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NOVEL *FOXC2* MISSENSE MUTATION IDENTIFIED IN PATIENT WITH LYMPHEDEMA-DISTICHIASIS SYNDROME AND REVIEW

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ABSTRACT

Lymphedema-distichiasis (OMIM 153400) is a dominantly inherited disorder typically presenting with lymphedema at puberty and distichiasis at birth. The condition has been decisively linked to mutations in the forkhead transcription factor FOXC2 which have been primarily frameshift mutations truncating the protein. We report here a novel missense mutation along with a literature review summarizing reported mutations.

Keywords: Lymphedema-distichiasis, *FOXC2*, primary lymphedema

Lymphedema-distichiasis (LD) (OMIM 153400) is a highly penetrant, autosomal dominant disorder characterized by peripheral edema and aberrant eyelashes arising from the meibomian glands (1,2). LD patients usually show signs of lymphedema around puberty; however, the age of onset and severity can vary (1,2). In contrast to patients with Milroy lymphedema, LD patients are reported to exhibit an increased number (or upper limit of normal) of lymphatic vessels and lymph nodes by oil contrast lymphography (1,3,4). Patients with LD may also exhibit other clinical abnormalities such as cleft palate, extradural cysts, ptosis, and cardiovascular defects (2). Mirroring the human condition, the mouse model of lymphedema-distichiasis displays an extra row of eyelashes, lymphatic hyperplasia, lymphatic valve defects, and abnormal coverage of lymphatic vessels with smooth muscle cells (5,6).

Mutations in the transcription factor *FOXC2* have been demonstrated in numerous patients with LD (2,4,7-13). Interestingly, most *FOXC2* mutations are frameshift mutations (insertions/deletions) that prematurely truncate the FOXC2 protein *(Table 1)*. Nonsense mutations in FOXC2 also occur, however, to a lesser extent (*Table 2*). In contrast to *FOXC1* (a related gene mutated in Axenfeld-Rieger Syndrome), missense mutations in FOXC2 are extremely rare (*Table 2*). Here we report a novel *FOXC2* missense mutation in a patient with LD.

CASE SUMMARY

The patient was a 36 year old man with onset of bilateral leg swelling at age 15. There was a family history of lower extremity lymphedema including the father and paternal grandfather with age of onset between 8 and 20 years. Lymphedema was sometimes accompanied by episodes of lymphangitis. No lymphatic imaging studies were performed. Six of eleven male members (one with scrotal edema) were affected and none of three at risk females were affected. Extra eyelashes (distichiasis) were documented in two of the six affected but not determined in the others. One of the affected

TABLE 1
Reported Insertions and Deletions in *FOXC2* in Patients with Lymphedema-Distichiasis Syndrome

Position	Mutation	Reference
nt201-202	2 bp Insertion	Bell et al., 2002
nt209-210	1 bp Insertion	Bell et al., 2002
nt290-300	11 bp Deletion	Bell et al., 2002
nt323	1 bp Deletion	Brice et al., 2002
nt333	1 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt412-413	1 bp Insertion	Bell et al., 2002
nt474	1 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt505*	1 bp Deletion	Finegold et al., 2001
nt589-590*	1 bp Insertion	Finegold et al., 2001
nt595-596	1 bp Insertion	Brice et al., 2002
nt595-596	1 bp Insertion	Bell et al., 2002
nt609-610*	1 bp Insertion	Finegold et al., 2001
nt638-639	2 bp Insertion	Bell et al., 2002
nt683-684	1 bp Insertion	Erickson et al., 2001
nt747	1 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt792-793	1 bp Insertion	Erickson et al., 2001
nt818-819	1 bp Insertion	Erickson et al., 2001
nt818-819	1 bp Insertion	Bell et al., 2002
nt854	1 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt866	1 bp Deletion	Bell et al., 2002
nt866-867	1 bp Insertion	Bell et al., 2002
nt871-872	1 bp Insertion	Brice et al., 2002
nt902*	1 bp Deletion	Finegold et al., 2001
nt902-921*	19 bp Deletion	Finegold et al., 2001
nt914-921	8 bp Deletion	Bell et al., 2001; Erickson et al., 2001; Bahuau et al., 2002; Houdayer et al., 2002
nt922-929	8 bp Deletion	Brice et al., 2002
nt983*	1 bp Insertion	Finegold et al., 2001
nt1006-1007	1 bp Insertion	Yildirim-Toruner et al., 2004
nt1024	1 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt1093-1094	4 bp Insertion	Fang et al., 2000
nt1140	1 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt1142-1143	1 bp Insertion	Brice et al., 2002
nt1229-1253	25 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt1238-1254*	16 bp Deletion	Finegold et al., 2001
nt1298	1 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt1331	1 bp Deletion	Bell et al., 2001; Sholto-Douglas-Vernon et al., 2005
nt1418	1 bp Deletion	Sholto-Douglas-Vernon et al., 2005
nt1420-1426	7 bp Deletion	Sholto-Douglas-Vernon et al., 2005

* Indicates position based on FOXC2 cDNA GenBank accession no. NM_005251

had scoliosis. There were no family members described with cleft palate, congenital cardiac defects, stillbirths, or venous-related disease.

To determine whether *FOXC2* was mutated in this LD patient, genomic DNA was isolated from blood and used for DNA

TABLE 2
Reported Missense and Nonsense Mutations in *FOXC2* in Patients with Lymphedema-Distichiasis Syndrome

Mutation	Reference
Q84Stop*	Finegold et al., 2001
I85N	Present Study
Y99Stop	Fang et al., 2000
Q100Stop	Erickson et al., 2001
W116R	Sholto-Douglas-Vernon et al., 2005
W116Stop	Brice et al., 2002
R121H	Brice et al., 2002
S125L	Bell et al., 2001
Y145Stop	Brice et al., 2002
W146Stop	Sholto-Douglas-Vernon et al., 2005
S235I	Sholto-Douglas-Vernon et al., 2005
Y313Stop	Traboulsi et al., 2002
C317Stop	Erickson et al., 2001

* Indicates position based on FOXC2 cDNA GenBank accession no. NM_005251

TABLE 3
Primers Used To Amplify and Sequence FOXC2.

Primer Name	Primer Sequence	Size of PCR Product
FOXC2F1	TCT-CTC-GCG-CTC-TCT-CGC-TC	806 bp
FOXC2R1	GCC-CTG-CAG-CGC-GCT-CTC-GG	
FOXC2F2	TCA-CCT-TGA-ACG-GCA-TCT-AC	847 bp
FOXC2R2	GCG-AGG-TTG-AGA-GCG-CTC-AGG	
FOXC2F3	CGA-GCG-ATG-AGC-CTG-TAC-ACC	602 bp
FOXC2R3	CTT-TTT-TGC-GTC-TCT-GCA-GCC-C	

sequencing. *FOXC2* was amplified and then sequenced using the primers listed in *Table 3* and a GC-Rich PCR system (Roche, 12140306001). Sequence analysis of PCR products revealed that the subject was heterozygous for a T → A transversion mutation at nucleotide position 254 in the open reading frame of *FOXC2* (*Fig. 1A*). This mutation was confirmed on both the sense and antisense strands of DNA and

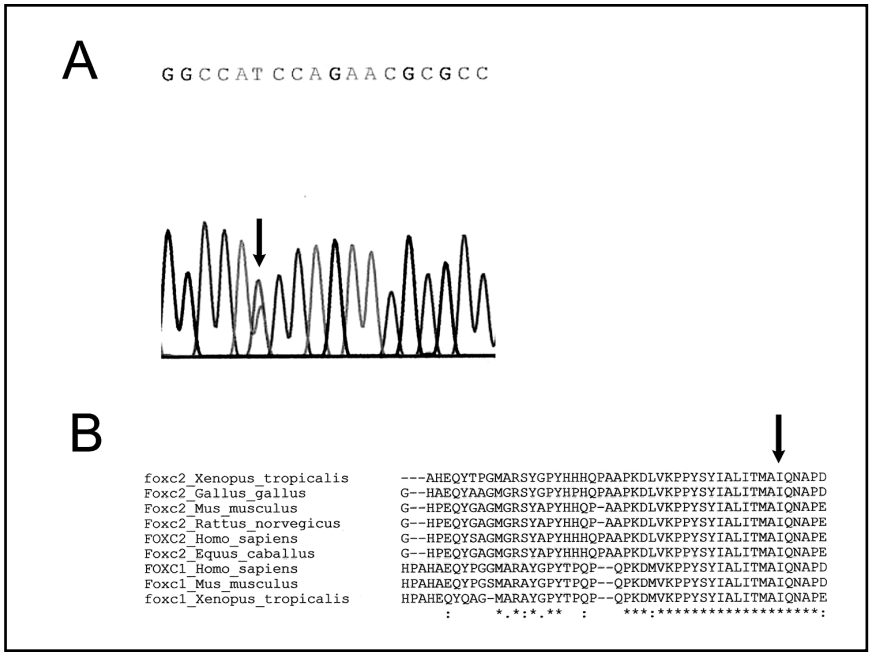

Fig. 1. Novel missense mutation identified in patient with lymphedema-distichiasis. A) Chromatogram showing heterozygosity for a T → A transversion mutation (arrow) at nucleotide position 254 in the open reading frame of FOXC2. B) CLUSTALW (http://align.genome.jp) was used to generate a multiple sequence alignment using FoxC1 and FoxC2 protein sequences from multiple species. The isoleucine (arrow) in the forkhead domain of FoxC proteins is conserved among all species.

was not present in the human SNP database (http://www.ncbi.nlm.nih.gov/SNP). Interestingly, this mutation is predicted to change amino acid 85 in the forkhead domain of FOXC2 from isoleucine to asparagine. Isoleucine is conserved at this position in FoxC1 and FoxC2 proteins in multiple species and, therefore, likely important for their function (*Fig. 1B*).

The forkhead domain of FOXC2 folds into a unique winged helix structure that binds to DNA and can activate or repress the transcription of target genes (14). LD causing missense mutations in the forkhead domain of FOXC2 can affect FOXC2's ability to bind DNA and/or localize to the nucleus (14), and the I85N missense mutation in this LD patient may act in a similar manner. Further exploration of this mutation may better define the importance of this key residue and shed light on the biochemical details of FOXC2.

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