WHAT ARE COMPLEX LYMPHATIC ANOMALIES?

CLAs are a group of rare developmental diseases characterized by abnormal growth of lymphatic vessels. They may involve multiple organ systems, including the lung, spleen, soft tissue, and bones. CLAs can manifest anywhere in the body and are associated with severe morbidity and health complications, including pain, swelling, infection, and lymphatic leakage (Reference 1).

CLAs include:

- Gorham Stout Disease (GSD)
- Generalized Lymphatic Anomaly (GLA)
- Kaposiform Lymphangiomatosis (KLA) and
- Central Conducting Lymphatic Anomaly (CCLA).

CLAs have a broad spectrum of symptoms and phenotypes. Consequently, patients with the same diagnosis may have different symptoms based on the location of the body that is involved.

It is essential to promote awareness of CLAs, provide resources and support to patients, and assist clinicians in gaining more information about these rare diseases to help patients reach an earlier diagnosis and receive optimum care.

For more information on the classification of CLAs, you can visit the International Society for the Study of Vascular Anomalies (ISSVA) (Reference 2).

THE LYMPHATIC SYSTEM

To understand CLAs and their manifestations, one must understand normal lymphatic structure and function.

The lymphatic vasculature is a unidirectional conducting system that returns filtered interstitial fluid and tissue metabolites to the blood circulation and plays major roles in immune cell trafficking and lipid absorption. Initial lymphatics consist of a single layer of loosely connected lymphatic endothelial cells (LECs) and are responsible for the uptake of excess fluid and macromolecules in tissues.

Initial lymphatics first drain into pre-collecting lymphatic vessels that merge with larger collecting lymphatics. The smaller lymphatic vessels, which take up the fluids, are called lymph capillaries. The lymphatic capillaries are composed of a single layer of endothelial cells (LECs). They drain into collecting lymphatics, which are distinguishable by the presence of a smooth muscle layer and one-way bicuspid valves to prevent retrograde fluid flow. Eventually the collecting lymphatics throughout the body coalesce into the larger lymph trunks, of which the largest, the thoracic duct and right lymph duct, empty directly into the subclavian veins.

The lymph from the lower limb terminates at the para-aortic nodes. They join with the lymph from the viscera of the pelvis and form bilateral lumbar trunks. The lumbar trunks, hepatic and intestinal lymphatics drain into the cisternae chyli, a structure that marks the beginning of the thoracic duct. Anywhere from 50% to 90% of lymphatic fluid is derived from the intestine and liver. Intestinal lacteals contain mostly dietary fat, and ingesting a fatty meal can increase the lymph flow in the lacteals ≥200-fold compared with a fasting state.

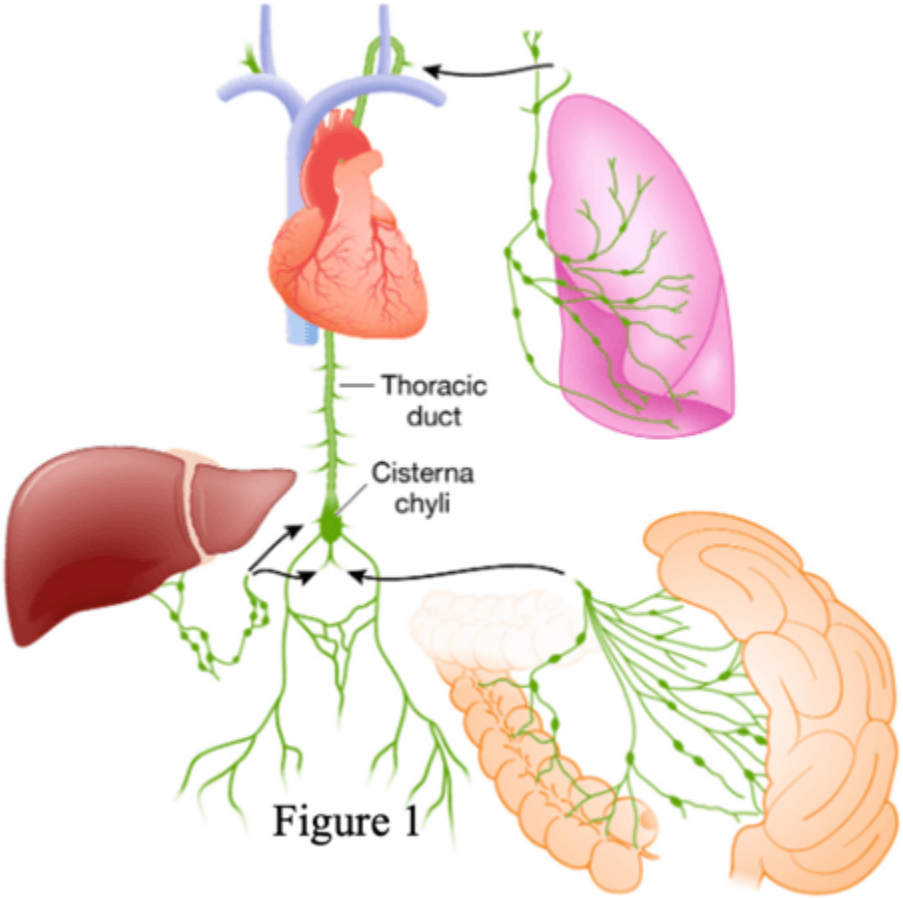


Figure 1

Important parts of the lymphatic system are:

- Lymph: Clear fluid containing white blood cells that helps clear toxins and waste
- Chule: A milky fluid consisting of fat droplets and lymph. It drains from the lacteals of the small intestine into the lymphatic system during digestion
- Lymphatic vessels: Small tubes (vessels) that carry lymph throughout the body.

GENETICS OF CLAS

Vascular anomalies are caused by post-zygotic (somatic) (Figure 2) or germline (Figure 3) pathogenic variants in genes that regulate cell growth and vascular development (Figure 4). The clinical variability seen in CLAs is believed, in part, a reflection of the somatic nature of these driver mutations and is highly dependent on when the variant occurs during development, where the affected cells and tissues are located, which specific alleles are present, and how the variant affect the function and expression of the altered genes (Reference 3).

Acquired somatic variant or mutation: An alteration in DNA that occurs after conception. Acquired somatic variants can occur in any of the cells of the body except the germ cells and therefore are not passed on to children. (Figure 2)

Germline (or inherited) variant: A DNA alteration within a germ cell during conception. Germline variants therefore become incorporated into the DNA of every cell in the body. Germline variants are passed on from parents to offspring. (Figure 3)

The majority of CLAs are caused by acquired somatic gene variants

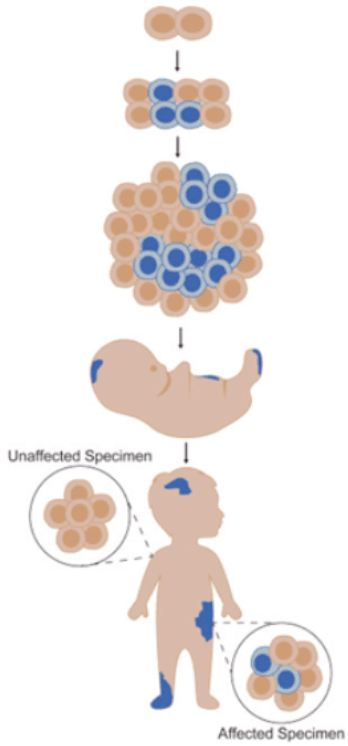


Figure 2: Somatic Gene Variants

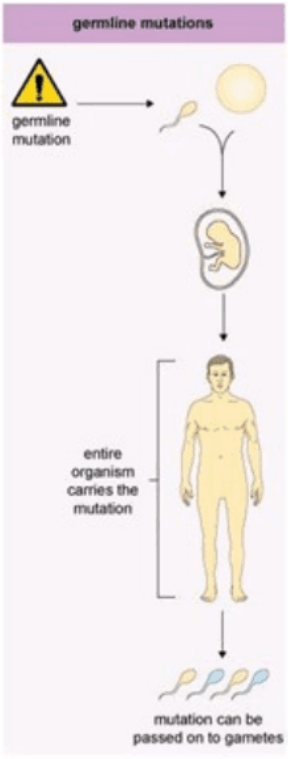


Figure 3: Inherited Gene Variants

CELL SIGNALING PATHWAYS

These genetic variants directly change the activities of intracellular signaling pathways, thereby influencing various downstream cellular processes. The pathways most affected in CLAs are the PI3K/AKT/mTOR and RAS/MAPK signaling pathways.

PI3K/AKT/mTOR pathway:

PI3K-Akt-mTOR pathway is an intracellular signal transduction pathway that promotes metabolism, proliferation, cell survival, growth, and angiogenesis in response to extracellular signals. It is commonly called the "anti-apoptosis pathway" because it prevents cell death.

Activation of PI3K typically occurs because of direct stimulation by the regulatory subunit bound to an activated receptor or indirectly by an adaptor molecule such as the insulin receptor substrate (IRS) proteins. Activation of PI3K converts PIP2 into PIP3. The second messenger PIP3 further stimulates protein kinase D (PDK), which activates Akt, starting the PI3K-Akt-mTOR signaling pathway that regulates cell proliferation. PI3K can also be activated by a GTP-bound RAS protein.

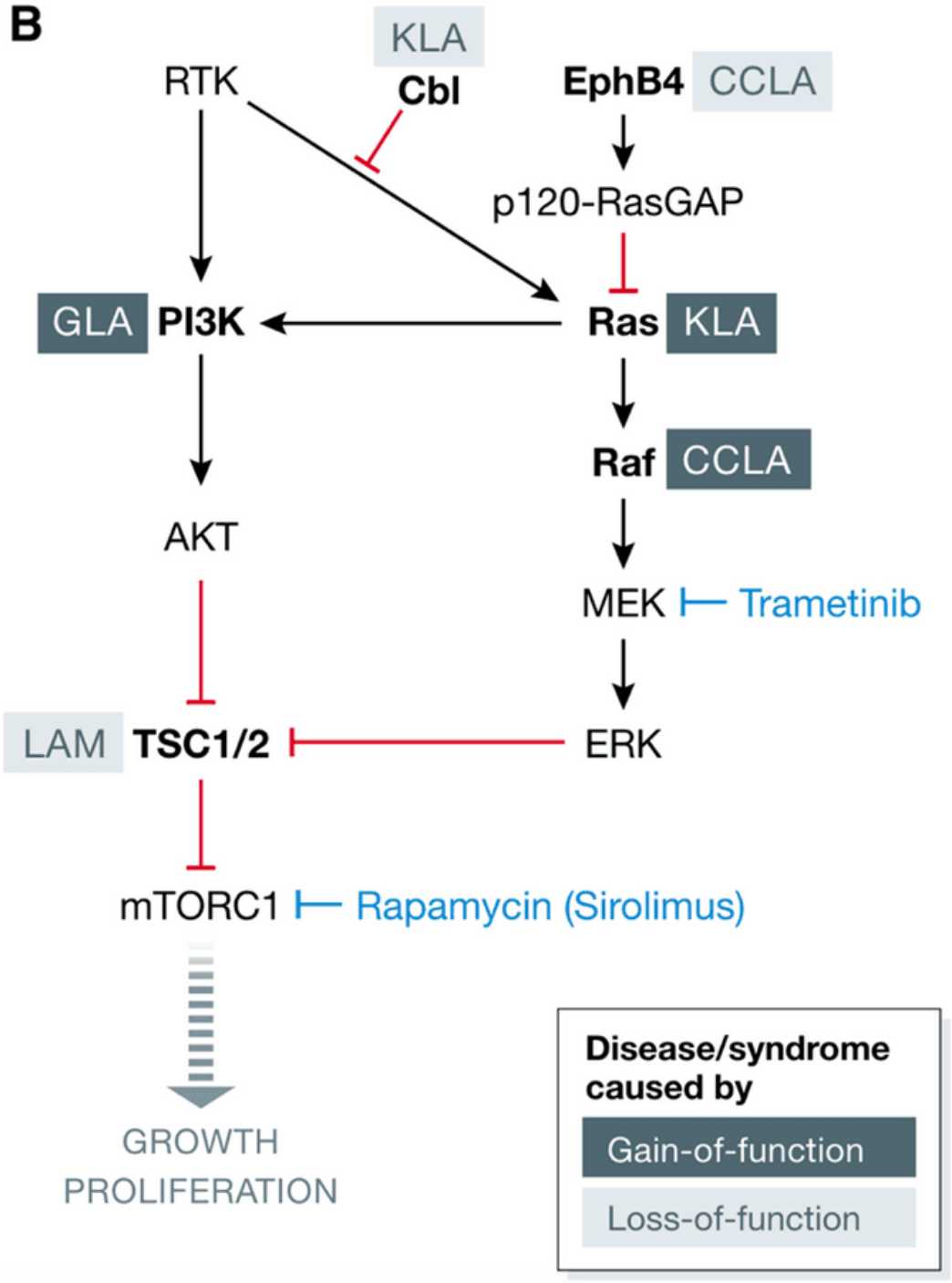
The general cellular impact of activating the PI3K-AKT-mTOR pathway is to stimulate cell growth and proliferation. Over-activation of this signaling pathway can overstimulate cells resulting in abnormal/excessive cell proliferation. Abnormalities in the PI3K pathway are common in cancer and lymphatic anomalies. PIK3CA-Related Overgrowth Spectrum (PROS) is a group of diverse overgrowth disorders caused by activating PIK3CA mutations. There are 13 disorders recognized as PROS including Klippel-Trenaunay syndrome (KTS), CLOVES (congenital lipomatous overgrowth, vascular malformations, epidermal nevi, and scoliosis/skeletal/spinal anomalies), and Proteus syndrome. (Reference 4)

The RAS/MAPK pathway:

The Ras-Raf-MEK-ERK signaling pathway regulates cell proliferation, differentiation, and survival. Overexpression and overactivation of members within the signaling cascade cause many human syndromes and diseases, including cancer.

The master regulator of the classical MAPK cascade is the Ras protein, which is encoded by three related genes, NRas, HRas, and KRas. Normally, Ras combines with GDP, which is in an inactive state. When Ras protein is released from Ras/GDP and binds to GTP, Ras is activated. Active Ras binds and activates Raf. Activated Raf phosphorylates and activates MEK1/2. MEK 1/2 phosphorylates ERK1/2 to activate signaling. Activation of ERK1/2 leads to its translocation into the nucleus and activates the expression of many downstream genes to promote cell proliferation and differentiation. In addition, activated Ras can directly bind and activate PI3K.

Syndromes resulting from genetic changes in the RAS/MPK pathway are called “RASopathies”. RASopathies include other more common disorders such as Noonan syndrome, Costello Syndrome, and Neurofibromatosis. (Reference 5)



DISEASES CONSIDERED CLAS

Generalized Lymphatic Anomaly (GLA)

Formerly known as Lymphangiomatosis, is characterized by lymphatic malformations involving soft tissues, bones, and organs such as the spleen with disease present in multiple locations in the body. GLA may present at birth but is more frequently identified in childhood or young adulthood. GLA can

cause pericardial, pleural (Figures 5, 6), or peritoneal effusions. It can also cause protein-losing enteropathy and leukopenia. Bone disease is commonly seen, involving multiple bones of the axial and appendicular skeleton. The ribs are the most common site of involvement in GLA, followed by the spine. Bone involvement in GLA does not typically include cortical bone and rarely results in progressive bone disappearance/loss. Fractures resulting from bone disease are uncommon. Activating somatic variants, or mutations, in the PIK3CA gene are thought to cause GLA. (Reference 6) PIK3CA variants are also found in other diseases, such as cancer and PIK3CA-related overgrowth spectrum (PROS).

Gorham-Stout Disease (GSD)

Is also called vanishing bone disease, is characterized by progressive loss of the cortex of the bone. GSD can progress rapidly but can also spontaneously stabilize. It typically extends beyond the original site of disease, involving nearby bones, and is much more common in the axial skeleton. Migration of lymphatic endothelial cells (LECs) into bone from soft tissue and their proliferation and expansion cause bone loss. Symptoms caused by GSD vary depending on the extent of the loss of cortical bone (Figure 7) and its location in the body. Pathologic fractures, the buildup of pericardial and pleural effusions (Figure 8) secondary to rib involvement, leaks of cerebral spinal fluid (CSF) resulting from skull-based damage, and neurologic symptoms including paralysis have all been reported. Activating somatic KRAS gene variants, or mutations, are thought to cause GSD. KRAS variants have also been seen in other diseases such as cancer and other vascular anomalies. (Reference 7)

Kaposiform Lymphangiomatosis (KLA)

Is a complex lymphatic anomaly with features of both neoplasia and malformation. KLA has characteristics that overlap with both GLA and CCLA, although the disease is often more aggressive. Symptoms are dependent on the location and severity of disease. Chest involvement is common in KLA. Affected tissues include the thoracic cavity, including the mediastinum, lung, and heart, and may be associated with pleural (Figure 9) and pericardial effusions. (Reference 8) Unique pathological features of KLA include abnormal lymphatic channels surrounded by spindle shaped LECs, rapid and progressive growth, and consumptive coagulopathy and hemorrhage. Hemorrhage, even in the absence of coagulopathy, can be a useful diagnostic characteristic. Markers to help diagnose KLA include elevated blood levels of angiopoietin 2 (Ang2), a protein involved in endothelial cell growth and function. Activating somatic variants, or mutations, in NRAS, CBL, or HRAS are thought to cause KLA.

Central Conducting Lymphatic Anomaly (CCLA)

Is characterized by dilated and dysfunctional lymphatic vessels in the torso leading to the backflow of lymphatic fluid into tissues. The dysfunction, in part, results from the abnormal lymphatic valves in the large vessels and dysplasia or hypoplasia of the thoracic duct resulting in chylous reflux, valve incompetence, and lymphatic leak (Figure 11). Patients often present with chylous effusions, ascites, protein-losing enteropathy, and swelling in the legs and feet. Both inherited (germline) variants in EPHB4 and MDFIC and somatic variants in ARAF, KRAS, and BRAF genes, may cause CCLA. (Reference 6) Syndromes in which CCLA was observed most frequently included RASopathies (both germline and mosaic), chromosomal abnormalities (e.g., trisomy 21), PIEZO1 lymphatic dysplasia, and metabolic disorders, confirming the involvement of RAS/MAPK signaling pathway.(Reference 9) Given the genetic variation in CCLA, genotype-phenotype correlations will be essential to identify subcategories under the general diagnosis of CCLA.

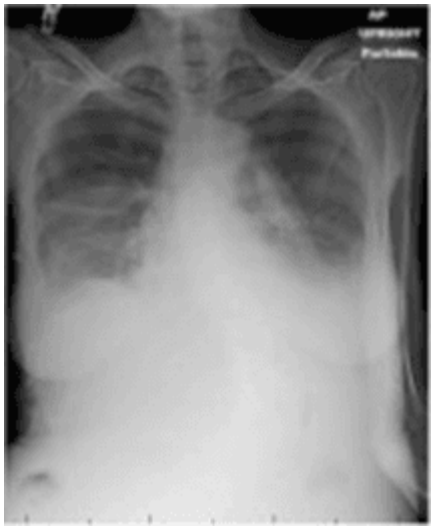


Figure 5
GLA Chest XR showing bilateral pleural effusions

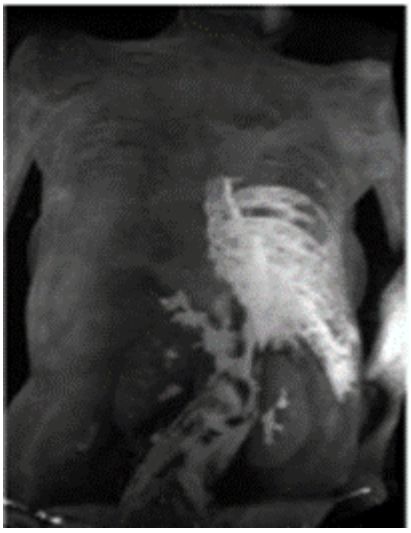


Figure 6
GLA MRI: left chest wall leak

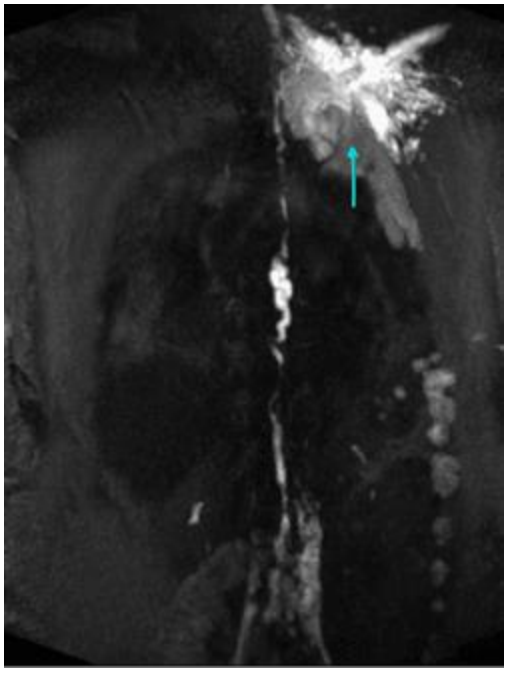


Figure 8
GSD MRL shows lymphatic leak

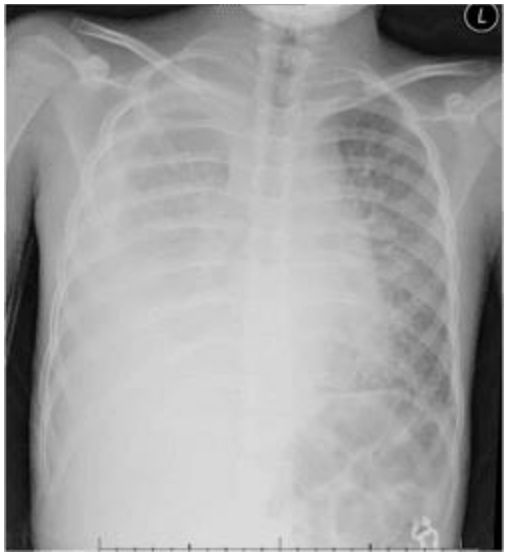


Figure 9
KLA Chest XR shows right pleural effusions

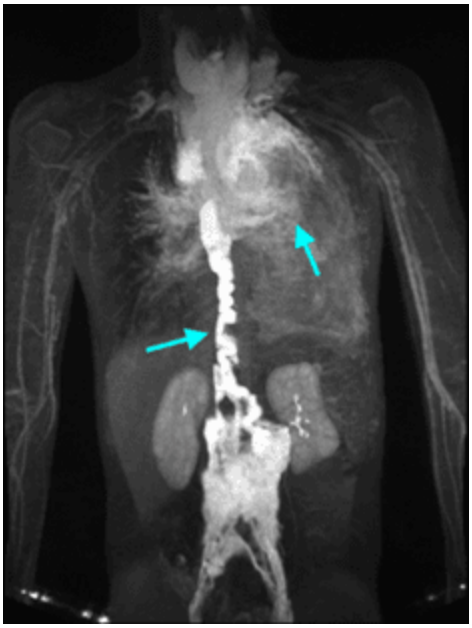


Figure 10
KLA MRL shows tortuous central lymphatics and mediastinal leak

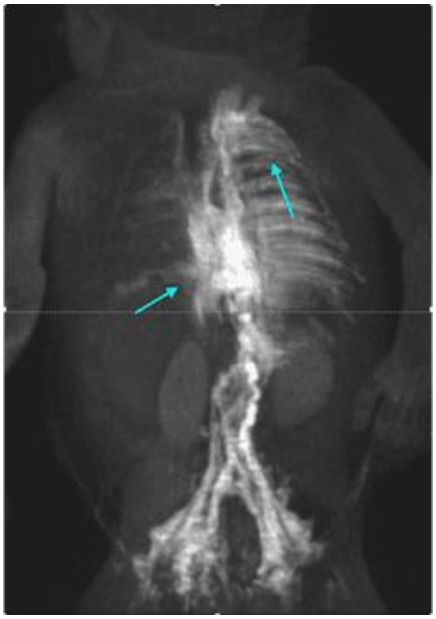
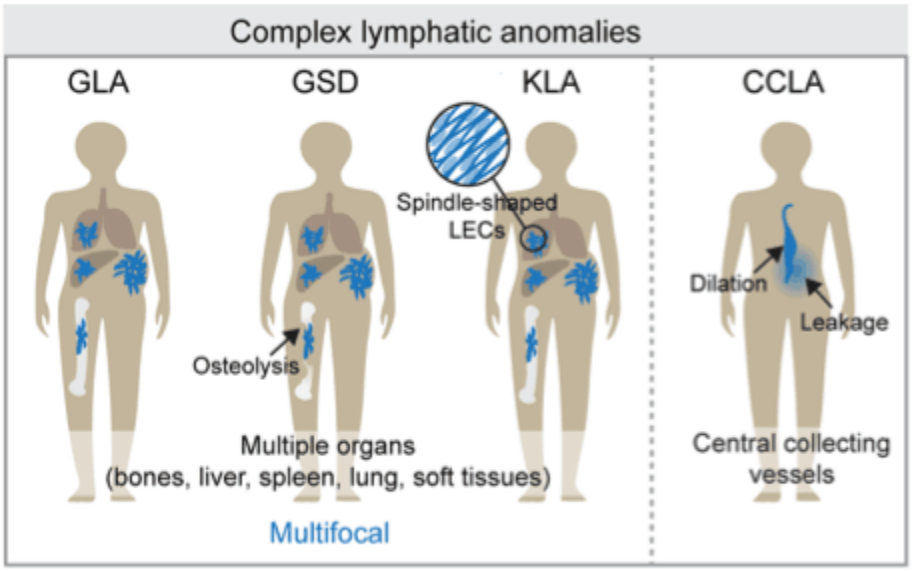


Figure 11
CCLA MRL shows abnormal central lymphatics with lymphatic leaks

TABLE I: SUMMARY OF CLAS

	GLA	GSD	KLA	CCLA
Older terminology	Lymphangiomatosis, Diffuse Lymphatic Malformation	Vanishing bone disease	Lymphangiomatosis	Lymphangiectasia, channel type, Lymphatic anomaly
Distinguishing feature	Disease affecting multiple parts of the body	Progressive bone loss	Spindled LEC, low platelets, hemorrhages, High Ang2	Abnormal structure and function of central lymphatic vessels
Genetics	Somatic PIK3CA	Somatic KRAS	Somatic NRAS; Somatic CBL; Somatic HRAS	Somatic ARAF; Somatic BRAF; Somatic KRAS; Germline EPHB4; Germline MDFIC
Pathway	PI3K/AKT/mTOR and RAS/MAPK	RAS/MAPK pathway	RAS/MAPK pathway	RAS/MAPK pathway

Figure 12: Overview of CLA



SYMPTOMS OF CLAS

While symptoms vary between conditions and individuals, the following is a list of symptoms most commonly reported by CLA patients:

- Persistent/progressive Coughing, wheezing, shortness of breath
- Persistent/progressive pain and swelling of affected areas.
- Bone fractures (spontaneous or after minor trauma)
- Recurring infection or respiratory disease
- Abdominal or pelvic pain
- Skin lesions
- Internal bleeding

DIAGNOSIS

Based on clinical characteristics, radiologic imaging, and pathologic findings, the International Society for the Study of Vascular Anomalies (ISSVA) created a standardized classification system in 1996, broadly categorizing vascular anomalies as tumors and vascular malformations. The ISSVA classification system was recently updated and can be accessed at https://www.issva.org/classification.

Initial presentation of CLA is variable, and the provider needs to have a high index of suspicion for and familiarity with these conditions to provide timely diagnosis and appropriate management. When in doubt referral to a specialized vascular anomalies center should be considered.

Imaging:
Radiologic evaluation should include chest, abdomen, pelvis, and total spine imaging with CT and/or MRI. If there is a suspicion of GSD, then skull imaging is indicated. A skeletal bone survey may be used to focus imaging needs. Dynamic, contrast-enhanced MR lymphangiography can also be helpful in certain situations.

Laboratory Evaluation:
Laboratory investigation should include complete blood count with differential, D-dimer, fibrinogen, prothrombin time (PT), partial thromboplastin time (PTT), albumin and total protein, immunoglobulins, liver and renal function tests, electrolytes, and bone turnover markers (alkaline phosphatase, C-telopeptide, etc.). Recently, angiopoietin-2 (ANGPT 2) have been identified as serum biomarkers in KLA and may be helpful in diagnosis and monitoring treatment response, especially to Sirolimus. (ANGPT 2 testing)

Genetic Testing:
Since mutations in CLA are often somatic, genetic testing of the affected tissue, not peripheral blood, should be considered to guide therapy. Biopsy of a soft tissue lesion is recommended over bone biopsy. Biopsy of ribs and vertebrae should be avoided as these can result in persistent pleural effusions and CSF leaks. Genetic testing of circulating free DNA (cfDNA) in blood and effusions is emerging as a potential diagnostic aid (spotSeq).

TREATMENT

Most patients with CLAs have extensive and invasive disease burden, necessitating systemic therapeutic approaches. Originally there was not a clear understanding of the underlying pathobiology and so medical therapies for patients with CLAs focused on agents that interfered with known mechanistic pathways such as angiogenesis (interferon-alpha, radiation) or the function of specific cells involved in disease pathogenesis such as LEC (mTOR and VEGF inhibitors) and osteoclasts (bisphosphonates). Symptom control has been the goal of treatment.

Recent advancements have identified pathognomonic gene variants occurring in low allelic frequency in a growing number of patients with CLAs.3 As a result, targeted medical therapies based upon identified gene variants are central to managing these diseases (reference 12) Many of the identified genetic variants in CLA patients are similar or identical to those identified in many cancers, offering significant potential for drug repurposing.

Medical Therapies:
Medications that have been commonly used to treat CLAs include the following (reference 13):

mTOR inhibitors (Sirolimus/Rapamycin)

- A serine/threonine kinase in the PI3K/AKT/mTOR signaling pathway.
- Reduction of mTOR signaling decreases the proliferation and function of the LECs in CLAs.
- Can be used to treat GLA, GSD, CCLA, KLA
- First-line therapy for KLA and studies have found that treatment results in a decrease in serum Ang-2 levels
- Originally approved as an immunosuppressant in 1999
- Dose is approximately 2 mg daily for adults and 0.8 mg/m2 twice daily for children, adjusted to target sirolimus blood concentrations of 8-12 ng/mL.

MEK inhibitors (Trametinib, Selumetinib)

- Block MEK and so cause reductions in ERK activation in the RAS/MAPK signaling pathway.
- Has been used to treat some individuals with GSD, KLA, and CCLA, although no clinical trials to date.
- Various levels of therapeutic improvement but has been observed in the remodeling of the lymphatic system and subsequent improvement of symptoms.

PIK3CA inhibitor (Alpelisib)

- A p110a-specific PI3K inhibitor.
- Used for the treatment of PIK3CA-mutated breast cancer.
- Has been used to treat PIK3CA related overgrowth spectrum (PROS).
- Has been shown to reduce organ dysfunction and improve vessel normalization.
- Effective at decreasing lesions related to CLAs.

AKT inhibitor (Miransertib)

- Targets AKT in PI3K/AKT/mTOR pathway.
- Has been successful in treating some PROS patients and is being investigated as a cancer therapeutic.
- Decreases cell proliferation resulting from activation of the PI3K/AKT/mTOR signaling pathway.

Bisphosphonates (Zoledronic acid, Pamidronate)

- Bisphosphonates slow bone resorption by reducing osteoclast function, improving bone mineral density.
- Nitrogen-containing bisphosphonates affect the RAS/MAPK pathway by reducing isoprenylation and subsequent activation of small GTPases.
- Has been used for the treatment of GLA, GSD, and KLA, as a single agent and in combination with Sirolimus.

Interferon-alfa 2B

- Interferon-alpha 2B has been reported to reduce lymphangiogenic activity as a single agent and in combination with zoledronic acid.

Surgery/Interventional Radiology

Surgical resection, sclerotherapy, and laser therapy can provide local control and symptomatic relief to CLA patients with recurrent effusions, infections, and pain. Surgical resection is often not possible due to the localization and extent of the lesions. Minimally invasive, image-guided procedures such as sclerotherapy, embolization, and recanalization can sometimes be used, but as the lesions are often extensive and located in challenging locations, these procedures can be difficult. Lymphovenous anastomosis, an innovative microsurgical technique developed to correct lymphedema, is being used more frequently to treat CCLAs (reference 14)

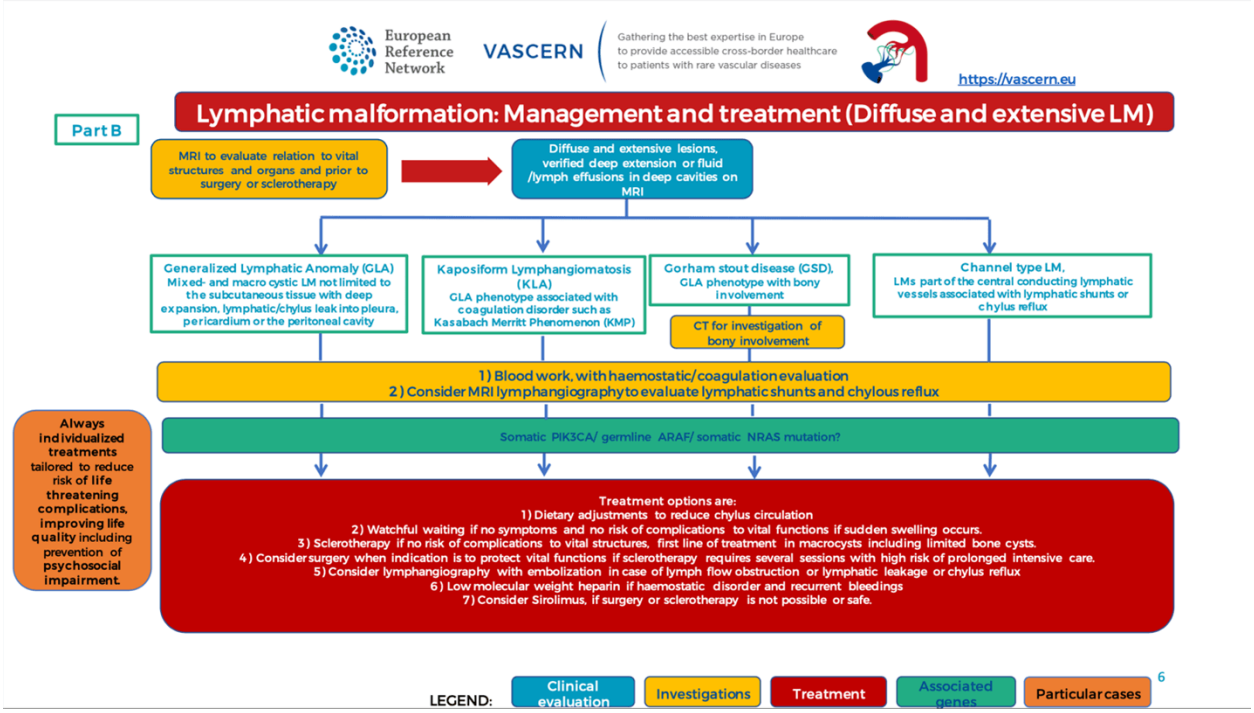
Nutrition

- Patients with chylous (lymph fluid with fat) effusions may be prescribed a low-fat diet.
- Patients treated with Sirolimus or Alpelisib may be prescribed a low carbohydrate diet as both medications can raise blood sugar.

SUMMARY DIAGNOSIS AND MANAGEMENT

The Vascular Anomalies Working Group (VASCA-WG) of VASCERN developed a diagnostic and management pathway for complex lymphatic anomalies (reference 15). Below is the summary care pathway developed and published by VASCERN.

Figure 13: Diagnostic and Therapeutic Summary [(click here to download image)]



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