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A NOVEL FLT4 GENE MUTATION IDENTIFIED IN A PATIENT WITH MILROY DISEASE

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ABSTRACT

Milroy disease is an autosomal dominant disorder generally presenting with below the knee lymphedema at birth. It is linked to mutations in the tyrosine kinase domain of the VEGFR3 protein which is encoded in the FLT4 *gene. Here we report a case of Milroy disease in a patient with a dominant pattern of inheritance, classical physical findings, and lymphatic system imaging demonstrating lack of tracer transport in the lower limbs. Genetic analysis revealed a novel missense mutation compared to a summary of reported mutations causing Milroy Disease.*

Keywords: Milroy's Disease, primary lymphedema, *FLT4*, novel gene mutation

Milroy Disease is a rare autosomal dominant disorder caused by a mutation in the FLT4 gene coding for VEGFR3 protein (1-3). The tyrosine kinase domains of VEGFR-3 are conserved units made up of exons 17-20 and 22-26. The receptor tyrosine kinase complex autophosphorylates after the ligand, VEGF-C, binds to it, and mutations in the tyrosine kinase domain can inhibit the transmission of VEGF-C binding and its downstream effects on lymphatic development and maintenance (4). The result of these mutations is seen clinically as Milroy disease. Patients with Milroy disease generally present with lymphedema at birth, and the swelling is confined below the knee (5). Other associated signs and symptoms include: hyperkeratosis, upslanting toenails, and recurrent cellulitis (6).

Fifty-eight mutations in the receptor tyrosine kinase of VEGRF3 are currently reported in the *FLT4*-Leiden Open Variation Database (7). Of the 58 mutations, 54 are missense, 1 is a splicing variant, and 3 are deletions. Presently, there have been no mutations reported outside the two receptor tyrosine kinase domains in exons 17-20 and 22-26.

Case Summary

Clinical

The patient presented as a 16 year old male who was born with bilateral, below the knee swelling. A family history of lower extremity lymphedema at birth included the patient's mother, grandmother, brother, and sister (*Fig. 1*).

Imaging

Lymphscintigraphy was performed using filtered 99m technetium labeled human serum albumin to examine the functioning of the lymphatic system. Following bilateral single intradermal injection (0.05 ml, 500 &Ci) into each foot, early static and late whole-body images were obtained using a collimated

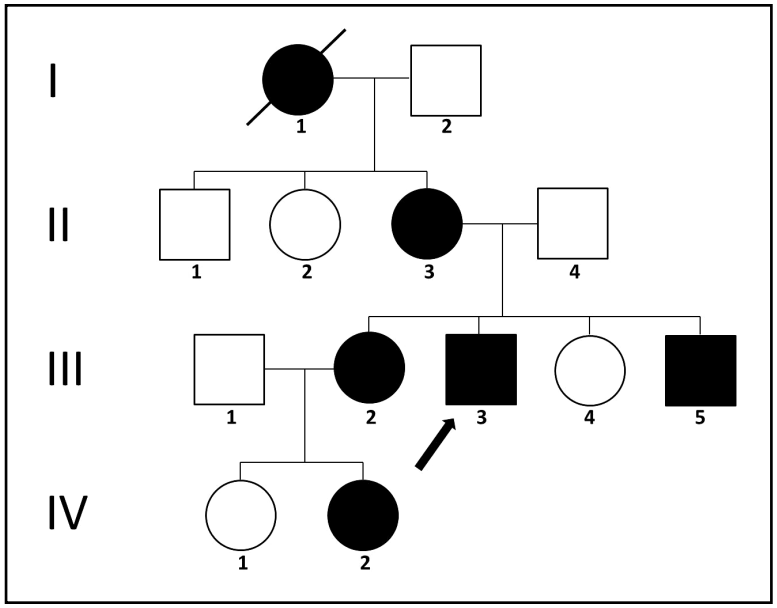

Fig. 1. Family pedigree displaying pattern of autosomal dominant inheritance (affected by history). Proband who was examined, imaged, and sequenced is III-3 (arrow).

gamma camera. Lymphscintigrams displayed an absence of lymphatic vessels in the legs with lack of tracer transport from the feet (*Fig. 2*).

DNA Analyses

A blood sample was taken, DNA prepared and PCR amplification of exons 17-20 and 22-26 of *FLT4* (exons encoding the tyrosine kinase domains) were submitted for Sanger sequencing. Sequence analysis revealed that the subject was heterozygous for a T>G missense mutation at position 3243 in exon 24 of *FLT4*, changing amino acid #1081 from methionine to arginine. This amino acid is conserved in mouse and chicken, although it is not in the rat. The rat, however, has about 10 extra amino acids in this stretch and is not, therefore, homologous to other sequenced species in this coding area. This base pair change does not occur as a single nucleotide polymorphism (SNP) on either the SNPper (snipper.chip.org) or HapMap (hapmap.org) SNP databases.

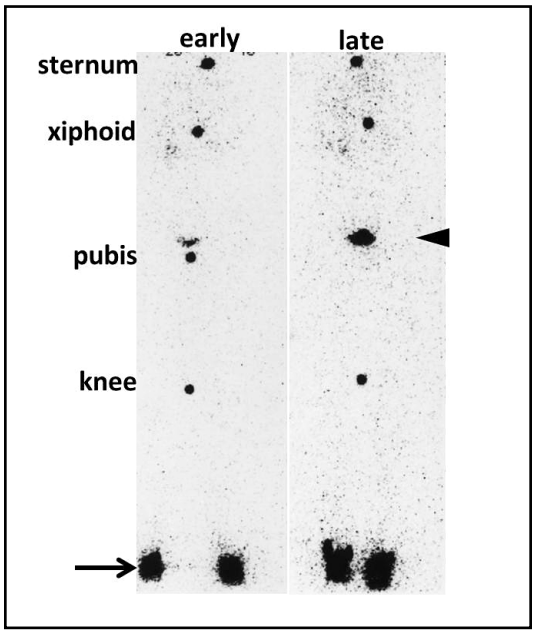

Fig. 2. Lymphscintigram sequence (early after massage on left and late on right) showing little to no tracer uptake after intradermal injection into the dorsum of the feet (arrow). Markers indicate the sternum, xiphoid, pubis, and knee; arrowhead indicates bladder.

TABLE 1
Reported Mutations in VEGFR-3 Causing Milroy Disease from Leiden Open Variation Database (7)

Mutation (reference)		
ARG844Pro (13)	Arg943Pro (13)	Ile1053Phe (8)
Gly852Ser (13)	Ser1018Arg (8)	Asp1055Ala (13)
Gly852Arg (13)	Phe1019Ser (8)	Asp1055Val (21)
Gly852Val (8)	Gln1020Leu (19)	Gly1057Asp (8)
Gly854Ser (14)	Gly1024Arg (13)	Glu1084Lys (8)
Gly854Arg (13)	Gly1024Glu (13)	Ile1086Thr (17)
Ala855Thr (15)	Met1025Ile (8)	Leu1103Pro (8)
Gly857Arg (4)	Cys1033Gly (8)	Leu1104Phe (8)
Ala877Thr (16)	His1035Arg (20)	Glu1106Lys (22)
Val878Met (17)	His1035Gln (17)	Glu1106Asp (8)
Leu893Val (15)	Asp1037His (13)	Phe1108Ser (8)
Met894del (8)	Asp1037Tyr (13)	Phe1108del (14)
Leu913del (18)	Asp1037Glu (8)	Pro1114Leu (1,4)
Gly914Arg (8)	Ala1040Asp (8)	Tyr1115Cys (13)
Ala915Pro 14)	Arg1041Trp (14)	Gly1131Arg (13)
Ala915Glu (8)	Arg1041Gln (14)	Pro1137Leu (14)
Cys916Trp (14)	Arg1041Pro (4)	Arg1145Cys (8)
Thr917Pro (8)	Asn1042Ser (13)	
Gly933Arg (14)	Leu1044Pro (4)	
Asn934Try (8)	Val1051Met (13)	

DISCUSSION

The patient presented a classical picture of Milroy disease by history, physical examination, and imaging findings. He is heterozygous for a novel mutation in *FLT4*/VEGFR3 which is highly likely to be inactivating as are other mutations in Milroy disease (7) (*Table 1*) (8,9). It has been shown that haploinsufficency of Vegfr3 results in lymphedema by studies in mice and that mice heterozygous for a loss-of-function mutation in the tyrosine kinase domain (10) or deleted for 1 copy of the gene (11) show lymphatic dysplasia. The similarities of *FLT4* kinase domain mutations in Milroy to haploinsufficency in the mouse highlights the crucial role of the tyrosine kinase domain in phosphorylating downstream targets-possibly including NF-kappaB (12).

REFERENCES

1. Ferrell, RE, KL Levinson, JH Esman, et al: Hereditary lymphedema: Evidence for linkage and genetic heterogeneity. Hum. Mol. Genet. 7 (1998), 2073-2078.

2. Witte, MH, R Erickson R, M Bernas, et al: Phenotypic and genotypic heterogeneity in familial Milroy lymphedema. Lymphology 31 (1998), 145-155.
3. Evans, AL, G Brice, V Sotirova, et al: Mapping of primary congenital lymphedema to the 5q35.3 region. Am. J. Hum. Genet. 64 (1999), 547-555
4. Karkkainen, MJ, RE Ferrell, EC Lawrence, et al: Missense mutations interfere with VEGFR-3 signaling in primary lymphoedema. Nat. Genet. 25 (2000), 153-159.
5. Milroy, WF: An undescribed variety of hereditary oedema. NY Med. J. 56 (1892), 505-508.
6. Brice, G, AH Child, A Evans, et al: Milroy disease and the VEGFR-3 mutation phenotype. J. Med. Genet. 42 (2005), 98-102.
7. Leiden Open Variation Database (LOVD). www.lovd.nl/flt4 (assessed December 09, 2013).
8. Gordon, K, SL Spiden, FC Connell, et al: FLT4/VEGFR3 and Milroy disease: novel mutations, a review of published variants and database update. Human Mutation 34 (2013), 23-31.
9. Michelini, S, D Degiorgio, M Cestari, et al: Clinical and genetic study of 46 Italian patients with primary lymphedema. Lymphology. 45 (2012), 3-12. Erratum in: Lymphology. 45(2012), 87. PubMed PMID: 22768468.
10. Karkkainen, M, A Saaristo, L Jusssila L, et al: A model for gene therapy of human hereditary lymphedema. PNAS 98 (2001), 12677-12682.
11. Dellinger, MT, RJ Hunter, M Bernas, et al: Chy-3 mice are Vegfc haploinsufficient and exhibit defective dermal superficial to deep lymphatic transition and dermal lymphatic hypoplasia. Dev. Dyn. 236 (2007), 2346-2355.
12. Aradhya, S, DL Nelson: NF-kappaB signaling and human disease. Curr. Opin. Genetics Dev. 11 (2001), 300-308.
13. Connell, FC, P Ostergaard , C Carver, et al: Analysis of the coding regions of VEGFR3 and VEGFC in Milroy disease and other primary lymphoedemas. Hum. Genet. 124 (2009), 625-631.
14. Evans, AL, R Bell, G Brice, et al: Identification of eight novel VEGFR-3 mutations in families with primary congenital lymphoedema. G. J. Med. Genet. 40 (2003), 697-703.
15. Ghalamkarpour, A, W Holnthoner W, P Saharinen, et al: Recessive primary congenital lymphoedema caused by a VEGFR3 mutation. J. Med. Genet. 46 (2009), 399-404.
16. Futatani, T, E Nii, M Obata, et al: Molecular characterization of two novel VEGFR3 mutations in Japanese families with Milroy's disease. Pediatr. Int. 50 (2008), 116-118.
17. Ghalamkarpour, A, S Morlot, A Raas-Rothschild, et al: A hereditary lymphedema type I associated with VEGFR3 mutation: the first de novo case and atypical presentations. Clin. Genet. 70 (2006), 330-335.
18. Carver, C, G Brice G, S Mansour S, et al: Three children with Milroy disease and de novo mutations in VEGFR3. Clin. Genet. 71(2007), 187-189.
19. Butler, MG, SL Dagenais, SG Rockson, TW Glover: A novel VEGFR3 mutation causes Milroy disease. Am. J. Med. Genet. A. 143A (2007), 1212-1217.
20. Irrthum, A, MJ Karkkainen, K Devriendt, et al: Congenital hereditary lymphedema caused by a mutation that inactivates VEGFR3 tyrosine kinase. Am. J. Hum. Genet. 67 (2000), 295-301.
21. Yu, Z, J Wang, S Peng, et al: Identification of a novel VEGFR-3 missense mutation in a Chinese family with hereditary lymphedema type I. J. Genet. Genomics. 34 (2007), 861-867.
22. Daniel-Spiegel, E, A Ghalamkarpour, R Spiegel, et al: Hydrops fetalis: An unusual prenatal presentation of hereditary congenital lymphedema. Prenat. Diagn. 25 (2005), 1015-1018.

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