

Primary lymphedema and HGF mutation

Analysis of the phenotype in 11 patients with primary lymphedema associated with mutation in HGF gene & review of literature

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Abbreviations list

<i>ADAMTS3</i>	A Disintegrin-Like and Metalloproteinase with Thrombospondin motifs 3
<i>AKT</i>	Protein Kinase B
<i>CCBE1</i>	Collagen and Calcium Binding Epidermal Growth factor Domaine 1
<i>CDT</i>	Complete or Complex decongestive therapy
<i>CLOVES</i>	Congenital Lipomatous Overgrowth, Vascular malformations, and Epidermal nevi Syndrome
<i>c-MET</i>	Tyrosin-protein kinase Met or Hepatocyte Growth factor receptor (HGFR)
<i>CT</i>	Computed tomography
<i>DNA</i>	Deoxyribonucleic Acid
<i>EC</i>	Endothelial cell
<i>F</i>	Female
<i>FGF</i>	Fibroblast Growth Factor
<i>FLT4</i>	FMS-like tyrosine kinase 4
<i>FOXC2</i>	Forkhead box C2
<i>GATA2</i>	Gata binding Protein 2
<i>GEHU</i>	Human Molecular Genetics, de Duve Institute laboratory
<i>GJA1</i>	Gap Junction Protein, Alpha 1 (CX43)
<i>GJC2</i>	Gap Junction Protein, Gamma 2 (CX47)
<i>HGF</i>	Hepatocyte growth Factor
<i>HLC</i>	Hereditary Lymphedema Cholestasis
<i>IGF</i>	Insulin Growth Factor
<i>IKBKG</i>	Inhibitor of Kappa light polypeptide gene enhancer in B cells
<i>ITGA9</i>	Integrine alpha 9
<i>KIF11</i>	Kinesin Family Member 11
<i>LD</i>	lymphedema-distichiasis syndrome
<i>LE x</i>	Patient's number in GEHU classification database
<i>LEC</i>	Lymphatic endothelial cell
<i>M</i>	Male
<i>MAPK</i>	Mitogen-Activated Protein Kinase
<i>MCLMR</i>	Microcephaly with or without Chorioretinopathy, lymphedema, or Mental Retardation
<i>MD</i>	Milroy-disease
<i>MHC</i>	Major histocompatibility complex
<i>MLD</i>	Manual lymph drainage
<i>MRI</i>	Magnetic resonance imaging
<i>mTOR</i>	mechanistic Target Of Rapamycin
<i>NF-Kb</i>	Nuclear Factor Kappa-B
<i>OLEDAID</i>	Ectodermal Dysplasia, Anhidrotic, with Immunodeficiency, Osteopetrosis, and Lymphedema
<i>OMIM</i>	Online Mendelian Inheritance in Man
<i>PI3K</i>	Phosphatidyl Inositol 3-kinase
<i>PTEN</i>	Phosphatase and Tensin Homolog
<i>PTPN11</i>	Protein Tyrosine Phosphatase Non-receptor type 11
<i>PTPN14</i>	Protein Tyrosine Phosphatase Non-receptor type 14
<i>RAS</i>	Retrovirus-Associated DNA Sequences originally isolated from murine sarcoma viruses
<i>RASA1</i>	RAS P21 Protein Activator 1
<i>SOX18</i>	SRY-Box18
<i>TSC1</i>	Tuberose Sclerosis Complex1
<i>TSC2</i>	Tuberose Sclerosis Complex2
<i>VEGF-C</i>	Vascular endothelial growth factor – C
<i>VEGFR3</i>	Vascular endothelial growth factor Receptor 3

2 Introduction

2.1 Lymphatic system

2.1.1 Structure

Parallel to the cardiovascular system, the lymphatic organ system is unique to vertebrates and is composed of draining lymphatics vessels, lymph nodes, and associated lymphoid organs. Lymphatic vessels are found in almost every vascularized tissue except neural tissue, bone marrow, bone and cartilage ¹. On the basis of their morphology, function, and hierarchy, lymphatic vessels are classified into capillaries, pre-collectors, and collectors. The capillaries converge into the larger collecting vessels, which drain via chains of lymph nodes, leading eventually to the thoracic duct and the right lymphatic trunk; these two drain into the venous circulation via four lymphovenous valves located at the junction of the subclavian and internal jugular veins ¹ (cf figure n°1)

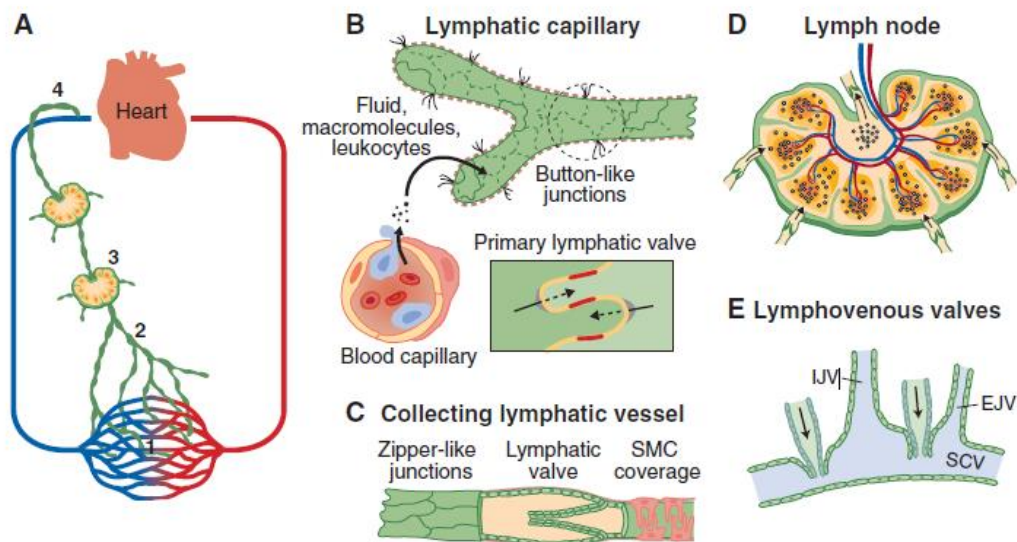


Figure n° 1. Organization of the lymphatic vascular tree.

A, In overview, the unidirectional lymphatic vascular system consists of (1) lymphatic capillaries, (2) collecting lymphatic vessels, (3) lymph nodes, and (4) the thoracic duct and right lymphatic trunk. B, Lymphatic capillaries absorb interstitial solutes, macromolecules, and immune cells that extravasate from the blood vascular system. Adapted from Baluk et al.¹⁷ with permission. C, Collecting lymphatic vessels contain zipperlike junctions, lymphatic valves, and contractile smooth muscle cells (SMCs) that enable the unidirectional propulsion of lymph. D, Organization of the lymph node with afferent lymphatic vessels and a single efferent lymphatic vessel. E, Lymph drains into venous circulation through 4 distinct lymphovenous valves located where the internal jugular vein (IJV) and external jugular vein (EJV) drain into the subclavian vein (SCV).

Aspelund A, Robcius MR, Karaman S, Makinen T, Alitalo K. Lymphatic system in Cardiovascular Medicine. *Circ Res.* 2016;118:515-530.

The lymphatic vascular tree is a one-way system whose main role is to recycle interstitial fluids back to the circulatory system. These recycled interstitial fluids compose what is called “lymph” and are mainly composed of drained plasma. Daily, a major part of the plasma exits into the interstitium to provide nourishments to surrounding tissues. Ninety percent of this fluid is recycled via venous capillaries; the 10% remaining fluid, that has a molecular weight too large to pass through these vessels, is recycled via lymphatic capillaries. Lymphatic capillaries are designed to obtain drained

lymph fluid. In a normal situation, they are collapsed, contrarily to blood capillaries constantly subjected to hemodynamic pressure that forces them to stay open. When an inrush of fluid into the interstitial space occurs, the increase of interstitial pressure leads to the anchoring filaments pulling the lymphatic endothelial cells (LECs) apart and opening the capillaries, making the draining possible².

2.1.2 Functions

The lymphatic vasculature is an essential system, the absence of which is incompatible with life ³. Its main roles are (1) maintaining fluid homeostasis, (2) acting as a conduit for migration and transport of immune cell, (3) regulating inflammatory responses and (4) enabling (dietary) fat absorption ².

2.1.2.1 *Tissue fluid homeostasis*

Water, solutes and proteins escape continuously from the arterial side of the capillary bed into the interstitial space through filtration and are not entirely reabsorbed at the venous side of the capillary bed ³. Return of leaked fluid and macromolecules is crucial for maintaining plasma volume and content, as well as preventing increases in tissue fluid volume and pressure ⁴. The lymphatic system has this vital role of maintaining fluid homeostasis in the body by draining the residual fluid from most tissues and transporting it back to the blood circulation ⁵.

2.1.2.2 *Absorption of dietary fats*

The lymphatic system is essential for the adsorption of hydrophobic nutrients, such as triglycerides, cholesterol, and other lipid molecules from the small intestine ³. This absorption is critically dependent on specialized lymphatic capillaries (lacteals) present in intestinal villi ¹.

2.1.2.3 *Immune cell trafficking*

The lymphatic vascular system serves as duct for many cells, mainly immune cells. Through the trafficking of leukocytes, activated antigen-presenting cells and soluble antigens, it has an important role in immune responses. It intervenes in the initiation of adaptive immune responses, especially in the case of humoral immunity, but also in the promotion of self-tolerance thanks to the expression of peripheral tissue antigens, major histocompatibility complex (MHC) molecules and a variety of immune-regulatory factors by the lymph nodes LECs ^{1 3}. Moreover, recent studies have revealed that lymphatic vessels also play an active role in the metastatic spread of malignant tumor by trafficking tumor cells to regional lymph nodes ⁶.

2.1.3 Development of lymphatic vasculature

The lymphatic vessels arise after the cardiovascular system is established and functional (A) ³. Lymphatic vessels development starts at about embryonic weeks 6-7 in humans when distinct subpopulations of endothelial cells commit to the lymphatic lineage. Lymphatic endothelial cells (LECs) were recently shown to have different origins that can be grossly classified into 2 categories: venous origin and non-venous origins ⁷. Venous derived LECs originate from a subpopulation of EC of the anterior cardinal vein ¹. Non-venous derived LECs (that notably contribute to the lymphatic vasculature of the skin, mesentery and heart) seem to have a multiple cellular origins (that are still partially unknown) which may contribute to the development of the diverse lymphatic vessel and LECs functions in the adult ⁷. Several signaling pathways are involved in the different steps of development of lymphatic vasculature (as explained on figure n°2).

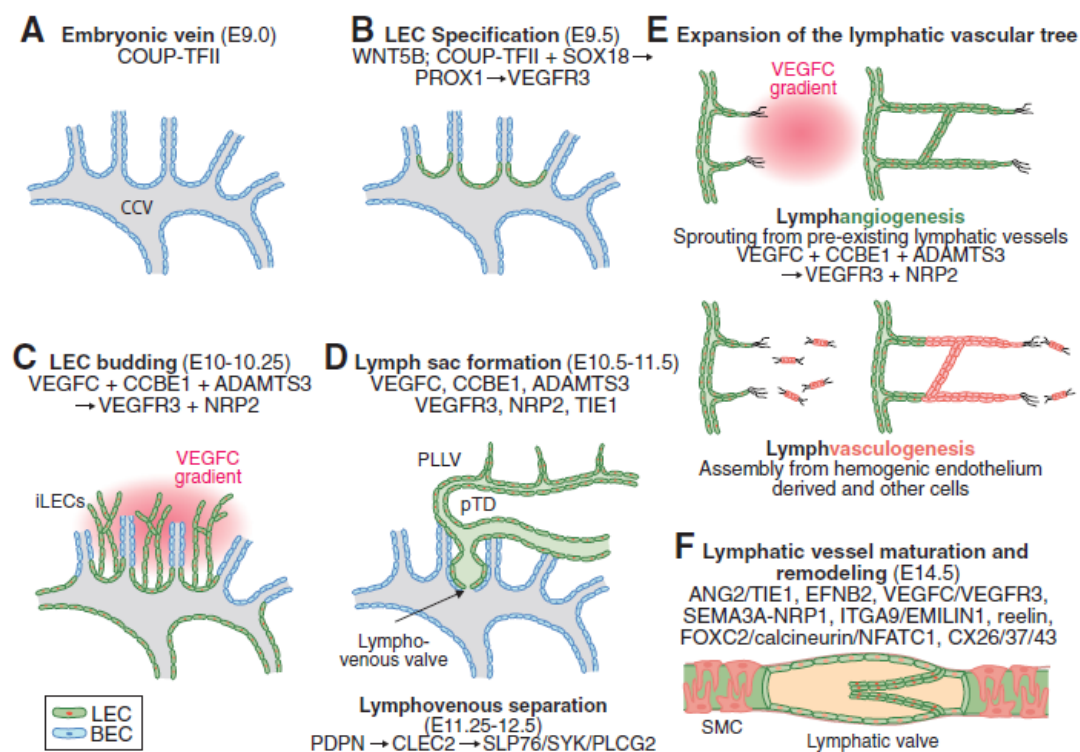


Figure n° 2. Development of the lymphatic vascular tree.

A, The common cardinal vein (CCV) at E9.0. B, Specification of lymphatic endothelial cells (LECs) at E9.5, identified by PROX1 expression in the CCV, in the intersomitic veins and in the superficial plexus. C, Budding of initial LECs (iLECs) from the CCV at E10 to E10.25. D, Formation of the lymph sacs, comprising the primordial thoracic duct (pTD) and primordial longitudinal lymphatic vessel (PLLV) between E10.5 and E11.5. E, Two mechanisms of expansion of the lymphatic vascular tree: lymphangiogenesis and lymphovasculogenesis. F, Lymphatic vessel maturation and remodeling from E14.5 onward: recruitment of smooth muscle cell (SMC) coverage and valve formation.

Panels A–D adapted from Hägerling et al⁵⁹ with permission. Panel E derived from Stanczuk et al.⁵⁵ BEC indicates blood endothelial cell.

Aspelund A, Robcius MR, Karaman S, Makinen T, Alitalo K. Lymphatic system in Cardiovascular Medicine. *Circ Res.* 2016;118:515-530

2.1.3.1 *Expansion – maturation*

The expansion of the lymphatic vascular tree occurs following 2 mechanisms: lymphangiogenesis and lympho-vasculogenesis. A major part of the peripheral lymphatic vasculature is generated by centrifugal sprouting of lymph vessels from pre-existing lymph structures, a process called lymphangiogenesis¹. When committed to lymphatic lineage, LECs sprout laterally from the anterior cardinal vein to form primordial lymphatic vascular structure, the lymph sacs. The peripheral vessels are then generated by centrifugal budding from those lymph sacs³.

A minor part of the (peripheral) lymphatic vascular tree, mainly the dermal and the mesenteric lymphatic vasculatures, develops through the process of lymphvasculogenesis, analogous to the process of vasculogenesis occurring during early establishment of the blood vasculature system^{1,7}. LECs migrate as single cells or as small groups of cells in the direction of interstitial flow, then later coalesce into individual short vessel fragments and eventually organize into an interconnected capillary network in the direction of lymphatic flow (distal to proximal)⁸.

After the establishment of the primitive lymphatic plexus, the lymphatic vessels undergo maturation to form a hierarchical tree composed of lymphatic capillaries, precollectors, and collecting vessels. Collecting lymphatic vessels, deeper vessels, recruit smooth muscle cells, deposit basement membrane and develop lymphatic valves¹.

2.1.3.2 *Disconnection from the blood circulation*

During the course of development, connections between the lymphatics and veins are lost, except at the sites where lymph mixes with the blood in the subclavian veins³. This disconnection process occurs concurrently to the expansion of lymphatic vessels. It involves the platelets, the aggregation of which blocks lymphatico-venous connections and helps to separate both systems.

2.1.3.3 *Lymphangiogenesis in adulthood*

In the adult, the lymphatic vasculature remains usually quiescent with some physiological and pathological exceptions. Lymphangiogenesis occurs physiologically during development of the corpus luteum and wound healing. Lymphatic vessel growth is also associated with a number of pathological conditions, including tumor metastasis, inflammation, and transplant rejection^{3,9}. Adult lymphangiogenesis occurs primarily by vasculogenesis, thus by sprouting from pre-existing vessels³.

2.2 Lymphedema

Lymphedema is a common disabling condition typically affecting the extremities, but it can also be associated with chylous ascites, chylothorax and cystic hygroma. It is the most frequent lymphatic anomaly, resulting from protein-rich interstitial volume overload, secondary to lymph drainage failure in the face of normal capillary filtration¹⁰. Estimates of the prevalence of lymphedema range widely and depend upon age, gender, and etiology but turn around 0,133 to 0,144% in the general population¹¹. This condition usually predominates in women. Lymphedema can be primary or secondary (acquired) (cf figure n°3): the secondary forms are the result of damage of a previously healthy lymphatic system (i.e., tumors, cancer treatment, trauma, etc.)¹². The primary forms result from genetic predisposition responsible for inadequate development or function of the lymphatic system. Until lately, primary lymphedema was classified in an oversimplified way into 3 groups, depending on the age of the onset of clinical manifestation: congenital (before age of 2 years old), praecox (between age 2 and 35 years old), and tarda (after the age of 35 years old)¹³. This antiquated classification has been progressively abandoned in favor of Connel et al¹² classification pathway based on the phenotypes which is considered as referential (cf figure n°4)

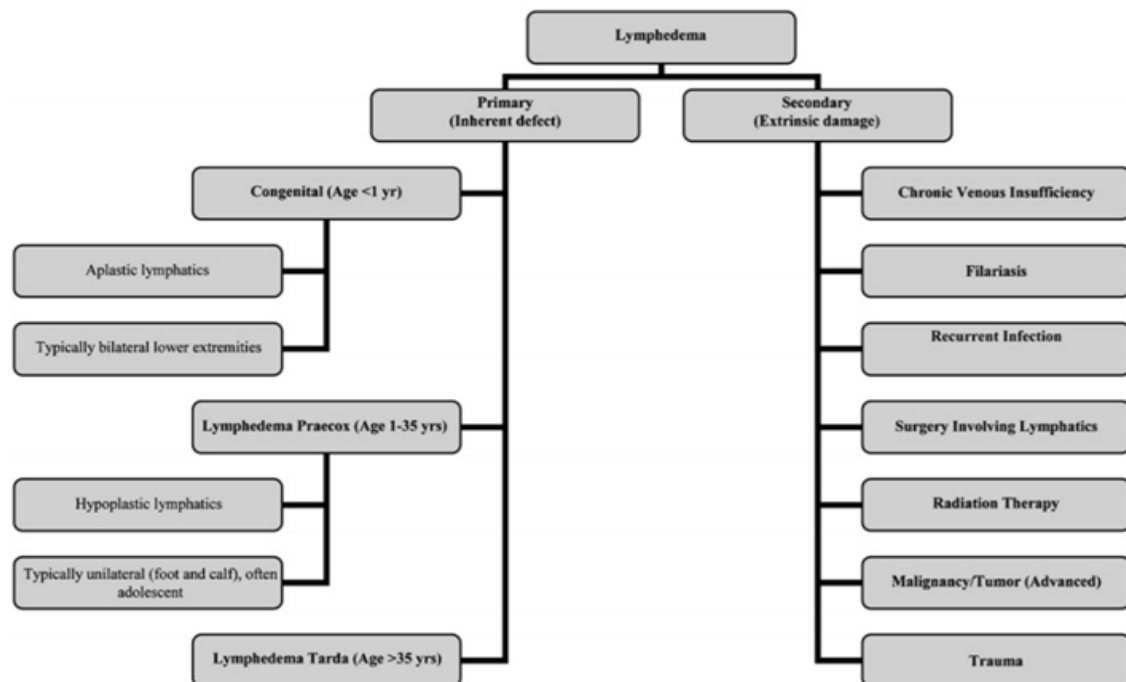


Figure n°3. Causes of Lymphedema.

Kerchner K, Fleischer A, Yosipovitch G. Lower extremity lymphedema - Update : Pathophysiology, diagnosis, and treatment guidelines. *J Am Acad Dermatol.* 2008; 59:324-331.

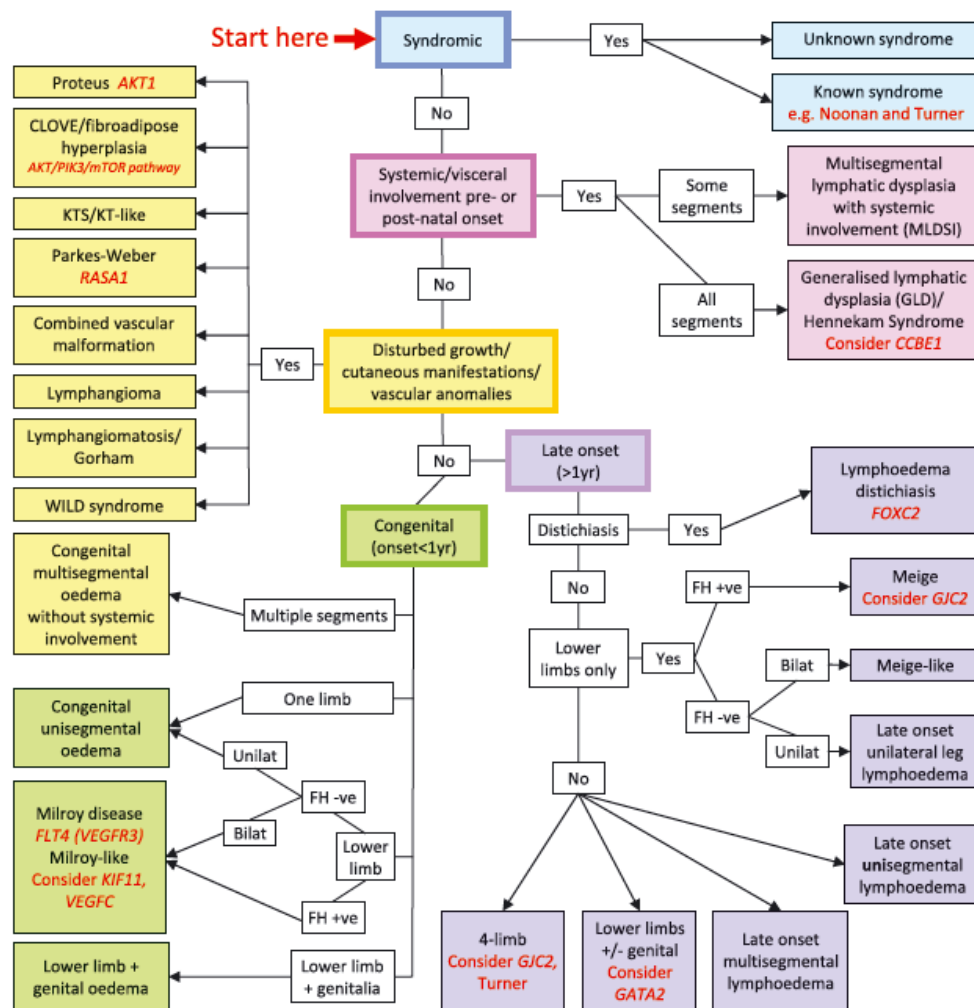


Figure n°4. Revised classification pathway for primary lymphedema.

Abbreviations: FH, family history; +ve, positive; -ve, negative; unilat, unilateral; bilat, bilateral. Text in red indicates suggested genetic test for the subgroup. Please refer to Table 1 for definition of other terminology used in the figure.

Connel F, et al. The classification and diagnostic algorithm for primary lymphatic dysplasia: an update from 2010 to include molecular findings. Clin Genet. 2013; 4(6):257-266

2.2.1 Pathophysiology

The pathophysiology of lymphedema is generally divided into 2 periods: The occult period and the symptomatic period. During the first period, the pathological changes occur mainly in the lymphatics; lymphedema symptoms are not apparent. After this stage, the pathological changes occur in the soft tissue (fat, connective tissue, skin, etc.) of the limbs, resulting in progressive swelling due to accumulation of lymph ¹⁴.

The complex biology of regional lymphatic disruption is a manifestation of the interplay between the 2 main roles of lymphatic vessels: circulatory function and immune function. The lymph stasis is associated with blunted regional immune trafficking, local inflammatory changes and a heightened propensity to infection, often with ensuing organ or tissue damage ¹⁵. Inflammation plays a central

role in lymphedema associated changes; its chronicity leads to massive proliferation of parenchymal and stromal elements with excessive deposition of extracellular matrix substances and often, adipose tissue ¹⁶. Besides, this situation of chronic inflammation impairs the local immune response, causing local immunosuppression and a high propensity to various infections, such as cellulitis.

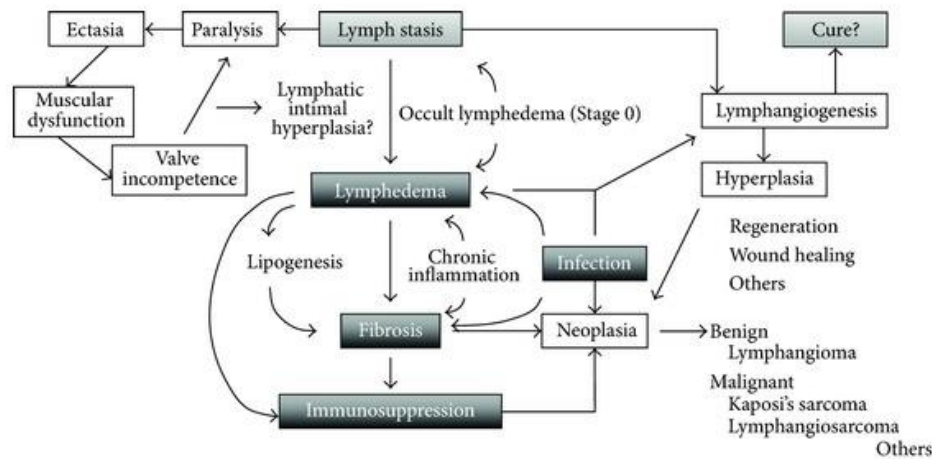


Figure n°5. Schematic diagram of the pathophysiology of lymphedema

Review Article : Saito Y, et al. Lymphedema and Therapeutic Lymphangiogenesis. Biomed Research International. 2013. DOI : 10.1155/2013/804675

2.2.2 Diagnosis & Differential diagnosis

The diagnosis of lymphatic vascular disease relies heavily on the physical examination.

2.2.2.1 Clinical presentation

Lymphedema is most often readily identified by its physical characteristics, including edema, peau d'orange, cutaneous fibrosis, positive "stemmer sign" (the inability of the examiner to tent the skin at the base of the digits in the involved extremity), hyperkeratosis, dermatitis, lymph vesicles, ulceration, etc. ¹⁷. The edema is initially a pitting oedema, but over time the subcutaneous tissue becomes fibrotic, resulting in non-pitting brawny edema. Eventually the involved skin becomes hyperkeratotic, hyperpigmented, and papillomatous or verrucous with increased skin turgor ¹⁰.

Clinical evaluation of patients with lymphedema of unclear origin must include a detailed medical case history (including family history) and physical examination. The history should address age at onset of symptoms and signs, and eventually, travels to tropical countries and detailed inquiry for all conditions that could result in secondary lymphedema. Any arteriovenous, venous, or capillary malformations should be recorded as well as any limb-length discrepancy, cellulitis, lymphangitis, malnutrition and immunodeficiency. Although less frequent possible malignancy (i.e., lymphangiosarcoma) should also be excluded ¹³.

2.2.2.2 Imaging investigation

Whenever there is doubt regarding clinical diagnosis or a need of better definition, diagnosis confirmation can be accomplished with imaging. Different types of imaging can be used including: radioisotope lymphography or lymphoscintigraphy, contrast lymphangiography, magnetic resonance imaging (MRI) and computed tomography (CT), as well as ultrasound scanning. Lymphoscintigraphy stands as the gold standard for the evaluation of lymphatic function and thus for the diagnosis of lymphedema, as it allows identifying the specific lymphatic abnormalities. Computed Tomography or MRI of the lower extremity can also identify lymphedema by detecting a specific pattern of the subcutaneous tissue. Additionally, ultrasound (duplex ultrasonography scan technique - DUS) might be useful by confirming or excluding venous anomalies or venous obstruction that can coincide with lymphedema. Besides, ultrasound helps differentiating lymphedema from lipedema, and quantifying dermal edema.^{10, 13}

2.2.2.3 Differential diagnosis

Lymphedema should be considered in the presence of painless and non-inflammatory peripheral edema. The main differential diagnosis are chronic venous insufficiency, lipedema and general causes of lower extremity edema (i.e.: renal failure, protein-losing nephropathy, hypoalbuminemia, congestive heart failure, pulmonary hypertension, obesity, pregnancy etc.), the exclusion of which should be a priority.

2.2.3 Complications

Lymphedema is a life-long disabling and disfiguring condition associated with multiple complications: infections, articular complications, skin changes, functional disability, psycho-social disorders and sometimes malignant transformations. Collectively these morbidities contribute to an overall poor quality of life.^{10, 18}

2.2.3.1 Infections

Lymphedema is associated with recurrent soft-tissue infections such as cellulitis, erysipelas, tinea pedis, and lymphangitis¹⁰. Different mechanisms are implicated in this propensity to infections: first of all, accumulated fluid and proteins provide a good substrate for bacterial growth. Secondly, lymphatic dysfunction impairs the local immune response, which plays a permissive role in the propagation of bacterial and fungal invasion¹⁴. Thirdly, stagnation of lymph may lead to impaired elimination of bacterial components, which are important triggers of the cytokine cascade and local and systemic inflammatory reactions¹⁹. The persistent inflammation leads to further lymphatic

damage, greater stagnation of lymph and further episodes of cellulitis, resulting in a vicious cycle of recurrent cellulitis. Due to this vicious cycle, lymphedema can be considered as a cause and a consequence of cellulitis. Lymphedema has been reported to be the most important risk factor in the development of recurrent cellulitis.²⁰

2.2.3.2 Articular complications

In some cases of evolved lymphedema, the major skin sclerosis and fat deposition that occur may have deleterious effects on ligaments and tendons. Consequently, an articular mobility reduction and a functional discomfort may appear.

2.2.3.3 Skin changes

Advanced lymphedema comes with various skin changes depending on the severity and duration of lymphatic damage. The cutaneous findings of lymphedema are common to all forms of lymphedema at whatever site or due to whatever cause. The earliest finding is an accentuation of skin creases and a peau d'orange appearance. It may evolve into the development of cobblestone like and hyperkeratotic, papillomatous plaques that characterize elephantiasis (nostra verrucosa)²¹. Elephantiasis nostra verrucosa, also called "lymphostatic papillomatosis cutis", is an uncommon and singular array of dermatologic manifestations that can complicate chronic lymphedema²². It is a benign skin growth of epithelial neoplasm that may contain villous or fibrous vascular outgrowth. The plaques of elephantiasis can be complicated by fissures, ulcers, loosely adherent crust, oozing of clear or yellow fluid (lymphorrhea) with a foul-smelling odor. These signs of skin barrier breakdowns, associated with lymphostatic dermopathy, lead to infections (i.e. cellulitis).²¹

2.2.3.4 Malignancy in Patients with lymphedema

Generally, lymphedema is not considered to influence prognosis of mortality. In rare cases, however, chronic lymphedema may entail the development of malignant tumors such as lymphangiosarcoma (Stewart-Treves syndrome), Kaposi's sarcoma, squamous cell carcinoma, malignant lymphoma, and melanoma. The pathophysiology of carcinogenesis is unclear but seems to deal with the chronicity state of lymphedema.¹⁴

2.2.3.5 Psycho-social disorders

The presence of lymphedema markedly affects the quality of life and self-perception of the patients and may lead to depression, anxiety and social adjustment difficulties. Therefore psychosocial support with a quality of life assessment-improvement program is an integral component of any lymphedema treatment.^{16, 17, 23}

2.2.4 Treatment

The treatment of lymphedema aims to minimize the associated swelling, to restore the functionality of the limb, and to prevent potential complications associated with lymphedema with the ultimate goal of improving quality of life ^{10, 13}.

Therapy of peripheral lymphedema is divided into conservative (non-operative) and operative methods. Applicable to both methods is an understanding that meticulous skin hygiene and care (cleansing, low pH lotions, emollients) is of utmost importance to the success of virtually all treatment approaches ¹⁶. Patient's education in self-care is also primordial and is critical for successful long term outcomes ²³.

2.2.4.1 *Conservative methods*

2.2.4.1.1 Physical therapy

Physical therapy which is usually called "Complete or complex decongestive therapy" (CDT) is considered as gold standard treatment. It is based on 4 pillars: skin care, exercises, manual lymph drainage and compression therapy. It is aimed at improving lymphatic drainage through existing lymphatic vessels by applying normal physiological mechanisms, which stimulate lymph flow.²⁴

2.2.4.1.1.1 *Skin care*

Good hygiene and prevention of infection are paramount in the management of lymphedema, as mentioned in point 1.2.4. Skin infection should be treated early with antibiotics. Occasionally prophylactic antibiotherapy may be required in cases of recurrent erysipelas. Careful antiseptic dressings must be used immediately after minor injuries and, even without any context of injury, antiseptics should be applied to control skin surface microbial colonization. The control of tinea pedis, especially toe web involvement, which is a portal of entry for bacterial infections (e.g., recurrent erysipelas), is also very important. Salicylic acid ointment can be used as keratolytic and use of emollient is advised for xeroderma. ^{10, 24}

2.2.4.1.1.2 *Exercise*

Exercising is an essential element in the management of lymphedema. It enhances lymph flow and may improve protein resorption. It is even considered to represent the most important mode of treatment. Weight reduction is also a key element if obesity is contributory to the lymphedema. ¹⁰

2.2.4.1.1.3 Manual lymph drainage

Manual lymph drainage (MLD) is a lymphatic-specific massage technique. It enhances the filling of the cutaneous initial lymphatics and causes the lymph conduits to contract more often and to milk lymphatic fluid away from congested regions, and subsequently toward nearby functioning lymph basins. This technique may also stimulate the development of accessory lymph collectors. Complete decongestive therapy relies heavily upon MLD. ^{16, 17}

2.2.4.1.1.4 Compression therapy

There are multiple different methods of external compression, including multiple-layer short stretch compression bandages, elastic stockings, adjustable non-elastic compression leggings and pneumatic compression devices

2.2.4.1.2 Drug therapy

Drug therapy offers marginal benefits in the management of lymphedema. Different drugs have been tried but have been disappointing. Two medications are essentially used. The first one are the diuretics: they are of little, if any, benefit and should be reserved for patients with specific comorbid conditions or complications. The second one is coumarin, a benzopyrone, which has been found to have a small favorable effect; however, this remains to be proven. Neither diuretics nor coumarin constitute an alternative or substitute for CDT. ^{10, 17, 24}

2.2.4.1.3 Psychological support

As mentioned earlier, lymphedema can be associated with serious emotional consequences. Moreover, because treatments are time consuming, expensive and life-long, there is often a lack of participation and commitment of the patient to the rehabilitation effort, which is one of the major obstacles to successful management. For those reasons, social support and support groups may be useful to help patients handle their condition and their engagement and motivation.

2.2.4.2 Operative methods

Surgical treatment of lymphedema is generally reserved for patients with pronounced lymphedema who have failed standard treatment trials ²⁵. Some of the indications are the following: recurrent lymphangitis/cellulitis, refractory pain, impaired movement and function of the affected limb due to its size and weight. Although, surgery is not curative for lymphedema, it may help in some circumstances.

2.2.4.3 *Molecular methods*

A new option starts to emerge in the therapeutic arsenal of lymphedema: therapeutic lymphangiogenesis. Indeed, the acquired insights into the molecular regulation of lymphatic vascular development have fostered sustained interest in the therapeutic potential of modulating post-natal lymphatic vessel growth and remodeling. Therapeutic lymphangiogenesis is a promising gene therapy strategy that consists in the administration of lymphatic growth factors or related molecules. However, to date, the applicability of this treatment is still uncertain.^{14, 17, 24}

As stated before, lymphedema is a disabling condition with a tendency to worsen inexorably, explaining why treatments must be introduced as early as possible to optimize their efficacy. Even with early intervention, lymphedema usually becomes a chronic, generally incurable, ailment. Consequently, it requires lifelong care and attention along with psychosocial support, as do other chronic disorders. A multidisciplinary approach thus appears crucial with a combination of rehabilitative lymphedema therapy, dermatologic care, and occasionally surgery.¹⁰

2.3 **Genetics basis of primary lymphedema**

Lymphangiogenesis is a complex process that is regulated by multiple growth factors (figure n°6). Anomalies in the signals of any of those factors might cause lymphatic defects, and thus might be responsible for the development of lymphedema. This high number of regulatory factors explains the high genetic heterogeneity of primary lymphedemas. Currently, germline mutations have been identified in 28 genes that encode proteins acting around *VEGFR3* signaling but also downstream of other tyrosine kinase receptors. These mutations explain about 40% of familial forms and 12% of sporadic forms of primary lymphedema. The underlying genetic abnormality thus remains obscure for a majority of cases, representing a large field of research²⁷. Due to this genetic heterogeneity, a systematic approach is essential when facing a patient with primary lymphedema of unknown origin. As such, the classification of Connel et al¹² (figure n°4) represents an excellent technical support and will be thus used to present this genetic section.

2.3.1 **Syndromic lymphedema**

When approaching a patient with primary lymphedema of unknown origin, the first step consists in ruling out the many well-known syndromes associated with lymphedema, such as: Turner syndrome (Monosomy X), Noonan syndrome (*RAS* pathway anomalies), Prader Willi syndrome etc (cf figure

n°6). Primary lymphedema has also been reported in some cases of tuberous sclerosis; the significance of this association remains uncertain and will be discussed in point 4.3.5.

Known syndrome	Chromosome/gene
Aagenaes syndrome	Not known
Carbohydrate deficient glycoprotein types 1a, 1b, and 1h	<i>PMM2, PM1, ALG8</i>
Cardio-facio-cutaneous syndrome	<i>KRAS, BRAF, MAP2K1, MAP2K2</i>
CHARGE syndrome	<i>CDH7</i>
Choanal atresia-lymphoedema	<i>PTPN14</i>
Ectodermal dysplasia, anhidrotic, immunodeficiency, osteopetrosis and lymphoedema (OL-EDA-ID syndrome)	<i>IKBKG (NEMO)</i>
Fabry disease	<i>GLA</i>
Hennekam syndrome	<i>CCBE1</i>
Hypotrichosis-lymphoedema-telangiectasia	<i>SOX18</i>
Irons-Bianchi syndrome	Not known
Lymphoedema-myelodysplasia (Emberger syndrome)	<i>GATA2</i>
Macrocephaly-capillary-malformation (MCM)	<i>PIK3CA</i>
Microcephaly-chorioretinopathy-lymphoedema-mental retardation (MCLMR)	<i>KIF11</i>
Mucke syndrome	Not known
Noonan syndrome	<i>PTPN11, KRAS, SOS1, and others</i>
Oculo-dento-digital syndrome (ODD)	<i>GJA1</i>
Progressive encephalopathy, hypsarrhythmia, optic atrophy (PEHO)	Not known
Phelan McDermid syndrome	22q terminal deletion or ring chromosome 22
Prader Willi	15q11 microdeletion or maternal UPD 15
Thrombocytopenia with absent radius	1q21.1 microdeletion and <i>RBM8A</i>
Turner syndrome	45XO
Velocardiofacial syndrome	22q11 microdeletion
Yellow Nail syndrome	Not known

Figure n°6. List of known syndromes associated with lymphedema and the causative gene (or chromosomal abnormality)

Connel F, et al. *The classification and diagnostic algorithm for primary lymphatic dysplasia: an update from 2010 to include molecular findings. Clin Genet. 2013; 4(6):257-266*

2.3.2 Systemic/visceral involvement

When a known syndrome has been excluded, the second step is to investigate the possible presence of associated abnormal internal lymph vessels. Individuals with internal involvement are characterized by widespread development anomaly of the lymphatic system; consequently, they may present with specific manifestations such as hydrothorax/chylothorax, ascites, as well as hydrops fetalis. Onset of lymphatic dysfunction prenatally, characterized by hydrothorax or hydrops fetalis, evokes different possible causes notably Hennekam syndrome (OMIM 235510), linked to mutations in *CCBE1* and *ADAMTS3* genes. Hennekam syndrome is an autosomal recessive form of primary lymphedema characterized by generalized lymphatic dysplasia associated with intestinal lymphangiectasia, typical facial features (flat face, hypertelorism and broad nasal bridge), mental retardation and hydrops fetalis^{27, 28, 29}. Despite its rarity, a mutation of *ITGA9*, integrin $\alpha 9$, should also be considered. Indeed, mutations in *ITGA9* have been associated with severe chylothorax in human fetuses²⁷. Finally, Turner and Noonan syndromes should also be evoked as they are associated with hydrops fetalis in some cases¹².

Visceral lymphatic dysfunction revealed by the presence of ascites can present postnatally. In that case, Aagaenae Syndrome or Hereditary Lymphedema Cholestasis (HLC) should be evoked. It is a recessive disorder, associated to a locus in 15q, characterized by extended lymphedema of the lower extremities associated with malabsorption, growth retardation, rickets, cholestatic jaundice and hepatomegaly.^{27, 29}

2.3.3 Disturbed growth and/or cutaneous/vascular anomalies

The presence of any disturbed growth or birthmark associated with lymphedema must also be looked after to exclude an overgrowth “syndrome” characterized by a genetic anomaly usually confined to the tissue affected. The mutations responsible for those syndromes affect the *AKT-PIK3-mTOR* pathway which is critical as a progrowth and antiapoptosis facilitator in cancer. Different overgrowth syndromes are described: Proteus (*AKT-1*), CLOVES/Fibroadipose hyperplasia, Klippel Trenaunay syndrome and Klippel Trenaunay –like, Parkes Weber syndrome (*RAS1*), etc.¹²

2.3.4 Congenital or Late Limbs localized lymphedema

After all previous situations have been excluded, we can proceed to differentiating the different forms of primary lymphedema affecting the limbs and sometimes genitals. A diagnosis of a congenital lymphedema should evoke aberrations in several genes depending on clinical features: mainly *VEGFR3* (Milroy disease) but also *VEGFC* (Milroy-like disease) and less frequently *KIF11* (MCLMR syndrome), as well as *GJA1* (Oculodentodigital syndrome).^{12, 27}

Milroy disease and Milroy-like disease will be described in part 5. As for *KIF11*, mutations of this gene cause a specific syndrome called MCLMR. It consists of lower limb lymphedema of variable expressivity associated with microcephaly with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR; OMIM 152950)³⁰. Mutations of *GJA1* gene, which encodes for a connexin, have been associated to primary lymphedema as part of the oculodentodigital syndrome (OMIM 164200). This syndrome is a congenital condition, most often autosomal dominant, with phenotypic features most commonly affecting the face, eyes, dentition and digits. Characteristic findings include hypotelorism, hypoplastic alae nasi, microphthalmia, microcornea, microdontia etc. Lymphedema in this condition usually develops after birth³¹.

Likewise a diagnosis of post-natal lymphedema will be linked to specific gene aberration and distinct clinical features. Distichiasis (presence of an extra row of eyelashes) for instance will point to mutations in *FOXC2*. *FOXC2* mutations are associated with lymphedema-distichiasis (LD) syndrome

(OMIM153400) which is a rare, primary lymphedema of pubertal onset, associated with distichiasis and sometimes with ptosis, yellow nails, syndactyly, cleft palate and cardiac septal defects ³². This syndrome will be described more thoroughly in part 5.

If lymphedema is associated with immunological problems, two main genes will be considered: firstly, *GATA2* a transcription factor whose mutations are associated with dendritic cell, monocyte, B lymphocyte, and natural killer lymphocyte deficiency (OMIM 614172), with susceptibility to myelodysplastic syndrome (OMIM 614286) and acute myeloid leukemia (OMIM 601626), as well as Emberger syndrome^{33, 34}. Emberger syndrome is characterized by lymphedema associated with deafness, immune dysfunction and myelodysplasia (OMIM 614038) and will be detailed in part 5 ^{33, 34}. Secondly, one has to consider *IKBKG* gene which has been linked to a rare form of familial lymphedema named OLEDAID syndrome (Osteoporosis Lymphedema Anhidrotic Ectodermal Dysplasia with Immunodeficiency) (OMIM 300301)²⁷.

An association of lymphedema and choanal atresia hints at mutation of *PTPN14* gene which has been linked to lymphedema- choanal atresia syndrome (OMIM 608911)²⁷

When a patient presents with lymphedema, sparse hair and cutaneous telangiectasias, mutations of *SOX18* will be evoked. Variants in the transcription factor gene *SOX18* have indeed been identified in patients with hypotrichosis-lymphedema-telangiectasia syndrome (OMIM607823). This rare syndrome is usually inherited with an autosomal dominant or recessive pattern of transmission and is characterized by a variable onset lymphedema with reduced body hair and localized cutaneous telangiectasias.^{27, 35}

Finally, when facing a patient with a four-limb lymphedema, *GJC2* gene mutation should be considered. Gap junction protein gamma-2 is a gene encoding for another connexin (Connexin X47). Mutations in *GJC2* were discovered in a few families with late-onset autosomal dominant lymphedema (OMIM 613480) affecting all 4 extremities (“four-limb lymphedema”) and sometimes associated with saphenous vein insufficiency, blepharoptosis, and involvement of the face or genitalia. ^{27, 36, 37}

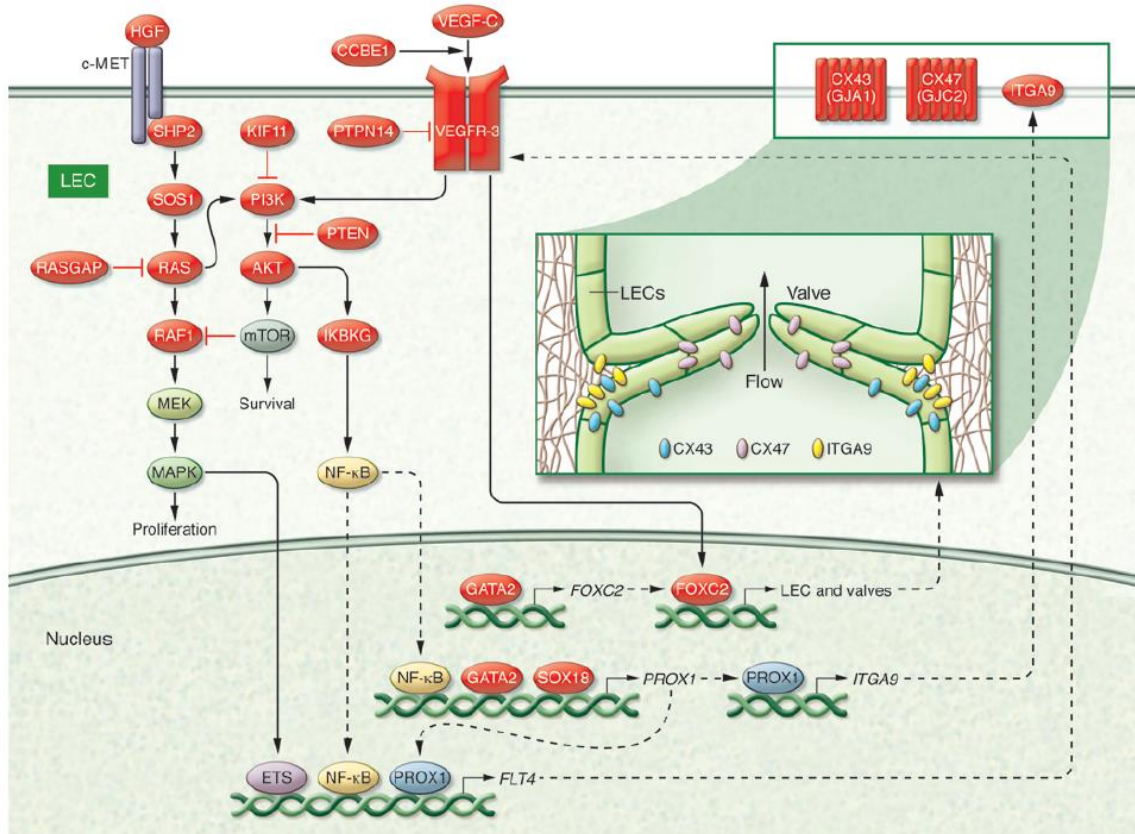


Figure n°7. Schematic view of a lymphatic endothelial cell and valve.

Lymphatic anomaly-associated proteins are frequently associated with the VEGF-C/ VEGFR-3 signaling pathway. Proteins mutated in lymphedema or other lymphatic disorders are shown in red. Arrows indicate direct or indirect interactions, red lines indicate inhibition, and dashed lines indicate transfer of protein. AKT includes AKT1; PIK3CA is part of PI3K; RAS includes HRAS and KRAS; RASA1 is one of the RASGAPs. LEC, lymphatic endothelial cell.

Brouillard P, Boon L, Viskula M. Genetics of lymphatic anomalies. *J Clin Invest.* 2014;124(3):898-904

As far as *HGF* - the main gene of interest - is concerned, its role as potential causal gene for lymphedema has only been revealed recently. The gene *HGF* codes for the “Hepatocyte growth factor” that regulates growth, motility and morphogenesis of different types of cells; this factor, associated with its receptor *MET* (Tyrosin-protein kinase Met), is also recognized to be a strong promoter of lymphatic vessel formation, as demonstrated by Kajiya *et al.* (2005)⁶ and confirmed by the observations of Saito *et al.* (2006)¹⁴ and Cao *et al.* (2006)³⁸. The role of *HGF* in primary lymphedema will be explored in details below in point 4.

3 Objectives

The understanding of the physiology of lymphangiogenesis and the pathogenesis of primary lymphedema are part of a booming research endeavor in the medical community. As mentioned earlier, 28 genes have already been found to be involved in primary lymphedema; however in a substantial number of patients the underlying cause has yet to be identified.

The main objective of the present work aims at phenotyping patients presenting *HGF* mutated primary lymphedema. A review of the principal genetic anomalies linked to primary lymphedema will also be offered and in doing so, a cohort of 7 cases followed at Saint Luc university hospital, Brussels, will be presented for illustration.

4 HGF Group

4.1 Material & Methods

4.1.1 Patient ascertainment

A cohort of 542 index patients with unrelated primary lymphedema was included in the study. Written informed consent was obtained from all patients and DNA samples were collected as approved by the Ethics Committee of the medical faculty at Université Catholique de Louvain, Brussels, Belgium.

4.1.2 Clinical phenotyping

Clinical information were collected by using a specific official lymphedema questionnaire from the Human Molecular Genetics (GEHU) de Duve Institute laboratory covering the most relevant features such as a detailed characterization of the lymphedema, family history and associated syndromes or anomalies frequently found in primary lymphedemas (see appendix n°1). The questionnaires were filled in by the clinician in charge of the patients before being analyzed anonymously. In addition, the medical records of the patients were reviewed, as well as the lymphoscintigraphy protocols, when available.

4.1.3 Molecular diagnosis (HGF group)

A panel of 66 genes known or suspected to be causal to lymphedema, including *HGF* was used to screen the DNA of the 542 index patients by targeted high-throughput sequencing.

4.2 Results

4.2.1 Clinical findings

4.2.1.1 Molecular results

Of the 542 screened index patients, 52 *HGF* variants were discovered but only 12 variants, carried by 11 patients, passed the filtering criteria used by the laboratory. Two more variants (p.G79R and p.Val631Met) passed the filtering criteria but were shown to have little or no impact on HGF, likely representing rare polymorphisms, underscored by the fact that a clear *GATA2* mutation was identified in one of patients (LE126) with an Emberger syndrome. The latter patient will be presented in the second part of this study, while focusing on lymphedema associated to *GATA2* mutations.

The 12 rare variants discovered in *HGF* were found to be pathogenic according to reviews by the host laboratory. *HGF*-burden analysis showed a significant association between lymphedema and the variants. The variants considered as probably pathogenic in lymphedema were all heterozygous. It includes 3 non-sense, 2 frameshifts, 6 missense and 1 splice-site alterations. Two changes were found as allelic changes in the same patient (LE47). Unfortunately no clinical data were available for this patient. Segregation analyses were available for 5 of our 11 patients, among them 3 were found to share their HGF variant with at least one of the relatives tested. In patient LE535, from the 2 relatives tested, his brother was found sharing the same HGF variant as him without being affected with lymphedema. In patient LE641, segregations studies showed the HGF variant was shared by the father, himself affected with lymphedema. In patient LE102, 2 variants were found, one in *HGF* (a missense mutation) and one in *VEGFR3* (pGly1131); the segregation analyses done in the DNA of the parents showed that mutation in *VEGFR3* was carried by his father, healthy carrier, and that his mother carried the same *HGF* mutation as him; none of the parents were suffering from lymphedema. Finally, from the 2 unaffected parents of patient LE197, the HGF variant was found to be shared with the father.

The in vitro functional analysis of the variants revealed various modified *HGF* function ranging from complete loss with premature stop codon (3 non-sense and 3 frameshifts) mutations to partial reduction of activity for amino acid substitutions (6 missense and 1 splice-site).

Table 2. Genetic information and clinical features per patient with mutation in HGF

	Sample ID	Nucleotide variant	Protein alteration	Activity of mutant allele*	Other gene	Gender	Inheritance	Age of onset	LE	Additional clinical features	Complications (I &/or P)	LS	Relatives informations
Premature stop codon	LE291	c.288_289delinsAA	p.Cys96*	-	-	F	Familial	Late (40 yo)	2L(i)	-	P	+	Mother
	LE169	c.1153dup	p.Met385Asnfs*	-	-	F	Sporadic	Late (9 yo)	4L	-	P	+	-
	LE535	c.1504C>T	p.Arg502*	-	-	M	Familial	Late (7 yo)	4L	-	I	+	1 Cousin
	LE563	c.1669G>T	p.Gly557*	-	-	M	Familial	Late (14 yo)	4L	-	I+P	-	Mother + 1 sister + 1 brother
	LE602	c.1853delG	p.Gly618Alafs*	-	-	M	Sporadic	Late (25 yo)	2L(i)	-	I	+	-
Splice-site change	LE641	c.1444+1G>A	-	-	-	F	Familial	Late (11 yo)	2L(i)	-	-	-	Father
Amino acid changes	LE153	C.559C>A	p.Pro187Thr	40%	-	F	Sporadic	Congenital	1L(i)	LE of genitals	P	-	-
	LE313	c.1525G>A	p.Val509Ile	70%	-	M	Sporadic	Late (44 yo)	1L(i)	LE of genitals + Dilated veins	I+P	+	-
	LE102	c.1814C>G	p.Thr605Arg	75%	VEGFR3	M	Familial	Congenital	2L(i)	Millroy disease	-	-	Father
	LE047	c.1872C>A	p.Asn624Lys	75%	-	M	Sporadic	Congenital	?	?	?	?	-
	LE197	c.1880G>A	p.Gly627Asp	70%	-	M	Sporadic	Late (10 yo)	1L(i)	-	-	+	-
	LE197	c.1947G>T	p.Lys649Asn	70%	-	M	Sporadic	Late (10 yo)	1L(i)	-	-	+	-

Abbreviations

F = Female, M = Male, LE = Lymphedema, L= limbs, (i) = inferior, I = Infection (cellulitis/erysipela), P = Papillomatosis, LS = lymphoscintigraphy protocol available, * = Activity estimated based on c-Met phosphorylation (E. Fastré, 2017)

Familial cases : informations available

LE291	Mother	information missing about lymphedema characteristics
LE535	Cousin	information missing about lymphedema characteristics
LE563	Mother	2L(i) - AOO : ?
	Brother	1L(i) - AOO : ?
	sister	1L(i) - AOO : ±30 y.o
LE641	Father	1L(i) - AOO : ±30 y.o
LE102	Father	information missing about lymphedema characteristics

4.2.1.2 Gender and family history

Eleven patients were found to carry pathogenic variant(s) of *HGF*, including 4 women and 7 males, of which 5 cases were suspected to be familial. Among the cases with a familial history, 2 were well documented (LE641 and LE563) but 3 remained unclear due of lack detailed history (LE291, LE535 and LE102). In patient LE641, the father, who was showed to share the same *HGF* variant as his daughter, had had a unilateral lower limb lymphedema of spontaneous onset since the age of 30 that remained stable without any intervention. Patient LE563 had 3 family members with lymphedema: his mother had a bilateral lower limb lymphedema of uncertain age of onset and evolution; he also had a sister with a unilateral lower limb lymphedema since the age of 30 and a brother with fluctuating episodes of swelling in one leg. Patient LE291 had a history of maternal lymphedema whose description remained approximate due to early death of the mother 30 years earlier. In patient LE535, there was a notion of uninvestigated lymphedema in one cousin. No information was available for patient LE102. (cf figure n°8)

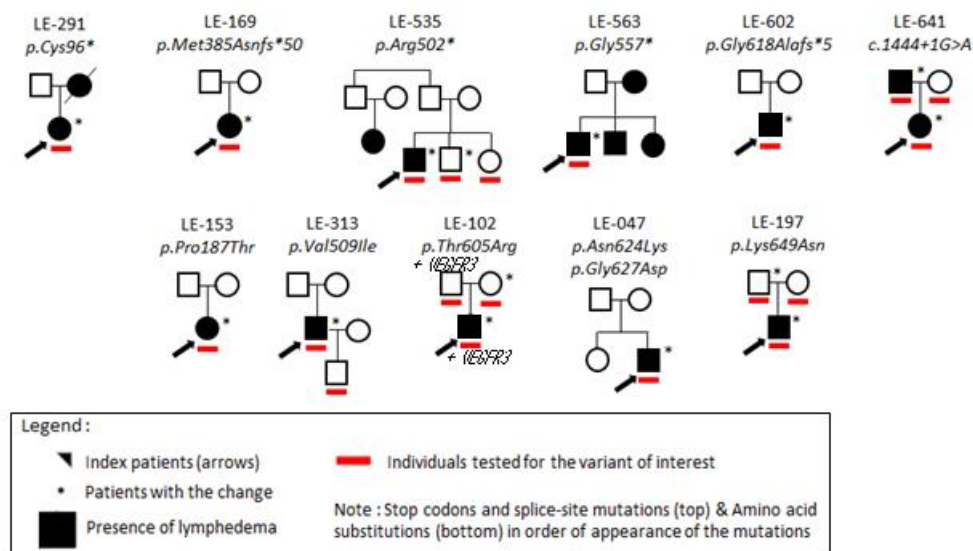


Figure n°8. Pedigrees of families with changes in *HGF*

4.2.1.1 Age of onset

Of the 11 patients ascribed to the study, 3 (2M/1F) reported swelling from birth (congenital), 6 during childhood or early adulthood (praecox), and 2 (1M/1F) at ages of 44 and 38 respectively. In the 2 late onset forms, lymphedema developed suddenly, without any known/obvious triggering factor in the female patient and following a sprain and an infection in the male patient. In most cases of early onset lymphedema, no trigger was identified.

4.2.1.2 *Distribution of swelling*

Concerning distribution of the swelling, 3 patients (3/11) presented with a four-limb lymphedema with a moved back unveiling of the upper limbs lymphedema compared to the lower limbs. In 2 of the patients, arms swelling had developed respectively 7 and 28 years after leg swelling. Among the 8 patients with lower-limb disease only, lymphedema was bilateral in 4 and unilateral in the other 4. Information was not available for 1 patient (LE47).

Among the patients with bilateral lower-limb lymphedema (7/11), the majority of them (6/7) had a limited swelling (below the knee) (cf figure n°9); the remaining patient had extended swelling (proximal extension - above the knee). In all of them, swelling of the limbs was asymmetrical. All of patients with unilateral lower-limb lymphedema (3/11) presented extended swelling (proximal extension – above the knee).

Lymphedema localized outside the limbs in 2 patients (1F/1M) who presented with concurrent involvement of the genito-urinary region: One female (LE153) had an edema of the vulva and perianal region and 1 male had perineal infiltration (LE 313). No patient had lymphedema localized in the neck, nor the face, nor had hydrops fetalis.



Figure n°9. Congenital bilateral lymphedema in a patient with HGF mutation. Photographs of the extremities of patient LE102, carrier of an amino acid change of HGF. The latter is suffering of a congenital bilateral lymphedema confined to the feet. The photographs are courtesy of Dr. G. M. Repetto

4.2.1.3 *Severity*

Severity of lymphedema is defined by the presence of different features such as pain, skin color change, bleeding of the limb affected, as well as presence of add-on problems: cellulitis, papillomatosis, upturned toenails, prominent veins, hydrocele etc. Only 2/11 patients reported their lymphedema as really painful, the others described a discomfort without real pain. Three patients (3/11) had a change of skin color in the affected limb(s), but none had bleeding in the affected area; of note, color skin change was difficult to attest in patients with papillomatosis.

Infections (Cellulitis & Erysipelas) had been reported in 4 males. Three were carrying a variant inducing a premature stop codon and one was a carrier of an amino acid substitution variant. It was reported as a recurring problem in 2 of them, leading to continuous antibiotherapy in one case. Papillomatosis cutis lymphostatica, a chronic and progressive skin manifestation of primary lymphedema, was reported in 5/11 cases among which 3 females and 2 males. Three of them (2F/1M) were carrying a variant inducing a premature stop codon; the other two were carrying an amino acid substitution variant. Commonly found in Milroy disease, upturned toenails, also called “ski-jump” toenails, were found in 2 patients (2/11) (1M/1F), predominantly on the big toe ³². Hydrocele was described in 1 patient. No patient had a history of prominent veins but one (1/11) had a history of venous insufficiency that had been operated on as well as a history of operated haemorrhoidal mariscas; in one patient (1/11), dilatations of pelvic veins were discovered on an abdominal-pelvic MRI study.

4.2.1.4 Associated phenotypic abnormalities

No specific phenotypic feature was found to be associated with *HGF* mutations. No patient had distichiasis. Neither hair abnormalities nor cutaneous abnormalities (ex: lipoma, hypo/hyperpigmentation etc.) were reported in any of our patients. No patient had any additional vascular anomalies a part of the lymphedema. None of them had any dysmorphism.

4.2.1.5 Treatment

All patients were subjected to “Complete/complex decongestive therapy” which, as a reminder, includes: skin care, exercises, manual lymph drainage and compression therapy. They were all using elastic stockings grade 3. One patient (LE153) had undergone lymphatic surgery consisting of a total superficial lymphangectomy.

4.2.1.6 Lymphoscintigraphy

Of the 11 patients with *HGF* mutations, 6 patients had undergone lymphatic system exploration. Lymphoscintigrams results were consistent with lymphedema in all the patients tested, with abnormally low or absence of uptake of radioactive colloid by peripheral lymphatic vessels in the foot and consequently no, or little, uptake in the ilio-inguinal nodes (cf figure n°10) . One of the 3 patients with 4-limbs lymphedema had undergone an upper limbs lymphoscintigraphy which showed a reduced flow and a limited uptake in the axillary nodes.

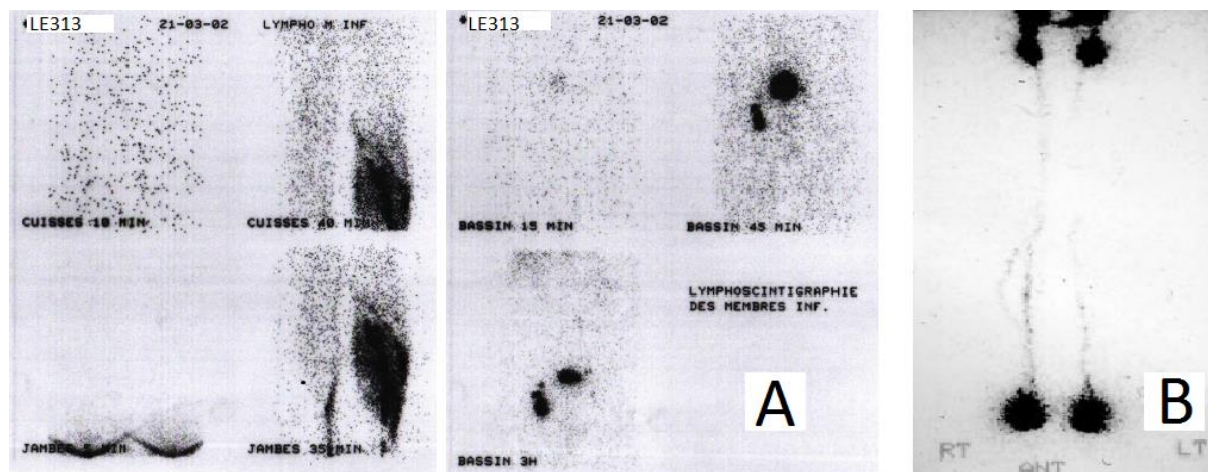


Figure n°10. Example of Lymphoscintigraphy results.

A: Lymphoscintigram of patient with HGF mutation (here patient LE313 with an amino acid change). The latter suffers of a unilateral left lower limb lymphedema of proximal extension. The lymphoscintigraphy's images show a clear reduction of the left lymphatic flow kinetics. **B:** Normal lymphoscintigram (copy of Brice. G et al figure n°2 ³²)

Table 1. Clinical features in patients with mutation in HGF gene

				Number	M/F	Premature stop/ AA substitution
Total patients with HGF mutation				11	7/4	5/6
Age of onset	Congenital			3/11	2/1	0/3
	Late (>1 yo)			8/11	5/3	5/3
** From here, informations missing for 1 patient LE47 --> Total patients/10						
Localisation	Upper Limbs			3/10	2/1	3/0
	Lower Limbs			10/10	6/4	5/5
		Extension	Distal*	6/10	3/3	4/2
	Proximal*		4/10	2/2	1/4	
	UL/BL **	BL	7/10	4/3	5/2	
		UL	3/10	2/1	0/3	
Associations	Pain			2/10	0/2	1/1
	Skin color change			3/10	2/1	2/1
	Hydrocoele			1/10	1/0	1/0
	Prominent veins			0/10		
	Upslanting toenails			2/11	1/1	1/1
	Cellulitis			4/10	4/0	3/1
	Papillomatosis			5/10	2/3	3/2
Familial history	YES			5/10	3/2	3/2
	NO			5/10	4/2	2/4

*Distal : under the knee limited swelling - Proximal : Above the knee swelling

**UL/BL : Unilateral or Bilateral lower limb lymphedema

4.2.2 Discussion - HGF group

The potential role of a *HGF* gene mutation as cause of primary lymphedema has only been evoked twice in the literature. *Finegold et al* (2008)³⁹ identified 4 *HGF* variants in a population of 234 index patients, all of whom were already screened for mutation in *FLT4*, *FOXC2* and *SOX18*, by screening *HGF* and *c-MET*. *Michelini S et al* (2016)⁴⁰ identified 4 additional changes in *HGF* by screening a cohort of 116 patients with primary lymphedema using a panel of various genes containing *HGF* and *c-MET*. In these 2 studies, *HGF* phenotype was briefly characterized. A detailed examination of the clinical features of primary lymphedema due to *HGF* mutation was lacking. In our study, we identified 12 pathogenic *HGF* variants and we focused on the associated phenotype in order to provide a more detailed clinical description.

HGF mutation is a rare cause of lymphedema. It causes an autosomal dominant disorder, with incomplete penetrance and probably variable expressivity. Its prevalence in a population of patients with primary lymphedema in which various well-known genes mutation already have been excluded is around 2%. As far as the gender is concerned, the usual female predominance affection does not seem to apply to *HGF* mutation associated lymphedema. Indeed, 7 out of the 11 in our *HGF* group were males which may hint to a male predominance; when including the relatives, the male predominance persists but is less significant with 9 males out of 17 individuals with lymphedema. However our cohort is too limited to make this result statistically significant. Larger studies are needed to support this observation.

The lymphedema is usually of late onset, swelling appearing mainly during childhood or early adulthood. Occasionally it presents from birth or in late adulthood showing that lymphedema of genetic origin can develop during adult life, as already shown by Brice, Manson, Bell et al³². The lymphedema predominantly affects the lower limbs; being mostly unilateral for patients carrying an amino-acid substitution, and bilateral for patients carrying a premature stop codon mutation. Some of the patients also present occasionally with four limbs lymphedema, which is rarely described in other types of primary lymphedema. Oddly, lymphedema is more extensive in unilateral compared to bilateral disease where a limited under-the-knee involvement seems more frequent. This observation remains to be explained; a unilateral under-knee lymphedema could be overlooked or it might be a coincidence. Limb swelling is in some case associated with a genito-pelvic lymphedema.

Curiously and unlike other syndromes associated with primary lymphedema, no specific anomaly, nor structural abnormalities, nor consistent dysmorphic features, are found to be associated with *HGF* mutation associated lymphedema phenotype. The mutations only seem to affect the process of

lymphangiogenesis. As a consequence, evoking *HGF* mutation as the causal gene of lymphedema by sole reference to clinical presentation seems impossible

HGF mutation associated lymphedema would appear relatively severe with a significantly high prevalence of cellulitis (4/10 patients) and papillomatosis (5/10 patients) found, as well as the presence of 4-limbs swelling cases. The majority of the severity factors are found in males which might be the sign that this condition affects males more severely than women, similar to what had been described in lymphedema-distichiasis syndrome by Brice et al.³²; however, this observation is of uncertain significance and may be linked to the predominance of males in our population.

There seem to be a correlation between the severity of the phenotype and the genotype of the patients, that is to say, the percentage of residual *HGF* activity: indeed, we observed a more pronounced and severe phenotype in patients with premature stop codon mutations which coincided with the results of the functional study of *HGF* variants. The latter showed a complete loss of activity of the mutant allele for the patients with a premature stop codon, responsible for haplo-insufficiency. Whereas the patients with amino-acid substitutions had only a partial loss of function, correlating with a less severe phenotype with almost no prevalence of cellulitis or papillomatosis and no 4-limb affection, predominantly 1 lower limb lymphedema. Those amino-acid substitutions can thus be called hypomorphic.

As far as the lymphoscintigram results are concerned, they point to a hypoplasia or aplasia of lymphatic vessels, similar to what has been described in *VEGFR3* linked lymphedema.

Regarding the genetic causes of primary lymphedema as described in point 2.3 following Connel et al¹² classification, *HGF* mutation should be evoked in front of a non-congenital lymphedema affecting the lower limbs only, without any associated anomaly; it should also be considered as a causal gene in a patient with non-congenital four-limb lymphedema, jointly to *GJC2* mutation. Finally, it can also be integrated in the differential diagnosis of congenital lymphedema – together with *VEGFR3*, *VEGFC*, *KIF11* and *GJA1*- mutation since the swelling is sometimes present from birth.

In conclusion, we have identified 12 *HGF* variants in 11 patients, associated to isolated primary lymphedema of congenital or late onset, affecting the lower limbs or the 4-limbs, without pathognomonic associated anomaly. The severity of the phenotype seems to correlate with residual *HGF* activity. Although this study was limited due to its retrospective design and small sample size, the thorough clinical characterization of our patients permits a more accurate delineation of the phenotypic spectrum than the previous publications^{39, 40}. This study places *HGF* in the differential diagnosis of the heterogeneous primary lymphedema group.

5 Non-*HGF* Group

In the second part of this study, we will report on several patients with mutated primary lymphedema followed at Cliniques Universitaires Saint-Luc, Brussels, Belgium, to illustrate some of the well-known genes responsible for primary lymphedema. Among those patients, we will present 1 case with *GATA2* mutation, 2 cases with *FOXC2* mutation, 2 cases with *VEGFR3* mutation, 1 case with *VEGF-C* mutation and finally 1 case with *TSC2* mutation (See Table 3)

5.1 Materials & Methods

Seven cases with mutations in genes known to cause varying forms of primary lymphedema, from GEHU's Lymphedema clinical database were included to the study. Clinical information were collected by consulting the medical records and using a specific lymphedema questionnaire (see annexe) developed by the host laboratory.

5.2 Case reports

Table 3: Genetic information and clinical features of non-HGF group.

Genes	Sample ID	Nucleotide variant	Protein alteration	Protein effect	Other gene	Gender	Inheritance	Age of onset	LE*	Additional clinical features
GATA2	Index 1	-	p.Arg396Trp	-	HGF	F	Sporadic	Late (14 yo)	2L(i)	Emberger syndrome
FOXC2	Index 2A	c.128C>T	p.Gln420*	STOP	-	M	Familial?	Late (17 yo)	2L(i)	
	Index 2B	?	?	?	-	M	Sporadic	Congenital	2L(i)	
VEGFR3	Index 3A	c.3179G>C	p.Arg1060Pro	AA change	-	M	Sporadic	Congenital	2L(i)	ski-jump toenails
	Index 3B	c.2740G>A	p.Gly914Arg	AA change	-	F	Familial	Congenital	1L(i)	
VEGFC	Index 4	c.552G>A	p.Ser1211Ilefs*3	STOP	-	M	Familial	Congenital	2L(i)	
TSC2	Index 5	c.5056C>T	p.Gln1686*	STOP	-	M	Sporadic	Congenital	1L(i)	Tuberous sclerosis syndrome

* LE = Lymphedema --> 1/2 L(i) = 1 or 2 inferior limb(s)

5.2.1 GATA2

5.2.1.1 Case report

We present a young woman (Index 1, ref. LE126) recently diagnosed with Emberger Syndrome at Cliniques universitaires Saint-Luc, Brussels. She was diagnosed with lymphedema at the age of 14, characterized by bilateral asymmetrical lower limb swelling and recurrent episodes of cellulitis and erysipelas. Also, she presented with recurrent respiratory infections of the upper and lower airways. At the age of 15, an immune deficiency syndrome was diagnosed (Immunoglobulin G, Immunoglobulin A and phagocyte function deficiency). The genetic analyses were done using the panel of 66 genes mentioned in Materials & Methods p.21 and two mutations were found, one on *GATA2* gene, causal of her syndrome, and one on *HGF* gene, with a probable minor effect or any, as the *HGF* variant was showed to have little or no impact on *HGF* activity (Fastré E.)⁴¹. She had no family history of lymphedema, or immune deficiency; and the segregation studies didn't find any of these 2 variants in the tested relatives, the parents, pleading a "de novo" mutation, even if a germinal mosaicism is not excluded. (cf figure n°11)

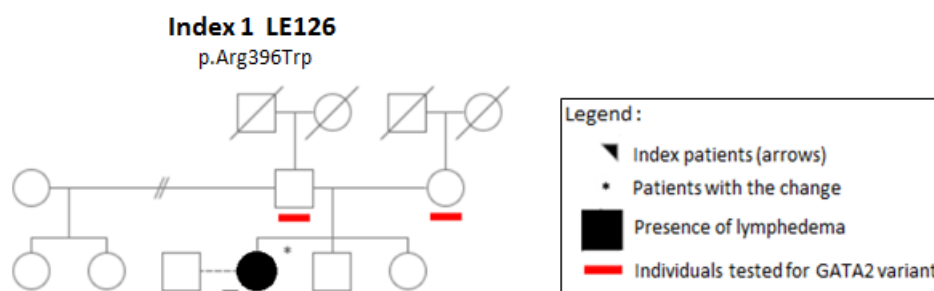


Figure n°11. Pedigree of family LE126 with mutations in GATA 2. Patient index 1 is also carrier of a rare HGF polymorphism (p.Val631Met) identified by the host laboratory.

5.2.1.2 Review of *GATA2* phenotype^{33, 34}

GATA2 is a transcription factor essential for embryonic and definitive hematopoiesis as well as lymphatic angiogenesis. *GATA2* deficiency is a germline disease which causes a wide spectrum of phenotypes including viral and bacterial infection, cytopenias, myelodysplasia, myeloid leukemia, pulmonary alveolar proteinosis and Emberger syndrome (OMIM 614038), without obvious genotype-to-phenotype correlation.^{33, 34}

Emberger syndrome is characterized by primary lymphedema associated with a predisposition to myelodysplasia. This syndrome segregates as a dominant autosomal trait with incomplete

penetrance. It has been reported as a sporadic disorder, which is the case for our patient whose *GATA2* variant appeared de novo. The lymphedema in this condition is usually confined to one or both of the lower limbs and can affect genitalia. As already described, our patient (Index1 LE126), has a bilateral lower limb swelling without genitalia involvement. Age of symptom onset ascribed to *GATA2* deficiency varies ³² and the most frequent initial symptoms are infections. Usually, lymphedema precedes the onset of hematological abnormalities³³, which is the case of our patient who is at an increased risk of developing myelodysplasia and acute myeloid leukemia and who will thus require close hematological follow-up in order to detect and manage these life threatening malignancies as early as possible.

5.2.2 FOXC2

5.2.2.1 Case report

We report 2 index patients with *FOXC2* mutations (Index 2A and 2B – Table 3). Both were males. Age of onset was 17 in one patient (2A) whereas it was congenital in the second one (2B). The lymphedema affected lower limbs in both patients; the first patient (2A) had an asymmetrical bilateral below the knee lymphedema without any other localization; the second patient (2B) had a unilateral congenital lower limb lymphedema that became bilateral at age 30, without any known trigger. Patient 2A had recurrent cellulitis for which he was treated daily by staphycid. As associated anomaly, both patients were known to have varicose veins in the lower limbs; patient 2B had undergone a bilateral saphenectomy for a suspicion of varicose veins. The second patient had a history of urethral valve syndrome. When exploring their familial history, patient 2B had no notion of any family member known with lymphedema whereas patient 2A had 2 possible affected relatives: his mother had important varicose veins that had been operated upon and a maternal grand uncle would have had asymmetrical bilateral under the knee lymphedema. No segregation analyses were performed in both patients' families. (cf figure n°12)

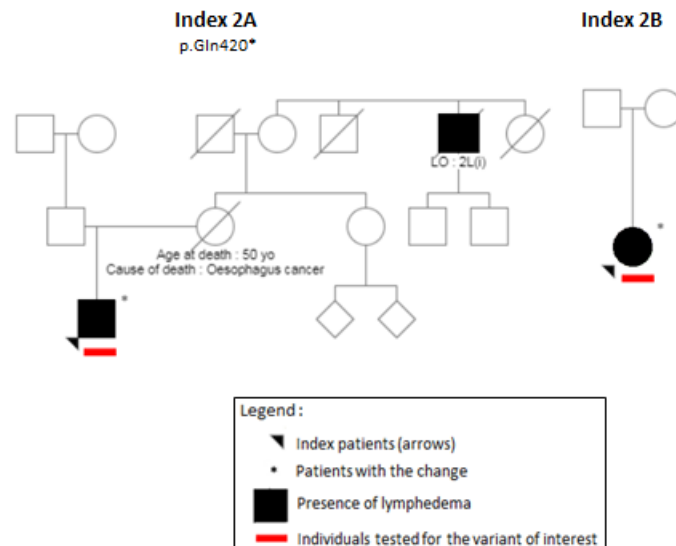


Figure n°12. Pedigree of families with changes in FOXC2

5.2.2.2 Review of FOXC2 phenotype ³²

FOXC2, Forkhead box C2, is a transcription factor involved in the maturation and maintenance of the collecting lymphatic vessels; the deficiency of this factor is associated with lymphedema-distichiasis syndrome (OMIM153400). The lymphedema-distichiasis (LD) is a rare primary lymphedema of pubertal onset associated with distichiasis. Its condition is an autosomal dominant disorder, with complete penetrance and variable expression within and between families affected. From the 2 index cases, one seemed sporadic which is infrequent, whereas the other had a familial form. As mentioned earlier, LD generally appears during or after puberty; occasionally it may appear in childhood. The congenital onset as observed for case 2B is an unexpected observation. In the majority of cases, lymphedema affects lower limbs bilaterally in an asymmetrical pattern, a characteristic found in our 2 patients. Rarely, is the swelling unilateral. As for the severity, Brice et al ³² showed that males seem to be more likely to develop cellulitis or infection in the edematous limb than women. The recurrent cellulitis in one of our patients supports this observation. It suggests that LD syndrome affects males more severely than women. Lymphedema distichiasis syndrome is associated with various anomalies. Although absent in our patients, the most highly penetrant associated feature is distichiasis. Distichiasis is a congenital anomaly characterized by an aberrant double row of extra-eyelashes and is often the first indicator of the affection. Another frequent anomaly is early onset varicosities unrelated to any predisposing factor; this trait was observed in our 2 patients. This association hints to a developmental role for *FOXC2* in both venous and lymphatic system. Other common associations are cleft palate, ptosis, congenital heart disease and extradural cysts, which were not found in our patients.

5.2.3 VEGFR3

5.2.3.1 Case report

We report on 2 patients with primary lymphedema and mutated *FLT4*, the gene coding for *VEGFR3* (1M/1F). Both presented with congenital lymphedema. The boy (3A) had bilateral swelling affecting the dorsum of the feet symmetrically; the girl (3B) had unilateral lower limb swelling confined to the right foot and ankle. None of them had any other disease localization, except for patient 3A who presented a facial edema and a hydrocele at birth that spontaneously resolved. They had no associated anomaly except for the boy who developed ski-jump toenails. As far as their family history is concerned, there was no history of lymphedema in index 3A but his mother had developed pre-eclampsia with lower limb edema during the last 3-4 months of her pregnancy for the index patient, which wasn't the case when was pregnant of his healthy brother. Also upslanting toenails were observed in the mother, in 2 maternal aunts and in the maternal grand-father without any suggestion of lymphedema. The parents have been tested negative for the mutation of case 3A. As for the girl (3B), the mutation was found in her mother as well as her elder sister who presented with a congenital lymphedema of the right foot which regressed spontaneously at 6 month of age. (cf figure n°13)

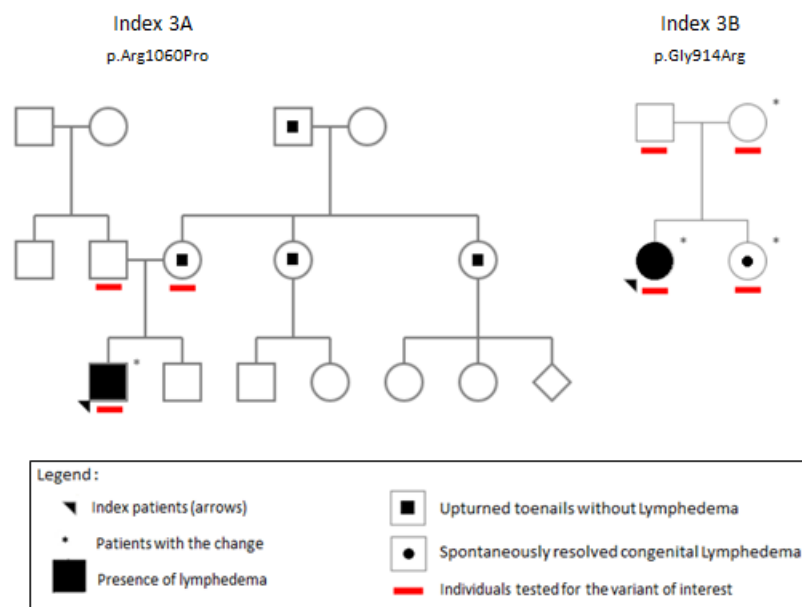


Figure n°13. Pedigree of families with changes in VEGFR3

5.2.3.2 Review of VEGFR3 Phenotype ^{42, 43, 44}

Vascular Endothelial Growth Factor Receptor 3 (*VEGFR3*, gene *FLT4*) is one of the most important genes responsible for lymphangiogenesis. Mutations in *VEGFR3* have been associated with a well-characterized form of isolated primary lymphedema, called Milroy disease (OMIM 153100). However, *VEGFR3* mutations explain 70% of the patients with the classic Milroy disease phenotype, suggesting genetic heterogeneity⁴⁶. Milroy disease usually shows an autosomal dominant pattern of inheritance with incomplete penetrance (85%). Its incidence is estimated to be 1:6000 births⁴³. Occasionally, it has a recessive inheritance pattern or develops de novo like in case 3A. According to the literature⁴⁴, de novo mutations of *VEGFR3* are not infrequent. Family history is thus not always mandatory to make the diagnosis of Milroy disease. The most consistent clinical pattern of Milroy disease is a bilateral below-the-knee swelling of congenital onset. Lymphedema may also sometimes occur in the first year of life. The lymphedema is typically confined to the feet and ankles, but may sometimes reach the knees. Clinical features in our index cases were perfectly matching with the usual presentation of Milroy disease, except for the unilateral swelling of case 3B. As for clinical severity, episodes of cellulitis seem to be more common in males, as is reported in lymphedema-distichiasis syndrome (LD) ³². Unlike other forms of primary lymphedema, only few anomalies are associated with lymphedema in Milroy disease; in descending order of frequency, the associated anomalies are hydroceles, large caliber leg veins, upslanting toenails and urethral malformations. Some cases may also present with prenatal pleural effusion or hydrops fetalis⁴⁴. In males, hydrocele is usually the next commonest manifestation after lower limb swelling, affecting 1/3 of them, as observed in index case 3A during childhood. The second most frequent associated anomaly, absent in our 2 cases, is prominent wide caliber veins of the legs, the presence of which is often a good clue to the underlying diagnosis as they are not seen with other causes of congenital lymphedema ⁴³. As far as the other associated anomalies are concerned, none were found in our 2 patients except for upslanting “ski-jump” toenails which were observed in case 3B with a positive familial history.

5.2.4 VEGFC

5.2.4.1 Case report

We present one multigenerational pedigree (cf figure n°14) in which Milroy-like lymphedema segregates in a pattern consistent with autosomal dominant inheritance with reduced penetrance (like the first two patients with *VEGF-C* mutation quoted by Gordon K et al (2013)⁴⁶ and Balboa-Beltran E et al (2014)⁴⁷. The index patient is a male with bilateral congenital lower limb lymphedema, located below the knees. The clinical presentation was suggestive of Milroy disease, but the *VEGFR3*

screening was normal. Consequently, their DNA was screened using the specific designed panel of 66 genes including *VEGF-C* (see above) within the framework of the GEHU project. A novel heterozygous *VEGF-C* mutation was identified (Fastré E et al, 2017)⁴⁸. The change was also found in six other family members with autosomal dominant inheritance of primary lymphedema, as well as in the index patient's young sister, despite the fact that she did not show any sign of lymphedema at the time of examination.

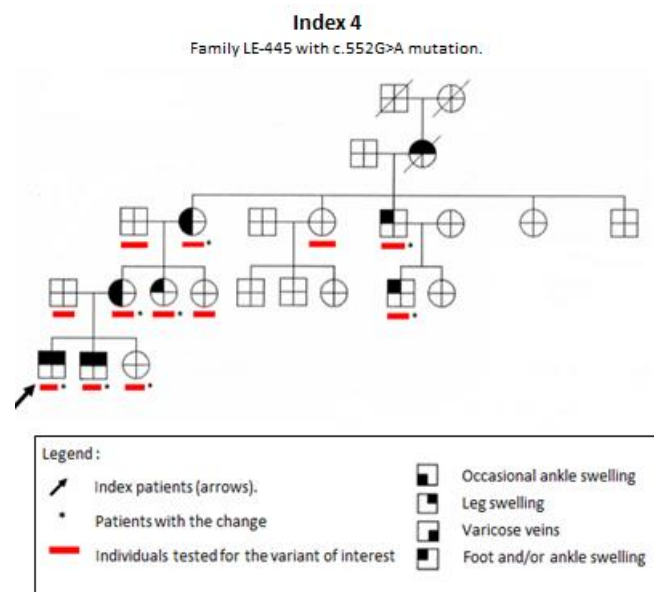


Figure n°14. Pedigree of a family with changes in VEGFC

5.2.4.2 Review of VEGFC Phenotype ^{45, 46, 47, 48}

As previously mentioned, Milroy disease phenotype is not entirely explained by *VEGFR3* mutations (*FLT4* gene), suggesting that other genes may be responsible for the same phenotype. Individuals with congenital lower limb lymphedema who screen negative for *FLT4* mutations are classified as having Milroy-Like disease. Vascular endothelial growth factor C (*VEGFC*), the main ligand of *VEGFR3*, has been associated with Milroy-like disease ⁴⁵. However, until recently, only two *VEGFC* variants had been reported in patients with Milroy-like disease ^{46, 47}. Clinically these patients are indistinguishable from those with a *FLT4* (*VEGFR3*) mutation. The two novel mutations in *VEGF-C* identified by Fastré E. et al⁴⁸ in GEHU laboratory corroborate the role of *VEGFC* mutations as cause of Milroy-like primary lymphedema, making it appropriate to consider *VEGFC* screen in patients with consistent phenotype. Moreover, the discovery of these 2 novel variants is pointing indirectly to genetic heterogeneity of Milroy-like disease, considering the rarity of *VEGFC* mutations associated with primary lymphedema.

This allows us to infer that unknown ligands and ligand-independent signaling via *VEGFR3* are still to be discovered as cause of Milroy-like disease⁴⁶.

5.2.5 *TSC2*

5.2.5.1 *Case report*

We report the case of a 14 years-old boy who presented with congenital right lower limb lymphedema associated with tuberous sclerosis syndrome. Diagnosis of tuberous sclerosis was made shortly after birth based on adequate clinical criteria (including the presence of cardiac rhabdomyoma). Genetic analysis was performed and confirmed a de novo *TSC2* mutation (cf figure 15). He developed lymphedema in childhood, characterized by a unilateral swelling of his right lower limb.

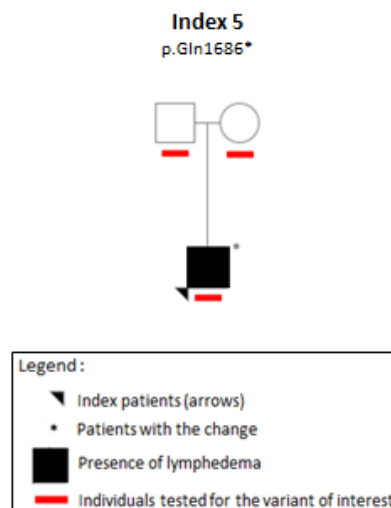


Figure 15. Pedigree of a patient with mutation in *TSC2*

5.2.5.2 *Background* ^{49, 50, 51}

Tuberous sclerosis (TSC) is a neurocutaneous disorder with multiorgan involvement characterized by numerous hamartomatous tumors including giant cell astrocytoma, renal angiomyolipoma, cardiac rhabdomyoma. The causal genes of this affection are *TSC1* and *TSC2*, genes encoding for 2 proteins, hamartin and tuberin, affecting the regulation of cellular proliferation and dedifferentiation. It is a variously expressed autosomal dominant condition, although 2/3 of the patients have spontaneous mutations. The incidence is approximately 1:6000. In some rare cases, tuberous sclerosis is associated to lymphedema with an estimated prevalence of 4% of patients with TSC, according to Geoffrey et al⁴⁹. Most of the cases reported with associated lymphedema are females with a potential

gender-specificity (female) of congenital forms suggested by Geffrey et al⁴⁹, although a sole congenital male case was recently reported in literature ⁵⁰. By the identification of a second male case with congenital lymphedema onset (index 5), we refute the hypothesis of female-specificity introduced by Geffrey et al⁴⁹. Lymphedema associated to tuberous sclerosis mainly appears during childhood or early adulthood, and is usually characterized by a unilateral lower limb swelling. The lymphedema features in the presented index case complies perfectly with this usual clinical description. Most cases of lymphedema associated with tuberous sclerosis have *TSC2* mutation, as our index case, consistent with *TSC2* predominance. However this percentage is similar to that of the percentage of patients with *TSC2* mutation in the general TSC population ⁴⁹.

6 Conclusion

The lymphatic system is an essential component of the circulatory system. Disruption of the lymphatic vasculature results in lymphedema, a life-long disabling condition associated with high morbidities contributing to an overall poor quality of life. Lymphedema occurs as a result of number of causes including genetic mutations (primary forms). Primary lymphedema is clinically and genetically heterogeneous. Different genes can produce a similar form of primary lymphedema and one gene is often responsible for different clinical presentations of lymphedema, making the clinical differential diagnosis very difficult. A number of causal genes have been identified in the pathogenesis of primary lymphedema; however there are still a substantial number of patients where the underlying causal gene has yet to be discovered.

In the first part of this study, we focused on the gene *HGF*, a gene suggested as potential new causal gene for primary lymphedema but only reported twice in the literature as associated to this condition. Thanks to the identification by the host laboratory of 12 *HGF* variant in 11 patients with unexplained primary lymphedema and a thorough clinical characterization of these, this study permits a more accurate delineation of the phenotypic spectrum associated to *HGF* mutations than the previous publications^{39, 40}. We observed that *HGF* mutations were associated to an isolated lymphedema of congenital or late onset, affecting the lower limbs or the 4-limbs, without pathognomonic associated anomaly and which severity varied depending on the level of *HGF* loss. In conclusion, this study places *HGF* in the clinical differential diagnosis of the heterogeneous group of primary lymphedema.

The second minor part of our study was devoted to the analysis of 7 cases of mutated primary lymphedema followed at Cliniques Universitaires Saint Luc, Brussels, illustrating some of the well-known genes responsible for primary lymphedema. The objective was to review the literature and to emphasize some key messages.

Gene identification helps delineate the phenotype more specifically and deepen the understanding of lymphedema etiopathogenesis and pathophysiology. It is hoped that advances in our knowledge of genetics of primary lymphedema will help to develop effective therapies, as to date no curative treatment is yet available.

7 Annexes

ANNEXE N°1. QUESTIONNAIRE LYMPHEDEMA GEHU (3pages)

LYMPHEDEMA QUESTIONNAIRE: GEHU-LAB

General Information

Our/GEHU ref.:

Name of the referring physician:

E-mail of the referring physician:

Name of the patient (or your ref.):

Sex: Male ☐ / Female ☐ Date of birth:

Ethnic origin (country):

Clinical working diagnosis:

Lymphedema

Age at Onset: In utero ☐ , Birth ☐ , After birth ☐ , at age:

Location of Lymphedema:

- Lower extremity Right ☐ , position: below the ankle ☐ , knee ☐ , inguinal ligament ☐
Left ☐ , position: below the ankle ☐ , knee ☐ , inguinal ligament ☐
- Upper extremity Right ☐ , position: below the wrist ☐ , elbow ☐ , shoulder ☐
Left ☐ , position: below the wrist ☐ , elbow ☐ , shoulder ☐
- Neck Yes ☐ /No ☐
- Face Yes ☐ /No ☐
- Ascites/chylothorax Yes ☐ /No ☐ , specify:
- Other parts of the body Yes ☐ /No ☐ , specify:
- Hydrops fetalis Yes ☐ /No ☐ , explain:

Severity of Lymphedema:

- Is the lymphedema painful? Yes ☐ /No ☐
- Is there any color changes on the skin? Yes ☐ /No ☐ , specify:
- Does the lymphedema bleed? Yes ☐ /No ☐
- Has any of the following clinical problems been superimposed to the lymphedema?
Cellulitis ☐ , Papillomatosis ☐ , Up-turned toenails ☐ , Prominent veins ☐
Hydrocele (if Male) ☐ , Other ☐ (please specify):

Varicosities

Age at Onset: Birth ☐ , After birth ☐ , at age:

- For females: did they appear only after pregnancy: Yes ☐ /No ☐
- Other inducing factors, such as trauma : Yes ☐ /No ☐ , specify :

Location of Varicosities:

- Lower extremity Right ☐ , position:
Left ☐ , position:
- Upper extremity Right ☐ , position:
Left ☐ , position:
- Other parts of the body (please specify):

LYMPHEDEMA QUESTIONNAIRE: GEHU-LAB

Associated Anomalies:

Presence of any associated anomaly? Yes ☐/No ☐:

Hair abnormalities ☐, (sparse hair, absent of eyebrows/eyelashes, age at onset), specify:

Cutaneous abnormalities ☐, lipoma ☐, hypo/hyperpigmentation ☐, specify:

Vascular anomalies: Telangiectasia ☐, Angiokeratoma ☐, Capillary malformation ☐, Venous malformation ☐, Lymphatic malformation ☐, Arteriovenous malformation ☐, (position, size, age at onset), specify:

Facial dysmorphism ☐: Epicanthal folds ☐, Low implanted ears ☐, other ☐, specify:
Cleft lip/palate ☐, Dental anomaly ☐, Nasal Atresia ☐, Deafness ☐, specify:

Micro/macrocephaly ☐, explain (head circumference at birth, – or +SD):

Lissencephaly ☐, other structural anomaly ☐, specify:

Developmental delay/learning difficulty ☐, specify:

Ptosis ☐, Distichiasis ☐, Photophobia ☐, Chorioretinal dysplasia ☐, explain (retinal folds, punched out lesions, visual deficit):

Cardiac anomaly ☐: ASD ☐, VSD ☐, tetralogy of Fallot ☐, specify:

Cysts ☐, specify: where: kidney ☐, brain ☐, heart ☐, lung ☐, specify:

Skeletal: polydactyly ☐, syndactyly ☐, other ☐, specify:

Diabetes ☐, type: Autoimmune disease ☐, specify:

Protein-losing enteropathy ☐, specify:

Membranoproliferative glomerulonephritis ☐, explain (hematuria, proteinuria, plasma urea and creatinine levels, creatinine clearance, serum complement profile including CH50, C3, C4, renal biopsy results):

Hematological abnormalities ☐: Neutropenia ☐, monocytopenia ☐, thrombopenia ☐, macrocytosis ☐, Hypogammaglobulinemia ☐. If possible, an available hemogram.

- Hb (g/dL):
- MCV (fL):
- WBC absolute counts (mm³/L):
- Other parts of the body (please specify):
 - Neutrophils (polynuclear):
 - Monocytes:
 - Lymphocytes:
 - Platelets (mm³/L):

LYMPHEDEMA QUESTIONNAIRE: GEHU-LAB

Syndrome:

Does the patient have signs of a syndrome? Yes ☐/No ☐:

Turner ☐, Noonan ☐, Tuberous sclerosis ☐, Hennekam ☐, MCLMR ☐, HTLS ☐,
Oculodentodigital ☐, OLEDAID ☐, HTLS ☐, Other ☐, specify:

Family History

Does the father have lymphedema or varicose veins?

Yes ☐/No ☐

If yes, please mention age at onset, location, phenotype and follow up:

Does the mother have lymphedema or varicose veins?

Yes ☐/No ☐

If yes, please mention age at onset, location, phenotype and follow up:

Does anybody else in the family have lymphedema or varicose veins?

Yes ☐/No ☐

If yes, please mention the relationship, age at onset, location, phenotype and follow up:

Does anybody in the family have been diagnosed as non-immune hydrops fetalis? Yes ☐/No ☐

If yes, please mention the relationship and follow up:

Is their consanguinity within the family Yes ☐/No ☐, specify:

Diagnosis, Treatment, Follow up

Have any complementary diagnostic exams been performed? Yes ☐/No ☐

If yes, specify:

Can we get a copy of the report (e.g. lymphoscintigraphy)? Yes ☐/No ☐, if yes, please attach.

Can we get clinical photographs? Yes ☐/No ☐, if yes, please attach.

Have any genetic studies been performed? Yes ☐/No ☐

Karyotype ☐, Molecular karyotype ☐, specify/attach report:

Gene ☐, VEGFR3 ☐, FOXC2 ☐, GATA2 ☐, PTPN11 ☐, SOS1 ☐, other ☐, specify:

Treatment :

Compression therapy: Yes ☐/No ☐

Surgery : Yes ☐/No ☐

Modified fat diet: Yes ☐/No ☐

Drainage: Yes ☐/No ☐

Antibiotics: Yes ☐/No ☐

Other, specify:

Follow up:

Progressed spontaneously ☐, explain:

Resorbed spontaneously ☐, explain:

No change with time ☐

Any other change/s with or without treatment:

Thank You Very Much for Your Time !

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9 Résumé

Le lymphœdème est une affection invalidante constituant l'anomalie lymphatique la plus fréquente. Il se caractérise par un gonflement chronique des tissus, touchant principalement les membres. Cette condition peut être primaire ou secondaire (acquise) : les formes primaires résultent d'un développement ou fonctionnement inadéquat du système vasculaire lymphatique. À l'heure actuelle, 28 gènes impliqués dans le lymphœdème primaire ont été identifiés. Cependant, ceux-ci n'expliquent que 40% des formes familiales et 12% des formes sporadiques, suggérant qu'il existe d'autres gènes responsables.

Le gène codant pour l'Hepatocyte growth factor (*HGF*) a été proposé comme nouvel acteur dans la genèse du lymphœdème primaire, notamment grâce à la découverte de son rôle important de stimulateur/promoteur dans la lymphangiogenèse^{6, 26, 38}. Cependant l'association entre mutation *HGF* et lymphœdème primaire n'a été rapportée que 2 fois dans la littérature et le caractère succinct des observations rapportées rendait souhaitable de clarifier la contribution d'*HGF* dans l'étiopathogenèse du lymphœdème. Dans ce but, notre laboratoire d'accueil a passé au crible l'ADN d'une cohorte de 542 patients avec lymphœdème primaire à l'aide d'un panel de 66 gènes, leur permettant d'identifier 12 variants *HGF* chez 11 patients.

Les caractéristiques cliniques n'ayant été que brièvement mentionnées dans les 2 études précitées, une définition précise du phénotype associé aux mutations *HGF* faisait défaut. La première partie de cette étude a donc été consacrée au projet de caractérisation du phénotype *HGF* par le biais d'une analyse détaillée des données cliniques des patients et l'utilisation d'un questionnaire spécifique.

Nous avons observé que les mutations *HGF* étaient associées à un lymphœdème sporadique isolé des membres inférieurs apparaissant le plus souvent dans l'enfance et dont la sévérité semblait varier en fonction du niveau résiduel d'activité de la protéine *HGF*.⁴¹.

Grâce à cette étude, nous avons délimité le spectre phénotypique associé à *HGF*, nous permettant de positionner ce gène dans le diagnostic différentiel de ce groupe hétérogène que constituent les lymphœdèmes primaires. Nous avons également pu mettre en évidence l'existence d'une corrélation phénotype-génotype jusqu'à présent absente de la littérature.

La deuxième partie de cette étude a été dévolue à l'analyse de 7 cas index muté avec lymphœdème primaire, suivis aux Cliniques Universitaires Saint Luc, à Bruxelles ; ceci a permis d'illustrer le rôle de certains gènes impliqués dans cette affection, l'objectif étant de réaliser une revue de la littérature tout en insistant sur certains points clés.

10 Summary

Lymphedema is a common disabling condition and is also the most frequent lymphatic anomaly. It is characterized by a chronic tissue swelling mostly in the limbs. Lymphedema can be primary or secondary (acquired): the primary forms result from inadequate development or function of the lymphatic system. Currently, germline mutations have been identified in 28 genes. However these only explain about 40% of familial forms and 12% of sporadic forms of primary lymphedema hinting at the responsibility of other genes.

The hepatocyte growth factor (*HGF*) was suggested as potential new causal gene for primary lymphedema. Indeed, it had been recognized as strong promotor of lymphangiogenesis by various studies. An association between *HGF* and primary lymphedema had been reported in the literature but only in 2 studies. Considering the paucity of information available, there was a need to clarify the contribution of *HGF* in the etiopathogenesis of lymphedema. Thus, the host laboratory screened a cohort of patients for mutation and identified 12 *HGF* variants in 11 patients.

An accurate description of the phenotype associated to *HGF* mutation was lacking, as clinical features were only summarily mentioned in the 2 studies reporting an association between primary lymphedema and *HGF*. A major part of our study was thus consecrated to the innovative project of characterizing *HGF*-phenotype. To accomplish this task, patient's clinical features were analyzed in detailed using a specific official lymphedema questionnaire as well as the medical records when available.

We observed that *HGF* mutations were associated to an isolated lower limbs lymphedema of late onset, the severity of which varied depending on the level of *HGF* loss.

This study permitted an accurate delineation of the phenotypic spectrum likely to manifest in patients with *HGF* mutation, positioning *HGF* in the clinical differential diagnosis of the heterogeneous group of primary lymphedema, as well as a demonstration of the existence of a correlation between the phenotype and the genotype.

The final part of our study was devoted to the analysis of 7 cases of mutated primary lymphedema followed at Cliniques Universitaires Saint Luc, Brussels, illustrating some of the well-known genes responsible for primary lymphedema. The objective was to review the literature and to emphasize some key messages.

« Je déclare sur l'honneur que ce mémoire a été rédigé sur base de données non falsifiées. Il a été écrit de ma plume, sans avoir sollicité d'aide extérieure illicite, qu'il n'est pas la reprise d'un travail présenté dans une autre institution pour évaluation, et qu'il n'a jamais été publié en tout ou en partie. Toutes les informations (idées, phrases, graphes, tableaux...) empruntées ou faisant référence à des sources primaires ou secondaires sont référencées adéquatement selon la méthode en vigueur. Je déclare avoir pris connaissance et adhérer au Code de déontologie pour les étudiants en matière d'emprunts, de citations ou d'exploitation de sources diverses et savoir que la falsification de données ainsi que le plagiat constituent des fautes graves ».

