loss. The session ended with **Dr. Cameron Trenor** (USA) presenting treatment and outcomes data for GLA and GSS patients treated at BCH. This information was obtained from a retrospective review of 85 GLA and 43 GSS patients in the Vascular Anomalies Center database. This review revealed that interferon and bisphosphonates were the most commonly prescribed therapies at BCH. Importantly, a few patients showed signs of bone remineralization when they were on both drugs. However, it took at least two years of therapy before signs of bone remineralization were observed. Dr. Trenor ended his presentation with his future plans, which are to continue to collect retrospective data from GLA and GSS patients and to prospectively collect information for a lymphatic anomalies registry at BCH. He also urged investigators to **avoid taking biopsies of rib lesions** whenever possible since these biopsies may lead to chronic pleural effusions.

Session IV: Current and emerging therapies for treating GLA and GSS

A standard protocol for treating GLA and GSS does not exist and most therapies are aimed at reducing symptoms. The purpose of this session was to discuss the various therapies used to treat GLA and GSS. **Dr. Juan Carlos Lopez-Gutierrez** (Spain), the first speaker of the session, reviewed several therapies used to treat GLA and GSS. Chylothorax is a serious complication in GLA and GSS patients and can cause respiratory distress and failure. Dr. Lopez-Gutierrez discussed various treatments for chylothorax such as diet modulation, thoracentesis, pleurodesis, thoracic duct embolization and thoracic duct ligation. The loss of skeletal stability is also a serious problem in GLA and GSS. Dr. Lopez-Gutierrez showed how surgery could be used to stabilize affected regions of the skeleton in individuals with GLA or GSS. He also stated that pain control, rehabilitation, and psychological support are important components of treatment for GLA and GSS patients. Next, **Dr. Manish Patel** (USA) presented preliminary findings from an ongoing trial evaluating the effect of sirolimus on complex vascular anomalies, many of which had lymphatic components (<http://clinicaltrials.gov/ct2/show/NCT00975819>). 61 patients are presently enrolled with 36 patients now eligible for evaluation. Final conclusions from this study will be available in the spring. Preliminary data both from patients on study and those treated prior to the study suggest that sirolimus is an effective therapy for lymphatic anomalies involving the bone. However, this is an ongoing trial and additional patients are needed before any firm conclusions can be made. Following this presentation, **Dr. Erik Eklund** (Sweden) captivated the audience with a gripping tale of a GSS patient treated at his hospital. This patient presented with multiple lytic lesions in the skeleton and chylothorax and was treated with surgery, radiation and several pharmaceutical agents. Eventually, the patient was treated with a heparin analog that does not possess anti-coagulation properties (tafoxiparin). Remarkably, the patient markedly improved over time while on tafoxiparin (7). A combination of surgery, radiation and tafoxiparin was used to successfully treat a second GSS patient at his hospital (7). Dr Eklund also presented work on the search for biomarkers to monitor disease activity in his two patients. He found that serum levels of VEGF-A were high in both patients when their disease was in an active state and that these levels normalized when their disease was in remission (7). This finding is in agreement with other studies exploring VEGF-A as a biomarker of disease activity in GLA and GSS (8, 9). Additional studies will help determine whether VEGF-A can function as a biomarker of disease activity in GSS. Lastly, **Dr. Eva Sevick-Muraca** (USA) wrapped up the session by discussing approaches to image the lymphatic vasculature. Her lab primarily uses near-infrared fluorescence lymphatic imaging (NIRFLI) to evaluate the architecture and function of lymphatic vessels in mice and patients. She reported how this imaging modality was recently used to characterize the lymphatic defects in the skin of *Rasa1* mutant mice and in a Parkes Weber Syndrome patient (10). In the future, NIRFLI could be used to determine whether the superficial lymphatic network is altered in some GLA and GSS patients.

Session V: Breakout Session

The conference featured a breakout session that allowed participants to discuss hypotheses and strategies aimed at developing solutions to many of the basic and clinical research questions and therapeutic challenges related to GLA and GSS. During the breakout session, participants segregated into groups focused on either: 1) the terminology and classification of GLA and GSS; 2) the etiology of GLA and GSS; 3) therapies for treating GLA and GSS; or 4) the identification of biomarkers for GLA and GSS. At the end of the breakout session, the discussion leader for each group presented the group's recommendations for advancing in these areas of research. **Dr. Cameron Trenor** (USA) led the terminology and classification group. Several different names are used to describe GLA and GSS and an agreed upon set of criteria for classification has not been established. A standardized nomenclature would facilitate communication among specialists and patients. The group felt that the International Society for the Study of Vascular Anomalies classification system, with anticipated updates at the 2014 congress in Melbourne, should be the guiding nomenclature in the field. **Dr. Matthew Warman** (USA) was the leader of the etiology group, which focused on the genetic basis of GLA and GSS. His group recommended that DNA from prospectively collected tissue samples and cell types such as LECs be used in exome sequencing projects. This type of analysis would greatly assist in the identification of the genetic cause(s) of GLA and GSS. Additionally, he encouraged groups to collaborate and share samples to tackle this important and challenging endeavor. **Dr. Erik Eklund** (Sweden) led the biomarker discussion group, which recommended that standards be in place for properly collecting and analyzing samples. These standards should accelerate the discovery of prognostic and/or predictive biomarkers for GLA and/or GSS. This group also recommended that sera and lymph fluid be banked for future use. **Dr. Francine Blei** (USA) was the leader of the therapy discussion group. This group recommended that GLA and GSS patients be enrolled in a registry and that clinicians work together to establish standards for evaluating, following and staging GLA and GSS patients. Importantly, several of the individuals from this group are working together to initiate the first clinical trial specifically for GLA and GSS.

Session VI: Search for the genetic cause(s) of GLA and GSS

Advances in DNA sequencing technology have greatly facilitated genetic studies of rare and sporadic diseases. The presentations during this session focused on approaches to identify the genetic underpinnings of GLA and GSS. **Dr. Nisha Limaye** (Belgium) started the session by reviewing the genetics of venous malformations and discussed how these studies may help guide the search for the genetic basis of GLA and GSS. She reported that inherited venous malformations require a 2nd (somatic) hit in the causative gene in order for a lesion to form whereas sporadic venous malformations are caused entirely by somatic mutations (11). Continuing with this theme, **Dr. Matthew Warman** (USA) described how his lab identified somatic *PIK3CA* mutations in CLOVES (Congenital, Lipomatous, Overgrowth, Vascular Malformations, Epidermal Nevi and Spinal/Skeletal Anomalies and/or Scoliosis) syndrome by performing exome sequencing of DNA isolated from affected tissue (12). He suggested that this approach might help uncover the causative gene(s) of GLA and GSS. Next, **Carmen Lorenzo** (Spain) presented preliminary work

showing genetic imbalances in individuals with GLA and GSS. Comparative genomic hybridization of DNA samples isolated from blood revealed that 19 % of GLA/GSS patients show copy number variations (CNVs) not found in normal individuals. Her future plans are to determine the significance of these CNVs and to perform next generation sequencing with DNA isolated from blood and affected tissue from GLA and GSS patients. The last speaker of the conference was **Dr. Michael Levine** (USA). He presented results from exome sequencing projects that used DNA isolated from blood and from cells collected from a pleural effusion from a GLA patient. Unfortunately, the analysis of genomic DNA from blood did not yield an obvious candidate gene for GLA. However, 18 candidates were identified in the analysis of the cells from the pleural effusion. More work is required to confirm the significance of the variants identified in this study.

Conclusions

The 1st International Conference on Generalized Lymphatic Anomaly and Gorham-Stout Syndrome served as a platform for investigators from diverse areas of research to share their published and unpublished work on, or relevant to, GLA and GSS. These presentations revealed that substantial progress has been made in generating research tools to study GLA and GSS and in characterizing these diseases. Additionally, **Jack Kelly** (President, LGDA) announced during the meeting that an international registry for GLA and GSS would be opening soon (www.LGDARegistry.org). This registry, as well as the BCH lymphatic anomalies registry (contact the Vascular Anomalies Center at BCH for more information), will greatly facilitate research by the scientific community. Despite the recent progress made in GLA and GSS research, much remains unknown about these diseases. **Dr. Steve Groft** (Director, ORDR/NIH) emphasized during his address to the meeting that future studies focused on GLA and GSS will require close collaboration among experts from different fields. We hope that this inaugural conference served as a catalyst to foster new partnerships and the development of innovative projects designed to better understand and treat GLA and GSS. We look forward to the next International Conference on Generalized Lymphatic Anomaly and Gorham-Stout Syndrome to learn of the new developments in the field.

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