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

M.T. Dellinger, K. Thome, M.J. Bernas, R.P. Erickson, M.H. Witte

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

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

* 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

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

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

TABLE 3
Primers Used To Amplify and Sequence FOXC2.

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

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
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.

REFERENCES

6. Petrova, TV, T Karpanen, C Norrmen, et al: Defective valves and abnormal mural cell...


Marlys Hearst Witte, MD
Professor of Surgery
University of Arizona College of Medicine
1501 N. Campbell Avenue, Room 4406
P.O. Box 245200
Tucson, AZ USA 85724-5200
Telephone: 520-626-6118
Fax: 520-626-0822
e-mail: lymph@u.arizona.edu