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PHENOTYPIC AND GENOTYPIC HETEROGENEITY IN FAMILIAL MILROY LYMPHEDEMA

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ABSTRACT

Familial Milroy lymphedema (ML) is classified as an autosomal dominant disorder characterized by peripheral edema of the lower extremities at birth or in early childhood. The variety of phenotypes are not well described, and the genomic location and functional expression of the gene or genes underlying this and related familial lymphedema syndromes remain largely unknown. In this collaborative study between the University of Arizona and the University of São Paulo, we collected clinical pedigrees on 6 ML families, carried out clinical examination of affected and unaffected individuals, and, in representative affected members of two of the families performed dynamic lymphangioscintigraphy (LAS) of the lower and upper limbs to delineate further the ML lymphangiodysplastic phenotype. To localize the gene for ML, we conducted a genome-wide search in 4 of the families using 387 polymorphic dinucleotide-repeat markers at approximate l0 cM spacing in 54 subjects (affected, unaffected bloodline relatives, and spouses). In all 6 families (86 subjects), we specifically examined the suggested linkage to the vascular endothelial growth factor (VEGF)-C receptor (Flt4) gene localized to the chromosome region 5q34-q35. The findings provide evidence for a spectrum of ML clinical and LAS phenotypes and also suggest ML locus heterogeneity.

Beginning in l865, Letessier (1), Nonne (2), and Milroy (3) successively reported family clusters of patients with lower limb swelling beginning at birth or later in childhood but before puberty (usually termed Milroy or Nonne-Milroy syndrome). Post-pubertal forms are usually classified as Meige (4) or Letessier-Meige syndrome. In Milroy's original description (3), family members generally exhibited bilateral leg edema at birth which was most prominent below the knee and often associated with repeated limb infections throughout life. Later case reports (reviewed in 5-8) expanded the clinical spectrum to include swelling of the genitalia, upper limbs, and face and, infrequently, also involvement of other organ systems including the heart and blood vasculature. It was not, however, until the development of contrast lymphography and its wide application to the delineation of congenital and acquired disorders of the lymphatic system by John Kinmonth in the l950's that an underlying lymphatic structural defect in Milroy lymphedema (ML) was clearly documented (5). Typically, lymphatic truncal aplasia or hypoplasia with minimal or no dermal collateral formation is depicted but the level and degree of lymphatic dysplasia varies and occasionally different imaging patterns have been described using conventional lymphography, fluorescent lymphangiography (9), and lymphangioscintigraphy (10,11).

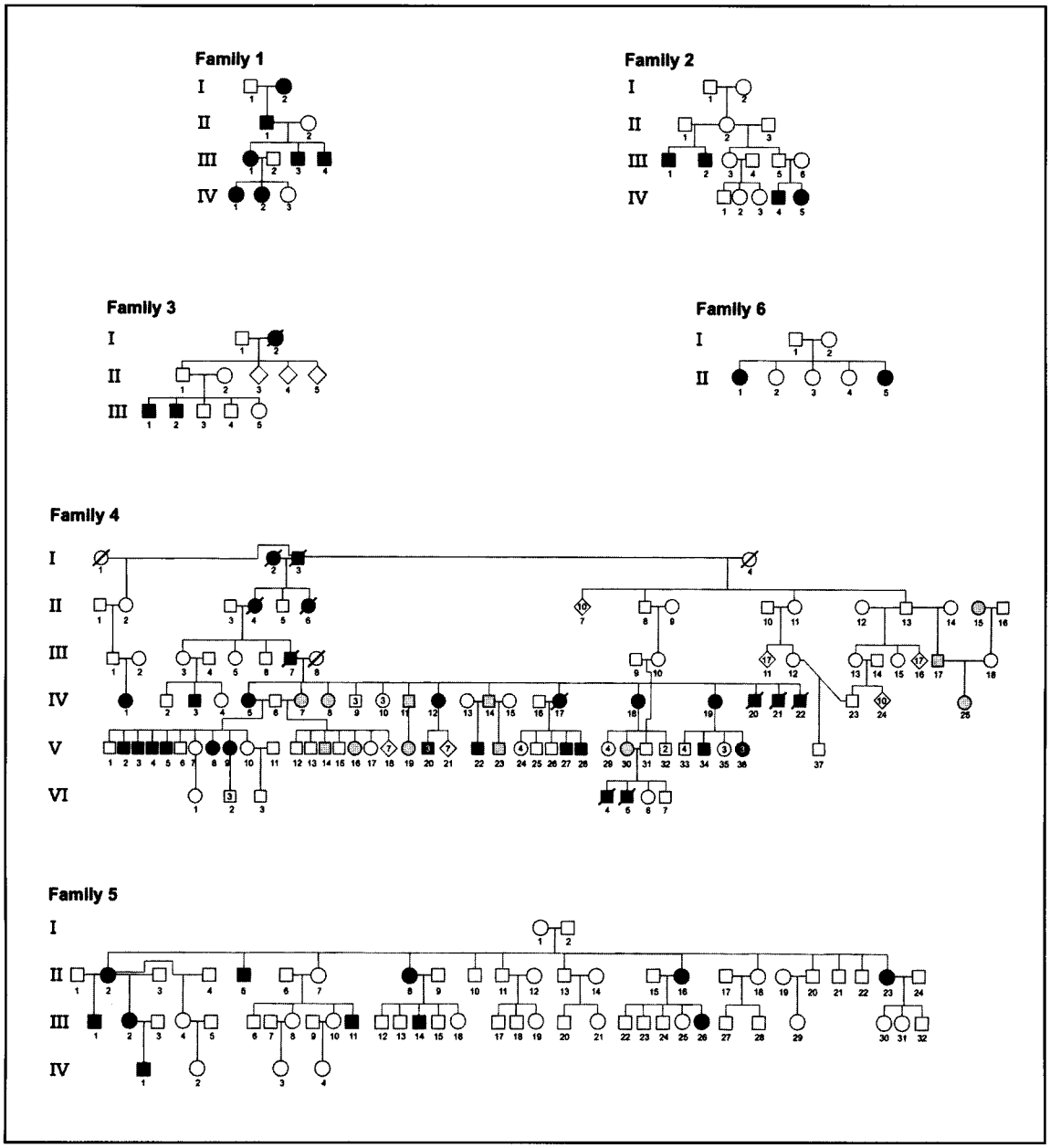

Fig. 1. Pedigrees of the 6 families used for linkage analysis. Affected individuals are indicated by blackened symbols, shaded grey for questionably affected, diagonal cross for deceased individuals, female (○), male (□) , and unknown sex (Δ).

Hereditary lymphedema of the Milroy type is classified as a rare (1:6000 births) autosomal dominant condition with incomplete penetrance (5). Whereas the gene(s) for this condition have not been previously localized to a specific gene locus or even chromosome, a recent brief communication suggests that in several families with hereditary lymphedema, there is a close linkage to the 5q34-q35 region of chromosome

5 (12). Interestingly, mutations in the gene for Flt4, the receptor for VEGF-C (termed the lymphatic growth factor), were identified only in affected and at risk individuals.

The current report describes the pedigrees and phenotypic features of 6 families of diverse ethnic origin with hereditary lymphedema of the Milroy type. A genome-wide search using polymorphic markers spaced at approximately 10 cM with linkage analysis was carried out in 4 of the families and specific linkage with a marker at the suggested 5q34-q35 region of interest examined in all 6 families. The findings, taken in the context of the complex sequence of events in the development of the lymphatic and blood vasculature, support the likelihood of genotypic as well as phenotypic heterogeneity in hereditary lymphedema syndromes.

SUBJECTS AND METHODS

Studies were carried out according to guidelines approved by the Institutional Review Boards of The University of Arizona and University of São Paulo, and informed consent was obtained from all subjects.

Six families with hereditary lower limb lymphedema (2 families from The University of Arizona and 4 from the University of São Paolo) were studied. Clinical pedigrees *(Fig. 1)* were established by detailed history and physical examination and potential usefulness for linkage studies (estimated maximum and mean LOD scores) calculated by SIMLINK analysis (13,14). In 7 affected individuals from the two University of Arizona families, lymphangioscintigraphy (tracer 99m-Tc human serum albumin) was performed in the lower limbs and in 3 of the 7 individuals also in the upper limbs as previously described (15) to evaluate structural and functional abnormalities of the peripheral lymphatic system. In 1 of these 7 patients, Doppler ultrasound venography was performed to evaluate patency and competence of the deep and superficial venous systems of the lower limbs.

Blood was collected from 86 members of the 6 families, and DNA extracted by standard techniques. Genome-wide genotyping was performed in Families 1-4 of the 6 families in collaboration with the Marshfield Medical Research Foundation under a Mammalian Genotyping Service subcontract from the National Heart, Lung, and Blood Institute of the National Institutes of Health. A panel of 387 polymorphic dinucleotide repeat markers spaced at approximate 10cM intervals with average 0.74±0.07 heterozygosity was used for genome-wide screening by polymerase chain reaction (PCR) amplification in samples from 54 members of these 4 families.

Based on the report of Kimak et al (12) of linkage with markers at 5q34-q35, we examined the data from our genome-wide search and also typed 2 new families (Families 5 and 6) (total 86 individuals) for marker 164xb8 (a short tandem repeat) at locus D5S408 in the 5q34-q35 chromosome region of interest. Two-point linkage analysis was carried out using the computer program MLINK from the Linkage Package version 5.1 (16) assuming autosomal dominant inheritance with 50% penetrance and 0.001 disease gene frequency. Positive LOD scores were tested for robustness with respect to variation in penetrance, prevalence of phenocopies and variable marker-allele frequency.

RESULTS

Family Pedigrees

Fig. 1 displays the pedigrees for the family members in this study. The University of Arizona component consisted of Families 1 and 4, the latter a large kindred. The University of São Paulo component consisted of 4 families (2,3,5, and 6). The cumulative SIMLINK estimated maximum LOD scores at $\theta=0$ for all pedigrees was 15.355 *(Table 1)*.

Whereas the clinical features of the affected individuals within each family were quite similar and basic similarities were

TABLE 1
Estimated Maximum and Mean LOD Score* (SIMLINK) for Any Marker and Two-Point LOD Score (MLINK) for D5S408 Marker at θ = 0 by Family

Family	1	2	3	4	5	6
Estimated Maximum LOD score	1.479	1.483	0.589	5.286	5.336	1.182
Estimated Mean ± SE LOD score	0.490±0.022	0.549±0.024	0.183±0.013	1.759±0.068	2.108±0.068	0.381±0.025
Two-point LOD score	-0.870	0.900	-3.176	-0.679	-3.159	NI**

*LOD score of 1.0 indicates ten-fold greater probability of the observed result due to linkage rather than chance.
**Non-informative

TABLE 2
Summary of Clinical Features of ML-Affected Family Members Studied

Family	Clinical Features
1	Childhood onset (pre-pubertal) (5/5)*; bilateral leg (4/5) and genital (2/2 males) involvement, severe (4/5); recurrent cellulitis (5/5)
2	Congenital (4/4), bilateral leg involvement (2/4), severe (0/4)
3	Congenital (2/2), bilateral leg involvement (1/2), severe (1/2)
4	Congenital (9/12), age 6-13 onset (3/12); bilateral leg (10/12), genital (3/7 males), arm or face involvement (7/12), severe (5/12); recurrent cellulitis (11/12); juvenile varicose veins (5/12)
5	Congenital (6/6), bilateral leg involvement (5/6), severe (0/6)
6	Congenital (2/2), bilateral leg involvement (1/2), severe (0/2)

*Numbers in parentheses represent individuals exhibiting feature/total affected individuals studied.

shared among the families, distinctive features were also found *(Table 2)*. Lymphedema appeared almost exclusively at birth or in infancy in Families 2-6 and somewhat later in childhood but prior to puberty in Family 1 (mean age 9). Males and females were equally affected, and lower limb involvement was bilateral in almost all cases. In Families 1 and 4, genital lymphedema was common in the males but absent in Families 2, 3, 5, and 6.

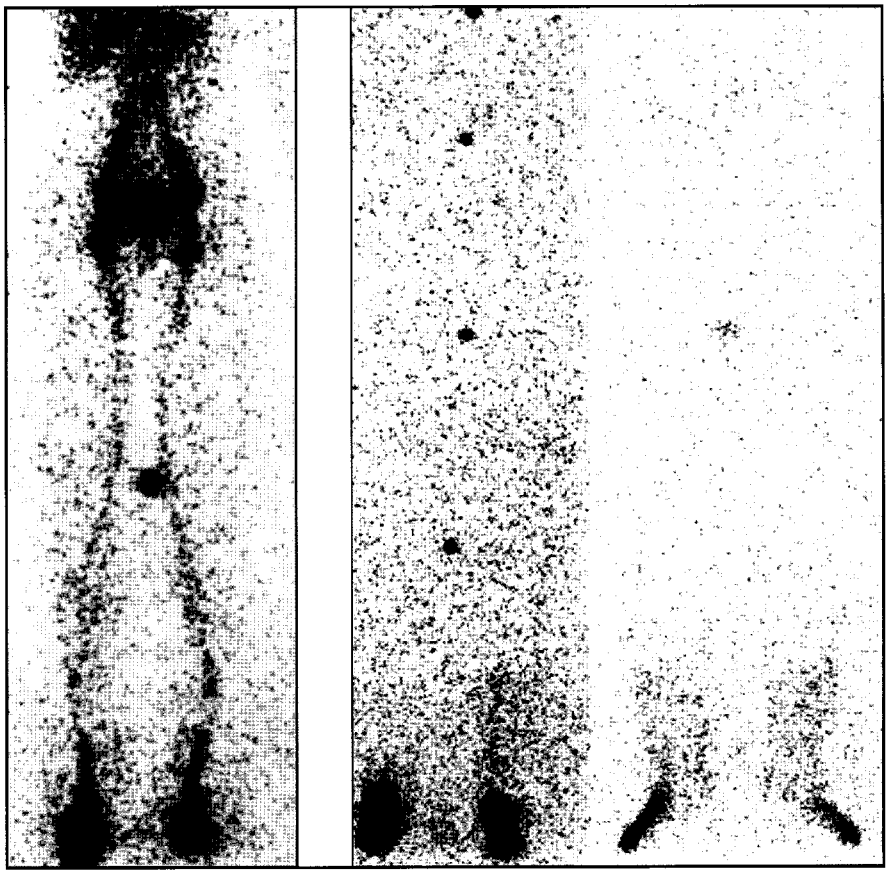

Fig. 2. Left, lower extremity ("whole body") lymphangioscintigram(LAS) in a normal subject showing intact peripheral and retroperitoneal lymphatic trunks (solitary bands) and "hot spots" representing regional lymph nodes. Marker is located at the knees and tracer filling the bladder is noted on this delayed film. Right, LAS (early-late image sequence) of the lower extremities in a 40-year old man with bilateral lymphedema of the legs showing minimal tracer transport consistent with severe truncal aplasia/hypoplasia typical of ML. Markers located at knees, pubis, xiphoid, and sternal notch. This patient's son, father, grandfather and male cousin, but none of the female family members, also exhibited lymphedema of the legs since infancy.

Only Family 4 displayed swelling of the upper limbs and face in some members. Families 1 and 4 reported frequent episodes of cellulitis of the involved limbs. Only Family 4 ML affected individuals (5 of 12 studied) also displayed prominent varicose veins of the lower limbs present since infancy or early childhood. One "unaffected" member of Family 4 (IV-25) exhibited right forequarter hypertrophy with macrodactyly (a Proteus or Klippel-Trenaunay syndrome variant associated with lymphatic and venous dysplasia).

Fig. 2 shows a "whole body" lymphangioscintigram (LAS) in a normal subject (left) for comparison with an LAS (right, early followed by late image) displaying truncal aplasia/hypoplasia in a male member of another Arizona ML family not reported here.

In Family 1 *(Fig. 3)*, the characteristic brawny swelling of the lower limbs is displayed in two successive generations (left, upper and lower) along with a hypoplastic pattern in both lower limbs of one affected family member *(right)*.

In Family 4 *(Fig. 4)*, the clinical appearance (A) and lymphangiodysplastic

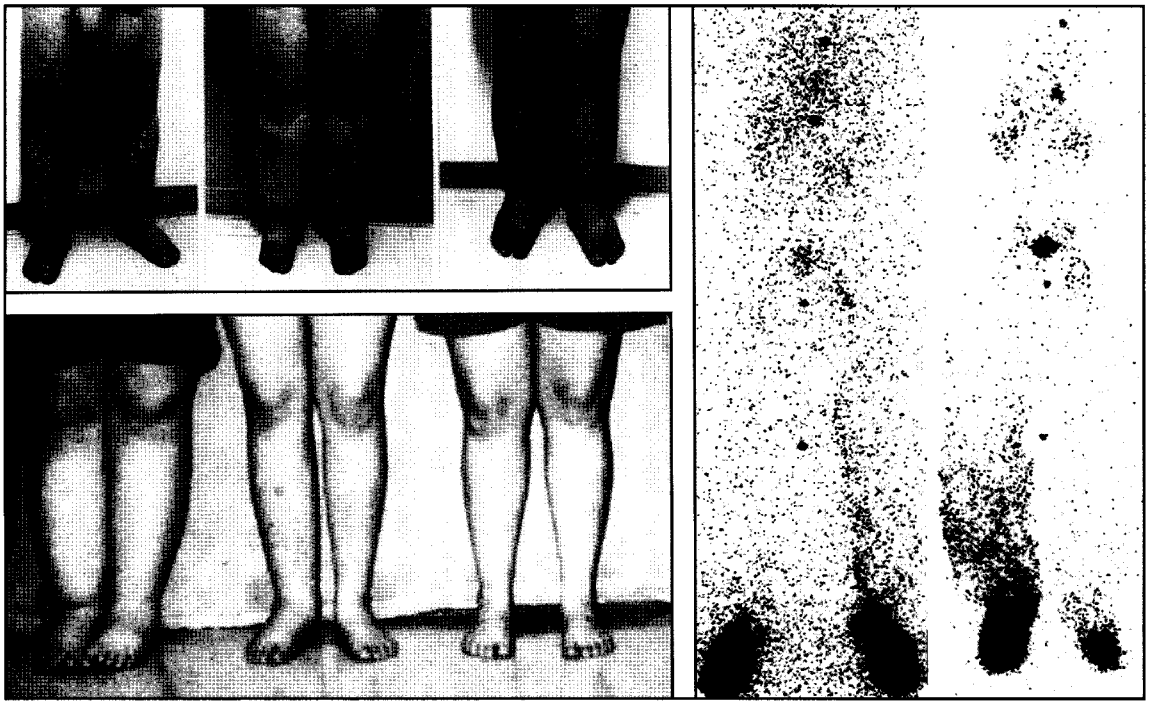

Fig. 3. Family 1: Left upper panel, lymphedema of legs and genitalia first appearing in early childhood in two brothers (III-4, III-3) and of legs alone in sister (III-1) (see Fig. 1 for pedigree). Left lower panel, two of three daughters (IV-1, IV-2) of the sister also have lower extremity lymphedema, which similarly first appeared in childhood and was associated with recurrent lymphangitis/cellulitis. One other daughter (IV-3) (right) is unaffected. Right, LAS (early-late image sequence) in subject III-4 showing aplasia (right leg) and hypoplasia (left leg) with only faint visualization of limb lymphatic trunks and lack of radiotracer in regional lymph nodes or retroperitoneal lymphatics.

LAS patterns (including irregular channels, dermal backflow and reflux) in the lower (B-F) and also the upper limbs (E,F) are shown in five affected individuals. In another brother of the subjects depicted in *Fig. 4 A-E*, who exhibited bilateral lower extremity lymphedema and prominent venous varicosities since infancy, a similar lymphangiodysplastic LAS pattern combined with an abnormal Doppler venogram was seen *(Fig. 5)*. These lymphatic and venous imaging studies document a combination of lymphatic dysfunction with deep and superficial venous reflux in both legs.

The genome-wide search strongly suggested locus heterogeneity because there were strong signals in some families with various markers while the signals were strongly negative in others (considering the size of the families). As seen in *Table 1*, only Family 2 showed a possible linkage to marker 164xb8 at locus D5S408 in the suggested 5q34-q35 region (LOD score 0.900 of 1.483 maximum possible). On the other hand, Families 3 and 5 were substantially negative, and Families 1 and 4 were slightly negative—the findings in Families 4 and 5 being the most significant given their large size and high maximum LOD scores by SIMLINK analysis. Family 6 was non-informative as the family members studied did not vary at the locus.

DISCUSSION

The collected literature on hereditary lymphedema syndromes (1-8; 17-21) describes a wide clinical spectrum not only in terms of

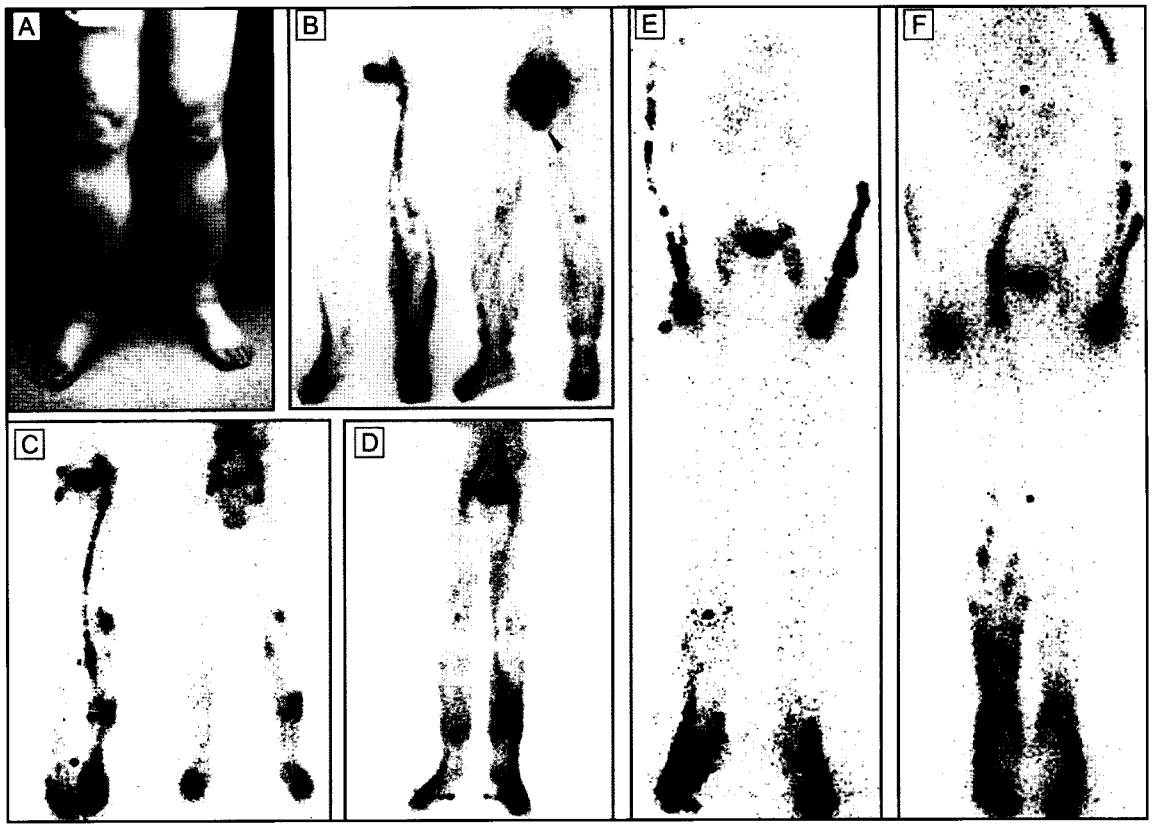

Fig. 4. Family 4: (A) Congenital lymphedema of the legs in a young man (V-3),whose LAS (early-late image sequence) (B) shows severe truncal aplasia/hypoplasia in the right leg and delayed transport, tracer pooling and faint truncal and regional lymph node visualization on the left with extensive dermal diffusion of tracer and late scrotal reflux (arrowhead). Three brothers V-5 (C, early-late sequence), V-4 (E), V-2 (see Fig.5, left) with ML show similar LAS features including in C scrotal reflux (arrowhead) and in E a normal appearing non-edematous left upper limb, in which no trunks or lymph nodes are visualized above the upper forearm. One sister (V-8) (D) shows a similar LAS pattern of delayed tracer transport, bizarre lymphatic trunks and late tracer pooling as does a distant relative (IV-1) (F), who also displays tracer pooling in the right forearm.

female sex predilection, age of onset, severity, bilateral or unilateral lower limb involvement but also accompanying swelling of the upper limbs, genitalia, and serous effusions as well as associated features such as distichiasis (double row of eyelashes), mental retardation, epilepsy, immunodeficiency, cholestasis, cardiac and blood vascular abnormalities. This study provides further support for the broad phenotypic spectrum even within the Milroy classification, while confirming the equal sex distribution and predominant bilaterality of lower limb involvement in families exhibiting lymphedema at birth or in early childhood in contrast to those with lymphedema precox and tarda, which show a strong predilection for the female sex and predominant unilateral lower limb involvement. Whereas simple single gene autosomal dominance with incomplete penetrance has been considered the likely mode of inheritance, genetic mechanisms may be more complex, heterogeneous and even polygenic.

Lymphedema is an overt manifestation of lymphatic circulatory "failure" stemming from an underlying lymphatic structural and functional disturbance (lymphangiodysplasia),

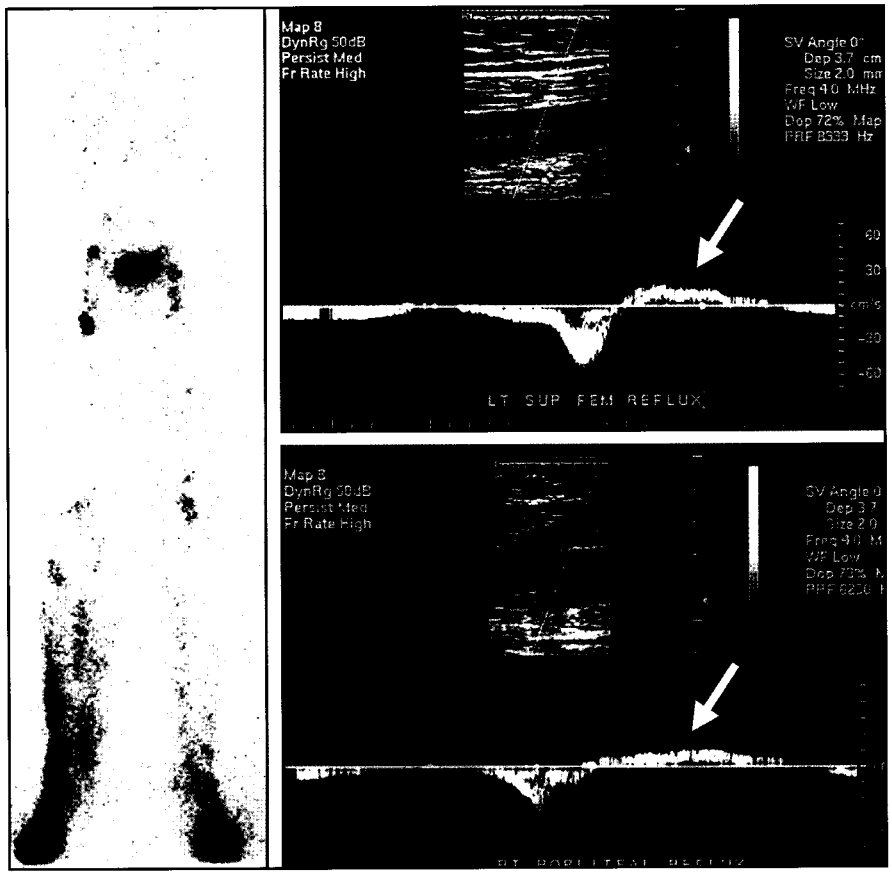

Fig. 5. Family 4: Abnormal LAS(left) in another ML brother (V-2) of the subjects depicted in Figs. 4A-E showing delayed tracer transport, irregular lymphatic channels, late tracer pooling and poor regional nodal visualization. Doppler ultrasound venogram (right) performed to evaluate prominent superficial venous varicosities of the legs since infancy, displays severe reflux throughout the superficial and deep venous system of the lower extremities including left femoral (upper) and right popliteal veins (lower) seen here as a prolonged reverse flow phase (arrows).

the phenotypic and genotypic spectrum of which remains to be better defined in the familial syndromes (11). In the absence of a more specific definition of the fundamental pathophysiologic disturbances, the assignment of "unaffected," "carrier," "age of onset," or "extent" of lymphatic involvement remains imprecise or even incorrect in pedigree analysis and may only be revealed by an "accident" of localized injury or infection precipitating overt swelling. Thus, a meaningful determination of the extent or level of lymphatic involvement, divergent structural or functional lymphatic capillary, truncal, and nodal defects, number of non-edematous limbs "affected," exact mode of inheritance, and degree of gene penetrance depends upon further evaluation beyond physical evidence of the characteristic brawny swollen leg.

Lymphatic imaging offers the next important step to define the "lymphangiodysplastic phenotype" in these families in terms of truncal aplasia/hypoplasia or hyperplasia, valvular incompetence with reflux, obstructive features, and lymph node abnormalities. The refined method of radioisotope lymphangioscintigraphy (15) provides a simple, safe, minimally invasive method to open a window on the

lymphangiodysplastic phenotype. The situation today with primary "lymphogenic" edema (i.e., lymphedema) is analogous to the designation of "cardiogenic" edema in bygone days. It remained for cardiovascular imaging and quantitative measurement of cardiovascular functions (beginning with cardiac catheterization and now elaborated by molecular techniques) to delineate in great detail a spectrum of cardiovascular "phenotypes" and most recently to provide the basis for "genotypes" of an increasing number of hereditary cardiovascular disorders. Several of these syndromes previously were described only by their nonspecific symptoms and signs when the heart "failed" and "cardiogenic" edema accumulated in the limbs, serous cavities, and viscera. In a real sense, the search for the genes involved in hereditary lymphedema syndromes is a search for "lymphangiodysplasia genes" or "lymphangiogenesis genes" rather than "lymphedema genes."

The range of lymphangiodysplastic phenotypes displayed on LAS in Families 1 and 4 included absence or paucity of lymphatic trunks, abnormal distribution of collectors, lymphatic reflux and incompetence, decreased and increased lymph nodal uptake, and mixed lymphatic and blood vascular disturbances (e.g., combined lymphatic and venous dysplasia/reflux). Of interest, "uninvolved" limbs which were either never swollen *(Fig. 4C, left leg)* or not swollen at the time of study *(Fig. 4E, left arm)* nonetheless exhibited distinct lymphatic structural and functional abnormalities that were not clinically apparent by history and physical examination alone. This situation of "asymptomatic" lymphatic disease is analogous to "asymptomatic" or "silent" heart disease and emphasizes the critical importance of lymphatic imaging in accurate phenotyping of family pedigrees.

Lymphangiodysplasia represents an abnormal pattern of development or growth of the lymphatic vasculature. Great attention has been directed toward understanding vasculogenesis (formation of primitive vascular networks from mesenchymal progenitors) and angiogenesis (capillary sprouting from preexisting vessels) over the past several decades since this phenomenon was reproduced *in vitro* in endothelial cell and mixed vascular tissue cultures. The focus, however, has been almost entirely on blood vessel growth (what we have termed "hemangiogenesis") (22). The analogous process in the lymphatic vasculature ("lymphangiogenesis") has received scant attention even though lymphatic (re)generation is both vigorous and essential, clinical disorders of lymphangiogenesis are not uncommon, and controversy persists as to whether lymphatics and veins arise from a common primordium in the jugular anlage (centrifugal theory) or independently from tissue mesenchyme (centripetal theory) (22-25). Only in the past few years, as the complex sequence of molecular events in angiogenesis and vasculogenesis is being unraveled, a cascade of vascular growth factor ligands and their respective transmembrane receptors has been identified (26). Moreover, a specific growth factor and its receptor have been isolated that target and characterize the lymphatic vasculature—namely, vascular endothelial growth factor (VEGF)-C and its receptor Flt4 on lymphatic endothelial cells (27-30). Nonetheless, some overlap in function with the blood vasculature at least during development or in pathologic states is likely.

Kimak et al (12) have recently shown in 3 large families a highly significant LOD score for linkage of hereditary lymphedema with the VEGF-C receptor (Flt4), which is located in the distal region of the long arm of chromosome 5 (31-35). Their abstract mentions mutation analysis in 9 additional families but mutations were only found in 3 families, presumably the same 3 showing the linkage. Thus, locus heterogeneity is suggested by this brief report. Although the mutations segregated with the phenotype were nonconservative (Pro 647 Ser, Pro 1126

Leu), whether these are gain or loss of function mutations is unclear. Overexpression of VEGF-C, driven by a keratin promoter in transgenic mice, resulted in lymphatic endothelial proliferation and lymphatic channel enlargement but not blood vessel prominence (34). It might be predicted that increased expression of the VEGF-C receptor (*VEGFR-3* is the name used in place of Flt4) could also lead to increased lymphangiogenesis. The homozygous knockout for *VEGFR-3* resulted in early embryonic death with many blood vascular abnormalities (35). However, the heterozygote was not noted to display any lymphatic abnormalities. Thus, it seems more likely that the two mutations identified in 3 families (12) are gain of function mutations. This narrow mutational spectrum may suggest that the range of tolerable mutations in *VEGFR-3* is limited. It is also of interest that of our Families 2, 3, and 5 who had similar clinical phenotypes of congenital onset, predominantly bilateral lymphedema of the lower limbs which did not become severe, only Family 2 has a possible linkage to *VEGFR-3*.

As the search for the "lymphangiogenesis gene(s)" continues, it will be of great interest to see what other genes are involved in hereditary lymphedema and mixed vascular syndromes (note Family 4 with combined lymphatic and venous abnormalities) as well as in chromosomal aneuploidies associated with lymphedema (11,36). How these genes interact with variations in the VEGF-C receptor, with the whole VEGF family and other vascular growth factors and their respective receptors, and with environmental influences should not only elucidate fundamental aspects of lymphatic and blood vascular growth and development but also provide the basis for new approaches to the detection, evaluation, prevention and treatment of these vexing, disabling, and at times life-threatening disorders.

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REFERENCES

1. Letessier, EE : *De l'elephantiasis des Arabes et de son hérédité.* Thèse à la Faculté de Médicine de Strasbourg, 1865.
2. Nonne, M: Vier falle von elephantiasis congenita hereditaria. Virch Arch Pathol Anat 125 (1891), 189-196.
3. Milroy, WF: An undescribed variety of hereditary edema. NY Med J 56 (1892), 505-508.
4. Meige, H: Dystophie oedemateuse héréditaire. Presse Méd 6 (1898), 341-343.
5. Kinmonth JB, ed. *The Lymphatics: Diseases, Lymphography and Surgery.* Edward Arnold, London, 1972, pp. 143-152.
6. Heinisch, H-M: Pediatric lymphangiology. In: *Lymphangiology.* Foldi, M, JR Casley-Smith (Eds.) Schattauer, Stuttgart and New York, 1983, pp. 777-783
7. Papendieck, CM: *Atlas Color: Temas de Angiología Pediátrica.* Editorial Médica Panamericana, S.A., Buenos Aires, Argentina, 1992.
8. Greenlee, R, H Hoyme, M Witte, et al: Developmental disorders of the lymphatic system. Lymphology 26 (1993), 156-168.
9. Bollinger A, G Isenring, UK Franzak, et al: Aplasia of superficial lymphatic capillaries in hereditary and connatal lymphedema (Milroy's Disease). Lymphology 16 (1983), 27-30.
10. Jackson, F, P Bowen, B Lentle: Scintilymphangiography with 99m Tc-antimony sulfide colloid in hereditary lymphedema (Nonne-Milroy Disease). Clin. Nucl. Med. 3 (1978), 296-298.
11. Witte, MH, R Erickson, FA Reiser, et al: Genetic alterations in lymphedema. Phlebolymphology 16 (1998), 19-25.
12. Kimak, MA, EC Lawrenc, KL Levinson, et al: Linkage and mutation in the vascular endothelial growth factor-C receptor (FLT4) gene in hereditary lymphedema. Am. J. Human Genetics 63 (1998), A34.

13. Ploughmnan, LM, M Boehnke: Estimating the power of a proposed linkage study for a complex genetic trait. Am. J. Hum. Genet. 44 (1989), 543-551.
14. Boehnke, M, LM Ploughman: SIMLINK: A program for estimating the power of a proposed linkage study by computer simulation. Version 4.1, April, 1990 and Version 4.2, April 2, 1997.
15. McNeill, G, MH Witte, C Witte, et al: Whole body lymphangioscintigraphy: The preferred method for the initial assessment of the peripheral lymphatic system. Radiology 172 (1989), 495-502.
16. Lathrop, GM, JM Lalouell, C Julier, et al: Strategies for multilocus linkage analysis in humans. Proc. Nat. Acad. Sci. 81 (1984), 3443-3446.
17. Esterly, J: Congenital hereditary lymphedema. J. Med. Gen. 2 (1965), 93-98.
18. Ersek, R, C Danese, J Howard: Hereditary congenital lymphedema (Milroy's disease). Surgery 60 (1966), 1098-1103.
19. Aagenaes, O, H Sigstad, R Bjorn-Hansen: Lymphedema in hereditary recurrent cholestais from birth. Arch.Dis. Child. 45 (1970), 690-695.
20. Dale, R: The inheritance of primary lymphedema. J Med. Gen. 2 (1985), 274-278.
21. Hoyme, HE, M Witte, L Hauck, et al: The primary familial lymphedema syndromes: Clinical classification revisited. Presented David Smith Morphogenesis-Malformation Conference, 8/97.
22. Witte, MH, DL Way, CL Witte, et al: Lymphangiogenesis: Mechanisms, significance and clinical implications. In *Regulation of Angiogenesis*. Goldberg ID, EM Rosen (Eds.), Birkhäuser Verlag Basel/Switzerland 1997, pp. 65-112.
23. Witte, MH, C Witte: Lymphangiogenesis and lymphologic syndromes. Lymphology 19 (l986), 21- 28.
24. Witte, MH, C Witte: Lymphatics and blood vessels, lymphangiogenesis and hemangiogenesis: From cell biology to clinical medicine. Lymphology 20 (l987), 171-178.
25. Witte, MH, C Witte, D Way, et al: Control of lymphangiogenesis. In: *Progress in Lymphology XVI*. Lymphology 31(Suppl) (1998), 37-40.
26. Hanahan, D: Signaling vascular morphogenesis and maintenance. Science 277 (1997), 48-50.
27. Joukov, V, K Pajusola, A Kaipanen, et al: A novel vascular endothelial growth factor is a ligand for the flt4 (VEGFR-3) and kDR (VEGFR-2) receptor tyrosine kinases. EMBO J. 15 (1996), 290-298.
28. Kaipanen, A, J Korhonen, T Mustonen, et al: Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. Proc. Nat. Acad. Sci. 92 (1995), 3566-3570.
29. Kukk, E, A Lymboussaki, S Taira, et al: VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. Development 122 (1996), 3829-3827.
30. Lee, J, A Gray, J Yuan, et al: Vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor Flt4. Proc. Nat. Acad. Sci.93 (1996), 1988-1992.
31. Warrington, J, S Bailey, E Armstrong, et al: A radiation hybrid map of 18 growth factor, growth factor receptor, hormone receptor, or neurotransmitter receptor genes on the distal region of the long arm of chromosome 5. Genomics 13 (1992), 803-808.
32. Galland, F, A Karamysheva, M Mattei, et al: Chromosomal localization of FLT4, a novel receptor-type tyrosine kinase gene. Genomics 13 (1992), 475-478.
33. Aprelikova, O, K Pajusola, J Partanen, et al: FLT4, a novel class III receptor tyrosine kinase in chromosome 5q33-qter. Cancer Res 52 (1992), 746-748.
34. Jeltsch, M, A Kaipainen, V Joukov, et al: Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science 276 (1997), 1423-1425.
35. Dumont, D, L Jussila, J Taipale, et al: Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. Science 282 (1998), 946-949.
36. Erickson, R, T Glover, G Brown, et al: Identification and cloning of genes involved in lymphedema. Presented David Smith Morphogenesis-Malformation Conference, 8/98.

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Editor's note: The Editor encourages ISL members and Lymphology readers from around the world to collaborate in the Hereditary Lymphedema project and contribute pedigrees, which will be entered in the developing ISL Lymphedema-Angiodysplasia Database-Registry, and further to assist in the detailed characterization of phenotypes and genotypes in these disorders according to standardized protocols.