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MEETING REPORT

First International Conference on Generalized Lymphatic Anomaly and Gorham–Stout Syndrome

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Meeting Report from the 1st International Conference on Generalized Lymphatic Anomaly and Gorham–Stout Syndrome, Bethesda, MD, USA, 7–8 June 2013

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 7–8 June 2013 in Bethesda, MD, and was chaired by Drs Bjorn Olsen and Michael Dellinger. Participants of the meeting included 20 invited speakers from seven 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 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.[1] Next, Dr Bjorn Olsen discussed the function of intracellular VEGF-A in controlling osteoblast differentiation.[2] Dr Lianping Xing 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 osteoclasts and that VEGF-C stimulates osteoclast-mediated bone resorption.[3] Together, these findings show that VEGF-C can stimulate lymphangiogenesis and osteolysis, two key features of GLA and GSS. The session ended with Dr Frank McCormack giving a presentation on the relationship between VEGF-D and the rare lung disease lymphangioleiomyomatosis.

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 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 describing a novel approach to isolate and culture LECs from GLA and GSS patients. In this method, lymph fluid is collected from lymphatic malformations and LECs are isolated from this fluid by fluorescence-activated cell sorting. 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 discussed the usefulness of zebrafish to study lymphangiogenesis and osteogenesis and reviewed several of the lymphatic and skeletal mutants discovered in his lab. Genetic manipulation of zebrafish could someday lead to a model of GLA and GSS. Presentations on animal models continued with Dr Donald McDonald describing a mouse model

of pulmonary lymphangiectasia and lymphangiomatosis. He showed that *CCSP-rtTA;TetO-Vegfc* transgenic mice exhibit a profound growth of pulmonary lymphatic vessels and develop chylothorax. In the future, this mouse model could potentially be used to test therapies for treating GLA. Dr Michael Dellinger 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 overexpresses 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 reviewing major milestones in GLA and GSS research. This included the successful culture of LECs from a GSS patient seen by Dr Witte over 20 years ago.[4] The next presentation was by Dr Gulraiz Chaudry 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 (VAC) database at Boston Children's Hospital (BCH) to determine whether GLA and GSS patients display differences in bone disease.[5] 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.[5] In addition, GLA patients typically had more bones affected than GSS patients and involvement of the appendicular skeleton.[5] Next, Dr Paula North described the histopathology of GLA and GSS. She showed that irregular lymphatic vessels are in affected soft tissues and bones in GLA and GSS patients and that affected bones do not appear to show evidence of osteoblast activity. The session ended with Dr Cameron Trenor reviewing treatment and outcomes data for 85 GLA and 43 GSS patients seen at BCH. This review revealed that interferon and bisphosphonates were the most commonly prescribed drugs at BCH. Importantly, a few patients showed signs of bone remineralization when they were on both drugs. Dr Trenor 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

The purpose of this session was to discuss the various 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 Juan Carlos Lopez-Gutierrez, the first speaker of the session, discussed various treatments for chylothorax such as diet modulation, thoracentesis, pleurodesis, thoracic duct embolization and ligation. Dr Lopez-Gutierrez also showed how surgery could be used to stabilize affected regions of the skeleton in individuals with GLA or GSS. Next, Dr Manish Patel presented preliminary findings from an ongoing trial evaluating the effect of sirolimus on complex vascular anomalies (http://clinicaltrials.gov/ct2/show/NCT00975819). Preliminary data from patients on the study indicate that sirolimus is an effective therapy for lymphatic anomalies involving the bone. However, this is an ongoing trial and additional patients are needed before firm conclusions can be made. Following this presentation, Dr Erik Eklund reported how he used a combination of surgery, radiation therapy and tafoxiparin to successfully treat two GSS patients at his hospital.[6] He also reported that serum levels of VEGF-A were high in both of his patients when their disease was in an active state and that these levels normalized when their disease was in remission.[6] Additional studies will help determine whether VEGF-A can function as a biomarker of disease activity in GSS. Finally, Dr Eva Sevick-Muraca 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. In the future, NIRFLI could be used to determine whether the superficial lymphatic network is altered in GLA and GSS patients.

Session V: Breakout Session

The conference featured a breakout session that allowed participants to discuss solutions to many of the basic and clinical research questions related to GLA and GSS. During the session, participants segregated into groups focused on either (1) terminology and classification; (2) etiology; (3) therapies; or (4) biomarkers. At the end of the breakout session, the discussion leader for each group presented the group's recommendations for advancing these areas of research. Dr Cameron Trenor 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. 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 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. Dr Erik Eklund led the biomarker discussion group, which recommended that standards be in place for collecting and analyzing samples and that sera and lymph fluid be banked for future use. Dr Francine Blei was the leader of the therapy discussion group, which recommended that clinicians work together to establish standards for evaluating, following and staging GLA and GSS patients and that GLA and GSS patients be enrolled in a registry. Importantly, 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 VAC at BCH for more information), will greatly facilitate research by the scientific community.

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 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 second (somatic) hit in the causative gene in order for a lesion to form whereas sporadic venous malformations are caused entirely by somatic mutations.[7] Continuing with this theme, Dr Matthew Warman described how his lab identified somatic *PIK3CA* mutations in CLOVES syndrome by performing exome sequencing of DNA isolated from affected tissue.[8] He suggested that this approach might help uncover the causative gene(s) of GLA and GSS. Next, Carmen Lorenzo 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 not found in normal individuals. Her future plans are to determine the significance of these copy number variations. The last speaker of the conference was Dr Michael Levine. 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.

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. However, a lot still 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.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Paquet-Fifield S, Levy SM, Sato T, Shayan R, Karnezis T, Davydova N *et al.* Vascular endothelial growth factor-d modulates caliber and function of initial lymphatics in the dermis. *J Invest Dermatol* 2013;**133**:2074–2084.
2. Liu Y, Berendsen AD, Jia S, Lotinun S, Baron R, Ferrara N *et al.* Intracellular VEGF regulates the balance between osteoblast and adipocyte differentiation. *J Clin Invest* 2012;**122**:3101–3113.
3. Zhang Q, Guo R, Lu Y, Zhao L, Zhou Q, Schwarz EM *et al.* VEGF-C, a lymphatic growth factor, is a RANKL target gene in osteoclasts that enhances osteoclastic bone resorption through an autocrine mechanism. *J Biol Chem* 2008;**283**:13491–13499.
4. Bowman CA, Witte MH, Witte CL, Way DL, Nagle RB, Copeland JG *et al.* Cystic hygroma reconsidered: hamartoma or neoplasm? Primary culture of an endothelial cell line from a massive cervicomediastinal hygroma with bony lymphangiomatosis. *Lymphology* 1984;**17**: 15–22.
5. Lala S, Mulliken JB, Alomari AI, Fishman SJ, Kozakewich HP, Chaudry G. Gorham-Stout disease and generalized lymphatic anomaly-clinical, radiologic, and histologic differentiation. *Skeletal Radiol* 2013;**42**:917–924.
6. Brodszki N, Lansberg JK, Dictor M, Gyllstedt E, Ewers SB, Larsson MK *et al.* A novel treatment approach for paediatric Gorham-Stout syndrome with chylothorax. *Acta Paediatr* 2011;**100**:1448–1453.
7. Limaye N, Wouters V, Uebelhoer M, Tuominen M, Wirkkala R, Mulliken JB *et al.* Somatic mutations in angiopoietin receptor gene TEK cause solitary and multiple sporadic venous malformations. *Nat Genet* 2009;**41**:118–124.
8. Kurek KC, Luks VL, Ayturk UM, Alomari AI, Fishman SJ, Spencer SA *et al.* Somatic mosaic activating mutations in PIK3CA cause CLOVES syndrome. *Am J Hum Genet* 2012;**90**: 1108–1115.