Published in final edited form as:

Am J Med Genet A. 2014 November ; 164(11): 2802–2807. doi:10.1002/ajmg.a.36736.

A Five Generation Family with a Novel Mutation in *FOXC2* and Lymphedema Worsening to Hydrops in the Youngest Generation

Carole Sargent¹, Julien Bauer¹, Muhamed Khalil², Parker Filmore³, Michael Bernas³, Marlys Witte³, M. Peggy Pearson⁵, and Robert P Erickson^{2,4}

¹ University of Cambridge, Dept of Pathology, Tennis Court Road, Cambridge, CB2 1QP, UK

² Dept of Pediatrics, University of Arizona, Tucson, Arizona

³ Dept of Surgery, University of Arizona, Tucson, Arizona

⁴ Dept of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona

⁵ Neonatology Associates, Ltd., Phoenix , Arizona

Abstract

We describe a five generation family with dominantly inherited lymphedema, but no distichiasis, in which 3/3 affected offspring in the fifth generation have died of fetal hydrops and related birth defects. Mutational analysis disclosed a novel mutation in *FOXC2* (R121C) in affected members. We searched for possible genetic influences on the greater severity of lymphedema (hydrops) in the fifth generation. Karyotypes disclosed an extra band in Xp in one affected fetus but this was also found in the mother. Copy number variation (CNV) studies on four members of the pedigree (mother of the three severely affected fetuses/infants; one severely affected; a full, and a half, unaffected sibs) did not detect the source of the Xp band or a possible influence on the severe phenotype. However, use of SNP arrays did allow identification of the portion of the maternal proximal Xp shared by a hydrops-affected daughter and son which was not shared by an unaffected daughter from the same sibship.

Keywords

Lymphedema; Fetal Hydrops; Lymphedema distichiasis; *FOXC2*; Xp

INTRODUCTION

Lymphedema-distichiasis (LD[OMIM 153400]) is a dominantly inherited disorder of lymphatics and other tissues, especially eyelids where a double row of eyelashes (disitichiasis) is frequently found [Erickson, 2008]. Of several 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* [Fang et al, 2000]. While identified by a translocation breakpoint in an 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 [Fang et al, 2000]. Such severe fetal hydrops in families of dominantly inherited lymphedema is rare enough that it warrants single case reports [Daniel-Spiegel et al, 2005]. Although large studies of congenital lymphedema have concluded that genetic causes are a rarity [Bellini et al, 2009], a recent report identified mutations in known familial lymphedema genes in 25% of surviving cases of generalized in utero lymphedema (two in *VEGFR3* and one in *FOXC2*) [Ghalamkarpour et al, 2009].

We have recently identified a second family with a *FOXC2* mutation and multiple cases of fetal hydrops. We identified a novel mutation in *FOXC2* in affected family members and searched for causes of fetal hydrops. An extra cytogenetic band on Xp was identified in one of the affected children and the mother, and studies of CNV and inheritance patterns of SNPs did not identify a unique cause of the fatal form of hydrops but were compatible with Xp involvement.

CLINICAL REPORT

Early generations of this family were briefly presented and illustrated [Family 1, Witte et al, 1998]. At that time five individuals (Individuals III 1,3,4 and IV 1,2) were examined with 5/5 having pre-pubertal onset of lymphedema and 4/5 with bilateral leg and genital (2/2 males) involvement. Four out of five of the cases were severe and all had recurrent cellulitis. The severe brawny swelling of the lower limbs was illustrated for Individuals III 2, 3 and 4, while lesser edema was exhibited for Individuals IV1 and 4 (then Individual IV 2) [Witte et al, 1998, Fig. 3]. Lymphangioscintigraphy in Individual III4 showed delayed tracer transport, lymphatic hypoplasia with extensive dermal diffusion, and lack of radiotracer in regional or retroperitoneal lymph nodes [Witte et al., 1998, Fig. 3]. Despite careful examination, none of the cases had distichiasis.

Since this earlier publication, Individual IV1 has a son with cleft palate and lymphedema (V1). Individual IV4 (previously IV2) has had two unaffected daughters (Individuals V2, 3) and three severely affected children who died of fetal hydrops and birth defects (Individuals V3-7; Fig. 1 shows the extended pedigree, Fig. 2 provides more recent illustrations of affecteds). Individual V 4 was born with tetralogy of Fallot, cystic hygroma, and hydrops and survived to 6 weeks. Individual V5 was terminated at 16 weeks due to cystic hygroma and lymphedema. Individual V6 was a “blighted ovum” which spontaneously aborted at 10 weeks gestation. Individual V7 was born with cleft palate and severe hydrops and died at 3 days of age. The family remains free of distichiasis.

MATERIALS AND METHODS

Informed consent for both participation and collection of blood samples was obtained from all family members in accordance with the Institutional Review Board of the University of Arizona. DNA was extracted from either venous blood or buccal swabs by standard

procedures. Sequencing of the single exonic, 70% GC rich *FOXC2* gene was performed as previously described [Dellinger et al., 2008]. The c.361C>T mutation found leads to a unique restriction site for *Nci I* in our PCR product. Thus, enzyme digestion was used to type many of the family members. The *Nci I* cutting created two smaller bands and left half of the larger band in the affected patients with no cutting in the unaffecteds.

Standard giemsa-banded karyotypes were performed on Individuals IV4 and V7 from the pedigree by a commercial lab.

First pass genome-wide genotyping and copy number analysis was carried out on Individuals IV4, V2, V3 and V4 using the IlluminaHumanCytoSNP-12 beadchip array (~300K SNPs from HapMap phases 1 and 2). This chip has a low SNP density on the X chromosome. A second pass analysis using Individuals IV 4, V3, V4, and V7 was performed with the IlluminaHumanOmni1-Quad (<10⁶ SNPs from HapMap phases 1, 2, 3), which has a mean SNP density of 1/ 2.4Kb, with 27,500 markers on X (~1/ 5Kb).

Copy Number Variants for each individual were identified using the Genome Studio software, using CNV partition 3.1.6, where a CNV is called when three consecutive SNPs show an abnormal allele count. CNV calls with a confidence value of <35 were excluded. Large LOH intervals (2 alleles, but no heterozygosity, over 1Mb in length) were manually re-inspected to assess their validity. All CNV intervals have been compared against only the control samples catalogued in dbVar (<http://www.ncbi.nlm.nih.gov/dbvar>: build 37) to determine if they were described previously. The size of each CNV interval, with an allele score of 3 or more, in samples from Individuals IV4 and V7 was scrutinized for the possible origin of the abnormal band on the X chromosome.

For analysis of potential X-chromosomal contributions to the phenotype, comparison of Individuals V3 and V4 (same paternal X chromosome) was performed in Genome Studio with genotype data from the IlluminaHumanCytoSNP-12, using the genome viewer, chromosome browser function. The differential interval on the X chromosome was confirmed as distal maternal Xp, and refined using SNP data from Individuals IV4, V3, V4 and V7, scored on the IlluminaHumanOmni1-Quad.

For the non-PAR region of the X chromosome, the genotype of Individual V7 was nominally assigned maternal allele 1 for each X-chromosomal locus (9960 markers for Xp). Maternal allele 2 was deduced from the genotype of the mother, IV4. The paternal X chromosomal alleles were imputed by comparing the genotypes of Individuals V3 and V4, as they have inherited the same allele for each locus from their father. By reconstructing the genotypes across the X chromosome, it was possible to determine if the full sibs, sisters V3 and V4, had inherited maternal allele 1 or 2 from the maternal X chromosomes. Data from the IlluminaHumanCytoSNP-12 were utilized to compare the half-sib V2 (2300 common alleles). Where Individual IV4 was heterozygous, but Individuals V2 and V4 were homozygous, it was possible to impute which maternal allele had been inherited by Individual V2. The alleles, as inherited by each offspring of Individual IV4 are summarized graphically in Figure 2.

RESULTS

Sequencing revealed a c.361C>T alteration which was confirmed by sequencing both strands. This results in a novel R121C substitution (mutations reviewed in Dellinger, et al, 2008; none further at HGMD.org) in the forkhead box of helix 3 and is a very highly conserved site. As indicated in Fig. 1, all affected members studied had the mutation.

Karyotyping disclosed an anomalous banding pattern in distal Xp in Individual V7 and his mother, IV4. Fluorescence *in situ* hybridization (FISH) with probes for the subtelomeric region of the X short arm (Xp,Yp, Vysis) and Xp22.32, the second most distal band on the X short arm, (STS, Vysis) showed normal localizations.

From the CNV analyses, multiple large regions, >1Mb, of homozygosity (two alleles, monozygosity) were detected, and are presented separately since losses are more important (Table I, and Supplementary Table I in supporting information online) from areas of copy number variation (loss, copy 0-1; gain, allele copy 3-4: gains are not shown. No intervals detected through these analyses were unique to the pedigree under investigation: all had been reported in the control samples catalogued in dbVar.

With the Illumina HumanCytoSNP-12 beadchip array, SNP studies showed that an unaffected daughter (V3) differed from her hydrops-affected sister (V4) by a portion of the mother's Xp from p11.3 to p22.2 (Fig 3A). Confirmation that this portion of the mother's X was shared by the hydrops affected son (V7) came from analysis of the Omni1-Quad data. Recombination breakpoints with respect to the maternal X chromosomes were identified from comparison of the genotypes of the offspring in the non-PAR region of Xp (Fig 3B). Individuals V3 and V4 share maternal markers on the X distal to rs5952572 (~44.2Mb): the breakpoint lies between markers rs1451511 and rs5952572, with the intervening SNP genotypes proving uninformative. The region of the maternal X chromosome common to individuals V4 and V7 extends from the PAR boundary to 22.9 Mb, with a breakpoint between markers rs5926147 and rs5926152. Its gene content is given in Supplementary Table II. The half-sibs V4 and V2 have inherited the opposite alleles in distal Xp, with a breakpoint between markers rs3764880 (12.9Mb) and rs5979833 (13.2Mb). If the abnormal cytogenetic pattern reported for V7 is diagnostic for the phenotype, and correlates with the defined maternal allele 1, then individual V2 should not carry this cytogenetic abnormality. We did not have cell lines to test whether the hydrops affected daughter (V5) also shared the maternal region in common with the other affected individuals, or has the cytogenetic anomaly observed in the hydrops affected son.

DISCUSSION

FOXC2 maps to 16q24.3 and lies in a cluster of three forkhead genes. *FOXC2* produces a 2.2 kb transcript with a 1.5 kb single exon coding region that is highly (~70%) GC-rich [Kaestner et al., 1996]. The *FOXC2* protein contains 501 amino acids. The one known domain in the gene is the forkhead domain. One functional *Foxc2* consensus forkhead domain-binding site has been defined in a reporter assay system [Miura et al., 1997]. However, binding specificity *in vivo* is thought to involve both the conserved forkhead

domain and flanking sequences [Miura et al., 1997]. It up regulates *Ang2* [Xue et al, 2008] and integrin beta 3 [Hayashi et al, 2008] and down regulates *Pax3* [Lagha et al, 2009].

The mutation found in the family is in the highly conserved DNA-binding, FOX box. Previously a mutation at the same amino acid site (R121H) was extensively studied by Berry et al [2005]. When *FOXC2* with the mutation was expressed in cultured cells following plasmid transfection, the mutant protein was stable but showed decreased phosphorylation and was grossly defective in nuclear localization. Gel shift mobility tests and luciferase response expression showed defective binding to a *FOXC2*, DNA-targeting sequence [Berry et al, 2005]. Amino acid 121 is located externally on the 3rd helix and the cysteine substitution would be accessible to disulfide linkage so different effects (no distichiasis) than other mutations might be explained by redox differences between tissues.

Karyotyping disclosed an apparent extra band on Xp which was not a telomeric duplication as studied with two probes. Xp has been strongly implicated as the location of gene(s) which are involved in lymphedema in Turner patients [Boucher, et al, 2001, Fig 4]. The most plausible candidate for the phenotype is *VEGFD*, the gene for a lymphatic growth factor, at ~15.4Mb.

The possible role for *VEGFD* variants as modifiers of lymphedema remains contentious. *VEGFD* is increased in the serum of patients with primary lymphedema [Fink et al, 2004], and in acute Kawasaki disease with edema [Ebata et al., 2011]: upregulation of protein expression also appears to correlate to increased lymphangiogenesis, increased rates of metastasis and poor prognosis in a number of studies of cancer patients [for examples, see Nakamura et al., 2003; Harris et al., 2011; Lin et al, 2011; Yanagawa et al., 2012]. In addition, over expression of *VEGFD* has been shown to promote neo-lymphangiogenesis in tumors, again supporting a role in human lymphangiogenesis [Stacker et al, 2001, Koch et al, 2009] but other work shows only *VEGFC* to have such a role [Shibata et al, 2013]. It seems not to be a critical gene for lymphedema, at least in mice, since the mouse knockout of *VegfD* has minimal effects on lymphangiogenesis [Baldwin et al, 2001] and the double knockout of *VegfC* and *VegfD* shows the same phenotype as the *VegfC* knockout [Haiko et al, 2008]. However, expression of *VegfD* in skin (targeted by a keratin 14 promoter) can rescue the lymphatic dysplasia seen in *VegfR3* heterozygous knockouts. This suggests functional redundancy of *VegfC* and *VegfD*. In a zebra fish model, *VegfD* activates Sox18 expression and plays a role in blood vasculogenesis as well [Duong et al, 2014].

Initially, given the evidence for correlation of elevated expression of serum *VEGFD* and lymphedema, and the abnormal appearance of the X chromosome in this pedigree, we wondered if the sub PAR interval of the X chromosome containing *VEGFD* (aka *FIGF*) could be duplicated. Thus, we used CNV studies to search for such a possible duplication. However, there was no evidence for a CNV gain involving this locus, or any other region of Xp, that was large enough to explain the abnormal banding pattern observed by cytogenetic analysis. There were many large intervals of apparent monozygosity probably arising from low minor allele (or extended haplotype) frequencies in these regions of the genome. In all of the examples listed, other family members show substantial overlapping regions of monozygosity below the 1Mb interval size used as a cut-off value in the software.

Furthermore, no regions of chromosomal gain were large enough (CNV size range 412bp to 23897bp) to explain the abnormal banding pattern of the maternal X inherited by her hydrops affected son.

There is still support for involvement of the abnormal X from analysis of the Illumina data. A difference was found between the affected and unaffected siblings: the hydrops affected infants shared a portion of the maternal Xp not inherited by an unaffected sibling. It is possible that this shared portion of Xp represents the portion of the chromosome involved in the cytogenetic differences, and covers the lymphedema critical regions that are causative for the fetal hydrops. Significantly this interval harbors the locus for *VEGFD*, the only gene between PAR and 22.9Mb currently known to be involved in lymphangiogenesis (See Supplementary Table II in supporting information online).

Although the additional band could be of autosomal origin, no region of the genome with a copy number gain was large enough to predict a cytogenetically visible alteration to the karyotype. Therefore, autosomal material can only be attributed to the cytogenetic abnormality if this reflects a balanced translocation which is not cytogenetically obvious. Finally, since the hydrops affected infants do share the same maternal X chromosome alleles, the banding pattern could reflect an intra-chromosomal rearrangement on Xp. This would not necessarily be detectable using SNP arrays, unless a structural rearrangement is accompanied by a substantial amplification or loss of neighboring genomic material. Unfortunately, all CNVs on the X chromosome are also seen in normal individuals, and do not appear to be unique to this family.

The same karyotypic finding for the X chromosome was present in the mother who did not show hydrops. However, there are possible scenarios to explain this and maintain its relevance: her chromosomal mutation could be somatic and include the germ-line or it might not have a zygotic effect, but an effect with maternal transmission. There may also be allelic variants of other genes which interact with *VEGFD* polymorphisms, of which we are unaware at this time, that have been inherited from the father of the affected offspring. Thus our CNV studies do not disclose a possible genomic cause of the increased severity of disease in this case from the fifth generation. Additionally, we are limited by not having cell lines and SNP analysis on more of the family. Currently, therefore, we do not have a definitive explanation for the occurrence of hydrops in three cases from this pedigree or for the two cases initially published [Fang et al., 2000].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

P Filmore was supported by NIH HL07479 (M Witte PI). Robert P Erickson was supported by the Hosclaw Family Professorship in Human Genetics and Inherited Diseases.

REFERENCES

- Baldwin ME, Halford MM, Roufail S, Williams RA, Hibbs ML, Grail D, Kubo H, Stacker SA Achen MG. Vascular endothelial growth factor D is dispensable for development of the lymphatic system. *Mol. Cell Biol.* 2005; 25:2441–2449. [PubMed: 15743836]
- Bellini C, Witte MH, Campisi C, Bonioli E, Boccardo F. Congenital lymphatic dysplasias: genetics review and resources for the lymphologist. *Lymphology.* 2009; 42:36–41. [PubMed: 19499766]
- Berry FB, Tamimi Y, Carle MV, Lehmann OJ, Walter MA. The establishment of a predictive mutational model of the forkhead domain through the analyses of FOXC2 missense mutations identified in patients with hereditary lymphedema with distichiasis. *Hum. Molec. Genet.* 2005; 14:2619–2627. [PubMed: 16081467]
- Boucher CA, Sargent CA, Ogata T, Affara N. Breakpoint analysis of Turner patients with partial Xp deletions: implications for the lymphoedema gene location. *J. Med. Genet.* 2001; 38:591–598. [PubMed: 11546827]
- Daniel-Spiegel E, Ghalamkarpour A, Spiegel R, Weiner E, Vikkula M, Shalev E, Shalev SV. Hydrops fetalis: an unusual prenatal presentation of hereditary congenital lymphedema. *Prenatal Diagn.* 2005; 25:1015–1018.
- Dellinger MT, Thome K, Bernas MJ, Erickson RP, Witte MH. Novel FOXC2 missense mutation identified in patient with lymphedema-distichiasis and review. *Lymphology.* 2008; 41:98–102. [PubMed: 19013876]
- Duong T, Koltowska K, Pichol-Thievent C, Le Guen L, Fontaine F, Smith KA, Truong V, Skoczylas R, Stacker SA, Achen MG, Koopman P, Hogan BM, Francois M. VEGFD regulates blood vascular development by modulating SOX18 activity. *Blood.* 2014; 123:1102–112. [PubMed: 24269955]
- Ebata R, Abe J, Yasukawa K, Hamada H, Higashi K, Suwazono Y, Saito H, Terai M, Kohno Y. Increased production of vascular endothelial growth factor-d and lymphangiogenesis in acute Kawasaki disease. *Circ J.* 2011; 75:1455–1462. [PubMed: 21483160]
- Erickson, RP. FOXC2 and Lymphedema Distichiasis. In: Epstein, CJ.; Erickson, RP.; Wynshaw-Boris, A., editors. *Inborn Errors of Development. The Molecular Basis of Clinical Disorders of Morphogenesis.* 2nd edition. Oxford University Press; NY: 2008. p. 841-845.
- Fang JM, Dagenais SL, Erickson RP, Arlt MF, Glynn MW, Gorski JL, Seaver LH, Glover TW. Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. *Am. J. Hum. Genet.* 2000; 67:1382–1388. [PubMed: 11078474]
- Fink AM, Kaltenegger I, Schneider B, Frühauf J, Jurecka W, Steiner A. Serum level of VEGF-D in patients with primary lymphedema. *Lymphology.* 2004; 37:185–189. [PubMed: 15693535]
- Ghalamkarpour A, Debauche C, Haan E, van Regemorter N, Sznajder Y, Thomas D, Revencu N, Gillerot Y, Boon LM, Vikkula M. Sporadic In Utero generalized edema caused by mutation in the lymphangiogenic Genes VEGFR3 and FOXC2. *J. Ped.s.* 2009; 155:90–93.
- Haiko P, Makinen T, Keskitalo S, Taipale J, Karkkainen MJ, Baldwin M, Stacker SA, Achen MG, Alitalo K. Deletion of Vascular Endothelial Growth Factor C (VEGFC) and VEGF-D is not equivalent to VEGF Receptor 3 deletion in mouse embryos. *Mo. Cell Biol.* 2008; 28:4843–4850.
- Harris NC, Paavonen K, Davydova N, Roufail S, Sato T, Zhang YF, Karnezis T, Stacker SA, Achen MG. Proteolytic processing of vascular endothelial growth factor-D is essential for its capacity to promote the growth and spread of cancer. *FASEB J.* 2011; 25:2615–2625. [PubMed: 21515745]
- Hayashi H, Sano H, Seo S, Kume T. The Foxc2 Transcription Factor Regulates Angiogenesis via Induction of Integrin beta3 Expression. *J. Biol. Chem.* 2008; 283:23791–23800. [PubMed: 18579532]
- Kaestner KH, Bleckmann SC, Monaghan AP, Schlondorff J, Mincheva A, Lichter P, Schutz G. Clustered arrangement of winged helix genes fkh-6 and MFH-1; possible implications for mesoderm development. *Development.* 1996; 122:1751–1758. [PubMed: 8674414]
- Koch M, Dettori D, Van Nuffelen A, Souffreau J, Marconcini L, Wallays G, Moons L, Bruyere F, Oliviero S, Noel A, Foidart J-M, Carmeliet P, Dewerchin M. VEGF-D deficiency in mice does not effect embryonic or postnatal lymphangiogenesis but reduces lymphatic metastasis. *J. Pathol.* 2009; 219:356–364. [PubMed: 19718705]

- Lagha M, Brunelli S, Messina G, Cumano A, Kume T, Relaix F, Buckingham ME. Pax3:Foxc2 Reciprocal Repression in the Somite Modulates Muscular versus Vascular Cell Fate Choice in Multipotent Progenitors. *Developmental Cell*. 2009; 17:892–899. [PubMed: 20059958]
- Miura N, Iida K, Kakinuma H, Yang XL, Sugiyama T. Isolations of the mouse (MFH-1) and human (FKHL-14) mesenchyme fork head-1 genes reveals conservation of their gene and protein structures. *Genomics*. 1997; 41:489–492. [PubMed: 9169153]
- Lin W, Jiang L, Chen Y, She F, Han S, Zhu J, Zhou L, Tang N, Wang X, Li X. Vascular endothelial growth factor-D promotes growth, lymphangiogenesis and lymphatic metastasis in gallbladder cancer. *Cancer Lett*. 2012; 314:127–136. [PubMed: 22071224]
- Shibata M, Shibata E, Morimoto J, Harada-shiba M. Therapy with siRNA for Vegf-c but Not for Vegf-d Suppresses Wide-spectrum Organ Metastasis in an Immunocompetent Xenograft Model of Metastatic Mammary Cancer. *Anticanc. Res*. 2013; 33:4237–4247.
- Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, Jackson DG, Nishikawa S, Kubo H, Achen MG. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. *Nat. Med*. 2001; 7:188–191.
- Witte MH, Erickson R, Bernas M, Andrade BM, Reiser F, Conlon W, Hoyme HE, Witte CL. Phenotypic and genotypic heterogeneity in familial Milroy lymphedema. *Lymphology*. 1998; 31:145–155. [PubMed: 9949386]
- Xue Y, Cao R, Nilsson D, Chen S, Westergren R, Hedlund E-M, Martijn C, Rondahl L, Krauli P, Walum E, Enerback S, Cao Y. FOXC2 controls Ang-2 expression and modulates angiogenesis, vascular patterning, remodelling, and functions in adipose tissue. *Proc. Nat'l Acad. Sci. USA*. 2008; 105:10167–10172. [PubMed: 18621714]
- Yanagawa T, Shinozaki T, Watanabe H, Saito K, Raz A, Takagishi K. Vascular endothelial growth factor-D is a key molecule that enhances lymphatic metastasis of soft tissue sarcomas. *Exp Cell Res*. 2012; 318:800–808. [PubMed: 22326461]

Figure 1. Pedigree of the family. Upper left hand corner, affected status (filled in if affected); lower right hand (speckled), whether examined; lower left hand mutation status (filled in if carrying the mutation, asterisk if tested and not carrying the mutation).

III-3, III-1, III-4

IV-1, IV-4, IV-6

Figure 2.

Photographs of legs of some of the affecteds in generations three and four (generation three previously published in Witte, et al, 1998, used by permission).

Figure 3.

Panel A shows comparison of the genotyping data from the Illumina HumanCytoSNP-12 beadchip array for sister V3 against V4. Light grey represents the designated maternal allele 1, and dark grey, maternal allele 2. The region differing between the siblings is shown as the variable plot from PAR to Xp11.3. Panel B is a schematic representation of maternal X alleles inherited by all offspring of IV-4 based on genotyping data from the Illumina HumanOmni1-Quad. Data confirm allele sharing between the PAR boundary and 22.9Mb in the two affected offspring V4 and V7.

Figure 4.

Previous analyses of Turner patients with genomic clones for X and Y homologous sequences delineated regions of the X and Y chromosomes where deletions were more common in patients with lymphedema. Analysis of patient phenotypes also suggested the presence of a modifier distal to *DMD*, for which *FIGF* is a candidate. (Adapted from Boucher, et al, 2001).

Table I

List of all regions showing monozygosity over > 1Mb intervals in family members. Common/ overlapping intervals are shaded. All positions in the human genome relate to assembly GRCh37.

Individual	Chr	Start	End	Size
IV 4	1	27990613	29608356	1617743
V 7	1	35580706	36975893	1395187
IV 4	1	45166383	46795447	1629064
V 4	1	92380302	93470714	1090412
V 4	1	102747793	104161265	1413472
V 3, V4	2	135171675	137854860	2683185
V 7	2	135299531	137854860	2555329
V 4	2	186195271	187498562	1303291
IV 4	3	52070955	53133487	1062532
V 3	3	108635060	109765649	1130589
IV 4	5	68429784	70307386	1877602
V 7	5	128743894	131743465	2999571
V 3, V 7	7	121934655	122992488	1057833
V 3	8	48288683	51029371	2740688
V 4	8	57804484	58854051	1049567
IV 4	8	82048061	83590728	1542667
V 3, V-7	8	108912166	110691429	1779263
V 7	10	74046370	75118586	1072216
V 7	10	104192691	105362287	1169596
V 4	11	50149988	51342126	1192138
V 4, V-7	14	61947044	62968343	1021299
V 7	14	91107323	92403660	1296337