Chapter 14
Clinical Disorders of Primary Malfunctioning of the Lymphatic System

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Abstract Primary lymphedema is defined as lymphedema caused by dysplasia of the lymph vessels. This complex group of diseases is discussed in detail from a clinical perspective. A review of the epidemiology and classification of lymphedema on the backdrop of its clinical presentation reveals weaknesses of the present classification system, which, however, is the basis for the choice of optimal patient care. Non-syndrome and syndrome types of primary lymphedema are presented in detail and related molecular findings are summarized.

14.1 Introduction

Lymphedema is a chronic, often progressive swelling of subcutaneous tissue due to failure of the lymphatic system to drain fluid from the interstitial spaces, causing fluid accumulation. Clinically a distinction is made between "primary" and "secondary" lymphedema (Rockson and Rivera 2008). Primary lymphedema is defined as lymphedema caused by dysplasia of the lymph vessels. It is usually congenital and genetically determined. It can be either isolated, so without manifestations in other tissues or outside the lymph vessels, or be part of a disorder that shows other signs and/or symptoms as well (syndrome). The distinction between isolated forms of lymphedema and those that are part of a more generalized entity is not strict as it also depends on the detail of the studies in affected individuals to search for other characteristics next to lymphedema. For instance the presence of an additional row of eyelashes (distichiasis) can be easily missed if not specifically searched for. Primary lymphedema usually affects the extremities as a result of abnormal

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regional lymph drainage, although visceral drainage showing in lymphangiectasias of for instance gut or lung can also be impaired. Secondary lymphedema is acquired, typically as consequence of an infection, trauma, or malignancy, and will not be discussed any further in this chapter.

Primary lymphedema in children can cause considerable diagnostic difficulties to clinicians and distress to parents. It is essential to obtain a rapid diagnosis and to implement correct treatment at the earliest opportunity. It is estimated that many physicians and surgeons will see less than ten cases of lymphedema in a year (Tiwari et al. 2006). It is therefore imperative that patients are referred at an early stage to a clinic with wide experience and expertise in diagnostics and treatment. Primary lymphedema can also show in lymphangiectasia of internal organs. When affecting the intestines it produces a protein-losing enteropathy and severe malabsorption of lipids and other nutrients (Braamskamp et al. 2010) Congenital pulmonary lymphangiectasia is a rare developmental disorder involving the lung and characterized by pulmonary subpleural, interlobar, perivascular, and peribronchial lymphatic dilatation, complicated by chylous pleural effusion (Bellini et al. 2006). Lymphangiectasias can also occur in other internal organs such as the pericardium, kidneys, and thyroid gland (Van Balkom et al. 2002). Lymphedema should be discerned from lipedema. This is a poorly understood condition characterized by swelling and enlargement of the lower limbs due to abnormal deposition of subcutaneous fat. Lymphatic system involvement seems likely but is actually debated (Child et al. 2010).

The increased knowledge regarding the etiology and pathogenesis of inherited disorders involving the lymphatic system has offered further insight in lymph vessel formation in general. Developments in lymphatic biology and various pathways and mechanisms through which the lymphatic system contributes to the pathogenesis of disorders have been reviewed elsewhere extensively (Tammela and Alitalo 2010; Alitalo 2011; Martinez-Corral and Makinen 2013) and are out of the scope of the present chapter.

14.2 Epidemiology

According to World Health Organization, lymphedema has a worldwide incidence of 300 million cases (~1 in every 20 individuals). Almost half of lymphedemas are of primary origin, due to congenital lymphatic dysplasia and subsequent poor functioning of lymph nodes and/or lymphatic vessels. Some 70 million are of parasitic origin (especially Filaria Bancrofti); 50 million are postsurgery cases, often following breast cancer surgery. The remaining 30 million cases are likely caused by functional problems related to water overload on lymphatic circulation (http://www.chirurgiadeilinfatici.it/en/lymphatic-diseases/lymphedema/epidemiology). The exact prevalence of primary lymphedema is unknown. Within the USA, it has been estimated to be 1.15 per 100,000 children (Smeltzer et al. 1985). A population prevalence of 1.33 per 1,000 for all ages has been reported, but it is probably an

underestimation of the true burden of disease (Moffatt et al. 2003). A female preponderance (M:F = 1:3) is documented, although in part this may represent ascertainment bias.

14.3 Classifications

Primary lymphedema is chronic edema, in which fluid accumulates due to abnormal structure or functions of the lymphatic system (Mortimer 1995). In most cases, edema will be present from birth, but in some cases the lymphedema develops at a later age despite the lymphatic dysplasia being present congenitally. Possibly an increased need of lymphatic functioning due to increased body size and weight or other factors such as hormones or external influences plays a role. Primary isolated lymphatic dysplasias constitute a spectrum of disorders that may manifest by a variety of clinical presentations: lymphedema, chylous effusions, lymphangiomatous malformations with cystic masses and localized gigantism, intestinal lymphangiectasia with malabsorption, lung lymphangiectasia, and lymphangiectasias of other internal organs and glands. Clinical classification of the various types of primary isolated lymphedema has historically been into three groups: lymphedema congenita, lymphedema praecox, and lymphedema tarda. Such classification based purely on the age of onset of the lymphedema does not take into account many other aspects of lymphedema and hinders a refined, detailed classification of phenotypes.

Anomalies of the lymphatic system should be considered as part of vascular anomalies. Mulliken and Glowacki (1982) proposed a classification system for vascular anomalies based on the clinical manifestations and endothelial cell characteristics into two main groups, i.e., hemangiomas and vascular malformations. This classification was adopted by the International Society for the Study of Vascular Anomalies (ISSVA) and with some subsequent modifications widely accepted. At present the two main types of vascular anomalies are vascular tumors (the most common type being hemangioma) and vascular malformations (Enjolras and Mulliken 1997) (Table 14.1). The term malformation that is used in this and other publications to indicate this is mostly wrong as the anomalies are in fact no malformations but dysplasias, although exceptions exist (Hennekam et al. 2013). However, to avoid confusion, we have chosen to use here the terminology used by the ISSVA and will discuss the right terminology with the ISSVA. Vascular malformations include slow-flow malformations which contain the lymphatic malformations (LM). The ISSVA uses a similar classification, splitting the congenital lymphatic dysplasias in truncular (T) or extratruncular (ET), depending on endothelial cells characteristics and the embryonic stage at which the defect was produced. The ET-LMs are embryonic remnants, which have occurred during early stages of vasculogenesis. The immature mesenchymal tissue (in fact dysplastic tissue), of which these malformations are formed, maintains a proliferative capacity. In the case stimuli occur such as pregnancy, hormonal stimulation, trauma, or

Table 14.1 Main characteristics of primary isolated lymphatic malfunctioning

Slow-flow malformations
Divided into truncular or extratruncular lesions
Extratruncular lesions are cystic and divided into microcystic, macrocystic, and mixed type
Extratruncular lesions maintain a proliferative capacity
Truncular lesions are linked to primary lymphedema, lymphangiectasia, and lymphangiomatosis
Truncular lesions have no proliferative capacity but behave as malformations
Extratruncular and truncular lesions may coexist within a single patient

surgery, ET-LMs can be stimulated and develop into (micro- and/or macro-) cystic lesions: lymphangioma. The T-LMs develop later during embryogenesis. The vascular tissue is mature and no longer has proliferative capacity. Primary lymphedema is linked with such malformations (Lee et al. 2005).

The Hamburg classification (7th international ISSVA workshop on vascular anomalies, Hamburg, 1988) distinguishes congenital vascular malformations (CVMs) in truncular (T) and extratruncular (ET) forms (Belov 1993; Lee et al. 2005). The Hamburg classification includes a further group of mixed venous malformations (VMs), identified as hemolymphatic malformation (HLM), making the classification of lymphatic malformations (LM) difficult and confusing. The majority of LM lesion exists as an "independent" form of the CVM, either as primary lymphedema representing "truncular" LM lesion or as cystic, cavernous, or capillary lymphangioma representing "extratruncular" LM lesion. Extratruncular LM lesion and truncular LM lesion co-occur together infrequently. In our opinion the Hamburg classification causes considerable problems. It is often extremely difficult to determine whether a finding is truncular or extratruncular as in fact this asks for detailed embryological studies in animal models and in fact also in humans, and the subdivision as is made now is build only for a small part on solid embryological grounds. The various terms used in the classification are often not sufficiently carefully chosen as malformations and dysplasias are insufficiently discerned from one another while this distinction has significant consequences in patient care. Lastly, the subdivision of the various forms of lymphatic malfunctioning is in our opinion not helpful in diagnostics or in providing optimal care to patients, and these issues should be the main determinants in any classification of disorders. A new classification of lymphatic malfunctioning is urgently needed.

Clinically, lymph reflux (backflow of lymph) may occur localized or at systemic level. The backflow of chyle from intestine is characteristic of intestinal lymphangiectasia. Intestinal lymphangiectasia results in protein-losing gastroenteropathy, but it can also affect the abdominal lymphatics or the thoracic duct; if the latter occurs, a chylothorax may develop. The primitive backflow at the level of pleural and pulmonary lymphatics results in pulmonary lymphangiectasia. Lymphangiectasia presents as dilated lymphatics and is usually associated with lymphedema. The pressure in the dilated lymphatics is increased, causing leakage of lymph into surrounding tissues. Lymphangiomatosis is characterized by

well-differentiated lymphatic capillaries which are dilated forming cysts and are not always associated with lymphedema. This collection of dilated lymphatics is typically isolated from the remainder of the lymphatic system, which can be completely normal. These are thought to arise from inappropriate connection of the embryonic lymph sacs with the lymphatic system during embryogenesis. The lymphangioma may be uni- or multilocular and macro- or microcystic and may occur in any part of the body. The most common site is the neck (cystic hygroma). Lymphangiomatosis is the widespread, multifocal occurrence of lymphangioma, which can grow aggressively. Lymphangiomatosis can be difficult to diagnose.

In general, LM may coexist with a wide spectrum of CVM and thus be part of complex disorders, affecting the entire circulation system: arteries, veins, lymphatics, and capillaries. This occurrence can be demonstrated in Klippel-Trenaunay syndrome, in which LM may coexist with venous and capillary malformation, or in Parkes Weber syndrome, in which LM presents in association with AV malformation. Indeed, in the entity that goes along with the most widespread lymphatic dysplasia, Hennekam syndrome, the co-occurrence of anomalies of other parts of the vascular system has been described (Van Balkom et al. 2002; Alders et al. 2013). Table 14.1 summarizes the main characteristics of the LMs.

14.4 Isolated Types

Primary congenital lymphedema (*Milroy syndrome*) is an autosomal dominant disorder of the peripheral lymphatics characterized by lower limb lymphedema, typically affecting the dorsum of the feet. It is usually bilateral and present at birth or evident soon thereafter. Milroy syndrome can also present as lymphedema of the upper limbs, or in markedly affected individuals, the lymphedema can start at the lower limbs and become present at the upper limbs later in life. In such individuals also the external genitalia may become affected, and the differentiation with more marked lymphedema as can be present in Hennekam syndrome may be difficult. Indeed molecularly proven cases with chylothorax and hydrops fetalis have been described (Daniel-Spiegel et al. 2005). Usually the lymphedema in Milroy syndrome becomes gradually more marked during life although rarely it can improve during life as well. The severity of lymphedema shows a marked variability, also intrafamilial, and careful evaluation of family members is regularly needed to establish whom in the family is affected or not. Milroy syndrome can be caused by mutations in *FLT4* and *GJC2* and may also be caused by *VEGFC* mutations.

Primary lymphedema at an older age (*Meige syndrome*) is an autosomal dominant disorder characterized by peripheral lymphedema predominantly in the lower limbs with onset around puberty. It is thought that the lymphatic system normally functions at ~10 % capacity (Connell et al. 2009). It is assumed to be caused by underdevelopment of the lymphatic vessels, which is however still sufficient in the first years of life but becomes functionally insufficient with increased body size and due to other such as puberty. Upper limb and facial involvement can also present.

The lymphedema, which occurs in Meige syndrome, is clinically indistinguishable from that found in the lymphedema-distichiasis syndrome. Indeed there have been publications reporting on *FOXC2* mutations in Meige syndrome, but likely this was a family with lymphedema-distichiasis syndrome. Until now no causative gene for Meige syndrome has been reported. It should be noted there is also an adult-onset segmental dystonia that is termed Meige syndrome (OMIM #128100).

Primary intestinal lymphangiectasia (Vignes and Bellanger 2008) is a disorder with unknown prevalence but which seems to occur only infrequently. It is characterized by hypoproteinemia, edema, and lymphocytopenia, resulting from loss of lymph fluid into the gastrointestinal tract due to intestinal lymphatic vessels dilatation, thus resulting in protein-losing gastro-enteropathy. The loss of lymph fluid can be confirmed by the elevated 24-h clearance of alpha-1-antitrypsin in stools. Bilateral lower limb edema and diarrhea are typical clinical signs that are secondary to the gastro-enteropathy. Lymphocytopenia, hypogammaglobulinemia, hypocalcemia, trace metal deficiency due to malabsorption, and chylous pleural effusions and chylous ascites may occur as secondary consequences in long-standing lymphangiectasia. Primary limb lymphedema may be present as well which can be difficult to distinguish from edema. If this occurs, more generalized lymphatic dysplasia such as occurs in Hennekam syndrome must be considered.

Primary pulmonary lymphangiectasia (Bellini et al. 2006) is an infrequently described developmental disorder involving the lung and characterized by pulmonary subpleural, interlobar, perivascular, and peribronchial lymphatic dilatation. The prevalence is unknown. Pulmonary lymphangiectasias typically present at birth with severe respiratory distress, tachypnea, and cyanosis, with a very high mortality rate at birth or within a few hours of birth. Most reported cases are sporadic and the etiology remains unexplained. Patients affected by PL who survive infancy present medical problems which are characteristic of chronic lung disease. Pulmonary lymphangiectasias also develop at a later age (often puberty of adolescence) in more generalized lymphatic dysplasias such as Hennekam syndrome. Also in such individuals the course is typically unpredictable but eventually fatal.

Chylothorax is defined as an accumulation of chyle in the pleural space. Chylothorax should be considered as a common endpoint for a variety of pathological processes including intrinsic abnormalities of the lymphatic system or disruption of the thoracic duct via trauma, surgery, malignancy, or cardiovascular disease. Congenital defects of the thoracic duct, either isolated or associated with generalized lymphatic vessel dysplasia, are the most frequent cause of congenital chylothorax. Congenital chylothorax is a rare cause of respiratory distress in the newborn but is the most common form of pleural effusion in the neonatal period. Reported incidence ranges from 1:1,000 to 1:15,000 pregnancies (Dubin et al. 2000). The actual incidence in man is probably higher, as intrauterine fetal death as well as stillbirth might well be underestimated. Although familial occurrence has been reported the exact pattern inheritance is not yet known. There is a 2:1 male to female predominance. Both X-linked and autosomal recessive inheritance have been suggested (Straats et al. 1980) and in our opinion it is likely genetically heterogeneous. It has been suggested that congenital pulmonary

lymphangiectasia is a constant pathological finding in congenital chylothorax and that this may imply a common pathogenesis for these disorders (Bellini et al., 2006; Bellini et al., in press).

The course of congenital chylothorax varies widely and the prognosis is unpredictable. Overall mortality for congenital chylothorax has been reported as high as 50 %. The presence of hydrops fetalis has significant prognostic implications (Dubin et al. 2000). In cases of chylothorax complicated by hydrops fetalis, a decrease in survival from 100 to 52 % has been reported. Still, nonimmune hydrops caused by chylothorax carries a better prognosis than nonimmune hydrops in general. The frequency of spontaneous resolution, which may occur either before or after birth, is still unknown. Lymphangiomatosis and lymphangiectasia are the two main anomalies of lymphatic development that cause chylothorax (Fox et al. 1998).

14.5 Syndrome Types

We performed a literature search to obtain an overview of syndromic forms of primary lymphatic malfunctioning. We used the online edition of Mendelian Inheritance in Man and the Winter-Baraitser Dysmorphology Database (WBDD), using as search terms lymphedema, lymphangiectasia, and chylothorax. OMIM is the comprehensive compendium of human genes and genetically determined phenotypes. OMIM contains information on all known Mendelian disorders and over 12,000 genes (http://www.ncbi.nlm.nih.gov/omim). The Winter–Baraitser Dysmorphology Database currently contains information on ~6,000 entities characterized by one or more morphologic abnormalities. It includes Mendelian disorders, chromosomal imbalances, sporadic conditions, and those caused by environmental agents (http://www.lmdatabases.com/).

The thus retrieved entries are summarized in Table 14.2, which contains the main characteristics of each entity such as chromosomal locus, gene involved, pattern of inheritance, and major clinical manifestations. It is impossible to discuss each entity listed in detail. Therefore we will only provide short descriptions of three entities that we consider paradigmatic of the various form of lymphatic maldevelopment.

Lymphedema-distichiasis syndrome is a single gene disorder caused by *FOXC2* (forkhead transcription factor) mutations (Sutkowska et al. 2012). Distichiasis (from Greek "distikhos," meaning two rows) is a congenital anomalous growth of eyelashes from the meibomian glands of the eyelid, causing the presence of a double row of eyelashes. It has been suggested that the lymphatic vessel malfunction may be linked to lymphatic valvular insufficiency which causes marked lymphatic reflux (Brice et al. 2002). The severity of lymphedema varies among families and among affected individuals of a single family and is linked to the grade of lymph reflux. "Yellow nail syndrome" has been published as a separate entity characterized by lymphedema and yellow, dystrophic, thick, and slowly growing

Table 14.2 Main characteristics of primary lymphatic malfunctioning as part of a syndrome

Syndrome	Pattern of inheritance	Prevalence	Location	Gene	OMIM number	Orphanet identifier	Clinical synopsis
Aagenaes (recurrent cholestasis, lymphedema)	AR AD	Rare	15q	?	214,900	ORPHA1414	Chronic lymphedema, recurrent neonatal cholestasis
Al-Gazali-Bakalinova (macrocephaly, multiple epiphyseal dysplasia)	AR	Rare	15q26.1	KIF7	607,131	ORPHA166024	Macrocephaly, multiple epiphyseal dysplasia, unusual face, lymphedema distal limbs
Amor (Adams–Oliver-like)	AR	Rare	19p13	DOCK6	614,219	ORPHA974	Intellectual disability, transverse limb anomalies, aplasia cutis scalp, cortical dysplasia, limb lymphedema
Avasthey (pulmonary hypertension, cranial arteriovenous malformations, lymphedema)	AD	Rare	?	?	152,900	OPHA86914	Cranial av malformations, pulmonary hypertension, childhood distal limb lymphedema
Bronspiegel (aplasia cutis congenita, intestinal lymphangiectasia)	AR	Rare	?	?	207,731	ORPHA1116	Aplasia cutis scalp, coloboma optic disk, heterotopia, intestinal lymphangiectasia
Cantu (hypertrichosis, osteodysplasia, cardiomyopathy)	AD	Unknown	12p12	ABCC9	239,850	ORPHA1517	Hypertrichosis, mildly coarse face, wide ribs, flat vertebrae, pericardial effusion, cardiomyopathy, limb lymphedema in adulthood
Cerebellar hypoplasia, lissencephaly, lymphedema	AR	Rare	7q22	RELN	257,320	ORPHA89844	Lissencephaly, seizures, hypotonia, ataxia, small cerebellum, limb lymphedema
Choanal atresia-lymphedema	AR	Rare	1q41	PTPN14	613,611	–	Choanal atresia, small nipples, childhood limb lymphedema

Chromosome 5p13 duplication	Chromosomal	Rare	5p13	?	–	–	Intellectual disability, obesity, macrocephaly, hypertelorism, loose skin neck, lymphedema
Chromosome 6q27 deletion	Chromosomal	Rare	6q27	?	–	–	Intellectual disability, autism, seizures, small callosal body, pulmonary lymphangiectasia
Chromosome 8q24 deletion	Chromosomal	Rare	8q24	?	–	–	Intellectual disability, sparse hair, broad nose, fractures, brachydactyly, limb lymphedema
Costello	AD	Unknown	11p15.5 12p12.1	HRAS KRAS	218,040	ORPHA3071	Intellectual disability, short stature, curly hair, coarse face, nasal papillomata, cardiomyopathy, pulmonary lymphangiectasia
Dahlberg (hypoparathyroidism, lymphedema)	AR/XL?	Rare	?	?	247,410	ORPHA1563	Hypoparathyroidism, ptosis, telecanthi, nephropathy, brachytelephalangy, congenital limb lymphedema,
Da Silva Lopes (frontonasal dysplasia, neuronal migration defect, lymphedema)	?	Rare	?	?	136,760	ORPHA250	Intellectual disability, cortical dysplasia, broad forehead, hypertelorism, congenital limb lymphedema
Distichiasis-lymphedema	AD	Unknown	16q24.1	FOXC2	153,400	ORPHA33001	Double row of eyelashes, ptosis, cleft palate, limb lymphedema in puberty
Ectodermal dysplasia, immune deficiency (OLEDAID)	XL	1:250,000 males	Xq28	IKBKG	300,301	ORPHA98813	Sparse hair, oligodontia, dry skin, immunodeficiency, osteopetrosis, congenital lymphedema
Emberger (myelodysplasia, lymphedema)	AD	Rare	3q21.3	GATA2	614,038	ORPHA3226	Myelodysplasia, leukemia, deafness, lymphedema of lower limbs/genitalia in infancy-puberty

(continued)

Table 14.2 (continued)

Syndrome	Pattern of inheritance	Prevalence	Location	Gene	OMIM number	Orphanet identifier	Clinical synopsis
German (hypotonia, arthrogryposis, unusual face, lymphedema)	AR	Rare	?	?	231,080	ORPHA2077	Hypotonia, arthrogryposis, metopic ridge, cleft palate, hand lymphedema
Gilewski (unusual face, eventration diaphragm, pulmonary lymphangiectasia)	?	Rare	?	?	–	–	Broad nasal bridge, small nose, small mouth, eventration diaphragm, small penis, pulmonary lymphangiectasia
Hennekam (intestinal lymphangiectasia, lymphedema, intellectual disability)	AR	Unknown	18q21.32	CCBE1	235,510	ORPHA2136	Intellectual disability, flat face, hypertelorism, flat nasal bridge, glaucoma, intestinal lymphangiectasia, congenital severe lymphedema of limbs, genitalia, and face
Hennekam (epidermal nevus, arteriovenous malformations, intestinal lymphangiectasia)	?	Rare	?	?	–	–	Epidermal nevus, lipoma, intramedullary hemangioma, intestinal lymphangiectasia
Hypotrichosis, lymphedema, telangiectasia	AR	Rare	20q13.33	SOX18	607,823	ORPHA69735	Progressive hypotrichosis, telangiectasia, childhood-pubertal lower limb lymphedema
Irons-Bianchi (congenital heart anomaly, unusual face, lymphedema)	AR	Rare	?	?	601,927	ORPHA86915	Congenital heart defects, prominent forehead, flat nasal bridge, congenital limb lymphedema
Jaeken (CDGIa)	AR	1/20,000	16p13.3	PMM2	212,065	ORPHA79318	Intellectual disability, small cerebellum, retinitis pigmentosa, abnormal fat pads, stroke-like episodes cardiomyopathy, congenital limb lymphedema

Klippel-Trenaunay	AD	1/25,000	?	?	149,000	ORPHA90308	Cutaneous hemangioma, arteriovenous malformations, lymphangioma, asymmetry, childhood-adulthood lymphedema
Lipedema	AD	Unknown	?	?	614,103	ORPHA77243	Fat legs, orthostatic edema, lower limb lymphedema
Lymphedema, agenesis corpus callosum	AR	Rare	?	?	613,623	–	Intellectual disability, callosal body agenesis, congenital resolving limb lymphedema
Mandibulofacial dysostosis, polydactyly, lymphedema	?	Rare	?	?	–	–	Underdeveloped malae, small nose, small ears, deafness, postaxial polydactyly hands and feet, congenital limb lymphedema
Meige	AD	Unknown	?	?	153,200	ORPHA90186	Pubertal lymphedema in lower limbs
Microcephaly-capillary malformation	AR	Unknown	2p13	STAMBP	614,261	ORPHA294016	Intellectual disability, microcephaly, cortical dysplasia, small distal phalanges, multiple capillary malformations, limb lymphedema
Microcephaly, cutis verticis gyrata, lymphedema	AR	Rare	?	?	–	–	Intellectual disability, cutis gyrate scalp, microcephaly, enlarged liver and spleen, congenital lymphedema
Microcephaly, chorioretinal dysplasia, lymphedema	AD	Rare	10q23.33	KIF11	152,950	ORPHA2526	Intellectual disability, microcephaly, chorioretinal pigmentation, congenital distal limb lymphedema
Milroy	AD	Unknown	5q35.3 1q42.13 4q34.3 6q16.2	FLT4 GJC2 VEGFC	153,100 613,480 601528	ORPHA79452	Congenital limb/genitalia lymphedema

(continued)

Table 14.2 (continued)

Syndrome	Pattern of inheritance	Prevalence	Location	Gene	OMIM number	Orphanet identifier	Clinical synopsis
Njolstad (pulmonary lymphangiectasia, lymphedema)	AR	Rare	?	?	265,300	ORPHA2414	Flat face, hypertelorism, flat nasal bridge, glaucoma, congenital pulmonary lymphangiectasia, congenital lymphedema
Noonan	AD	1/2,500	12q24.13 12p12.1 2p22.1 3p25.2 1p13.2 7q34 1q22	PTPN11 KRAS SOS1 RAF1 NRAS BRAF RIT1	163,950 190,070 610,733 611,552 613,224 613,706 615,355	ORPHA648 ORPHA98733	Short stature, unusual face, webbed neck, pulmonic stenosis, pectus carinatum, intestinal lymphangiectasia, limb lymphedema
Opitz Frias	XL Chromosomal	Rare	Xp22.2 22q11.2	MID1 deletion	145,410	ORPHA2745	Intellectual disability, hypertelorism, cleft palate, laryngeal cleft, hypospadias, pulmonary lymphangiectasia
PTEN hamartoma tumor	AD	1/200,000	10q23.31	PTEN	153,480	ORPHA109	Macrocephaly, hemangioma, lipoma, tumors, pulmonary lymphangiectasia
Pulmonary stenosis, lymphedema	XL	Rare	Xp11	?	–	–	Peripheral pulmonary stenosis, distal limb lymphedema
Prolidase deficiency	AR	Rare	19q13.11	PEPD	170,100	ORPHA742	Intellectual disability, short stature, photosensitivity, recurrent limb ulcers, childhood lymphedema
Schindler disease (alpha-*N*-acetylgalactosaminidase deficiency)	AR	Rare	22q13.2	NAGA	104,170	–	Intellectual disability, cortical blindness, deafness, seizures, limb lymphedema

Silver (acro-osteolysis, intestinal lymphangiectasia)	?	Rare	?	?	–	–	Clubbing, acro-osteolysis, bowed radius and ulna, intestinal lymphangiectasia
Stoll (brachydactyly, tachycardia, lymphedema)	AD	Rare	?	?	–	–	Brachydactyly, syndactyly paroxysmal tachycardia, pubertal limb lymphedema
Tuberous sclerosis	AD	1/10,000	9q34.13 16p13.3	TSC1 TSC2	191,100 613,254	ORPHA805	Depigmented skin lesions, angiofibroma, hamartoma in heart/kidneys/lung/brain, congenital lymphedema
Turner	Chromosomal	1/1,000	X	?	–	ORPHA881	Short stature, ovarian failure, variable visceral manifestations, congenital limb and sometimes facial lymphedema
Urioste	AR	Rare	?	?	235,255	ORPHA1655	Oligodactyly, polydactyly, vertebral anomalies, aplasia of internal organs, congenital intestinal lymphangiectasia
Yellow nail	AD	Unknown	16q24.1	FOXC2	153,300	ORPHA662	Yellow nails, chylothorax, congenital adulthood limb lymphedema
Zellweger	AR	1/25,000–50,000	7q21.2	PEX1	214,100	ORPHA912	Intellectual disability, hypotonia, seizures, unusual face, enlarged liver, intestinal lymphangiectasia

AD autosomal dominant, *AR* autosomal recessive, *XL* X-linked recessive, *?* not known

nails. However, it has become likely that the thickening and yellow discoloration of the nails is not a distinctive sign and can occur in several marked forms of distal limb lymphedema, and most patients with "yellow nail syndrome" may in fact have had lymphedema-distichiasis syndrome (Rezaie et al. 2008).

Hennekam syndrome is a form of very marked lymphatic dysplasia, in which lymph vessels in all body areas are affected (Van Balkom et al. 2002; Alders et al. 2013) and which is caused by mutation in *CCBE1* (Alders et al. 2009). These are also already affected prenatally, and the facial manifestations are thought to be explainable this way. According to the original description (Hennekam et al. 1989) the main characteristics are lymphedema, intestinal lymphangiectasia, intellectual disability (which can be markedly different, also within a single sibship, varying from moderate to severe intellectual disability to completely normal development), and facial signs. The lymphedema has always been congenital, sometimes markedly asymmetric, and after initial decrease in the first years of life has become often gradually progressive with age. Lymphangiectasias are not limited to the intestines but can also be found in the lungs, pleura, pericardium, thyroid gland, and kidneys.

Noonan syndrome is a well-known and frequent entity that is mainly characterized by short stature, unusual face, webbing of the neck, a combined pectus carinatum and excavatum, pulmonic stenosis and later on an increased chance to develop a cardiomyopathy, and a host of further major and minor anomalies. Congenital lymphedema of the distal upper and lower limbs is often but not always present. Some infants and children develop more marked lymphatic malfunctioning including intestinal lymphangiectasias and chylothorax. Within families the variability of the lymphatic system involvement can vary very widely. Indeed even newborns with fatal hydrops have been born to an affected parent with only limited manifestations of the syndrome. Noonan syndrome can be caused by a series of genes that all act in the same MAPK pathway and is one of the entities that form the rasopathies (Rauen 2013).

14.6 Molecular Findings

In isolated and syndromic primary lymphedema various patterns of inheritance can be recognized. The etiology and pathogenesis of the group of disorders is only partially understood (Table 14.2), but research in this field has improved significantly in recent years due to advances in sequencing techniques. Mutations in *VEGFR3*, *FOXC2*, and *SOX18* are known to cause Milroy disease, lymphedema-distichiasis syndrome, and hypotrichosis-telangiectasia-lymphedema syndrome, respectively (Ferrell et al. 1998; Fang et al. 2000; Irrthum et al. 2003; Brice et al. 2005) and *VEGFC* mutations have been found in a Milroy-like disorder (Gordon et al. 2013). *CCBE1* has been reported to be mutated in a proportion of patients with Hennekam syndrome and the analysis of this gene should be studied in every patient with a Hennekam syndrome phenotype or otherwise marked lymphatic

malfunctioning (Alders et al. 2009, 2013; Connell et al. 2010), irrespective of the cognitive functioning of affected individuals or the presence or absence of other abnormalities. Chromosome imbalances often result in multiple organ defects and the lymphatic system can be part of this as well, although this is not common, except for Turner syndrome. The number of genes known to cause isolated primary lymphatic malfunctioning is still relatively small compared to the number of genes known to cause similar vascular malfunctioning, and we may expect several other genes to be recognized as being involved in lymphangiogenesis or lymphatic functioning. Indeed sequencing of a series of biologically plausible candidate genes such as *PROX1*, *EMILLIN1*, *LCP2*, *LYVE1*, *NRP2*, *PDPN*, and *SYK* has been suggested as these may be involved in primary lymphedema families.

14.7 Diagnostic Work-Up

The approach to establish the diagnosis in the often complex and sometimes confusing lymphatic disorders can cause difficulties. We suggest a general scheme to provide help in the diagnostic process that can be generally applied in disorders that go along with lymphatic malfunctioning. In all patients a detailed family history of at least two generations and including information to the existence of a possible consanguinity between the parents should be obtained. The examination of a fetus with a suspected lymphatic dysplasia may comprise amniotic fluid examination and chorionic villi study, in particular searching for inherited metabolic disease, lysosomal storage diseases (LSD) included. The examination of a deceased fetus may include autopsy, including babygram, photo-documentation, immunohistochemical studies, and also a skin biopsy (both for DNA collection and to have access to cultivated cells), samples of other tissues, examination of the placenta, and obtaining and evaluation of fetal urine for metabolic disorders.

Lymphedema in an infant or child is usually diagnosed clinically, but in case of doubt lymphoscintigraphy is the main instrument to establish the diagnosis of lymphedema and to visualize peripheral lymphatic vessels. Lymphoscintigraphy relies on one of the essential functions of the lymphatic system, i.e., to transport lymph. Thus, it can demonstrate each defect in lymphatic functions, as delayed lymphatic drainage, asymmetric or absent visualization of regional lymph nodes, dermal backflow, and interrupted lymphatic structures (Keeley 2006). It can study both superficial and deep lymphatic circulation. Lymphoscintigraphy has been demonstrated to be safe and effective in newborns and in children (Bellini et al. 2008). The diagnostic approach in an individual with visceral lymphangiectasia is usually complex. Lymphoscintigraphy may be helpful and is often combined with computerized tomography scanning and interstitial magnetic resonance imaging. These combined methods of studying lymphatic anomalies are nor widely available however. Additional laboratory exams are usually indicated if lymphangiectasias are present. Determining fecal excretion of alpha-1-antitrypsin helps in diagnosing a protein-losing syndrome and points to intestinal lymphangiectasias. If visceral

effusions are present, such as chylothorax, chylopericardium, and chylous ascites, the abnormal fluids should be obtained and examined to evaluate the triglyceride level (pointing to lymph if >110 mg/dL [= 1.1 mmol/L]), presence of chylomicrons (Sudan III staining), determining the absolute cell count (pointing to lymph if >1,000 cs/mcL, with a lymphocyte fraction of >75–90 %), and the cholesterol level (abnormal if 60 mg/dL or above) (Bellini et al. 2012).

Recognizing congenital lymphatic disorders and diagnosing reliably lymphatic malfunctioning are still challenging. It still starts with clinical recognition, but likely other tools that are patient-friendly such as molecular genetics and other biomarkers can be developed in the near future.

References

Alders, M., Hogan, B. M., Gjini, E., Salehi, F., Al-Gazali, L., Hennekam, E. A., et al. (2009). Mutations in CCBE1 cause generalized lymph vessel dysplasia in humans. *Nature Genetics, 41*, 1272–1274.

Alders, M., Mendola, A., Adès, L., Al Gazali, L., Bellini, C., Dallapiccola, B., et al. (2013). Evaluation of clinical manifestations in patients with severe lymphedema with and without CCBE1 mutations. *Molecular Syndromology, 4*(3), 107–113.

Alitalo, K. (2011). The lymphatic vasculature in disease. *Nature Medicine, 17*, 1371–1380.

Bellini, C., Boccardo, F., Bonioli, E., & Campisi, C. (2006). Lymphodynamics in the fetus and newborn. *Lymphology, 39*, 110–117.

Bellini, C., Boccardo, F., Campisi, C., Villa, G., Taddei, G., Traggiai, C., et al. (2008). Lymphatic dysplasias in newborns and children: The role of lymphoscintigraphy. *J Pediatric, 152*, 587–589.

Bellini, C., Ergaz, Z., Boccardo, F., Bellini, T., Bonioli, E., & Ramenghi, L. A. (2013). Dynamics of pleural fluid effusion and chylothorax in the fetus and newborn: Role of the lymphatic system. *Lymphology*. in press.

Bellini, C., Ergaz, Z., Radicioni, M., Forner-Cordero, I., Witte, M., Perotti, G., et al. (2012). Congenital fetal and neonatal visceral chylous effusions: Neonatal chylothorax and chylous ascites revisited. A multicenter retrospective study. *Lymphology, 45*, 91–102.

Belov, S. T. (1993). Anatomopathological classification of congenital vascular defects. *Seminars in Vascular Surgery, 6*, 219–224.

Braamskamp, M. J., Dolman, K. M., & Tabbers, M. M. (2010). Clinical practice. Protein-losing enteropathy in children. *European Journal of Pediatrics, 169*, 1179–1185.

Brice, G., Child, A. H., Evans, A., Bell, R., Mansour, S., Burnand, K., et al. (2005). Milroy disease and the VEGFR-3 mutation phenotype. *Journal of Medical Genetics, 42*, 98–102.

Brice, G., Mansour, S., Bell, R., Collin, J. R., Child, A. H., Brady, A. F., et al. (2002). Analysis of the phenotypic abnormalities in lymphoedema-distichiasis syndrome in 74 patients with FOXC2 mutations or linkage to 16q24. *Journal of Medical Genetics, 39*, 478–483.

Child, A. H., Gordon, K. D., Sharpe, P., Brice, G., Ostergaard, P., Jeffery, S., et al. (2010). Lipedema: An inherited condition. *American Journal of Medical Genetics Part A, 152A*, 970–976.

Connell, F., Brice, G., Mansour, S., & Mortimer, P. (2009). Presentation of childhood lymphoedema. *Journal of Lymphoedema, 4*, 65–72.

Connell, F., Kalidas, K., Ostergaard, P., Brice, G., Homfray, T., Roberts, L., et al. (2010). Linkage and sequence analysis indicate that CCBE1 is mutated in recessively inherited generalised lymphatic dysplasia. *Human Genetics, 127*, 231–241.

Daniel-Spiegel, E., Ghalamkarpour, A., Spiegel, R., Weiner, E., Vikkula, M., Shalev, E., et al. (2005). Hydrops fetalis: An unusual prenatal presentation of hereditary congenital lymphedema. *Prenatal Diagnosis, 25*, 1015–1018.

Dubin, P. J., King, I. N., & Gallagher, P. G. (2000). Congenital chylothorax. *Current Opinion in Pediatrics, 12*, 505–509.

Enjolras, O., & Mulliken, J. B. (1997). Vascular tumors and vascular malformations (new issues). *Advances in Dermatology, 13*, 375–423.

Fang, J., Dagenais, S. L., Erickson, R. P., Arlt, M. F., Glynn, M. W., Gorski, J. L., et al. (2000). Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. *American Journal of Human Genetics, 67*, 1382–1388.

Ferrell, R. E., Levinson, K. L., Esman, J. H., Kimak, M. A., Lawrence, E. C., Barmada, M. M., et al. (1998). Hereditary lymphoedema: Evidence for linkage and genetic heterogeneity. *Human Molecular Genetics, 7*, 2073–2078.

Fox, G. F., Challis, D., O'Brien, K. K., Kelly, E. N., & Ryan, G. (1998). Congenital chylothorax in siblings. *Acta Paediatrica, 87*, 1010–1012.

Gordon, K., Schulte, D., Brice, G., Simpson, M. A., Roukens, M. G., van Impel, A., et al. (2013). Mutation in vascular endothelial growth factor-C, a ligand for vascular endothelial growth factor receptor-3, is associated with autosomal dominant milroy-like primary lymphedema. *Circulation Research, 112*(6), 956–960.

Hennekam, R. C., Biesecker, L. G., Allanson, J. E., Hall, J. G., Opitz, J. M., Temple, I. K., et al. (2013). Elements of morphology: General terms for congenital anomalies. *American Journal of Medical Genetics.* [epub ahead of print].

Hennekam, R. C. M., Geerdink, R. A., Hamel, B. C., Hennekam, F. A., Kraus, P., Rammeloo, J. A., et al. (1989). Autosomal recessive intestinal lymphangiectasia and lymphoedema, with facial anomalies and mental retardation. *American Journal of Medical Genetics, 34*, 593–600.

Irrthum, A., Devriendt, K., Chitayat, D., Matthijs, G., Glade, C., Steijlen, P. M., et al. (2003). Mutations in the transcription factor gene SOX18 underlie recessive and dominant forms of hypotrichosis-lymphedema-telangiectasia. *American Journal of Human Genetics, 72*, 1470–1478.

Keeley, V. (2006). The use of lymphoscintigraphy in the management of chronic oedema. *Journal of Lymphoedema, 1*, 42–57.

Lee, B. B., Kim, Y. W., Seo, J. M., Hwang, J. H., Do, Y. S., Kim, D. I., et al. (2005). Current concepts in lymphatic malformation. *Vascular and Endovascular Surgery, 39*, 67–81.

Martinez-Corral, I., & Makinen, T. (2013). Regulation of lymphatic vascular morphogenesis: Implications for pathological (tumor) lymphangiogenesis. *Experimental Cell Research, S0014–4827*(13), 00034–00037. doi:10.1016/j.yexcr.2013.01.016 [Epub ahead of print].

Moffatt, C. J., Franks, P. J., Doherty, D. C., Williams, A. F., Badger, C., Jeffs, E., et al. (2003). Lymphoedema: An underestimated health problem. *Quarterly Journal of Medicine, 96*, 731–738.

Mortimer, P. S. (1995). Managing lymphoedema. *Clinical and Experimental Dermatology, 20*, 98–106.

Mulliken, J. B., & Glowacki, X. X. (1982). Classification of pediatric vascular lesions. *Journal of Plastic and Reconstructive Surgery, 69*, 412–422.

Rauen, K. A. (2013). The RASopathies. *Annual Review Genomics Human Genetics, 14*, 355–369.

Rezaie, T., Ghoroghchian, R., Bell, R., Brice, G., Hasan, A., Burnand, K., et al. (2008). Primary non-syndromic lymphoedema (Meige disease) is not caused by mutations in FOXC2. *European Journal of Human Genetics, 16*, 300–304.

Rockson, S. G., & Rivera, K. K. (2008). Estimating the population burden of lymphoedema. *Annals of the New York Academy of Sciences, 1131*, 147–154.

Smeltzer, D. M., Stickler, G. B., & Schirger, A. (1985). Primary lymphedema in children and adolescents: A follow-up study and review. *Pediatrics, 76*, 206–218.

Straats, B. A., Ellefson, R. D., Budahn, L. L., Dines, D. E., Prakash, U. B., & Offord, K. (1980). The lipoprotein profile of chylous and nonchylous pleural effusions. *Mayo Clinic Proceedings, 11*, 700–704.

Sutkowska, E., Gil, J., Stembalska, A., Hill-Bator, A., & Szuba, A. (2012). Novel mutation in the FOXC2 gene in three generations of a family with lymphoedema-distichiasis syndrome. *Gene, 498*, 96–99.

Tammela, T., & Alitalo, K. (2010). Lymphangiogenesis: Molecular mechanisms and future promise. *Cell, 140*, 460–476.

Tiwari, A., Myint, F., & Hamilton, G. (2006). Management of lower limb lymphoedema in the United Kingdom. *European Journal of Vascular and Endovascular Surgery, 31*, 311–315.

Van Balkom, I. D. C., Alders, M., Allanson, J., Bellini, C., Frank, U., De Jong, G., et al. (2002). Lymphoedema-lymphangiectasia-mental retardation (Hennekam) syndrome: A review. *American Journal of Medical Genetics, 112*, 412–421.

Vignes, S., & Bellanger, J. (2008). Primary intestinal lymphangiectasia (Waldmann's disease). *Orphanet Journal of Rare Diseases, 3*, 5.