Lymphology 51 (2018) 85-88

FROM CHILDHOOD ONSET LYMPHEDEMA TO FATAL FETAL HYDROPS: POSSIBLE MODIFYING GENES FOR A FOXC2 MUTATION

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ABSTRACT

We performed whole exome sequencing in a family with FOXC2 *mutation where the phenotype in one generation was strikingly more severe. Although there were 3 mutations shared by 2 fatal fetal hydrops cases and not the mildly affected mother, none of them were likely to be the cause of the marked phenotypic change.*

Keywords: lymphedema, fatal fetal hydrops, *FOXC2*, modifying genes

Lymphedema-distichiasis (LD [OMIM 153400]) is a dominantly inherited disorder of lymphatics and other tissues, especially eyelids where a double row of eyelashes (distichiasis) is frequently found (1). Of many inherited forms of lymphedema, LD is the one which has most frequently been found with other congenital abnormalities including cardiac defects, cleft palate, and spinal extradural cysts. Infrequently it has presented as fetal hydrops.

The causative gene was cloned in 2000 and found to be a member of the Forkhead transcription factor family, FOXC2 (2). While identified by a translocation breakpoint in a singleton affected child, it is of note that two children in one of the two initial families (both families showing a dominant inheritance pattern) presented with severe, in utero, fetal hydrops (2). Such severe fetal hydrops in families of dominantly inherited lymphedema is rare enough that it warrants single case reports (3). Although large studies of congenital lymphedema have concluded that genetic causes are a rarity (4), heterozygous mutations in EPHB4 provide a single gene cause of severe fetal hydrops with survivors passing on the gene in a dominant fashion (4). A recent report identified mutations in known familial lymphedema genes in 25% of surviving cases of generalized in utero lymphedema [two in FLT4 (also known as *VEGFR3*) and one in *FOXC2*] (5) gene in a dominant fashion (6).

Twenty years ago, we reported a four generation family with a late onset but prepuberty (average age 9 years), lymphedema phenotype (7). The lymphedema was moderately severe and occurred in lower limbs and male genitals. The family did not show distichiasis by careful external examination and lack of symptoms but was found to have a novel mutation in *FOXC2*: NM_005251.2:c.361C>T:p.Arg121Cys (8). In the fifth generation of the family, an affected female (IV-4; *Fig.*) had one non-mutant child in a first marriage but 3 mutant (and 1 possibly mutant – a spontaneous abortion at 10 weeks) children/fetuses with severe and fatal hydrops (8).

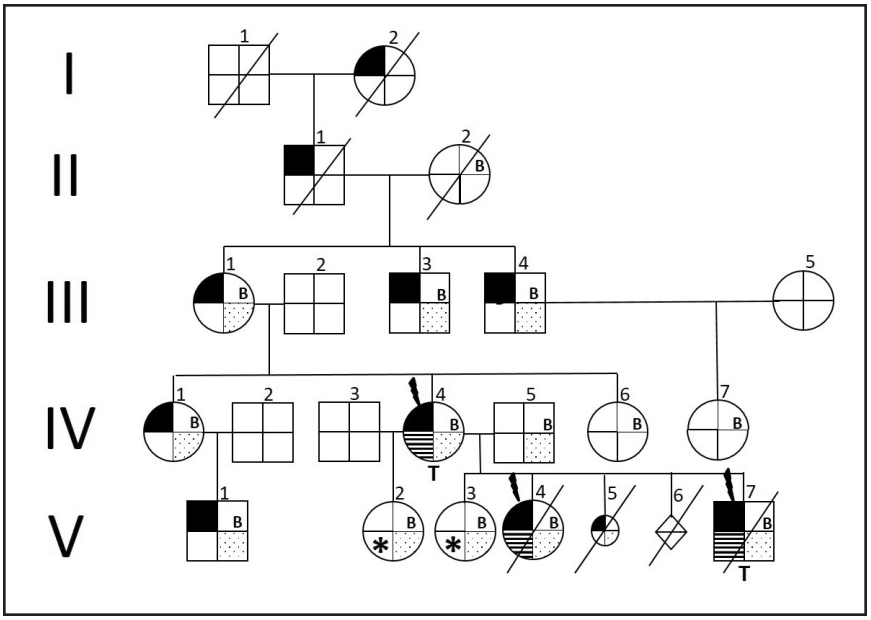

Fig. Pedigree of family, modified from Sargent, et al, 2014. Upper left quadrant: affected status, black if affected. Upper right quadrant: B if blood or tissue available for DNA. Lower left quadrant: FOXC2 mutation status: asterisk if studied and no mutation, horizontal bars if mutation positive. Lower right quadrant: stippled if personally examined. Lightning bolt above indicates individuals on whom WES was performed. T below indicates individuals with extra karyotypic band on Xp

This family (*Fig.*) was extensively studied in a search for causes of the extreme change in phenotype. Our initial focus was on the X chromosome because of an extra band on Xp found in the mother and V-7, who died at 3 days of age with cleft palate and severe hydrops (8). SNP arrays did not detect the cause of the extra X band but identified a portion of the maternal Xp which was shared by the fetal hydrops-affected daughter and son but not shared by an unaffected daughter. While of interest, since the mother had such a different phenotype, the possible contribution of this finding was an enigma.

We have now used whole exome sequencing (WES) on previously studied members of the family in a search for modifying genes which could be responsible for altering the phenotype from childhood onset lymphedema to fatal fetal hydrops.

MATERIALS AND METHODS

Genomic DNA was extracted from peripheral blood DNA samples from mother (IV-4) and daughter (V-4) and from cultured skin fibroblasts from male newborn (V-7). This study was approved by the Human Subjects Committee at the University of Arizona. Whole exome sequencing on IV-4 and V-7 was performed at University of Arizona Genome Center (Tucson, AZ) using the Agilent SureSelect Exon V6 target enrichment kit followed by 2X100 bp paired-end sequencing on the Illumina HiSeq 2500. Patient V-4 was sequenced at the University of California, San Francisco using the SeqCap EZ Human Exome Library v3.0 (Roche Nimblegen) followed by 2X100 bp paired-end sequencing on the Illumina HiSeq 2500. Sequences were aligned to the human genome (GRCh37). Base quality recalibration, indel realignment, and calling of SNVs and small indels were performed using the Genome Analysis Toolkit, v.3.3-0.

The mean coverage over the target regions of the candidate genes ranged from 70- to 90-fold with ~90% of RefSeq exonic base positions covered at least 20-fold. The VCF files were uploaded to Ingenuity Variant Analysis (Qiagen, Redwood City, CA) and the variants were filtered using the predicted deleterious filters. Disease association of the variant was computed according to the ACMG variant classification guidance. In addition, any HGMD annotated variant was called. The net gain-of-function in a gene was predicted based on establishment in the literature, inferred activating mutation by Ingenuity, predicted gain of function by BSFIT (9), microRNA Binding Site and copy number gain. The net loss-of-function in a gene was predicted based on frameshift, in-frame indel, or stop codon change; missense predicted to be tolerated or not by SIFT, PolyPhen-2 and CADD Score; loss of splice site; deleterious to a microRNA; structural variant; promoter loss with ENCODE TFBS; loss of enhancer; and evolutionary conservation with PhyloP score.

RESULTS AND DISCUSSION

We performed WES on the mother (IV-4, pedigree in ref. 8) and 2 infants affected with fatal fetal hydrops (V-4 and V-7). We found only 3 "mutations" which were shared by both affecteds and not found in the mother, thus, necessarily from the father. It is hard to make a strong case for any of the 3 mutations as a cause for the worsening of the lymphedema although the gene product of one of the three has an important role in VEGFR signaling. However, it is important to point out that a modifying gene might not be pathogenic per se. The three genes are:

1) KCNE2, Potassium channel, voltage-gated, ISK-related sub-family, member 2. The mutation (NM_172201.1:c.22A>G:p.Thr8Ala, rs2234916), with minor allele frequency (MAF) of 0.0038 (ExAC), changes a tyrosine to alanine at position 8 in the protein and 4 different damage-prediction programs (see Methods) all considered it as likely benign. KCNE2 mutations have been associated with arrhythmias, especially long QT syndrome, and sudden infant death (10). However, it is also essential for thyroid function (11). Hypothyroidism is frequently diagnosed by a puffy face and is known to be associated with myxedema but maternal euthyroidism prevents the fetus from the effects of congenital hypothyroidism. Nonetheless, it is a possibility that the position 8 substitution of alanine could be a dominant-negative gain-of-function mutation causing decreased thyroid function by interaction with the *FOXC2* mutation. To our knowledge, none of the fetal hydrops patients in this family had thyroid studies because, as said, the fetus is protected from hypothyroidism by the mother's thyroid.

2) PLCD3, Phospholipase, delta-3 isoform codes for an enzyme which catalyzes the hydrolysis of phosphotidyl 4,5 biphosphate to inositol 3,4,5 triphosphate, an important second messenger, and diacyl glycerol (12). The mutation (NM_133373.4:c.310C>G:p.Pro104Ala,rs200792069, MAF=0.0013 (ExAC)) changes proline 104 to alanine and the 4 damage-predicting programs predict it to be benign despite the elimination of the fold-promoting proline. This second message is used in VEGF signaling (13) so alterations in levels could have a negative effect on lymphatic vessel formation. Nonetheless, given the likelihood of a benign mutation in one of many isoforms, it is hard to make a strong case for this mutation as the cause of progression to fatal fetal hydrops. If this isoform was found to be unique to lymphatics, a stronger case might be made.

3) SLC19A1, Solute carrier family member 19 (folate transporter), member 1. This gene encodes the major intestinal folate transporter (14) and variation in it has been much studied in regard to the relationship of folate to spina bifida (15). Lack of it can make lymphedema worse (16) but a deficiency is unlikely to occur with this rare mutation (rs373685390, MAF=0.0003 (ExAC), NM_001205206.1:c.926C>T:p.Ala269Val) or to cause such a severe worsening of the phenotype.

CONCLUSION

We performed whole exome sequencing of 2 children affected with severe neonatal hydrops and a FOXC2 mutation which had not caused fatal neonatal presentations of lymphedema-distichiasis in previous generations. The family had also been extensively studied using comparative genome hybridization without detecting a cause for the change in phenotype in the fifth generation. Three mutations shared by the affecteds were unlikely to be causative for the new phenotype although a variant could be a modifier without being pathogenic Thus, the mystery of the sometimes fatal presentation of *FOXC2* mutations remains unsolved until these variants have been found in other families or studied in cells or animals.

ACKNOWLEDGMENTS

Supported in part by a University of Arizona Translational Imaging Program Project Stimulus Award (MHW).

CONFLICT OF INTEREST AND DISCLOSURE

All authors declare that no competing financial interests exist.

REFERENCES

1. Erickson, RP: *FOXC2* and lymphedema distichiasis. In: Inborn Errors of Development. The Molecular Basis of Clinical Disorders of Morphogenesis. Epstein, CJ, RP Erickson RP, A Wynshaw-Boris (Eds). 2nd edition Oxford University Press. NY: 2008. pp. 841-845.
2. Fang, JM, SL Dagenais, RP Erickson, et al: Mutations in F*OXC2* (*MFH-1*), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. Am. J. Hum. Genet. 67 (2000), 1382-1388.
3. Daniel-Spiegel, E, A Ghalamkarpour, R Spiegel, et al: Hydrops fetalis: An unusual prenatal presentation of hereditary congenital lymphedema. Prenatal Diagn. 25 (2005), 1015-1018.
4. Bellini, C, MH Witte, C Campisi, et al: Congenital lymphatic dysplasias: Genetics review and resources for the lymphologist. Lymphology 42 (2009), 36-41.
5. Ghalamkarpour, A, C Debauche, E Haan, et al: Sporadic in utero generalized edema caused by mutation in the lymphangiogenic genes *VEGFR3* and *FOXC2*. J. Pediatr. 155 (2009), 90-93.
6. Martin-Almedina, S, I Martinez-Corral, R Holdhus, et al: EPHB4 kinase-inactivating mutations cause autosomal dominant lymphatic-related hydrops fetalis. J. Clin. Invest. 160 (2016), 3080-3088.
7. Witte, MH, R Erickson, M Bernas, et al: Phenotypic and genotypic heterogeneity in familial Milroy lymphedema. Lymphology 31 (1998), 145-155.
8. Sargent, C, J Bauer, M Khalil, et al: A five generation family with a novel mutation in *FOXC2* and lymphedema worsening to hydrops in the youngest generation. Am. J. Med. Genet. Part A, 164A (2014), 2802-2807.
9. Lee, W, Y Zhang, K Mukhyala, et al: Bi-directional SIFT predicts a subset of activating mutations. PLoS One 4 (2009), e8311. doi: 10.1371/journal.pone.0008311.
10. Liu, W, J Deng, G Wang, et al: *KCNE2* modulates cardiac L-type Ca(2+) channel. J. Molec. Cell. Cardiol. 72 (2014), 208-218.
11. Roepke, TK, EC King, A Reyna-Neyra, et al: *Kcne2* deletion uncovers its crucial role in thyroid hormone biosynthesis. Nature Med. 15 (2009), 1186-1194.
12. Ghosh, S, T Pawelczyk, JM Lowenstein: Phospholipase C isoforms delta 1 and delta 3 from human fibroblasts. High-yield expression in Escherichia coli, simple purification, and properties. Protein Expr. Purif. 9 (1997), 262-278.
13. Cross, MJ, J Diselius, T Matsumoto, et al: VEGF-receptor signal transduction. Trends Biochem. Sci. 28 (2003), 488-494.
14. Nguyen, TT, DL Dyer, DD Dunning, et al: Human intestinal folate transport: Cloning, expression, and distribution of complementary RNA. Gastroenterology 112 (1997), 783-791.
15. Shaw, GM, EJ Lammer, H Zhu, et al: Maternal periconceptional vitamin use, genetic variation of infant reduced folate carrier (A80G), and risk of spina bifida. Am. J. Med. Genet. 108 (2002), 1-6/
16. Földi-Börcsök, E, M Földi: Lymphedema and vitamins. Am. J. Clin. Nutr. 26 (1973), 185-190.

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