LYMPHSPIRATION

DEVELOPMENTAL DISORDERS OF THE LYMPHATIC SYSTEM

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ABSTRACT

Approximately 67% of human conceptuses die prenatally. Of these, a significant number involve a disorder of the lymphatic system. A small number of live-born children also exhibit congenital lymphatic malformations, including an estimated 60% of patients with Turner syndrome. These observations have prompted a search for the genetic and dysmorphologic basis and the different patterns of congenital lymphedema and associated anomalies. In this article, we attempt to summarize available pertinent information on congenital disorders of the lymphatic system and to propose a conceptual overview of lymphatic development.

Fetal malformations may be separated into major categories based on their pathogenetic mechanisms of origin. Chromosomal aneuploidy affects 50% of human embryos (1), accounts for approximately 10% of congenital malformations in newborn infants (2), and affects approximately 1/200 live-births (2). Several of these aneuploidy syndromes involve a lymphatic abnormality such as Turner (3), Klinefelter (XXY), and trisomy 21 or Down syndromes. Single gene abnormalities and teratogenic exposure(s) during embryonic and/or fetal development may lead to malformation as well. Some "multiple malformation syndromes" are sporadic and of unknown cause. The Klippel-Trenaunay-

Weber and congenital lymphedema syndromes are among the most illustrative examples which are associated with developmental anomalies of the lymphatic system. These disorders are examined here along with other rarer multiple malformation syndromes in which lymphatic abnormalities may be a noteworthy feature.

CHROMOSOMAL ANEUPLOIDY AND LYMPHATIC MALFORMATION

Turner Syndrome

The XO karyotype is linked to a collection of anomalies known as Turner syndrome and occurs in as many as 4% of human conceptuses (2). The nonmosaic XO chromosome complement, however, is present in only 1/10,000 newborn females (4), implying that most XO conceptuses abort spontaneously during gestation. Among multiple malformation syndromes, Turner syndrome is most often associated with congenital lymphatic disorders (5-7). The manifestations may include cystic hygroma, hydrops fetalis and peripheral edema (Fig. 1). It is hypothesized that lymph channels distend secondary to delayed or failed emptying of jugular lymph sacs into central veins during embryonic development (8). In addition, some have suggested that many of the heart anomalies associated with Turner syndrome (aortic

Fig. 1. Aborted fetus with Turner syndrome demonstrating massive cystic hygroma (nape) and generalized hydrops. These in utero abnormalities likely relate to the web neck and peripheral lymphedema of newborns and children with XO Turner karyotypes.

coarctation, enlarged pulmonary artery, persistent ductus arteriosus, anomalous pulmonary venous connection, and bicuspid aortic valve) are traceable to encroachment *in utero* by adjacent distended or dysplastic lymphatics (8-10). Similarly, the characteristic webbed or wry neck of "Turner patients" may represent redundant skin which was once stretched by distended underlying lymphatics but later was relieved when truncal lymphatics finally joined with the venous system at an abnormally late time *in utero* (8). Other investigators have suggested that disordered intramyocardial lymphatic development leads to a cardiomyopathy and resultant altered hemodynamics, leading to observed congenital heart defects (11). Enlarged lymphatics of the thoracic cage may also contribute to the

Fig. 2. Forty-year old man with trisomy 21 chromosomal abnormality with massive lymphedema of the right leg. The dark discoloration is melanin pigmentation (? non-hairy nevus).

characteristic "shield chest" and widely-spaced nipples in these females (10). Finally, an increased incidence of urinary tract malformation may, at least in part, emanate from developmental hemodynamic changes brought about by lymphatic abnormalities at a critical period during maturation of the urogenital ridge.

Klinefelter Syndrome

Klinefelter syndrome, most often associated with a 47XXY karyotype, occurs in 1/500 live newborns (12). Although some external features may be apparent early in life, the diagnosis is usually made at puberty with its characteristic microorchidism and sterility.

Fig. 3. Klippel-Trenaunay syndrome in a 25-year-old man involving the right leg. Typically these patients have atresia or hypoplasia of the deep venous system, often lymphatic dysplasia, a diffuse portwine dermal hemangioma and bulky overgrowth of the affected extremity.

Recently, Hoyme and Byrne-Essif (13) described three children with Klinefelter syndrome, each of whom had initially presented with prenatal edema. Taken in conjunction with earlier observations, they suggest that lymphatic blockage or fetal hydrops is an occasional manifestation of this syndrome (13).

Trisomy 21

Down syndrome characteristically arises from a trisomy 21 chromosomal complement but translocations and mosaicism account for a small percentage. Cystic hygroma and lymphedema *(Fig. 2)* have occasionally been noted in these children (12).
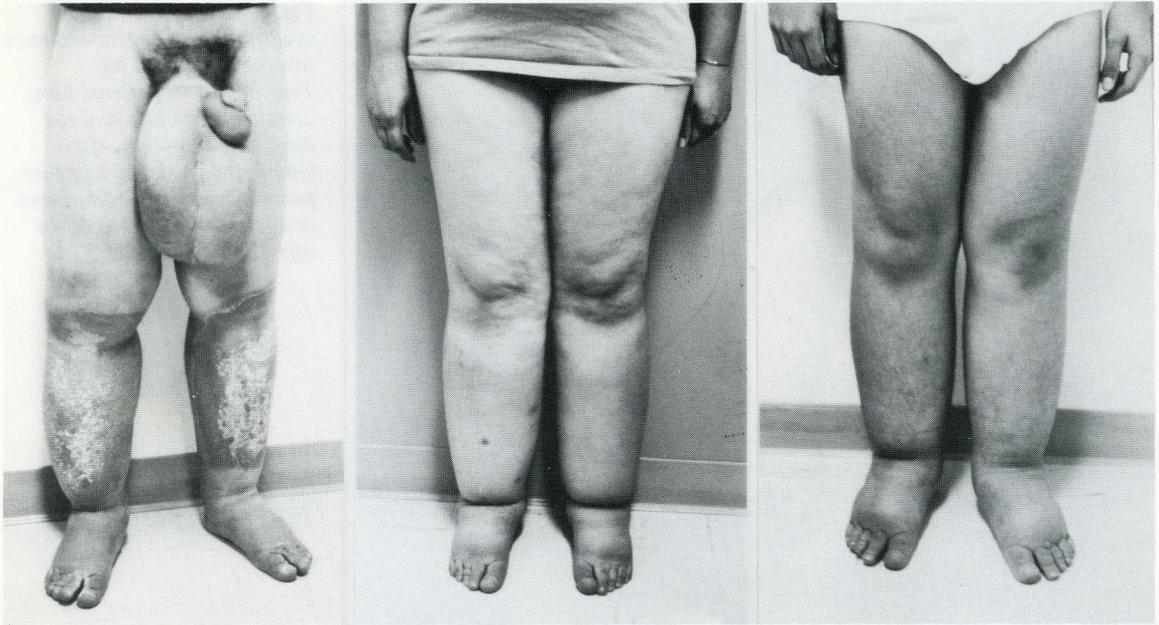
Fig. 4. Hereditary lymphedema (Milroy Disease) in brothers (right and left) and sister (middle) present since time of birth. Father, but not the mother, had lymphedema.

OTHER MULTIPLE MALFORMATION SYNDROMES AND LYMPHATIC ABNORMALITIES

Klippel-Trenaunay-Weber Syndrome

Klippel-Trenaunay (KT) and the rarer Parkes Weber (W) syndromes are panangiopathies associated with localized overgrowth of bone and soft tissue of a limb or portion of the trunk (Fig. 3). A variety of blood and lymph vascular malformations may be seen, including hemangiomas, arteriovenous malformations, port-wine stains, varicose veins, lymphangiomas, and lymphedema (2). Less commonly, extensive lymphatic dysplasia may occur in KTW (14,15).

Noonan Syndrome

Noonan syndrome has been suggested to have a "Turner-like" phenotype with similar manifestations that include peripheral lymphedema, hypoplastic nails, and shield chest. Other common features of Noonan syndrome include short stature, mental retardation, and downslanting palpebral fissures. Like Turner syndrome, patients with Noonan syndrome commonly exhibit congenital heart abnormalities. Most often these are right sided, whereas in Turner syndrome the cardiac abnormalities usually involve the left side of the heart. Noonan patients may also display abnormalities of the urinary and reproductive tracts, as well as intestinal lymphangiectasia and lymphangiomata (16). Despite similarities, Noonan syndrome is etiologically and clinically distinct from the Turner syndrome. Males and females are equally affected. Most cases are likely due to altered autosomal dominant gene(s), although a "Noonan phenotype" has been described in some patients with neurofibromatosis and with prenatal exposure to the anticonvulsant primidone (Mysoline®) (2,12).

Lymphedema I (Noone-Milroy-Type Hereditary Lymphedema)

This disorder presents as brawny edema usually of the lower extremity (Fig. 4). The diagnosis is made at birth, in contrast to Lymphedema II (see below). Tissue swelling occurs distally or proximally in the involved limbs, and either hypoplasia or hyperplasia of the lymphatics has been found. The described incidence is 1 per 6000 births. Lymphedema I may occur in association with microcephaly, with an incidence of 1-2 per 50,000. Less commonly, it occurs with distichiasis (supernumerary eyelashes), intestinal lymphangiectasia, or extradural cysts. The pattern of inheritance is autosomal dominant with variable expressivity. Father to son inheritance, excluding X linkage, has been reported.

Lymphedema II (Meige-Type Lymphedema)

This syndrome is similar to Lymphedema I but the onset of peripheral edema occurs during the second to the fifth decades. Like Lymphedema I, the legs are most commonly involved. Associated findings include distichiasis, cleft palate, and dystrophic yellow nails. Etiologic heterogeneity is likely, with most cases being sporadic, while some cases suggest an autosomal dominant pattern of inheritance. Lymphangiography reveals aplasia and/or hypoplasia of peripheral lymphatics with dilation of lymphatic trunks. About six kindreds have been reported with the inherited form but the primary type is more common (12).

Lymphedema-Hypoparathyroidism Syndrome

The major diagnostic criteria for this syndrome include congenital lymphedema (which develops soon after birth), hypoparathyroidism, nephropathy, mitral valve prolapse, and brachytelephalangy. Additional traits are short stature, dry thickened skin, and "bizarre" facies. From two adult males it has been suggested that it is transmitted as an autosomal recessive or X-linked trait (12,17). Chromosomal and Dysmorphogenic-Genetic disturbances associated with lymphatic disorders are summarized in *Tables 1 and 2*, respectively.

WHAT CAUSES CONGENITAL LYMPHEDEMA?

To develop a theory to explain lymphatic dysplasia *in utero*, it is first appropriate to examine normal lymphatic development. Early in this century, several investigators suggested that lymphatics initially arise independently in the mesenchyme and later form connections with the venous system (18). This scheme conforms with the explanation of Graham and Smith (19) for the morphogenesis of several varieties of congenital lymphedema. Termed the "jugular lymphatic-obstruction sequence," they propose that cystic hygromas, fetal hydrops, neck webbing and peripheral edema are the end result of malcommunication between the embryonic jugular lymph sacs and the central venous system (19). This hypothesis was later used by Hoyme and Byrne-Essif to explain rare cystic hygroma or fetal hydrops in children having a 47,XXY chromosome complement (13).

The theory of jugular sac lymphatic-obstruction in the morphogenesis of congenital lymphedema is supported by several observations. Among these is hypoplasia of lymphatics as seen on direct lymphangiography in many patients with these syndromes (21,22). In addition, Shaub et al (23) described a fetus having unilateral left-sided cystic hygroma and generalized edema *except for the right arm*, suggesting isolated blockage of the left jugular lymph sac and thoracic duct system but with retained intact lymphatic drainage from the right side. Moreover, in examination of seven fetuses with nuchal cystic hygromas, van der Putte (24) was unable to find a connection between the venous system and lymphatic network near the jugulo-subclavian junction.

TABLE 1: Chromosomal Aneuploidy Associated With Lymphatic Disorders

Syndrome	Lymphatic Malformation(s)	Other Features	Incidence/Other
XO Turner Syndrome	cystic hygroma, lymphedema, webbed neck, fetal hydrops, "shield chest" with widely-spaced nipples, intestinal lymphangiectasia, heart anomalies	streak ovaries, urinary tract malformation	1:5000 LB
Trisomy 18	cystic hygroma, lymphedema, webbed neck, widely-spaced nipples	developmental and mental retardation, failure to thrive, cryptorchidism, heart defects, renal and hip abnormalities	1:6600 LB; higher incidence with increased maternal age
Trisomy 13	cystic hygroma, lymphedema	triad of microphthalmia, cleft lip and palate, polydactyly; developmental retardation; undescended testes; heart, GU, GI and brain abnormalities, apnea	1:8000 LB; higher incidence with increased maternal age
Trisomy 21	cystic hygroma, lymphedema	characteristic facies hypotonia, short broad hands, heart defects, mental retardation maternal age	1:770 LB overall; higher incidence with increased maternal age
Duplication 11p	cystic hygroma, lymphedema, widely-spaced nipples	mental and growth retardation, heart and kidney abnormalities, umbilical or inguinal hernia	only 30 patients described; patient may carry a balanced reciprocal translocation
Triploidy	cystic hygroma, lymphedema	multiple congenital anomalies	1:10000 LB, (1% of all conceptuses)
Klinefelter (XXY) Syndrome	cystic hygroma, lymphedema	micro-orchidism, sterility	1:500 LB; higher incidence with increased maternal age
Trisomy 22	cystic hygroma, webbed neck	hemidystrophy, cardiovascular and renal abnormalities, streak gonads	not compatible with long life; 10% of abortions
13q-	cystic hygroma	low birth weight, microcephaly, severe psychomotor retardation, heart abnormalities	72:100,000 LB for ring chromosome 13; parent may carry a balanced reciprocal translocation
11q-	cystic hygroma	trigonocephaly, severe speech impairment, psychomotor retardation, heart abnormalities	just over 30 patients reported; parent may carry a balanced reciprocal translocation

LB = live births; GU=genitourinary; GI=gastrointestinal

TABLE 2: Dysmorphogenic-Genetic Disturbances Associated With Lymphatic Disorders			
Syndrome	Lymphatic Malformation(s)	Other Features	Incidence/Other
Klippel-Trenaunay (K-T)-Weber — (W)	lymphedema, lymphangioma	bone and soft tissue overgrowth, hemangiomas, A-V malformations, port wine stain, varicose veins	Common (K-T); rare (W); sporadic occurrence
Maffucci (Enchondromatosis and Hemangiomas)	lymphangiomas	coexisting hemangiomas and enchondromas, vitiligo, benign and malignant tumors	>100 reported patients (1990); sporadic occurrence
Neurofibromatosis, Type I (von Recklinghausen Type)	lymphedema (may coexist with a Noonan phenotype)	cafe-au-lait spots, neurofibromas, distinctive eye and bone findings, learning disabilities	1:3000 LB; autosomal dominant
Overgrowth, Bannayan Type	lymphangioma	megalocephaly; hamartomas; transient motor, speech and coordination delays	reported in ~15 kinships; autosomal dominant
Proteus	lymphangioma	lipomas, hemangiomas, megalencephaly, mental retardation, seizures, choristomas	undetermined - rare; sporadic occurrence
Noonan	lymphedema, "shield" chest, intestinal lymphangiectasia	heart abnormalities, GU abnormalities	1:2500-1:1000LB; autosomal dominant; may be etiologically heterogeneous
Lymphedema I (Nonne-Milroy Type)	lymphedema, intestinal lymphangiectasis	microcephaly, distichiasis, extradural cysts	1:6000 LB; autosomal dominant
Lymphedema II (Meige Type)	lymphedema	cleft palate, dystrophic yellow nails	Rarely genetic; sporadic instances more frequent
Distichiasis-Lymphedema	lymphedema	distichiasis, pterygium coli, vertebral anomalies	rare ~1:100,000; autosomal dominant
Lymphedema-Hypoparathyroidism	lymphedema	hypoparathyroidism, nephropathy, mitral valve prolapse, brachytelephangy	2 patients described

LB=live births; GU=genitourinary; GI=gastrointestinal

Hypoproteinemia as a causative factor in congenital lymphedema is not well substantiated, although Anders and Brace (25) found that hydropic fetuses produce plasma proteins at four times the normal daily rate. Theoretically the hyperproduction of protein is in response to a decreased plasma protein level compared with non-hydropic fetuses. On the other hand, Möise et al (26) found no difference in blood pressure, heart rate, hematocrit, serum osmolarity, pH and PaO_2 after a 41% reduction in plasma protein levels in six fetal sheep compared to twin controls. Furthermore, in examination of seven spontaneously aborted fetuses with cystic hygromas, van der Putte found the edematous fluid to be high in protein content (24). Hypoproteinemia alone also cannot readily explain the lymphatic disarray seen on oil-contrast lymphangiograms of fetuses with congenital lymphedema, including aplasia, hypoplasia, and even hyperplasia (12,22).

Van der Putte's meticulous examination of the development of the human lymphatic system in 1975 contradicted earlier work and complicated current understanding of the morphogenesis of lymphedema *in utero* (18). From multiple cross-sections of 40 human embryos between 8mm total length and 33mm crown-to-rump (CR) length (estimated age 35 to 65 days post-fertilization), van der Putte described "split-like communications" between primitive embryonic veins and sprouts which later were to become lymphatics. The endothelium lining the early veins and these sprouts also did not appear to differ. Van der Putte concluded that human lymphatics arise from primitive embryonic veins. According to this theory, the "sprouts" quickly acquire a lymphatic character, and the resultant "lymphatic primordia" go on to form interlymphatic connections. He further maintained that except for the early "split-like" communications between the primitive veins and the pre-lymphatic sprouts, no communication occurred between the venous and lymphatic systems. This description of lymphatic development conformed to earlier observations by Clark and Clark that ingrowth of new lymphatic vessels as seen in transparent chambers of a rabbit ear consistently occurred after the earlier development of blood vessels (27).

Van der Putte's observations raise a couple of hypotheses which are possible refinements of the jugular sac obstruction theory. One is that a proximal component of the prelymphatic "sprouts" and/or their connections with the venous system never sufficiently develops a bona fide channel, and thereby lymphedema occurs at the outset of gestation. Alternatively, lymphatics may develop early in the embryo but then are not maintained.

Whereas the morphogenesis of congenital lymphedema has yet to be firmly elucidated, even less is known about possible initiating events at a cellular level. A number of recently described growth factors, for instance, may exert substantial effects on newly developed endothelium. One intriguing substance is transforming growth factor beta (TGFß), which displays a wide range of effects depending on target cell type (28,29). In endothelial cells, for example, TGFß inhibits proliferation in vitro but stimulates it in vivo (30-33), suggesting that extracellular matrix, cell-cell interactions, or other circulating factors may contribute to the ultimate outcome on endothelial growth. TGFß has been isolated from a variety of tissue types, including bone marrow, fetal hepatic hematopoietic stem cells, thymus, and placenta (28,35). That TGFß is detected in both embryonic and placental tissue is particularly noteworthy with respect to congenital malformations. In light of the varied effects of TGFß on endothelium, it is conceivable that such a factor may be angiogenic in one microenvironment while anti-angiogenic in a different milieu.

Another growth factor incriminated in lymphangiogenesis is platelet-derived growth factor which stimulates endothelial cell growth and chemotaxis *in vitro* as well as angiogenesis *in vivo* (35). This substance has been detected in large amounts in human term placenta (36). Other angiogenic substances are fibroblast

growth factor (37-42), a human placenta-derived protein (43), angiogenin (44), wound fluid isolate (45), and prostaglandins PGE_1 and PGE_2 (44).

Macrophages also produce substances that are strongly angiogenic (46), while human lymphocyte-containing supernatants inhibit migration of endothelial cells (47). Indirect evidence also incriminates a lymphocyte-derived factor capable of initiating angiogenesis in vivo (48). The influence of the immune system in angiogenesis may be further deduced from the capillary proliferation that occurs in local graft-versus-host reactions and other immunological phenomena (45,49,50). Endothelial cells are also capable of antigen presentation as shown by the production of surface Ia antigens by endothelium after stimulation with gamma-interferon or activated T cells (51,52).

Mechanical disruption of the endothelium lining has long been known to increase microvascular leakiness, which with an inflammatory response causes further extravasation of plasma with its proteins into the surrounding tissue (52). Platelet-derived growth factor (PDGF) is also released from the damaged endothelial cells and has both mitogenic and vasoconstrictor properties (53). These findings taken together suggest that a genetic or other ubiquitous subcellular element could cause sufficient perturbation in endothelial cells to initiate a cascade of events similar to those observed in blood vascular injury. Lymphedema associated with either abnormal lymphangiogenesis secondary to released mitogenic factors and/or increased "leakiness" of damaged lymphatics is a plausible outcome of these phenomena.

Although a variety of known promoters and inhibitors of angiogenesis have been studied extensively with reference to the blood vascular system, such complex interactions may also be involved in lymphangiogenesis. Anders and Brace (25) note that hydrops fetalis in the sheep occurs in only one out of eleven fetuses after ligation of major lymphatics but in contrast appears in five out of five fetuses after excision of lymphatic trunks. They propose rapid growth of collaterals in the lymphatic-ligated fetuses as the explanation for circumvention of fluid accumulation (25). As for the role of the immune system in lymphatic (dys)function, Heinisch proposes (3) that acute skin infection (erysipelas) with involvement of cutaneous lymphatics (lymphangitis) initiates lymphedema in tissues already compromised by lymphatic dysplasia and suppressed immunity responsiveness. Of note, Patterson et al (54) described limb inflammatory changes in sixteen dogs with hereditary lymphedema. The rare occurrence of malignancy in these syndromes (12) may also reflect faulty immunity and in the case of congenital lymphedema, may be a factor in the origin of the lymphedema itself. Abnormalities of blood vessel walls in patients with lymphedema also occur (54), which would be expected if ubiquitous angiogenic factors, such as the ones just described, are involved in the dysmorphogenesis of congenital lymphedema.

Another critical consideration in the evolution of congenital lymphedema is the chromosomal complement contained within cells. For instance, the efforts of Anders and Brace (25) to produce hydrops fetalis via lymphatic ligation and excision along with numerous reports of spontaneous regression of congenital lymphedema suggest that genes may have greater bearing on the rate of lymphatic growth than on lymphatic morpho-genesis. In Turner syndrome, lymphatic "growth genes" may be located on the X chromosome and would conform to the phenotypic features of XO. In males, this "deficiency" may be offset by a lymphatic growth factor on the Y chromosome. On the other hand, the gene for human platelet-derived endothelial cell growth factor (hPD-ECGF) has been localized to chromosome 22 (55). Given the complexity of lymphangio-genesis, several chromosomes are probably involved and may account for the common manifestation of lymphedema in a variety of

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genetic disorders.

Despite the vast wealth of knowledge already amassed in lymphology, understanding the origin and development of congenital lymphedema at the subcellular, cellular, and even tissue level is still rudimentary. Research is hampered by the limited availability of human specimens. Whereas studies in other species have yielded notable leads, the findings can only be applied to humans with caution (54). Extrapolations from acquired to congenital lymphedema also must be cautionary as, for example, the flow dynamics of lymph in the human fetus is considerably different from that in the adult (56). Nonetheless, the occurrence, persistence, and disappearance of congenital lymphedema is inextricably linked to a wide array of biological phenomena and elucidation of their interaction is essential to formulating new strategies for its prevention and treatment.

ACKNOWLEDGMENTS

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Now a resident in pediatrics, Dr. Greenlee completed this review and concepts for a medical student thesis and American Heart Association research fellowship. The coauthors later embellished the manuscript.

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