Digital access to libraries

"No evidence of locus heterogeneity in familial microcephaly with or without chorioretinopathy, lymphedema, or mental retardation syndrome."

Schlögel, Matthieu ; Mendola, Antonella ; Fastre, Elodie ; Vasudevan, Pradeep ; Devriendt, Koen ; de Ravel, Thomy Jl ; Van Esch, Hilde ; Casteels, Ingele ; Arroyo Carrera, Ignacio ; Cristofoli, Francesca ; Fieggen, Karen ; Jones, Katheryn ; Lipson, Mark ; Balikova, Irina ; Singer, Ami ; Soller, Maria ; Mercedes Villanueva, María ; Revencu, Nicole ; Boon, Laurence ; Brouillard, Pascal ; Vikkula, Miikka

Abstract

BACKGROUND: Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation syndrome (MCLMR) is a rare autosomal dominant disorder with variable expressivity. It is characterized by mild-to-severe microcephaly, often associated with intellectual disability, ocular defects and lymphedema. It can be sporadic or inherited. Eighty-seven patients have been described to carry a mutation in KIF11, which encodes a homotetrameric motor kinesin, EG5. METHODS: We tested 23 unreported MCLMR index patients for KIF11. We also reviewed the clinical phenotypes of all our patients as well as of those described in previously published studies. RESULTS: We identified 14 mutations, 12 of which are novel. We detected mutations in 12 affected individuals, from 6 out of 6 familial cases, and in 8 out of 17 sporadic patients. Phenotypic evaluation of patients (our 26+61 earlier published=87) revealed microcephaly in 91%, eye anomalies in 72%, intellectual disability in 67% and ...

Document type : *Article de périodique (Journal article)*

Référence bibliographique

Genetic Causes of Lymphedema

3

Matthieu J. Schlögel, Pascal Brouillard,
Laurence M. Boon, and Miikka Vikkula

Key Points
- Careful patient examination for all signs and symptoms is important for precise clinical diagnosis.
- Primary lymphedema has a high underlying genetic heterogeneity. Currently, 20 genes are implicated.
- Neonatal edema, including non-immune hydrops fetalis, can also be caused by mutations in some of these genes.
- Genetic predisposing factors are unknown for a large fraction of patients.
- There is large variability in clinical expressivity and often incomplete penetrance for all signs and symptoms.

- Panel-based targeted next-generation sequencing is the most efficient approach for diagnostic screens.
- Secondary lymphedema may be influenced by genetic predisposition.

Introduction

Lymphedema is known since the middle of the nineteenth century, yet the first genes associated with this condition have been discovered only in the twenty-first century. Since then, more than 20 genes have been linked to the development of primary lymphedema. Originally discovered using linkage analysis in large families or animal models, the more recent approach using Next-Generation Sequencing (NGS) has allowed to discover genes using smaller families and even sporadic cases. In parallel, detailed in vitro and in vivo studies on molecular and cellular mechanisms involved in lymphangiogenesis have unraveled numerous novel functional candidate genes.

Primary lymphedema can be present as an inherited or a sporadic trait. It can be dominant, recessive (with consanguinity or not), or linked to the X-chromosome. There is important heterogeneity in the clinical appearance of lymphedema. Primary lymphedema can be the unique sign, affect different parts of the body (limb(s), arms, hands, head and neck, abdomen, etc.), be unilateral or bilateral, and appear at different ages at onset. It can also be part of a complex syndrome,

M.J. Schlögel, M.Sc. • P. Brouillard, Ph.D.
Laboratory of Human Molecular Genetics,
de Duve Institute, Université catholique
de Louvain, Brussels, Belgium

L.M. Boon, M.D., Ph.D.
Cliniques universitaires Saint-Luc, Center
for Vascular Anomalies, Universite catholique
de Louvain, Brussels, Belgium

M. Vikkula, M.D., Ph.D. (✉)
Laboratory of Human Molecular Genetics,
de Duve Institute, Université catholique
de Louvain, Brussels, Belgium

Walloon Excellence in Lifesciences
and Biotechnology (WELBIO), Brussels, Belgium
e-mail: miikka.vikkula@uclouvain.be

19

some of which are very rare, with only few cases reported.

Our current knowledge on the environmental and genetic variability as the cause of primary lymphedema is limited. Most of the Mendelian mutations have been identified in a limited number of patients or even only few families used in the original linkage study, several of the genes have been identified very recently limiting the time they have been used in clinical setting for diagnostic screening, the number of patients screened in reports is often very limited, and most screens have been done on gene-by-gene basis. Moreover, some of the clinical signs may be missed, and thus, the clinical classification is not necessarily correct. This renders it difficult to have a representative and a comprehensive overview of the current state of the art.

In this chapter, we make an extensive review of the medical literature. Clinical data was collected for all patients with a proven mutation, taking into account each mentioned sign and symptom. In the presentation, we divide the genes (and associated lymphedemas) into two groups. The first category includes the genes that cause lymphedema as the major sign, which is also the reason for medical consultation (Table 3.1). The second group contains the genes that are related to a usually well-known syndrome and for which lymphedema is minor sign (Table 3.2). Only a quarter of all cases are explained by mutations within the 20 genes. It appears that the historical classification based on the age of onset, i.e., congenital (at birth or early in life), praecox (teenage years), and tarda (late in life) is becoming irrelevant, as this does not correlate with the genetic background. Instead, the clinical presentation of the signs and symptoms can be helpful to associate the primary lymphedema to the most likely causative gene.

Lymphedema as a Major Sign

Phenotype of Patients

Lymphedema is the major sign for 14 of the genes currently known to cause primary lymph-

edema when mutated. The penetrance of lymphedema is though often incomplete, i.e., even though an individual carries a familial mutation, (s)he does not necessarily have lymphedema. This is also true for the other associated signs and symptoms listed in Table 3.1.

The cardiovascular system is often affected; varicose veins are not infrequent. Hydrocele can be present in at least four of the entities (Table 3.1). In the nervous system, symptoms range from hearing loss to learning difficulties and macrocephaly. Cutaneous and subcutaneous symptoms are frequent, including infection, papillomas, and cellulitis, many of which are considered secondary. Yet there are subtype-specific differences in prevalence (Table 3.1). In the musculoskeletal system, syndactyly or camptodactyly is observed. Mutations in GATA2 affect the respiratory system, generating pulmonary alveolar proteinosis. Mutations in GATA2, as well as IKBKG, also predispose to severe infections. Involvement of the digestive system is relatively rare (GJC2 and IKBKG), and renal abnormalities have been reported only in some cases (VEGFR3, FOXC2, and SOX18). Patients with a GATA2 mutation have a susceptibility to hematologic malignancies.

Genetic Differential Diagnosis: Which Gene to Screen?

The unraveled high genetic heterogeneity in primary lymphedema has resulted in a high number of clinical subcategories. Currently 21 genetically defined subgroups, as well as the subgroup of undefined ones, exist. Moreover, the latter mostly likely involves several genes. Some of the genetically defined 21 subgroups have typical signs, the presence of which can help in clinical diagnosis. In addition, familial history (Table 3.3) is an important factor in determining candidate genes for diagnostic screens.

The "unique" signs, i.e., those specific to one or two genes, are variable. Isolated lower limb lymphedema present at birth (the classic presentation of Milroy disease) suggests a FLT4/ VEGFR3 (FMS-like tyrosine kinase 4/vascular

Table 3.1 Signs and symptoms by mutated gene; lymphedema a cardinal sign

System	Sign	FLT4	VEGFC	FOXC2	PTPN14	GJC2	GJA1	KIF11	CCBE	FAT4	GATA2	SOX18	IKBKG	HGF	ITGA9
		(139)	(10)	(120)	(7)	(12)	(5)	(75)	(13)	(9)	(110)	(6)	(70)	(4)	(12)
Cardiovascular	Lymphedema	81 %	8/8	71 %	5/7	100 %	1/5	51 %	100 %	9/9	15 %	5/6	13 %	3/4	0/4
	Hydrops (fetalis)	¶	–	1/6	–	–	–	–	23 %	–	–	1/6	–	–	42 %
	Lymphangiectasia	–	–	–	–	–	–	–	100 %	7/9	–	–	–	1/4	100 %
	Lymphatic anomaly	36 %	–	¶	–	–	–	–	–	–	–	¶	–	–	100 %
	Varicose veins	7 %	–	49 %	–	42 %	1/5	–	–	–	–	–	–	–	–
	Vascular anomaly	28 %	4/7	–	–	25 %	–	–	23 %	–	25 %	¶	–	–	–
	Hemangioma	¶	–	–	–	–	–	–	–	–	–	–	–	–	–
	Hydrocoele	35 %	2/4	1 %	–	–	–	–	–	–	–	3/3	–	–	–
	Telangiectasia	–	–	–	–	–	–	–	–	–	–	3/5	1/2	–	–
	Heart defect	–	–	7 %	1/7	–	–	9 %	15 %	–	–	–	–	–	–
Nervous	Intellectual disability	5 %	–	3 %	–	–	–	69 %	69 %	7/9	–	¶	–	–	–
	Neurologic defect	–	–	¶	–	–	–	19 %	8 %	1/8	–	–	–	–	–
	Microcephaly	–	–	–	–	–	–	91 %	46 %	1/9	–	–	–	–	–
	Macrocephaly	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Ptosis	–	–	59 %	–	17 %	–	69 %	–	–	7 %	–	–	–	–
	Ophthalmologic problem	13 %	–	74 %	–	–	¶	69 %	–	–	–	¶	–	–	–
	Hearing loss	–	–	–	–	–	–	–	–	2/9	7 %	–	–	–	–
Integumentary	Papilloma (papule)	10 %	20 %	–	–	–	–	–	–	–	–	1/5	–	–	–
	Skin infection	–	–	¶	–	33 %	–	–	–	–	–	–	–	–	–
	Cellulitis	19 %	30 %	¶	–	50 %	–	–	–	–	–	–	–	–	–
	Eczema	–	–	–	–	8 %	–	–	–	–	–	1/5	1/2	–	–
	Skin problem	10 %	2/7	–	–	–	–	–	–	–	¶	3/5	78 %	–	–
	Hair problem	–	–	–	–	–	–	–	–	–	–	5/5	–	–	–
	Nail or toe anomaly	28 %	3/3	12 %	–	–	–	–	–	–	–	–	–	–	–
	Distichiasis	–	–	86 %	–	–	–	–	–	–	–	–	–	–	–
	Dental anomaly	–	–	–	–	–	¶	–	–	–	–	–	–	–	–

(continued)

Table 3.1 (continued)

System	Sign	FLT4 (139)	VEGFC (10)	FOXC2 (120)	PTPN14 (7)	GJC2 (12)	GJA1 (5)	KIF11 (75)	CCBE (13)	FAT4 (9)	GATA2 (110)	SOX18 (6)	IKBKG (70)	HGF (4)	ITGA9 (12)
Musculoskeletal	Reduced growth	–	–	–	–	–	–	–	69 %	5/9	–	–	–	–	–
	Cleft lip and Palate	–	–	21 %	¶	–	–	–	–	–	7 %	–	–	–	–
	Dysmorphic face	4/5	–	¶	–	–	¶	40 %	100 %	9/9	¶	1/5	–	–	–
	Thoracic/vertebral	–	–	7 %	¶	–	–	–	–	–	–	¶	–	–	–
	Joint	¶	–	–	–	–	–	–	–	–	–	–	–	–	–
	Syndactyly	–	–	–	–	–	4/5	27 %	31 %	2/8	–	–	–	–	–
	Choanal atresia	–	–	–	7/7	–	–	–	–	–	–	–	–	–	–
	Bone	–	–	–	–	–	–	–	38 %	6/9	–	–	10 %	–	–
Respiratory	Pulmonary	–	–	–	–	–	–	–	–	–	24 %	–	–	–	–
Immunitary	Infection	–	–	–	–	–	–	–	–	–	56 %	–	98 %	–	–
	Warts	2 %	–	–	–	–	–	–	–	–	53 %	–	–	–	–
	Hematological dysfunction	–	–	–	–	–	–	–	–	–	86 %	–	24 %	–	–
Digestive	Gastrointestinal	–	–	–	–	8 %	–	–	–	–	–	2/2	–	–	–
Urogenital	Genitourinary	4 %	–	3 %	–	–	–	–	–	–	–	¶	–	–	–
	Kidney	2 %	–	7 %	–	–	–	–	–	–	–	2/5	–	–	–
	M:F>2:1	–	–	¶	–	–	–	–	–	–	–	–	–	–	–
Malignancy	Tumor	–	–	1 %	–	8 %	–	–	–	–	73 %	–	–	–	–

The frequency of observation is translated in percentage when the number of examined patients was above 10. Number of patient studied is in parentheses. ¶ represents symptoms mentioned occasionally

Table 3.2 Signs and symptoms by mutated gene; lymphedema a non-cardinal sign

System	Signs	TSC1 (>100)	TSC2 (>100)	PTPN11 (31)	SOS1 (56)	KRAS (26)	Monosomy X (>500)	RASA1 (314)
Cardiovascular	Lymphedema	4 %	4 %	16 %	30 %	¶	36 %	¶
	Hydrops (fetalis)	–	–	–	–	–	–	0.60 %
	Lymphangiectasia	–	–	–	–	–	–	¶
	Lymphatic anomaly	–	–	–	–	–	¶	¶
	Vascular anomaly	71 %	82 %	–	–	¶	–	97 %
	Heart defect	–	–	90 %	77 %	¶	53 %	0.60 %
Nervous	Intellectual disability	49 %	83 %	27 %	10 %	92 %	11 %	0.60 %
	Neurological defect	91 %	91 %	23 %	–	–	¶	¶
	Macrocephaly	–	–	–	59 %	79 %	–	–
	Ptosis	–	–	69 %	7/8	–	–	–
	Ophthalmologic problem	10 %	37 %	31 %	3/8	3/5	–	0.60 %
	Hearing loss	–	–	19 %	–	–	–	–
Integumentary	Papilloma (papule)	–	–	–	–	–	–	–
	Skin problem	93 %	99 %	–	82 %	–	–	¶
	Nail or toe anomaly	45 %	34 %	–	–	–	–	–
	Dental anomaly	53 %	50 %	–	–	–	–	–
Musculoskeletal	Reduced growth	–	–	56 %	29 %	83 %	69 %	–
	Fetal macrosomia	–	–	–	31 %	–	–	–
	Dysmorphic face	–	–	63 %	82 %	60 %	–	–
	Thoracic/vertebral	–	–	38 %	44 %	4/5	–	–
	Limb defect	–	–	–	–	–	–	–
Respiratory	Pulmonary	–	–	–	–	–	–	–
Immune	Warts	–	–	–	–	–	–	–
	Hematological dysfunction	–	–	48 %	4/8	–	–	–
Digestive	Gatrointestinal	–	–	38 %	5/8	–	–	–
Urogenital	Genitourinary anomaly	–	–	27 %	3/6	2/3	22 %	–
	Renal defect	7 %	0.5 %	–	–	–	40 %	–
Malignancy	Tumor	42 %	42 %	¶	–	–	–	¶

The frequency of observation is translated in percentage when the number of examined patients was above 10. Number of patients studied is in parentheses. ¶ represents symptoms mentioned occasionally

Table 3.3 Diseases and genes associated with primary lymphedema

Diseases	Genes (proteins)	OMIM diseases	Inheritances	Lymphatic anomalies	Types of mutations	Patients[a]
Primary congenital lymphedema/Nonne–Milroy lymphedema	FLT4 (VEGFR3)	153,100	AD, AR, de novo	Bilateral congenital LE	Inactivating	139
Milroy-like disease	VEGFC	–	AD	Bilateral congenital LE	LOF	10
Lymphedema–distichiasis syndrome (LD)	FOXC2	153,400	AD, de novo	Late-onset LE	LOF	120
Choanal atresia/lymphedema syndrome	PTPN14	613,611	AR	LE	LOF	7
Hereditary lymphedema IC (Meige disease)	GJC2 (CX47)	613,480	–	4-limbs late-onset LE	Missense	12
Oculodentodigital dysplasia/lymphedema syndrome	GJA1 (CX43)	164,200	AD	LE	Missense	5
Microcephaly Chorioretinopathy Lymphedema Mental Retardation syndrome (MCLMR)	KIF11 (EG5)	152,950	AD, de novo	Lower-limbs LE	LOF	75
Hennekam lymphangiectasia–lymphedema syndrome	CCBE1	235,510	AR, de novo	4-limbs LE	LOF	13
Hennekam lymphangiectasia–lymphedema syndrome	FAT4	235,510	AR	4-limbs LE	LOF	9
Primary lymphedema with myelodysplasia (Emberger syndrome)	GATA2	614,038	AD, de novo	LE	LOF	110
Hypotrichosis–lymphedema–telangiectasia (renal defect) syndrome (HLTR)	SOX18	607,823	AD, AR	LE	LOF/D-N	6
Ectodermal dysplasia, anhidrotic, with immunodeficiency, osteopetrosis, and lymphedema (OLEDAID)	IKBKG	300,301	X-linked	LE	Hypomorphic	70
Lymphedema–lymphangiectasia	HGF	–	AD?	LE	LOF?	4
Fetal chylothorax	ITGA9	–	AR, de novo	Hydrops fetalis	Missense	12
Tuberous sclerosis-1 (TSC1)	TSC1	191,100	AD, de novo	LE	LOF	>100
Tuberous sclerosis-2 (TSC2)	TSC2	613,254	AD, de novo	LE	LOF	>100
Noonan syndrome 1 (NS)	PTPN11 (SHP2)	163,950	AD	Nuchal edema	GOF	31
Noonan syndrome 4 (NS)	SOS1	610,733	AD	Nuchal edema	GOF	56
Noonan syndrome 3 (NS)	KRAS	609,942	AD	Nuchal edema	GOF	26
Turner syndrome	Monosomy X	–	X-linked	Edema	–	>500
Capillary malformation—arteriovenous malformation (CM-AVM), including Parkes Weber syndrome	RASA1	608,354	AD	LE	LOF	314

AD autosomal dominant, *AR* autosomal recessive, *LOF* loss-of-function, *D-N* dominant-negative, *GOF* gain-of-function, *LE* lymphedema

Question marks indicate that mutation type and/or inheritance is unclear

[a]Number of patients studied

endothelial growth factor receptor 3) or VEGFC (vascular endothelial growth factor C) mutation. For VEGFR3, the intracellular part is most frequently mutated and should be sequenced first (exons 17–25), whereas any part of the VEGFC coding sequence can be mutated. Functional aplasia of the lymphatic vessels (failure of initial lymphatic absorption) in lymphoscintigraphy underscores the clinical diagnosis of VEGFR3 mutation. Patients with a mutation in VEGFC have reduced uptake with tortuous lymphatic tracts and evidence of rerouting [1–3].

When a patient has distichiasis (double rows of eyelashes, which is not always easy to notice) and lower limb lymphedema, the likely candidate is FOXC2 (forkhead box C2). The lymphedema is often of late onset, even if some patients with congenital lymphedema or hydrops fetalis have been described [4]. The presence of a cleft lip and/or palate also guide towards this gene. Individuals with FOXC2 mutation demonstrate reflux of lymph within the lower limbs as a result of valve failure within the hyperplastic lymphatic vessels [3].

Patients with lymphedema on all four extremities, whether early or late onset, evoke GJC2 (gap junction protein gamma-2) [5]. In lymphoscintigraphy, lymphatic tracts appear normal, but with a significant reduction in absorption by peripheral lymphatics in all four limbs [3]. Mutations in another gap junction protein GJA1 (gap junction protein alpha-1) have been found in patients with lymphedema and, among other signs, microdontia; a syndrome known as oculodentodigital dysplasia. The patient had clear lower limb lymphedema and subclinical upper limb lymphedema by lymphoscintigraphy [6].

Microcephaly can be helpful as a sign for differential diagnosis. Within the genetically defined groups, it is described, as a major sign, in patients with a mutation in KIF11 (kinesin family member 11). This phenotype combines microcephaly with or without chorioretinopathy, lymphedema, and intellectual disability into a syndrome, abbreviated as MCLMR. Microcephaly is present in 91 % (68/75) of the patients and two other major signs, intellectual disability and eye anomalies, both in 69 % of the patients (MJ Schlögel, Submitted). As for VEGFR3, failure of initial lymphatic absorption is observed in lymphoscintigraphy [3].

Facing a patient with microcephaly, 4-limb lymphedema and "unusual face" suggests CCBE1 (collagen and calcium-binding EGF domain-containing protein 1) and FAT4 (homolog of drosophila FAT tumor suppressor 4) as the mutated gene. This entity is known as the lymphedema–intestinal lymphangiectasia–intellectual disability syndrome or the Hennekam syndrome. There is no family history, or history is suggestive of recessive inheritance. The patient can be homozygous or compound heterozygous for the mutation(s) [7]. In one patient with a mutation in CCBE1, abnormal drainage in the upper and lower limbs, and the thoracic duct, was observed in lymphoscintigraphy [3].

GATA2 (gata-binding protein 2) and IKBKG (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma) are associated with severe immunological problems. Patients with a GATA2 mutation develop warts, and viral and/or bacterial infections [8]. Lymphoscintigraphy reveals hypoplasia of lymphatics within the affected lower limbs [3]. Similar features, as well as ectodermal dysplasia, are seen in patients mutated for IKBKG [9]. It is important to note that GATA2 predisposes to several cancers. This syndrome is known as primary lymphedema with myelodysplasia or Emberger syndrome.

There are some additional rare associations. In one family, lymphedema was associated with choanal atresia, and the affected individuals had a partial deletion (exons 7) in *PTPN14* (protein-tyrosine phosphatase nonreceptor-type 14) [10]. Another rare syndrome combines lymphedema with hypotrichosis and telangiectasias (HLT = Hypotrichosis–lymphedema–telangiectasia). It is caused by dominant or recessive mutations in SOX18 (SRY-Box 18) [11]. A particular stop codon in this gene causes severe glomerulonephritis leading to end-stage renal disease necessitating renal transplantation [12].

Lymphedema as a Minor Sign

In some well-known syndromes, such as tuberous sclerosis, Noonan syndrome and Turner syndrome, lymphedema can be present, although the diagnosis is made on the basis of other signs and symptoms. In some syndromes, such as in capillary malformation–arteriovenous malformation or CM-AVM, presence of lymphedema is only rarely reported, and/or it is subclinical, and thus not systematically looked for. Therefore, prevalence figures are only weak estimates. These genes are presented in Table 3.2.

Tuberous Sclerosis 1 and 2

Tuberous sclerosis (TSC) is an autosomal dominant disease characterized by hamartomas in different organ systems (skin, brain, heart, etc.). TSC affects between 1/6.000 and 1/10.000 individuals [13]. Compared to patients with a TSC1 mutation, those with a TSC2 mutation are more likely to have partial epilepsy, complex partial seizures, infantile spasms, subependymal giant-cell astrocytomas, and intellectual disability. Dental pits are often noted (Table 3.2). Primary lymphedema is present in less than 10 % of the cases.

Noonan Syndrome

Noonan syndrome (NS) is usually considered as a clinical diagnosis on the basis of the "typical face," including a broad forehead, hypertelorism, down-slanting palpebral fissures, ptosis, a high-arched palate, and low-set and posteriorly rotated ears. Cardiac anomalies and cryptorchidism can also be present. Mutations in PTPN11, SOS1, RAF1, KRAS, NRAS, SHOC2, or CBL cause this syndrome [14]. The overall incidence of lymphatic manifestations among NS is estimated to be ~20 % [15]. Lymphedema has only been reported in patients with a mutation in PTPN11, SOS1, or KRAS; thus, only these subtypes are included in Table 3.2. With enlarged screens it may become evident that the other genetic subtypes can also be associated with lymphedema. Patients with RAF1 (Raf-1 proto-oncogene, serine/threonine kinase) mutation have been reported with lymphangiectasia or microkystic lymphatic malformation. The Costello syndrome, caused by KRAS (Kirsten rat sarcoma viral oncogene homolog) mutations, shares many features with Noonan syndrome and patients can also present lymphatic anomalies. Patients with a PTPN11 (protein tyrosine phosphatase, non-receptor type 11) mutation could have thrombocytopenia, while mutations in SOS1 (son of sevenless homolog 1) or KRAS seem to commonly cause macrocephaly.

Turner Syndrome

Turner syndrome occurs in 1/2.500–1/3.000 live-born girls. About 50 % have monosomy X (45,X), and 5–10 % have a duplication of the long arm of one X (46,X,i(Xq)). Most of the rest are mosaic for 45,X [16]. The main signs are mental retardation, cardiac disease, renal malformation, short stature, and edema (puffy hands and feet, and redundant nuchal skin). Most cases of Turner syndrome are diagnosed prenatally, by the presence of edema. Karyotype can easily reveal the genetic defect in most cases.

Capillary Malformation–Arteriovenous Malformation Syndrome

Heterozygous mutations in *RASA1* (RAS p21 protein activator 1) cause multiple capillary malformations (CM) associated with fast-flow vascular malformations (CM-AVM). There is high intrafamilial phenotypic variability. Almost all patients with a RASA1 mutation have one or more capillary malformations (97 %) and 23 % have also a fast-flow lesion [17]. In a few patients with Parkes Weber syndrome and a RASA1

mutation, primary lymphedema is also present [18]. As for the other syndromes in this subclass, the signs of CM-AVM lead to the correct differential diagnosis.

Approach for Genetic Screening

Most diagnostic genetic tests have relied on Sanger sequencing of the exonic parts of a given candidate gene. When more than one possible candidate gene exists, a sequential approach has classically been used, starting with the gene that most often is mutated in the given clinical subtype. The high genetic heterogeneity within patients with primary lymphedema (so far 20 "candidate" genes) renders this approach time-consuming and labor intense. Only precise clinical diagnosis can help target the correct gene. Yet the incomplete penetrance of the associated signs and symptoms, and the high frequency of de novo cases for some of the genes may make differential diagnosis impossible. Targeted high-throughput sequencing now allows to screen several genes at a time using panels. This is replacing the sequential method. The panel approach allows the clinician to obtain results for multiple genes at once, which in turn helps in clinical diagnosis, even in the absence of associated symptoms.

Prenatal Testing

Many signs and symptoms associated with a mutation in the 20 genes and monosomy X are detectable only after birth. Thus, differential diagnosis is even more difficult in the prenatal period. Fetal edema may appear as nuchal edema, ascites, pleural effusion, chylothorax, pericardial effusion, cutaneous edema, or hydrops fetalis. These can be caused by mutations in VEGFR3, FOXC2, CCBE1, RASA1, PTPN11, and SOS1, and Monosomy X. In families at risk for lymphedema, detailed morphological ultrasound should be carried out paying attention to the signs in Tables 3.1 and 3.2. It is also important to search for familial history of the various signs, as they could help establish the correct differential diagnosis. The usefulness of the novel panel approach is particularly appreciated for prenatal genetic testing due to its completeness and rapidity.

Genetic Counseling

Diagnostic Genetic Testing

The identification of a mutation in one of the known genes allows more precise structure of the follow-up for the patient, especially regarding the signs and symptoms that develop with time. For example, myelodysplasia is not present at birth and necessitates a careful monitoring in patients with a GATA2 mutation (Table 3.1). Genetic counseling for risk calculation and prenatal genetic testing also become possible via the identification of the causative genetic mutation.

Despite an increased number of genes involved in lymphangiogenesis and/or in the etiology of primary lymphedema, a large proportion of lymphedema patients still remain unexplained after diagnostic genetic testing. Based on the analysis of more than 400 index patients, mutations in the known genes only explain about one third of the patients [19]. It could be that some of the mutations in these genes go undetected, as they are not in the parts that are classically screened (i.e., the exons) or they are not detected by the methods used. These could be intronic or promoter mutations, or large deletions or insertions. They would likely not explain the majority of the unexplained patients. Thus, additional genes should exist.

Genetic diagnosis is important to advance our knowledge on primary lymphedema. Precise sub-classification on the basis of genetic data is needed to better define the patient groups for genotype–phenotype correlations. Prognosis may also differ between the subgroups. Moreover, targeted therapies are best developed on detailed comprehension on the underlying pathophysiological mechanisms.

Next-Generation Sequencing and the Usefulness of a Panel-Based Approach

During the past few years, Next-Generation Sequencing (NGS) has opened a new era for mutation screens. Whole-exome sequencing (WES) allows to screen all the coding exons of the human genome in one experiment. However, not all genes are equally well covered. This may be due to for example inequalities in exome capture and difficulty in amplifying areas rich in G and C nucleotides. Moreover, the cost and ethical issues limit the WES approach in clinical settings.

Another interesting approach is targeted next-generation sequencing. This is based on capture or amplification of a certain, much more limited, number of exons from the human genome. For example, the 20 known "lymphedema genes" cover a total target size of around 117 kb. With a specifically designed panel, they can be analyzed in one experiment for a given patient often for a similar prize as a single gene screen using Sanger sequencing. For the Noonan syndrome, a study shows a sixfold reduction in cost using a RASopathy panel with a target area of 30 kb [20]. Therefore, it can be used as a primary genetic screen, which does not need a finalized, detailed clinical diagnosis to be efficient. The value of this technique also lies in its rapidity. Thus, genetic results do not only confirm a clinical diagnosis but help, in a timely manner, to establish it.

How to Identify the Causative Variant?

The Sanger-sequencing-based monogenic screens often reveal one probably pathogenic variant in one gene. If no such variant is identified, screening of a second gene is started. When a "causative mutation" is identified, screens are stopped. This differs fundamentally from the targeted NGS approach, which renders data available on all targeted genes at once. Thus, hundreds of variants can be analyzed at the same time, not limited to a single gene only. This allows to study possible interacting variants between the screened genes. However, software in diagnostic routine cannot address this question. Moreover, identifi-

cation of even the monogenic disease-causing mutation(s) is not without caveats, as it is often difficult to make the distinction between an amino acid changing polymorphism and a disease-causing mutation.

Co-segregation analysis of the identified changes within the family can give further proof for the implication of a given nucleotide change. This can be done using classic Sanger sequencing. Alternatively, several family members may be sequenced in parallel using a panel approach.

To predict the impact of a mutation at the protein level, different tools based on evolutionary conservation, structural constraints, or chemical qualities of the protein with the changed and unchanged amino acids have been developed. Moreover, databases, such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000Genomes (http://browser.1000genomes.org/index.html), and GoNL (http://www.nlgenome.nl/search/), collect reference sequence variants (most of which are polymorphisms) from different populations. These can be used to filter out known polymorphisms. In contrast, the absence, or a low allele frequency indicates that the variant is rare and may have a deleterious impact on the protein function. Other databases, such as HGMD (www.hgmd.org), regroup the known mutations in most genes, including small changes (Single Nucleotide Changes, Multiple Nucleotide Changes, insertions, and deletions) and larger structural variations, such as chromosomal deletions, insertions, or amplifications.

Even with these tools, many identified variants are reported as having "an unknown significance." For these, tests for in vitro and in vivo functional analysis would need to be developed. This is time-consuming and out of scope for routine diagnostic laboratories.

The Lymphedema-Causing Proteins

The genes that harbor mutations causing primary lymphedema and especially the proteins they encode can be grouped around the VEGFC–VEGFR3 ligand–receptor signaling complex and the downstream signaling pathways via PI3Kinase-AKT and MAPK [21].

VEGFC–VEGFR3 Axis

VEGFR3 was the first protein found to be mutated in primary lymphedema patients and the VEGFC–VEGFR3 signaling pathway is a major regulator of lymphangiogenesis. VEGFC and PTPN14 interact directly with VEGFR3. CCBE1 increases the capacity of VEGFC to activate VEGFR3 phosphorylation. Downstream of this complex, activation of the transcription factor FOXC2 ensues. GATA2 also regulates the expression of FOXC2, which plays a major role in the development of valves in lymphatic vessels by regulating the expression of connexin CX47/GJC2. Connexin, CX43/GJA1 is enriched on the upstream side of the lymphatic valves. Phosphorylation of FOXC2 is also linked to the expression of KIF11 [22]. The transcription factor SOX18 regulates PROX1 (prospero homeobox 1), a main factor for lymphangiogenesis, which regulates ITGA9, another valvular protein. So far, there is no clear link between VEGFR3 and FAT4, but the latter is regulated by the miRNA MIR31, linked to lymphangiogenesis [23].

Ras/MAPK Axis

Another major signaling pathway associated with lymphedema is the RAS-MAPK pathway. Mutations in this family of proteins can cause RASopathies. Mutations in each component of the pathway cause a distinct disease, but all the RASopathies share common features, such as craniofacial dysmorphology and cardiac malformations [14]. The pathway is involved in cell cycle, cellular growth, differentiation and senescence. The activation of the pathway can come from a membrane receptor that upon activation binds adaptors (PTPN11/SHP2, SOS1, and RASA1), which increase the proportion of the active form of a RAS protein (KRAS, HRAS). The activated RAS is able to activate the MAPK (RAF1) signaling cascade. The MAPK phosphorylates, among others, TSC1 and TSC2, and inhibits their function [24].

Therapeutic Targets

To date, no curative treatment exists for primary lymphedema. Symptomatic alleviation can be achieved by lymphatic drainage, elastic compression, and debulking surgery. The risk of infection is not negligible and should also be adequately managed. The numerous associated clinical features require their specific management.

Alternative treatments are being developed. Sorafenib, a tyrosine kinase inhibitor used in selected cancers, decreases vascular permeability by suppressing VEGFRs, and it is in a clinical trial for secondary lymphedema [25]. Another ongoing trial combines autologous lymph node grafts with adenoviral expression of VEGF-C [26]. It seems to improve the connectivity of the graft with the lymphatic system. Animal models are also developed and used to test inventive therapies. Plasmid-based expression of Hepatocyte growth factor (HGF) in rat-tail or in mice with induced upper limb edema inhibits the growth of swelling and lymphangiogenesis [27]. As there is high genetic heterogeneity within the causes of primary lymphedema, it would be interesting to identify if there is any common pathologic molecular alteration. This would allow a more general, targeted therapy, to be developed. For example, if the RAS/MAPK pathway would be altered in various patients in similar fashion independent of the underlying causative gene, molecules developed to treat cancer could be used for RASopathies and primary lymphedema [14].

Secondary Lymphedema

Secondary lymphedema is the most common form of lymphedema. It may be caused for example by infection, surgery, radiation, or injury. Secondary lymphedema occurs in approximately 30 % of breast cancer patients who undergo surgery or irradiation. Risk factors include the extent of surgery and irradiation, disease related factors (stage at diagnosis, pathological nodal status, and

number of dissected lymph node), and patient-related factors (age at diagnosis, body mass index, and presence of a sedentary lifestyle). A study suggests a link between germline mutations in CX47/GJC2 and the occurrence of secondary lymphedema [28]. Another study genotyped 155 patients and 387 controls without lymphedema for 17 candidate genes (including FOXC2, HGF, VEGFC, and VEGFR3, but not GJC2) [29]. A significant association was found with LCP2 (lymphocyte cytosolic protein 2), NRP2 (neuropilin 2), SYK (spleen tyrosine kinase), VCAM1 (vascular cell adhesion molecule 1), FOXC2, and VEGFC. However, other studies are needed to confirm the significance of these associations and to identify the nucleotide changes that are causative for the predisposition.

Conclusion

Although more than 20 genes have been found to be mutated in patients with primary lymphedema, they explain less than a third of all cases. However, all the 20 genes have never been exhaustively screened for any patient cohort, and the respective prevalences are likely underestimated. Moreover, detailed genotype–phenotype correlation studies have not been exhaustive, especially when lymphedema was not the major feature of the disease/syndrome. Although it remains important to look for all additional signs to orient diagnosis, the targeted panel approach, which allows to obtain results for all known genes at ones, will greatly help primary diagnosis and genotype–phenotype correlation studies.

References

1. Balboa-Beltran E, Fernandez-Seara MJ, Perez-Munuzuri A, Lago R, Garcia-Magan C, Couce ML, et al. A novel stop mutation in the vascular endothelial growth factor-C gene (VEGFC) results in Milroy-like disease. J Med Genet. 2014;51(7):475–8.
2. Gordon K, Spiden SL, Connell FC, Brice G, Cottrell S, Short J, et al. FLT4/VEGFR3 and Milroy disease: novel mutations, a review of published variants and database update. Hum Mutat. 2013;34(1):23–31.
3. Connell F, Brice G, Jeffery S, Keeley V, Mortimer P, Mansour S. A new classification system for primary lymphatic dysplasias based on phenotype. Clin Genet. 2010;77(5):438–52.
4. Finegold DN, Kimak MA, Lawrence EC, Levinson KL, Cherniske EM, Pober BR, et al. Truncating mutations in FOXC2 cause multiple lymphedema syndromes. Hum Mol Genet. 2001;10(11):1185–9.
5. Ferrell RE, Baty CJ, Kimak MA, Karlsson JM, Lawrence EC, Franke-Snyder M, et al. GJC2 missense mutations cause human lymphedema. Am J Hum Genet. 2010;86(6):943–8.
6. Brice G, Ostergaard P, Jeffery S, Gordon K, Mortimer PS, Mansour S. A novel mutation in GJA1 causing oculodentodigital syndrome and primary lymphoedema in a three generation family. Clin Genet. 2013;84(4):378–81.
7. Alders M, Al-Gazali L, Cordeiro I, Dallapiccola B, Garavelli L, Tuysuz B, et al. Hennekam syndrome can be caused by FAT4 mutations and be allelic to Van Malder gem syndrome. Hum Genet. 2014;133(9):1161–7.
8. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014;123(6):809–21.
9. Kawai T, Nishikomori R, Heike T. Diagnosis and treatment in anhidrotic ectodermal dysplasia with immunodeficiency. Allergol Int. 2012;61(2):207–17.
10. Au AC, Hernandez PA, Lieber E, Nadroo AM, Shen YM, Kelley KA, et al. Protein tyrosine phosphatase PTPN14 is a regulator of lymphatic function and choanal development in humans. Am J Hum Genet. 2010;87(3):436–44.
11. Irrthum A, Devriendt K, Chitayat D, Matthijs G, Glade C, Steijlen PM, et al. Mutations in the transcription factor gene SOX18 underlie recessive and dominant forms of hypotrichosis-lymphedema-telangiectasia. Am J Hum Genet. 2003;72(6):1470–8.
12. Moalem S, Brouillard P, Kuypers D, Legius E, Harvey E, Taylor G, et al. Hypotrichosis-lymphedema-telangiectasia-renal defect associated with a truncating mutation in the SOX18 gene. Clin Genet. 2014. Article first published online: 16 APR 2014, DOI: 10.1111/cge.12388.
13. Geffrey AL, Shinnick JE, Staley BA, Boronat S, Thiele EA. Lymphedema in tuberous sclerosis complex. Am J Med Genet A. 2014;164A(6):1438–42.
14. Rauen KA. The RASopathies. Annu Rev Genomics Hum Genet. 2013;14:355–69.
15. Romano AA, Allanson JE, Dahlgren J, Gelb BD, Hall B, Pierpont ME, et al. Noonan syndrome: clinical features, diagnosis, and management guidelines. Pediatrics. 2010;126(4):746–59.
16. Sybert VP, McCauley E. Turner's syndrome. N Engl J Med. 2004;351(12):1227–38.
17. Revencu N, Boon LM, Mendola A, Cordisco MR, Dubois J, Clapuyt P, et al. RASA1 mutations and associated phenotypes in 68 families with capillary malformation-arteriovenous malformation. Hum Mutat. 2013;34(12):1632–41.

18. Burrows PE, Gonzalez-Garay ML, Rasmussen JC, Aldrich MB, Guilliod R, Maus EA, et al. Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man. Proc Natl Acad Sci U S A. 2013;110(21):8621–6.

19. Mendola A, Schlogel MJ, Ghalamkarpour A, Irrthum A, Nguyen HL, Fastre E, et al. Mutations in the VEGFR3 signaling pathway explain 36% of familial lymphedema. Mol Syndromol. 2013;4(6):257–66.

20. Lepri FR, Scavelli R, Digilio MC, Gnazzo M, Grotta S, Dentici ML, et al. Diagnosis of Noonan syndrome and related disorders using target next generation sequencing. BMC Med Genet. 2014;15:14.

21. Brouillard P, Boon L, Vikkula M. Genetics of lymphatic anomalies. J Clin Invest. 2014;124(3):898–904.

22. Ivanov KI, Agalarov Y, Valmu L, Samuilova O, Liebl J, Houhou N, et al. Phosphorylation regulates FOXC2-mediated transcription in lymphatic endothelial cells. Mol Cell Biol. 2013;33(19):3749–61.

23. Wu YH, Hu TF, Chen YC, Tsai YN, Tsai YH, Cheng CC, et al. The manipulation of miRNA-gene regulatory networks by KSHV induces endothelial cell motility. Blood. 2011;118(10):2896–905.

24. Sevick-Muraca EM, King PD. Lymphatic vessel abnormalities arising from disorders of Ras signal transduction. Trends Cardiovasc Med. 2014;24(3): 121–7.

25. Moncrieff M, Shannon K, Hong A, Hersey P, Thompson J. Dramatic reduction of chronic lymphoedema of the lower limb with sorafenib therapy. Melanoma Res. 2008;18(2):161–2.

26. Honkonen KM, Visuri MT, Tervala TV, Halonen PJ, Koivisto M, Lahteenvuo MT, et al. Lymph node transfer and perinodal lymphatic growth factor treatment for lymphedema. Ann Surg. 2013;257(5):961–7.

27. Saito Y, Nakagami H, Morishita R, Takami Y, Kikuchi Y, Hayashi H, et al. Transfection of human hepatocyte growth factor gene ameliorates secondary lymphedema via promotion of lymphangiogenesis. Circulation. 2006;114(11):1177–84.

28. Finegold DN, Baty CJ, Knickelbein KZ, Perschke S, Noon SE, Campbell D, et al. Connexin 47 mutations increase risk for secondary lymphedema following breast cancer treatment. Clin Cancer Res. 2012;18(8):2382–90.

29. Miaskowski C, Dodd M, Paul SM, West C, Hamolsky D, Abrams G, et al. Lymphatic and angiogenic candidate genes predict the development of secondary lymphedema following breast cancer surgery. PLoS One. 2013;8(4):e60164.