Short Report

Hereditary lymphedema type I associated with VEGFR3 mutation: the first de novo case and atypical presentations


Mutations in the vascular endothelial growth factor receptor 3 gene, VEGFR3/FLT4, have been identified in a subset of families with hereditary lymphedema type I or Milroy disease (MIM 153100). Individuals carrying a VEGFR3 mutation exhibit congenital edema of the lower limbs, usually bilaterally and below the knees, sometimes associated with cellulitis, prominent veins, papillomatosis, upturned toenails, and hydrocele. In this study, we report the first de novo VEGFR3 mutation in a patient with sporadic congenital lymphedema. We also describe three other families with a VEGFR3 mutation. In each family, one individual had an atypical clinical presentation of hereditary lymphedema type I, whereas the others had the classical VEGFR3 mutation-caused phenotype. The atypical presentations included pre-natal pleural effusion, spontaneous resorption of lymphedema and elephantiasis. Three of the four identified mutations were novel. These data show that de novo VEGFR3 mutations may be present in patients without family history of congenital lymphedema. This has implications for follow-up care, as such individuals have nearly a 50% risk for occurrence of lymphedema in their children. Our findings also indicate that although most patients with a VEGFR3 mutation have the well-defined phenotype for hereditary lymphedema type I, there are exceptions that should be considered in genetic counseling. Because VEGFR3 mutation can cause generalized lymphatic dysfunction and can thus result in hydrops fetalis, VEGFR3 screening should be added to the investigation of cases of hydrops fetalis of an unknown etiology.

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Hereditary lymphedema type I was described more than a hundred years ago by Nonne and Milroy (1, 2). Milroy’s original definition included congenital, bilateral or unilateral, chronic hereditary edema in lower extremities, either throughout the limbs or limited to the feet or toes, and never above the inguinal ligament (3). The clinical spectrum was expanded in later case reports to include edema of the genitalia, upper limbs, face, and infrequently, involvement of pleura, peritoneum and pericardium (4–7). Hereditary lymphedema with pubertal onset
was described by Meige a few years after Milroy; it is known as hereditary lymphedema type II or Meige disease (8).

Hereditary lymphedema type I shows an autosomal dominant pattern of inheritance with incomplete penetrance, and it is estimated to occur in approximately 1:6000 births (7). Genetic studies in some families demonstrated linkage between hereditary lymphedema type I and a locus in the telomeric part of chromosome 5q (9, 10). Subsequently, dominant mutations were identified in the vascular endothelial growth factor receptor 3 gene, \( VEGFR3/FLT4 \), causing inhibition in receptor autophosphorylation (11, 12). To date, 14 \( VEGFR3 \) mutations have been reported in 17 distinct families (11–15). There seems to be locus heterogeneity, as linkage to 5q was excluded in some families (7, 9). To better define hereditary lymphedema type I, the phenotypes of 71 individuals with a confirmed \( VEGFR3 \) mutation were evaluated (16). Lymphedema was present in 90% of the patients (reduced penetrance); most cases were congenital (97%). The lymphedema was always confined to the lower limbs, usually bilaterally (85%) and below the knees (94%). Lymphedema was accompanied by prominent veins (23%), recurring cellulitis (20%), upturned toenails (10%), papillomatosis (10%), and hydrocele (37% of men).

In this study, we present four additional families with three novel \( VEGFR3 \) mutations. Most of the patients had the classical Milroy phenotype. However, in one family with sporadic congenital lymphedema, a \textit{de novo} \( VEGFR3 \) mutation was identified. In the second family, a mutation was identified in a 22-week-old fetus with pleural effusion, and in the other families, the phenotype included spontaneous resorption of lymphedema and elephantiasis.

**Materials and methods**

**Samples**

DNA samples of families LE-11 and LE-18 and blood samples of families LE-53 and LE-54 were collected as approved by the ethics committee of the medical faculty at the Université catholique de Louvain, Brussels, Belgium. DNA was extracted from blood samples using Gentra kit (Minneapolis, MN, USA) following the manufacturer’s instructions.

**Mutational screening**

Exons 16 to 27, which encode the intracellular tyrosine kinase (TK) domains of the \( VEGFR3 \) receptor, were amplified from genomic DNA. Primers were designed using GenBank accession number X68203 as the reference sequence and were synthesized using Eurogentec (Seraing, Belgium). The samples were screened using radioactive single-strand conformation polymorphism and heteroduplex analysis. Fragments with altered migration were reamplified, purified with polymerase chain reaction (PCR) purification columns (Qiagen, Hilden, Germany) and sequenced on a CEQ2000 capillary sequencer (Beckman Coulter, Fullerton, CA, USA). Cosegregation (in family LE-18) and screening of 110 unaffected controls for the novel mutations were performed either by restriction fragment length assay (c.2632G>A) or by allele-specific PCR (c.3105C>G and c.3257T>C).

**Paternity test**

In family LE-11, false paternity was excluded using 10 highly polymorphic Weber set 8 or laboratory-designed microsatellite markers. Genotyping was performed as described elsewhere (17).

**Results**

**Phenotypes**

LE-11 is a nuclear family, with a child presenting sporadic congenital lymphedema. Swelling was bilateral and below the knees, accompanied by upturned toenails and mild hydrocele. Neither of the parents had lymphedema. The pregnancy for the patient was uneventful and no factor that can provoke the onset of lymphedema was known.

The clinical findings in family LE-18 have been described in detail elsewhere (18). The index case (III.1) was discovered to have bilateral leg edema and bilateral pleural effusion at 22 weeks of gestation. Maternal evaluation for other causes of hydrops fetalis was negative. The couple chose to terminate the pregnancy because of pulmonary hypoplasia in the presence of bilateral hydrothorax. Pathological examination showed a female fetus with subcutaneous edema of the legs and feet, bilateral pleural effusion (mainly in the left cavity) and hypoplastic lungs. Other affected members in this family had congenital lymphedema of the legs with variable degree of severity.

The index case in family LE-53 (III.2) had bilateral elephantiasis up to the inguinal ligament with chronic venous ulcerations, cellulitis and papillomatosis. She had undergone several operations to prevent recurrent infections and to preserve function of the limbs. Other affected
family members had congenital unilateral or bilateral leg edema. Individuals III.4, III.8 and IV.6 were not clinically examined and could have minor signs of lymphedema or be carriers.

The index case in LE-54 family (III.3) had congenital lymphedema below the knees, most prominently below the ankles. His father, LE-54.II.3, had congenital lower limb lymphedema that spontaneously and permanently resorbed. No clinical history was available for I.1 and I.2.

The family history in LE-18, LE-53 and LE-54 was compatible with autosomal dominant inheritance, in contrast to LE-11, in which the family history was consistent with either autosomal or X-linked recessive inheritance, dominant mutation with reduced penetrance, dominant de novo mutation or false paternity. None of the affected members in these families had other anomalies, such as hypotrichosis, distichiasis, or microcephaly. Pedigrees are shown in Fig. 1.

Mutations

Three novel missense mutations were identified in families LE-11, LE-18 and LE-53, and the p.F1108del mutation in family LE-54. All mutations were heterozygous and located in the TK domain I or II of the receptor. VEGFR3 sequence alignment of multiple species showed a high evolutionary conservation of the amino acids (Fig. 2). None of the novel mutations were identified in 110 control individuals (220 alleles).

In family LE-11, the mutation was a c.3105C>G nucleotide change causing amino acid substitution p.H1035Q in the TK domain II of the receptor. The mutation was not identified in either parent, and false paternity was excluded using 10 informative microsatellite markers on various chromosomes. In family LE-18, a nucleotide change c.2632G>A was identified in all the affected individuals. This mutation results in an amino acid substitution, p.V878M, in the TK domain I. In family LE-53, the nucleotide change c.3257T>C causes an amino acid substitution p.I1086T in the TK domain II. In family LE-54, the deletion of three nucleotides, c.3323_3325del, results in a loss of phenylalanine at position 1108 of the receptor (p.F1108del). This mutation was published by Evans et al. in a distinct family with hereditary lymphedema type I (13). All the

Fig. 1. Pedigrees of families LE-11, LE-18, LE-53 and LE-54 (a–d). Arrows, probands; blackened symbols, lymphedema; hatched symbol, hemangioma; +, mutation in vascular endothelial growth factor receptor 3 (VEGFR3); −, no mutation in VEGFR3; bar above symbol, individual clinically examined.
previously reported and the novel mutations identified in this study are shown in Fig. 3.

Discussion

Identification of VEGFR3 mutations as the cause of hereditary lymphedema type I permits precise diagnosis and directs studies that someday may lead to targeted therapy. In this report, we present four additional families with a VEGFR3 mutation that helps to delineate the phenotypic spectrum of the disease. This has implications for genetic counseling and management of these patients. Mutation p.H1035Q was identified in individual LE-11.II.1 with congenital lymphedema and no family history. Histidine at position 1035 of human VEGFR3 belongs to the highly conserved –HRDLAARN– sequence in the catalytic loop. Histidine-to-arginine change in this position inhibits autophosphorylation of the receptor (11). Therefore, we conclude that a histidine-to-glutamine change in this conserved region also inhibits activity of the receptor. The mutation was heterozygous and not present in the non-affected parents, and thus constitutes a dominant de novo VEGFR3 mutation. This type of mutation...
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has not been reported before, however, in a single affected patient with nonconfirmed paternity was suggested (19). Because VEGFR3 mutations can be the pathophysiological cause of sporadic congenital lymphedema, VEGFR3 screening in such patients is necessary for specific diagnosis. Individuals with a VEGFR3 mutation carry nearly a 50% risk of lymphedema in their children, compared with the low incidence in the general population. Obviously, this molecular diagnosis has implications for genetic counseling.

The second mutation, p.V878M, was identified in family LE-18, in which a 22-week-old fetus presented with bilateral leg edema and bilateral pleural effusion. The fetus had pulmonary hypoplasia due to early severe bilateral hydrothorax. Another case of hydrops fetalis associated with a VEGFR3 mutation has been reported in a distinct family (15). This fetus presented with polyhydramnios, massive bilateral pleural effusion and minimal ascites at 33 weeks of gestation. Although hydrops fetalis is only occasionally seen in families with hereditary lymphedema type I, its occurrence indicates that patients with a germline VEGFR3 mutation are susceptible to systemic failure of lymphatic function. Generalized lymphatic deficiency also occurs in relation to two other genes implicated in lymphedema-associated disorders: the FOXC2 gene, a member of the forkhead family of transcription factors, involved in lymphedema–distichiasis syndrome (MIM 153400), and SOX18, a member of the Sry-related high mobility group (HMG) box containing transcription factors, mutated in hypotrichosis–lymphedema–telangiectasia syndrome (MIM 607823) (20, 21). Alterations in either of these genes can cause hydrops fetalis; however, the frequency of mutations in these three genes in hydrops patients is currently unknown. The occurrence of this severe phenotype could be due to the influence of modifier genes, epigenetic changes, environmental or other factors. Similar spectrum of phenotypic expression has been observed in Chy-mice, with a heterozygous mutation in Vegfr3 (p.I1053F). Approximately 10% of the affected pups develop a severe fluid accumulation in the abdomen during the first three post-natal weeks, whereas in the remaining mice, the chylous fluid spontaneously resorbs (22, 23).

Elephantiasis in individual LE-53.III.2 was associated with p.I1086T mutation, and spontaneous regression of lymphedema in individual LE-54.II.3 with p.F1108del mutation. These are rare presentations in patients with a VEGFR3 mutation; both have been reported twice (16).

The progress of lymphedema to elephantiasis is not a typical presentation of hereditary lymphedema type I and could result from poorly followed lymph therapy. Resorption of lymphedema has also been described in one patient with hypotrichosis–lymphedema–telangiectasia syndrome, with a mutation in SOX18. This suggests a self-healing activity, rather than a progressive deterioration, in patients with a limited localized lymphedema. This should be taken into consideration during discussion of prognosis for a newborn with lymphedema.

The VEGFR3 mutations identified in this study were located in exons 18, 23 and 24. When including the 14 previously reported mutations, they are all localized in six exons: 18, 19, 20, 23, 24 and 25. Analyses of these 6 exons should provide an efficient screening of the gene. These exons encode the two intracellular TK domains of the receptor. Loss of autophosphorylating capacity of the receptor has been shown for five of these mutations (11, 12). However, the absence of truncating mutations indicates that these mutations do not simply cause haploinsufficiency; a dominant-negative effect has been suggested in vitro (12).

In conclusion, we showed that de novo VEGFR3 mutations can occur and thus should be considered in sporadic congenital lymphedema. Similar to FOXC2 and SOX18 mutations, VEGFR3 alterations can also result in hydrops fetalis, known to be associated with a poor prognosis. Thus, a detailed pre-natal follow up should be offered for fetuses at risk for hereditary lymphedema type I.

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