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A NOVEL FLT4 GENE MUTATION AND MR LYMPHANGIOGRAPHY IN A CHINESE FAMILY WITH MILROY DISEASE

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ABSTRACT

Milroy disease is a congenital onset lymphedema linked to FLT4 gene mutations in the tyrosine kinase domain. So far, a total of 59 different FLT4 *variants have been identified. Here, we report a novel* FLT4 *gene mutation in a Chinese family with Milroy disease and present their clinical symptoms and MR lymphangiographic findings.*

Keywords: Milroy disease, *FLT4*, *VEGFR-3*, primary lymphedema, novel gene mutation, MR lymphangiogram, phenotype

Milroy disease (MD; MIM# 153100) is a rare autosomal dominantly inherited primary lymphedema. Patients with MD generally present with bilateral lower leg lymphedema at birth (1-3). The *FLT4* gene (also known as *VEGFR-3*), which encodes vascular endothelial growth factor receptor 3, was identified as being responsible for the majority of MD cases (4). So far, 59 mutations in *FLT4* have been reported (5,6), with most reported as missense mutations. All the mutations are located in exons 17-20 and 22-26 of *FLT4*, which are within the tyrosine kinase domain of the receptor (5). Here, we report a novel missense *FLT4* mutation in a Chinese family with MD and provide MR lymphangiographic characterization of the lymphatic phenotype.

METHODS

Two sisters (patient 1, 32 years old; and patient 2, 30 years old) both have congenital bilateral lower leg lymphedema. One of patient 1's two daughters, aged 3, also showed obvious edema in the bilateral dorsum of the foot at birth but the edema reduced spontaneously with time. The elder daughter, aged 5, has no clinical symptoms of edema. Patient 2 exhibits bilateral lower extremity lymphedema. The patients confirmed that no other family members had similar symptoms. Their parents are deceased (*Fig. 1A*).

Blood samples from the two sisters were collected and DNA prepared. Exons 17-20 and 22-26, encoding the tyrosine kinase domains, were amplified by PCR. The purified PCR products were submitted for Sanger sequencing (ABI, 3730XL, Perkin Elmer, Foster City, CA, USA) and nucleotide sequences compared with published *VEGFR-3* cDNA sequence (NCBI Reference Sequence: NG_011536.1).

Magnetic resonance (MR) lymphangiography was performed using a 3.0T MR unit (Philips Medical System, Best, The Netherlands) with gadobenate dimeglumine (Gd-BOPTA, MultiHance) contrast medium injected intradermally into the interdigital webs of the dorsal foot. For lymphatic channel imaging of the lower limbs, 3D fast spoiled gradient-recalled echo T1-weighted images were obtained using a fat saturation technique (8).

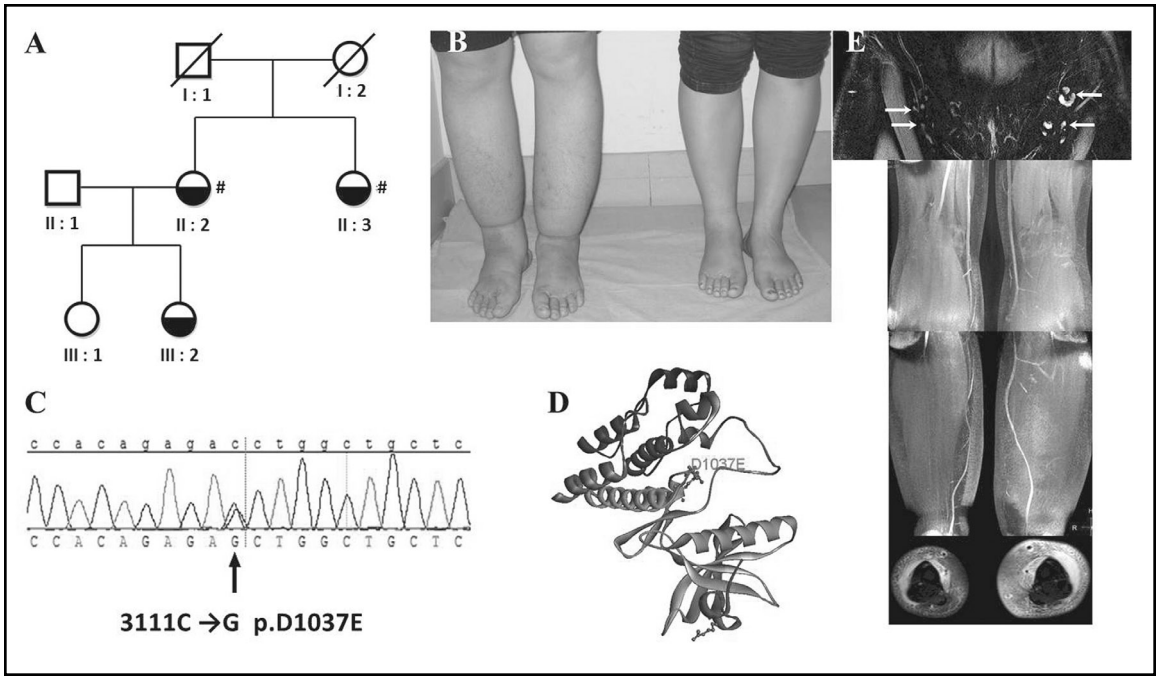

Fig. 1. Clinical and genetic findings in a Chinese family with MD. A: Pedigree of the family. Squares indicate males; circles, females; Affected subjects are shown as half-filled circles. The probands are indicated with symbol #. B: Bilateral below knee lymphedema and evident skin fibrosis in patient 1(left, II: 2) and bilateral lower leg lymphedema with much milder edema on the left side in patient 2 (right, II:3). C: Sequence analysis showed a C>G missense mutation at position 3111 in exon 23 of FLT4 changing amino acids # 1037 from arginine to glutamine. D: Alteration mapped to the structure of the FLT4 Pkinase Tyr domain. Alterations are highlighted in color according to the number of mutations targeting the corresponding amino acid. E: MR lymphangiography performed in patient 1 showing bilateral inguinal lymph nodes with normal structure (arrows) but no demonstrable lymph collector in the lower limbs after contrast injection.

This study was granted approval from Shanghai Ninth People's hospital medical ethics committee.

RESULTS

Clinical findings: Patient 1 presented typical MD symptoms with bilateral below knee non-pitting edema and evident skin fibrosis (*Fig. 1B*). The patient had not experienced erysipelas. Patient 2 also exhibited bilateral edema of the lower leg with much milder edema on the left side (limited to the dorsum of the feet) compared with the right side (up to the ankle). Skin fibrosis of her bilateral toes was evident (*Fig. 1B*).

Mutation screening: Sequence analysis revealed the two subjects were heterozygous for a C>G missense mutation at position 3111 in exon 23 of *FLT4* (*Fig. 1C*) resulting in a change in amino acid #1037 from arginine to glutamine. This missense mutation occurred within the VEGFR-3 tyrosine kinase domain. The novel C>G missense mutation in exon 23 was mapped to the structure of the *FLT4* tyrosine kinase domain built using Discovery Studio (accelrys.com) (*Fig. 1D*). This base pair change was not found in dbSNP, the 1000 genome project (http://www.1000genomes.org), Yanhuang project (http://yh.genomics.org.cn), or in the web-accessible Leiden Open Variation Database (LOVD, www.lovd.nl/flt4).

Imaging findings: T2-weighted MR imaging showed a significantly thickened subcutaneous layer with massively diffused water and fibrotic tissue of the bilateral lower legs of patient 1 (*Fig. 1E*). MR lymphangiogram showed that no lymph collector was visualized post-contrast injection although bilateral inguinal lymph nodes were demonstrated with normal structure (*Fig. 1E*).

DISCUSSION

Primary lymphedema is a disease caused by lymphatic system malformation. There are complicated types of lymphatic malformations in primary lymphedema (8), and the underlying pathology of the lymphatic system deformity remains largely unknown. *FLT4* is the first gene to be identified and associated with a specific type of inherent lymphedema i.e., MD. Until now, more than 50 different *FLT4* variants have been found. However, the clinical phenotype may vary even among patients with the same mutation (5). In this study, patient 1 demonstrated bilateral stage III lymphedema of the lower leg with evident skin fibrosis. One of her daughters exhibited bilateral edema on the dorsum of the foot at birth but the edema reduced spontaneously. Her younger sister demonstrated asymmetrical lymphedema of the two limbs, with much milder edema on the left feet, although skin fibrosis of her bilateral toes was evident. The *FLT4* mutation in this family is located in exon 23 of tyrosine kinase domain. A recent study found that mutations within the tyrosine kinase domain of *FLT4*/*VEGFR-3* are sufficient to reduce tyrosine kinase activity (9) and thereby affect lymphatic development. Here, MR lymphangiography failed to demonstrate collecting lymphatic in the affected lower limbs, consistent with the reported lymphoscintigraphy image results in MD patients. However, unlike typical lymphoscintigraphy images of MD, with no evidence of tracer uptake into the inguinal lymph nodes (6), MR lymphangiographic imaging clearly visualized the inguinal lymph nodes with a normal morphology in this study. Whether absence of observable lymphatic collectors in the affected limb in MD is due to lack of lymph vessel development or lymphatic dysfunction remains unclear. Some studies propose that MD is a disease caused by lymphatic dysfunction because: (1) there are primary lymphatic vessels in the skin of affected feet by immunohistochemical staining, and (2) the main functional lymphatic tract is displayed on lymphoscintigrams in a few MD patients (10). If so, a more complicated mechanism may underlie the pathology of MD, and more genes, other than *VEGFR-3*, may be involved in MD as modifier genes.

In summary, our study has further expanded the mutation spectrum of *FLT4* and confirms that FLT4 is important in MD.

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