## 5.10 Genetic causes of primary lymphedema

K. Mattonet, J. Wilting, M. Jeltsch

Primary lymphedema is treatable but not curable. In addition, diagnosis is often not clear due to heterogeneous phenotypes. To address these problems, we need to know the underlying genetic lesions, diagnose them and develop targeted therapies. The necessary technologies for these tasks are provided by new developments in the field of molecular biology. The genetic causes of many monogenic primary lymphedema conditions have been identified over the past few years, using techniques like exome sequencing. Multifactorial inheritance is suspected for a large proportion of those primary lymphedema conditions, for which the causative genetic lesions are still unknown.

This chapter provides an overview of our current knowledge about the genetic causes, the categorization and the molecular biology and etiology of primary lymphedema conditons.

### Problems of classification and causal research

The human genome can nowadays be sequenced quickly and efficiently. Despite this, not all genetic elements involved in the development of primary lymphedema have been identified.

On the one hand, most of the currently used genome-wide sequencing methods cover only the exome of the patient. With this method, we do not detect DNA modifications that do not affect protein sequences, such as promoter mutations or epigenetic changes. In addition, many of the diseases occur spontaneously, possibly caused by somatic mutations in the same genes that are responsible for familial cases (1). Spontaneous mutations often result in mosaic phenotypes in which only certain body parts or cell lineages harbour the mutation. Even with a complete genome sequencing of sample material from the 'wrong' body region such mutations would not be found.

For these reasons, mutations have only been found in a certain proportion

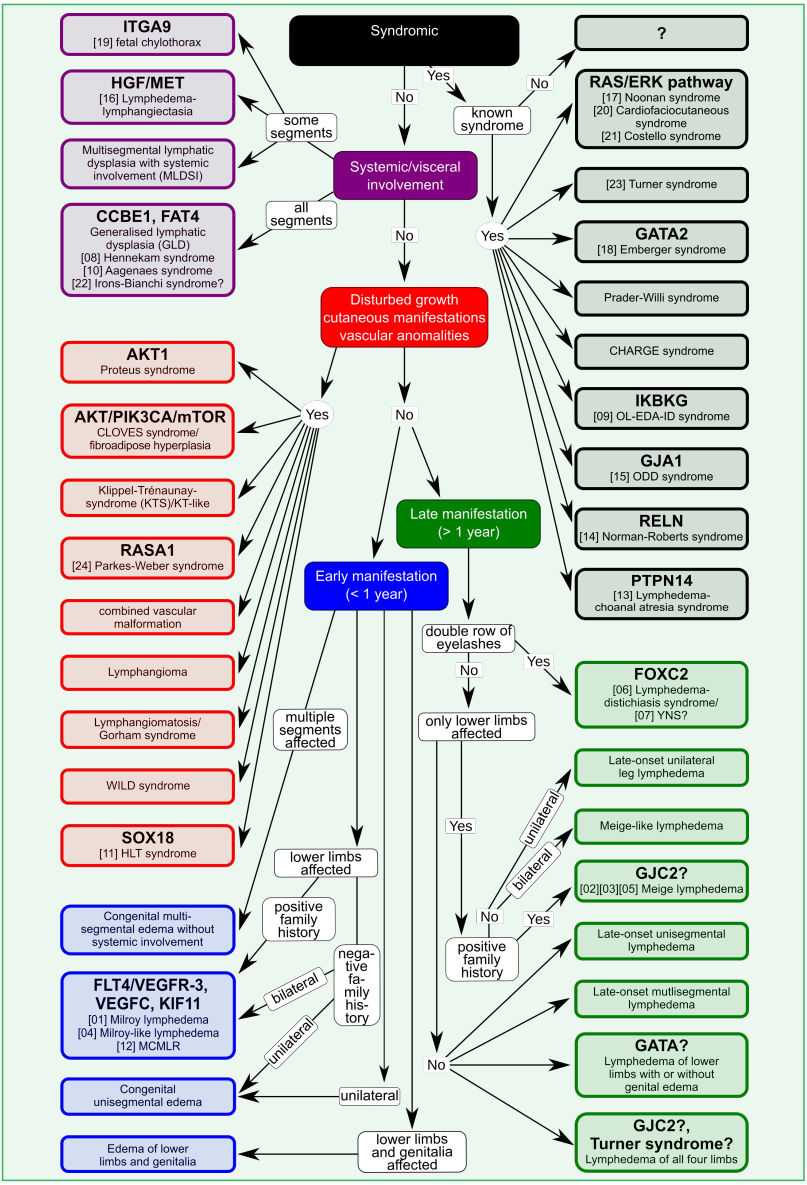


**Fig. 5.10-1**

*Modified diagnostic algorithm for primary lymphatic conditions according to Connell et al. 2013 (2). The numbers in square brackets refer to entries in Table 5.10-1 and Figure 5.10-2.*

of primary lymphedema, and the classification is still primarily based on phenotypic traits (2). These, however, are often polysemous and subject to the assessment of the treating physician (3). Nowadays, efforts are therefore being made to group diseases with the same genetic cause, to rearrange them and to establish a uniform system which places the genetic cause to the forefront (4). As an aid to clinical practice, Figure 1 shows a

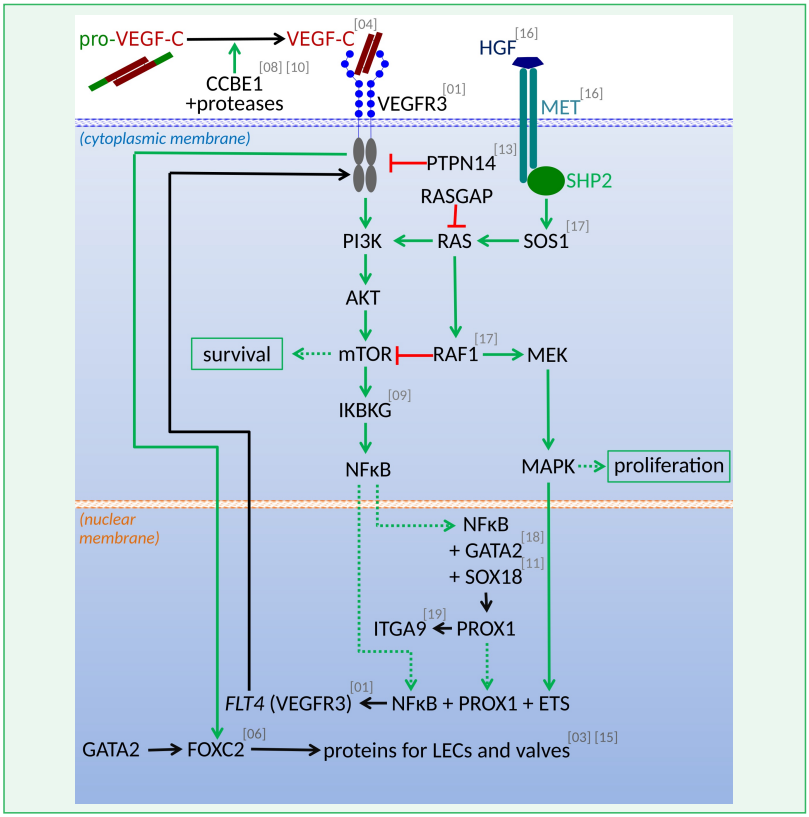


**Fig. 5.10-2**
*Overview of the most important signal transduction pathways. The arrows indicate an activating (green) or inhibitory (red) effect or an upregulation of protein synthesis due to the signaling (black) and should not be misinterpreted as an indication of direct physical interaction. The numbers in square brackets refer to lymphatic conditons described in Table 1, caused by a mutation in the numbered component.*

| [#] | Condition | Gene (Protein) | Characteristic symptoms | Temporal manifestation | Etiology | OMIM | Inheritance | Reference(s)* |
|---|---|---|---|---|---|---|---|---|
| [01] | **Hereditary lymphedema type IA (Milroy disease)** | FLT4 (VEGFR3) | LE of lower limbs, chylous ascites | congenital | The ability of the mutated VEGF receptor-3 to induce cell growth and division is compromised, resulting in lymphatic hypoplasia. | 153100 | AD, AR, de novo | (5, 6) |
| [02] | **Hereditary lymphedema type IB** | Locus 6q16.2-q22.1 | bilateral LE of lower limbs | childhood to puberty | unknown | 611944 | AD with reduced penetrance | (7) |
| [03] | **Hereditary lymphedema type IC** | GJC2 (Connexin 47) | LE of limbs | childhood to puberty | The connexin communication among LECs and between LECs and their environment is dysfunctional. | 613480 | AD | (8) |
| [04] | **Hereditary lymphedema type ID (Milroy-like disease)** | VEGFC (VEGF-C) | LE of lower limbs | congenital or early childhood | The mutated VEGF-C is not anymore efficiently secreted. Likley, its affinity for VEGFR-3 is also reduced. | 615907 | AD | (9, 10) |
| [05] | **Hereditary lymphedema type II (Meige lymphedema)** | - | LE of lower limbs | late childhood, puberty | unknown | 153200 | AD | (3, 11) |
| [06] | **Lymphedema-Distichiasis-Syndrom (LD-Syndrome)** | FOXC2 (FOXC2) | LE of lower limbs, distichiasis, varicose veins, sometimes ptosis | puberty or later | Lymphatic drainage is compromised by incompetent lymphatic vessels: Collecting vessels lack fully functional valves und lymphatic capillaries recruit an abnormal amount of pericytes. | 153400 | AD, de novo | (12) |
| [07] | **Yellow-Nail-Syndrom (YNS)** | - | LE of lower limbs, characteristic finger and toe nail changes, chronic respiratory infections | puberty or later (mostly in mid age) | unknown | 153300 | AD | (13) |

**Table 5.10-1** *(continued on the following pages)*

| [#] | Condition | Gene (Protein) | Characteristic symptoms | Temporal manifestation | Etiology | OMIM | Inheritance | Reference(s)* |
|---|---|---|---|---|---|---|---|---|
| [08] | **Hennekam lymphangiectasia lymphedema syndrome (HS)** | CCBE1 (CCBE1), FAT4 (FAT4) | LE of limbs, lymph-angiectasia of the gut, MR, CFA | congenital | The mutated CCBE1 protein cannot support ADAMTS3 in the activation of VEGF-C. The molecular etiologie is unknown for FAT4 mutations. | 235510 | AR | (14, 15) |
| [09] | **OL-EDA-ID syndrome (osteopetrosis, lymphedema, anhidrotic ectodermal dysplasia, and immunodeficiency)** | IKBKG (NEMO) | abnormal bone hardening, LE, sparse hair, facial dysmorphy, delayed eruption of teeth, sweat gland abnormalities, multiple infections | congenital, fatal in early childhood | A stop codon mutation prevents transcription of the regulatory IKBKG-gene, resulting in reduced NF-κB activity, which affects several essential signaling pathways, which are necessary for cell survial and cytokine production. | 300301 | XR | (16) |
| [10] | **Aagenaes syndrome (Cholestasis-lymphedema syndrome/CLS 1)** | locus in 15q, CCBE1 (CCBE1) | severe cholestasis, LE of lower limbs | neonatal and in early childhood | Hypoplasia of lymphatic vessles. The CCBE1 mutation features a missense mutation in the N-terminal domain. | 214900 | AR | (17-19) |
| [11] | **Hypotrichosis-lymphedema-telangiectasia syndrome (HLT syndrome)** | SOX18 (SOX-18) | alopecia, ectatic blood vessels, LE | congenital hypotrichosis, LE onset later (before end of puberty) | A recessive missense or a dominant frameshift mutation compromises the function of transcription factor SOX-18. | 607823 | AD, AR, de novo | (20) |
| [12] | **Microcephaly with or without chorio-retinopathy, lymph-edema, or mental retardation (MCLMR)** | KIF11 (EG5) | variable spectrum of ocular and central nerv-ous system develop-mental defects (e.g. microcephaly and chorioretinopathy), CFA, LE (mostly of the feet) | congenital | Different mutations in the Kinesin EG5 gene interfere with protein functions, that are likely required for the development and maintenance of retinal and lymphatic structures. | 152950 | AD | (21) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **[13]** | **Lymphedema-choanal atresia syndrome** | PTPN14 (PTPN14) | blockage of nasal passage, LE of lower legs | early childhood | A frameshift mutation disturbs the PTPN14 function. PTPN14 has been suggested to form a complex with VEGFR3 and thus regulate lymphangiogenesis. | 613611 | AR | (22) |
| **[14]** | **Norman-Roberts syndrome (lissencephaly 2)** | RELN (Reelin) | LE, MR and developmental defects of the central nervous system | congenital | The mutation in the RELN gene strongly reduces the amounts of Reelin, which results in abnormal migration of neuronal precursor cells and abnormal axonal connections. The LE phenotype suggests other, yet unknown functions for Reelin. | 257320 | AR | (23) |
| **[15]** | **ODD syndrome (oculodentodigital dysplasia/ lymphedema)** | GJA1 (Connexin 43) | CG, syndactyly (mostly 4/5), sometimes LE und MR | congenital | The mutated Connexin 43 interferes in a dominant negative fashion with the activity of wild type Conenxin 43. However, if the mutation results in a completely nonfunctional protein, the inheritance pattern becomes recessive, since a single functional Connexin 43 allele appears sufficient for normal physiological function. | 164200 257850 | AR, AD | (24, 25) |
| **[16]** | **Lymphedema/ lymphangiectasia** | HGF, MET | LE | unknown | unknown | - | AD (?) | (26) |

| [#] | Condition | Gene (Protein) | Characteristic symptoms | Temporal manifestation | Etiology | OMIM | Inheritance | Reference(s)* |
|---|---|---|---|---|---|---|---|---|
| [17] | **Noonan syndrome (NS1-NS8, NSLH, NSLL)** | PTPN11, RIT1, BRAF, KRAS, SOS1, RAF1, NRAS, SHOC2, CBL | LE, cardiovascular defects, characteristic age-dependent facial abnormalities, short stature (small phenotypic differences between the subtypes) | congenital | All mutations, that are associated with Noonan syndrome result in an increased activity of the RAS-MAPK signaling pathway. The disturbance of this central signaling pathway results in a characteristic and broad spectrum of developmental disorders. | 163950 605275 609942 610733 611553 613224 613706 615355 613563 607721 | AD (sometimes AR according to some case reports) | (27) |
| [18] | **Emberger syndrome (Primary lymphedema & myelodysplasia)** | GATA2 | LE of lower limbs (and genitalia), abnormal lymphocyte counts/ myelodysplasia (progressing to acute myeloid leukaemia), deafness | childhood to puberty | It is suspected that GATA2 plays a critical role in the development and maintanance of the lymphatic system, but the mechanism is unknown. | 614038 | AD | (28) |
| [19] | **Fetal chylothorax** | ITGA9 | Hydrops fetalis, chylothorax | prenatal | The G404S mutation in the ITGA9 protein is associated with a bad prognosis for chylothorax treatment using OK-432 pleurodesis. The mutation likely destabilizes the protein structure leading to misfolding. The exact biochemical mechanism is unknown. | - | AR, de novo | (29, 30) |
| [20] | **Cardiofacio-cutaneous syndrome (CFC1-CFC4)** | KRAS, BRAF, MAP2K1, MAP2K2 | CG, heart defects, MR | congenital | The MAPK/ERK signal transduction pathway is compromised (see Noonan syndrome). | 115150 615278 615279 615280 | AD | (31) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| [21] | **Costello syndrome/ chylous ascites** | HRAS | Characteristic hand posture, CG, growth disturbances, heart defects, flexible joints and loose skin folds | congenital | The MAPK/ERK signal transduction pathway is compromised (see Noonan syndrome). | 218040 | AD | (32) |
| [22] | **Irons-Bianchi syndrome** | unknown | LE of lower limbs, MR, CFA, atrial septal defect, hydrops fetalis | congenital/ prenatal | unknown, the phenotype is very similar to Hennekam syndrome | 601927 | AR | (33, 34) |
| [23] | **Turner syndrome** | Xp11.4 (critical locus for LE) | LE, reduced growth, gonadal dysgenesis, high risk of cardiovascular complications | congenital | unknown | (not inherited) | 45,X; 46,X,i(Xq); 45,X, mosaic type | (35, 36) |
| [24] | **Parkes-Weber syndrome (CM-AVM/lymphedema)** | RASA1 (RASA1) | vascular malfomations, LE of lower limbs | congenital | The mutated RASA1 results in dysregulated RAS activity, which might be the reason for the defects in lymhatic development. | 608354 | AD | (37) |

**Table 5.10-2.** *Selection of known disorders that are associated with primary lymphedema. If possible, a recent review article is referenced*, which provides an overview of the disease. Otherwise, the reference is made to the primary literature. Further references can be found via the OMIM number. This table is not a complete listing or definite categorization of known primary lymphedema conditions, but a preliminary suggestion based on current knowledge. AD, autosomal dominant; AR, autosomal recessive; CFA, characteristic facial abnormalities; LE, lymphedema; MR, mental retardation; XR, X-linked recessive.*

currently valid algorithm as an aid for the classification and diagnosis of primary lymphedema.

A detailed description of all primary lymphedema conditions is outside the scope of this article and is for many cases also not available. The focus of this review is therefore on diseases with a high number of cases and on cases with a known etiology. Without a claim to completeness, such a selection of lymphedema conditions is shown in Table 1. In particular, diseases defined by lymphatic malformations and tumors have been omitted. These are, however, shown in Figure 5.10-2, which ís a schematic representation of the signal pathways, which are explained in Table 5.10-1. The development and homeostasis of the lymphatic system are regulated by a complex network of signal transduction pathways. Mutations in the involved genes can lead to quantitative or qualitative (gain or loss of function) changes in signaling components and thus affect structure or function of the lymphatic system (38), which often results in lymphedema. The following sections present a selection of such signaling pathways.

## 5.10.2 The VEGF receptor-3 / VEGF-C signal axis

The most important growth factor for the development and homeostasis of the lymphatic system is VEGF-C (39). The deletion of both VEGF-C alleles is lethal during early embryonic development and is associated with generalized lymphedema (40). Therefore, a complete loss of function of the VEGF-C signal pathway does not occur clinically. However, a mutation in only one allele in some the involved genes can also have a severe phenotype (41).

A mutation in the gene for VEGF receptor-3 (VEGFR-3), the primary lymphangiogenic receptor for VEGF-C, is the main cause of hereditary lymphedema. VEGFR-3 is expressed on the endothelial cells of lymph vessels (Figure 5.10-3). It allows the survival, migration and proliferation of these cells and is thus essential for the outgrowth of new lymphatics (41). Primary lymphedema with mutations in this receptor is referred to as hereditary lymphedema type IA. Nevertheless, a mutation in VEGFR-3 does not necessarily lead to lymphedema. Only in approximately 85% of

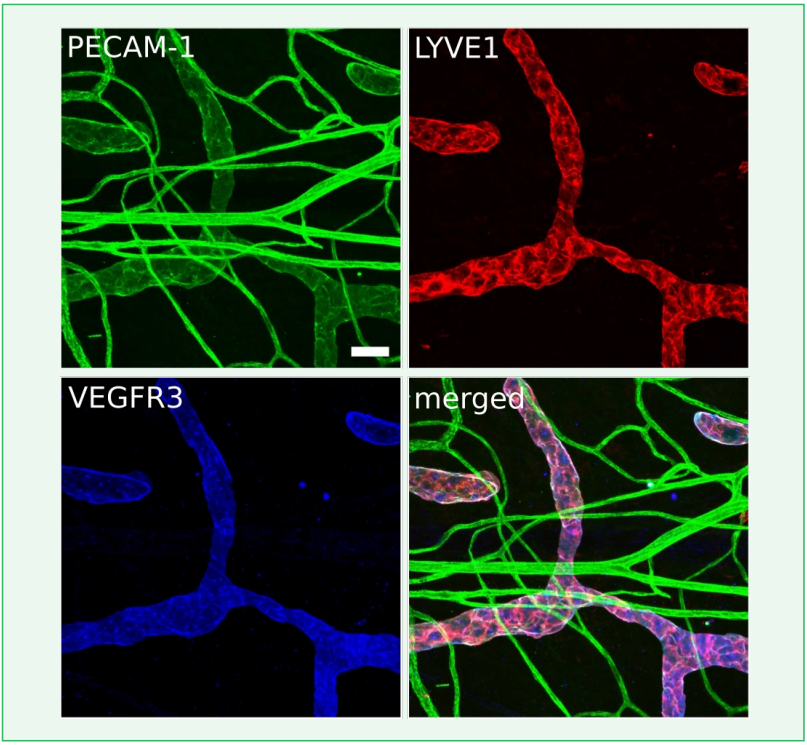


**Fig. 5.10-3**
*Immunohistochemistry of a mouse ear, scale bar 100 µm. On the upper left, in green, all vessels were stained with the endothelial cell marker PECAM-1 (aka CD31). On the upper right and on the lower left are images of the same tissue stained with the markers LYVE-1 (red) and VEGFR3 (blue) to visualize lymphatic endothelial cells. The bottom right image shows the overlay of all three stainings. We thank Georgia Zarkada for providing these images.*

patients with a mutated allele, lymphedema can be diagnosed (2). Thus, the inheritance pattern that leads to the disease is usually described as *dominant with reduced penetrance*. The reason for dominance is the so-called *dominant negative effect*, which results from the fact that the mutant receptor antagonizes functional receptors from the wild type allele, since heterodimers between mutant and wild type VEGFR-3 are nonfunctional. The mutation responsible for the symptoms mostly affects the phos-

phorylation sites of the receptor, preventing signal transduction (1, 42). However, individual recessive cases are also known in which only ATP binding is reduced, which results in a less severe phenotype (43). The leading symptom of hereditary lymphedema type IA is congenital lymphedema of the lower extremities. The is no obvious explanation why the edema is limited to the legs and why symptoms are rarely seen in the arms or internal organs. Increased hydrostatic pressure has been implicated (44), but the exact reasons remain unclear. Dominant mutations in the VEGF-C gene can lead to a phenotype that is clinically indistinguishable from hereditary lymphedema IA (9, 10). This disease, classified as a hereditary lymphedema type ID, greatly reduces the secretion of VEGF-C and its affinity for VEGFR-3 and thus its ability to stimulate the VEGFR-3 signaling pathway.

### 5.10.3 CCBE1 - Regulation of the VEGFR-3 pathway

Differently from VEGF-A, which is secreted as an active protein, VEGF-C is secreted as an inactive propeptide (45). It needs to go through a series of enzymatic cleavages until it assumes its fully active form and is able to stimulate VEGFR-3. The activation of VEGF-C is the subject of intensive research, but the process is not yet fully understood. However, the collagen-binding protein CCBE1 appears to play a crucial role by enhancing the activation of VEGF-C (15). In mouse and zebrafish models, it was shown that CCBE1, just like VEGF-C, is needed for the correct development of the lymphatic system (46, 47). CCBE1 probably immobilizes the inactive pro-VEGF-C on the cell surface and allows for effective activation by proteases such as ADAMTS3 (15). A few years ago, mutations in the CCBE1 gene have been found to cause Hennekam syndrome (14). CCBE1 appears essential for all lymphangiogenesis since lymphedema symptoms in Hennekam syndrome are observable in all body regions. In particular, the characteristic intestinal lymphangiectasia leads to severe complications by hypogammaglobulinaemia, hypoproteinaemia and lymphocytopenia. Additional symptoms include mental retardation and facial abnormalities of remarkably variable severity (18). It is suspected, that also other syn-

dromes with a lymphedema component, such as the Aagenaes syndrome, can be caused by mutations in the CCBE1 gene (19). However, the canonical classification based on phenotypic features reaches here again its limits. Recently, mutations in the FAT4 gene, which have so far only been associated with the Van Maldergem syndrome (48), were shown to manifest as Hennekam syndrome. However, this phenocopy still lacks a molecular explanation.

### 5.10.4 HGF and MET

Another relatively new discovery is the involvement of HGF (hepatocyte growth factor) and its receptor (high affinity hepatocyte growth factor receptor) MET in the formation of lymphedema. Mutations of these genes are not only suspected of being the cause for lymphoedema-lymphangiectasis, but could also be a risk factor for the occurrence of secondary lymphedema (26). The stimulation of MET by HGF results in a RAS/MAPK signal cascade, the disturbances of which are discussed in the following section.

### 5.10.5 Rasopathies

Disturbaces of the RAS/MAPK signal transduction pathways are commonly referred to as rasopathies. Characteristic of this group of disorders is a wide range of developmental defects (49). Since the lymphatic system is a highly ordered and complex system, it is not surprising that malformations of the lymphatics and a resulting lymphedema often belong to the list of symptoms. Although lymphedema is not a cardinal sign in these diseases, the high incidence of some of these syndromes justifies their discussion in the context of hereditary lymphedema. The most common rasopathy with an incidence of 1 in 1000-2500 births is the Noonan syndrome (50). A common cause are mutations in the PTPN11 gene, which encodes the phosphotyrosine phosphatase SHP2 (27). These and mutations in nine other genes trigger diseases that are collectively classified as Noonan syndrome, since they differ only slightly in their clinical

phenotype. All these mutations occur in genes that encode components of the RAS/MAPK signaling pathway (see Fig. 2) and lead to a phenotype similar to Turner syndrome (51). Interestingly, all known mutations that result in rasopathies increase the signal pathway activity (49). Other syndromes, that similarly to rasopathies occassionally present with primary lymphedema are Costello syndrome and Cardiofasciocutaneous syndrome.

### 5.10.6 FOXC2-Associated Syndromes

Both the RAS/MAPK signaling pathway and the VEGFR-3-stimulated PI3K/Akt pathway result in the activation of transcription factors, which effect changes in the expression of specific genes (41). Examples are shown in Figure 2. A central transcription factor for lymphatic development is FOXC2. Mutations in the FOXC2 gene are responsible for the lymphedema-distichiasis syndrome (52). Nevertheless, the distinction between this disease and two other diseases (Meige disease and Yellow Nail syndrome) is still controversial, and hence, FOXC2 mutations have been associated with all three diseases (3). The reports of FOXC2 mutations in Meige disease (11, 53) might include misdiagnosed lymphedema-distichiasis syndrome, since it is sometimes difficult to diagnose distichiasis. However, many different types of FOXC2 mutations can be observed. The hypothesis has been put forward, that activating FOXC2 mutations lead to hyperplasia and a phenotype that corresponds to the Meige phenotype, whereas inactivating mutations lead to hypoplasia and lymphoedema-distichiasis syndrome (11).

Yellow nail syndrome (YNS) is a rare disease, and its independence has recently been questioned. It could represent a particular manifestation of the lymphoedema-distichiasis syndrome (13). This presumption is supported by the fact that only one third of the cases show all three symptoms (yellow nails, lymphedema, respiratory involvement). For the diagnosis of YNS, two of the three cardinal symptoms suffice (54). In recent review articles, the YNS is often classified together with lymphoedema-distichiasis syndrome (1, 38). However, YNS has been included in the table as independ-

ent entity, since this chapter uses the OMIM (Online Mendelian Inheritance in Men) classification, which still lists it as an independent disease. The controversy surrounding lymphedema-distichiasis, Meige disease and YNS clearly underlines the need for an objective lymphedema classification based on genetic markers since the canonical phenotypic classification remains too often ambigous.

## 5.10.7 Perspectives in Diagnostics and Treatment

Similar to most hereditary diseases, we have no causal treatment for primary lymphedema. The elucidation of the molecular causes of the disease is the starting point for an understanding of lymphangiogenesis and the a search for potential therapeutic approaches. For example, the first pro-lymphangiogenic therapy with the VEGF-C growth factor is about to enter clinical trials. The aim of the therapy, developed under the name Lymfactin® is to increase the success rate of lymph node transplants for the treatment of postoperative lymphedema (55). Animal models show that if it is safe and well tolerated in phase I trials, a therapy with VEGF-C could also be suitable for the treatment of Milroy disease (44).

Screening for risk factors that favor the development of secondary lymphedema after surgery could also prove to be valuable in clinical practice once sufficiently reliable molecular markers become available. It remains a fact, that lymphedema remains often undiagnosed when the symptoms are not severe. The possibility to use blood tests to look at a wide variety of genetic markers and thus to arrive at a clear diagnosis could lead to better prevention and medical care for many patients. The investigation of genetic causes and biochemical mechanisms that result in primary lymphedema is a relatively recent field of research, since many of the necessary technological possibilities have not been available until recently and therefore, most of the studies on the genetics and biochemistry of primary lymphedema mentioned in this chapter are not older than a decade. In the next edition of this textbook we hope to draw a much more complete picture of the genetic causes of primary lymphedema.

A more detailed description of the genetics of primary lymphedema can be

| | |
|---|---|
| **allele** | Variant form of a gene. Somatic human cells have two alleles of each nuclear-coded gene. |
| **cytokines** | Group of extracellular, small signaling protein molecules, which e.g. play a central role in mediating inflammatory processes and the immune response. |
| **epigenetic modification** | A collection term for modifications of the genetic material that do not alter the base sequence of the DNA (e.g., histone modifications or base methylations). |
| **exome** | The totality of all transcribed sequences in the genome, which are found in the mRNA. |
| **LEC** | Lymphatic endothelial cell |
| **missense mutation** | Point mutation, that results in an incorrect amino acid or a premature stop codon being encoded. Depend-ing on the properties of the original and the mutated amino acid, this can have no effects, serious effects, or anything in between on the function of the protein. |
| **phenotype** | Observable result from expression of a gene/genome |
| **proliferation** | Cell growth and division |
| **promotor** | Sequence motifs in the genome, which mark in eukaryotic cells the binding site of transcription factors guiding the RNA polymerase **II** to its destination: the starting point of transcription (copying of DNA into mRNA sequence). |
| **propeptid** | Part of the polypeptide chain of a protein that is cleaved enzymatically to activate the protein or to induce other functional changes. |
| **proteases** | Protein enzymes that can cleave other proteins at specific amino acid sequence motifs. |
| **frameshift mutation** | Mutation that shifts the reading frame in translation. Since three base pairs code for an amino acid and reading proceeds without gaps, the insertion of one or two bases causes a shift of the reading frame and a completely new (wrong) amino acid sequence. On average, this altered sequence is terminated after about 20 amino acids by a stop codon and thus leads almost always to a shortened and dysfunctional protein. |
| **signaling path-way/signaling cascade** | Intracellular chain of sensors, effectors and signaling molecules that relay the signal from an activated receptor to the effectors (e.g., ion channels or trans-cription factors). |
| **somatic mutation** | Mutation that does not occur in the germ line (i.e. not in reproductive cells) and is therefore not inherited or passed on to the offspring. |
| **transcription factor** | A protein that controls transcription (copying of DNA sequence into mRNA sequence) and thus regulates protein expression levels. |

**Table 5.10-2**
*Glossary of selected biochemical terminology.*

found in the review by Brouillard et al. (1). The most recent classification with a description of the diagnostic procedures is provided by Connell et al. (2). An insight into the genetics of lymphatic development and maintenance including an overview of the existing mouse models can be found in the review by Schulte-Merker et al. (38) and a comprehensive review of the molecular foundations and diseases of the lymphatic system can also be found in the German literature by Krebs and Jeltsch (56, 57).

## 5.10.8. References

1. Brouillard P, Boon L, Vikkula M. Genetics of lymphatic anomalies. J Clin Invest 2014; 124: 898–904.

2. Connell F, Gordon K, Brice G et al. The classification and diagnostic algorithm for primary lymphatic dysplasia: an update from 2010 to include molecular findings. Clin Genet 2013; 84: 303–314.

3. Rezaie T, Ghoroghchian R, Bell R et al. Primary non-syndromic lymphoedema (Meige disease) is not caused by mutations in FOXC2. Eur J Hum Genet 2008; 16: 300–304.

4. Mortimer PS, Rockson SG. New developments in clinical aspects of lymphatic disease. J Clin Invest 2014; 124: 915–921.

5. Ghalamkarpour A, Morlot S, Raas-Rothschild A et al. Hereditary lymphedema type I associated with VEGFR3 mutation: the first de novo case and atypical presentations. Clin Genet 2006; 70: 330–335.

6. Irrthum A, Karkkainen MJ, Devriendt K et al. Congenital Hereditary Lymphedema Caused by a Mutation That Inactivates VEGFR3 Tyrosine Kinase. Am J Hum Genet 2000; 67: 295–301.

7. Malik S, Grzeschik K-H. Congenital, low penetrance lymphedema of lower limbs maps to chromosome 6q16.2–q22.1 in an inbred Pakistani family. Hum Genet 2008; 123: 197–205.

8. Kanady JD, Dellinger MT, Munger SJ et al. Connexin37 and Connexin43 deficiencies in mice disrupt lymphatic valve development and result in lymphatic disorders including lymphedema and chylothorax. Dev Biol 2011; 354: 253–266.

9. Balboa-Beltran E, Fernández-Seara MJ, Pérez-Muñuzuri A et al. A novel stop mutation in the vascular endothelial growth factor-C gene (VEGFC) res-

ults in Milroy-like disease. J Med Genet 2014; 51: 475–478.

10. Gordon K, Schulte D, Brice G et al. 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. Circ Res 2013; 112: 956–960.

11. Van Steensel MA, Damstra RJ, Heitink M et al. Novel missense mutations in the FOXC2 gene alter transcriptional activity. Hum Mutat 2009; 30: E1002–E1009.

12. Brice G, Mansour S, Bell R et al. Analysis of the phenotypic abnormalities in lymphoedema-distichiasis syndrome in 74 patients with FOXC2 mutations or linkage to 16q24. J Med Genet 2002; 39: 478–483.

13. Hoque SR, Mansour S, Mortimer PS. Yellow nail syndrome: not a genetic disorder? Eleven new cases and a review of the literature. Br J Dermatol 2007; 156: 1230–1234.

14. Alders M, Hogan BM, Gjini E et al. Mutations in CCBE1 cause generalized lymph vessel dysplasia in humans. Nat Genet 2009; 41: 1272–1274.

15. Jeltsch M, Jha SK, Tvorogov D et al. CCBE1 Enhances Lymphangiogenesis via A Disintegrin and Metalloprotease With Thrombospondin Motifs-3–Mediated Vascular Endothelial Growth Factor-C Activation. Circulation 2014; 129: 1962–1971.

16. Döffinger R, Smahi A, Bessia C et al. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-κB signaling. Nat Genet 2001; 27: 277–285.

17. Bull LN, Roche E, Song EJ et al. Mapping of the Locus for Cholestasis-Lymphedema Syndrome (Aagenaes Syndrome) to a 6.6-cM Interval on Chromosome 15q. Am J Hum Genet 2000; 67: 994–999.

18. Drivdal M, Trydal T, Hagve T-A et al. Prognosis, with evaluation of general biochemistry, of liver disease in lymphoedema cholestasis syndrome 1 (LCS1/Aagenaes syndrome). Scand J Gastroenterol 2006; 41: 465–471.

19. Shah S, Conlin LK, Gomez L et al. CCBE1 Mutation in Two Siblings, One Manifesting Lymphedema-Cholestasis Syndrome, and the Other, Fetal Hydrops. PLoS ONE 2013; 8: e75770.

20. Irrthum A, Devriendt K, Chitayat D et al. Mutations in the Transcription Factor Gene SOX18 Underlie Recessive and Dominant Forms of Hypotrichosis-Lymphedema-Telangiectasia. Am J Hum Genet 2003; 72: 1470–1478.

21. Ostergaard P, Simpson MA, Mendola A et al. Mutations in KIF11 Cause Autosomal-Dominant Microcephaly Variably Associated with Congenital Lymphedema and Chorioretinopathy. Am J Hum Genet 2012; 90: 356–362.

22. Au AC, Hernandez PA, Lieber E et al. Protein tyrosine phosphatase PTPN14 is a regulator of lymphatic function and choanal development in humans. Am J Hum Genet 2010; 87: 436–444.

23. Hong SE, Shugart YY, Huang DT et al. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat Genet 2000; 26: 93–96.

24. Richardson RJ, Joss S, Tomkin S et al. A nonsense mutation in the first transmembrane domain of connexin 43 underlies autosomal recessive oculodentodigital syndrome. J Med Genet 2006; 43: e37–e37.

25. Brice G, Ostergaard P, Jeffery S et al. A novel mutation in GJA1 causing oculodentodigital syndrome and primary lymphoedema in a three generation family. Clin Genet 2013; 84: 378–381.

26. Finegold DN, Schacht V, Kimak MA et al. HGF and MET Mutations in Primary and Secondary Lymphedema. Lymphat Res Biol 2008; 6: 65–68.

27. Roberts AE, Allanson JE, Tartaglia M et al. Noonan syndrome. The Lancet 2013; 381: 333–342.

28. Ostergaard P, Simpson MA, Connell FC et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet 2011; 43: 929–931.

29. Ma G-C, Liu C-S, Chang S-P et al. A recurrent ITGA9 missense mutation in human fetuses with severe chylothorax: possible correlation with poor response to fetal therapy. Prenat Diagn 2008; 28: 1057–1063.

30. Yeang C-H, Ma G-C, Shih J-C et al. Genome-Wide Gene Expression Analysis Implicates the Immune Response and Lymphangiogenesis in the Pathogenesis of Fetal Chylothorax. PLoS ONE 2012; 7: e34901.

31. Roberts A, Allanson J, Jadico SK et al. The cardiofaciocutaneous syndrome. J Med Genet 2006; 43: 833–842.

32. Gripp KW, Hopkins E, Sol-Church K et al. Phenotypic analysis of individuals with Costello syndrome due to HRAS p.G13C. Am J Med Genet A 2011; 155: 706–716.

33. Irons MB, Bianchi DW, Geggel RL et al. Possible new autosomal recessive syndrome of lymphedema, hydroceles, atrial septal defect, and characteristic

facial changes. Am J Med Genet 1996; 66: 69–71.

34. Van Steensel MAM, van Geel M, Schrander-Stumpel C et al. Lymphedema, cardiac septal defects, and characteristic facies: Possible new case of Irons–Bianchi syndrome. Am J Med Genet A 2007; 143A: 2448–2451.

35. Davenport ML. Approach to the Patient with Turner Syndrome. J Clin Endocrinol Metab 2010; 95: 1487–1495.

36. Sybert VP, McCauley E. Turner's Syndrome. N Engl J Med 2004; 351: 1227–1238.

37. Burrows PE, Gonzalez-Garay ML, Rasmussen JC et al. Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man. Proc Natl Acad Sci U S A 2013; 110: 8621–8626.

38. Schulte-Merker S, Sabine A, Petrova TV. Lymphatic vascular morphogenesis in development, physiology, and disease. J Cell Biol 2011; 193: 607–618.

39. Tammela T, Alitalo K. Lymphangiogenesis: Molecular Mechanisms and Future Promise. Cell 2010; 140: 460–476.

40. Karkkainen MJ, Haiko P, Sainio K et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol 2004; 5: 74–80.

41. Zheng W, Aspelund A, Alitalo K. Lymphangiogenic factors, mechanisms, and applications. J Clin Invest 2014; 124: 878–887.

42. Brice G, Child AH, Evans A et al. Milroy disease and the VEGFR-3 mutation phenotype. J Med Genet 2005; 42: 98–102.

43. Ghalamkarpour A, Holnthoner W, Saharinen P et al. Recessive primary congenital lymphoedema caused by a VEGFR3 mutation. J Med Genet 2009; 46: 399–404.

44. Karkkainen MJ, Saaristo A, Jussila L et al. A model for gene therapy of human hereditary lymphedema. Proc Natl Acad Sci 2001; 98: 12677–12682.

45. Joukov V, Sorsa T, Kumar V et al. Proteolytic processing regulates receptor specificity and activity of VEGF-C. EMBO J 1997; 16: 3898–3911.

46. Bos FL, Caunt M, Peterson-Maduro J et al. CCBE1 Is Essential for Mammalian Lymphatic Vascular Development and Enhances the Lymphangiogenic Effect of Vascular Endothelial Growth Factor-C In Vivo. Circ Res 2011; 109: 486–491.

47. Hogan BM, Bos FL, Bussmann J et al. ccbe1 is required for embryonic lymphangiogenesis and venous sprouting. Nat Genet 2009; 41: 396–398.

48. Alders M, Al-Gazali L, Cordeiro I et al. Hennekam syndrome can be caused by FAT4 mutations and be allelic to Van Maldergem syndrome. Hum Genet 2014; 133: 1161–1167.

49. Rauen KA. The RASopathies. Annu Rev Genomics Hum Genet 2013; 14: 355–369.

50. Mendez HM, Opitz JM. Noonan syndrome: a review. Am J Med Genet 1985; 21: 493–506.

51. Bhambhani V, Muenke M. Noonan Syndrome. Am Fam Physician 2014; 89: 37–43.

52. Fang J, Dagenais SL, Erickson RP et al. Mutations in FOXC2 (MFH-1), a Forkhead Family Transcription Factor, Are Responsible for the Hereditary Lymphedema-Distichiasis Syndrome. Am J Hum Genet 2000; 67: 1382–1388.

53. Finegold DN, Kimak MA, Lawrence EC et al. Truncating mutations in FOXC2 cause multiple lymphedema syndromes. Hum Mol Genet 2001; 10: 1185–1189.

54. Piraccini BM, Urciuoli B, Starace M et al. Yellow nail syndrome: Clinical experience in a series of 21 patients. JDDG J Dtsch Dermatol Ges 2014; 12: 131–137.

55. Lähteenvuo M, Honkonen K, Tervala T et al. Growth Factor Therapy and Autologous Lymph Node Transfer in Lymphedema. Circulation 2011; 123: 613–620.

56. Krebs R, Jeltsch M. Die lymphangiogenen Wachstumsfaktoren VEGF-C und VEGF-D. Teil 1. Grundlagen und Embryonalentwicklung. Lymphol Forsch Prax 2013; 17: 30–37.

57. Krebs R, Jeltsch M. Die lymphangiogenen Wachstumsfaktoren VEGF-C und VEGF-D. Teil 2. Die Rolle von VEGF-C und VEGF-D bei Krankheiten des Lymphgefäßsystems. Lymphol Forsch Prax 2013; 17: 96–104.