BMJ Connections Clinical Genetics and Genomics

Structural variants dysregulating *FOXC2* cause lymphoedema distichiasis syndrome: a series of case reports

Sara E Dobbins ,[1] Ruby Moy,[1] Alexandra J Noble,[2] Holm H Uhlig,[2,3,4] Emma Fowler,[5] Angela Davies,[5] Jing Yu,[3] Helena G Karstensen,[6] Christoffer Valdemar Nissen,[7] Bitten Schönewolf-Greulich,[6] Malene Djursby,[6] Silvia Martin-Almedina,[1] Kristiana Gordon,[1,8] Pia Ostergaard,[1] Alistair T Pagnamenta,[3,4,9] Sahar Mansour[1,10]

To cite: Dobbins SE, Moy R, Noble AJ, *et al*. Structural variants dysregulating *FOXC2* cause lymphoedema distichiasis syndrome: a series of case reports. *BMJ Connections Clinical Genetics and Genomics* 2025;**2**:e000033. doi:10.1136/bmjccgg-2025-000033

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjccgg-2025-000033).

SED and RM are joint first authors.

Received 24 June 2025
Accepted 31 October 2025

For numbered affiliations see end of article.

Correspondence to
Dr Sahar Mansour;
sahar.mansour@stgeorges.nhs.uk

ABSTRACT

Background Lymphoedema distichiasis syndrome (LDS) is an autosomal dominant inherited form of lymphoedema, typically presenting with lower limb lymphoedema from puberty and distichiasis from birth. For up to 97% of patients, a coding change in *FOXC2* is identifiable. However, a number of case studies identifying structural variants (SVs) outside of the *FOXC2* locus have been reported.

Methods Using a range of approaches, including genome sequencing, we investigated whether we could identify SVs, which may be impacting *FOXC2* in a series of unsolved cases. In silico tools were used to annotate these variants for potential insights into regulatory mechanisms.

Results We identified five families with SVs impacting *FOXC2*. One with a mosaic deletion causing a truncated protein, and four with SVs impacting the non-coding portion of the genome downstream of *FOXC2*, likely causing dysregulation of the gene. A review of 28 patients in the DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources (DECIPHER) database with 16q24 deletions, including the whole of *FOXC2*, identified only two reported to have lymphoedema or distichiasis.

Conclusion These additional cases bolster the evidence supporting *FOXC2* as a monogenic cause of LDS. The fact that these SVs are not detected through panel testing underscores the recommendation for employing genome sequencing or array-comparative genomic hybridisation (CGH) in patients with suspected LDS who lack a genetic diagnosis. Public databases of patients with 16q24 deletions, incorporating *FOXC2*, but without lymphoedema reported, demand caution when interpreting deletions affecting the entirety of *FOXC2*. Work is required to explore the role of these putative regulatory elements whose dysregulation may cause this syndrome.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ For 97% of patients with lymphoedema distichiasis syndrome (LDS), a coding change in *FOXC2* can be identified, although there have been several isolated case reports with structural variants downstream of *FOXC2* over the last two decades.

WHAT THIS STUDY ADDS

⇒ We identify an additional four families with structural variants likely impacting regulatory elements causing dysregulation of *FOXC2*, and an additional individual with a mosaic deletion overlapping *FOXC2*.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study underscores the importance of considering array-CGH, genome sequencing or other methods to detect structural variants in patients with LDS who lack a genetic diagnosis; However, public databases of patients with 16q24 deletions, incorporating *FOXC2*, but without lymphoedema reported, demand caution when interpreting deletions affecting the entirety of *FOXC2*.

INTRODUCTION

Lymphoedema distichiasis syndrome (LDS) is an autosomal dominant inherited form of primary lymphoedema. While distichiasis is typically present from birth, onset of lower limb lymphoedema may be during childhood, postpubertal or later. Distichiasis is a congenital anomaly, whereby there is aberrant growth of eyelashes from the meibomian glands of the inner eyelid causing corneal irritation, recurrent conjunctivitis and photophobia. The condition is also associated with other congenital abnormalities including congenital heart disease, cleft palate (with or without Pierre Robin Sequence), renal abnormalities, varicose veins and spinal extradural cysts.[1]

While LDS presentation is variable both within and between families, the genetic basis is homogenous with the vast majority of patients harbouring heterozygous, pathogenic variants in the *FOXC2* gene.[2] However,

some cases of structural variants (SVs) causing dysregulation of *FOXC2* have been reported, including balanced translocations,[3 4] deletions distal to *FOXC2*[5 6] and deletions and duplications overlapping both 5' and 3' untranslated regions of *FOXC2*.[7 8] It is likely that the distal events delete or disrupt cis-regulatory elements controlling the expression of *FOXC2*, with translocations disrupting the structure of local topological associated domains (TADs). Here, we document five families with LDS, each with SVs impacting *FOXC2*, through a range of mechanisms.

MATERIALS AND METHODS

Patient recruitment and clinical investigation

Four cases were recruited from the St George's University Hospitals NHS Foundation Trust Primary Lymphoedema clinic, UK; and one from Bispebjerg Hospital, Denmark. The Case Report guidelines were consulted.[9] Laboratory investigations, including genetic testing and imaging, were performed as clinically indicated. Routinely collected clinical data were retrospectively evaluated with ethical approval. Lower limb lymphoscintigraphy was performed according to standard local procedure by injecting radioactive isotope (technetium-99m-nanocoll) into the web spaces between the toes.[10 11] Images were taken at 15 min and 2 hours postinjection with a gamma camera. Quantification figures 2 hours postinjection were calculated, where possible, as percentage of tracer retention within right and left foot, and tracer uptake in the ilioinguinal nodes.

Sequencing and validation

Two individuals from family 1 and three from family 2 were recruited to the 100 000 Genomes Project (100kGP) and genome sequencing performed as previously described.[12] SVs were identified using Genomics England's SV pipeline, which employs CANVAS[13] and MANTA[14] for SV detection. Prioritisation of SVs used SVRare.[15] Reads were visualised using the Integrative Genomics Viewer (IGV).[16] Expression data were not available as part of the 100kGP for either family 1 or family 2 (V.19). The inversion in family 1 was further investigated via fluorescent in situ hybridisation (FISH) and Nanopore sequencing technology (online supplemental figure 1); the ligation sequencing kit (SQK-LSK114) with 1 µg of DNA was run on a PromethION flow cell. Each of the samples was sequenced for 72 hours. Read totals and coverage were reported for II.1 and II.2 (figure 1), respectively as follows: 9 604 000 and 9 030 000, 27.5x and 26x. The translocation in family 2 was confirmed in II.3 and III.2 (figure 1; not tested in I.2 or II.1) by in situ hybridisation using FISH probes Cytocell 5p subtel (5ptel48, TexasRed) and Cytocell 16q subtel (16qdel48, fluorescein isothiocyanate).

Array-CGH was performed on DNA from the proband of family 3 (II.1) using oligonucleotide arrays with approximately 60 000 probes across the genome (Agilent 085030, median resolution 120 kb).

A single nucleotide polymorphism (SNP) array was performed on DNA from affected members of family

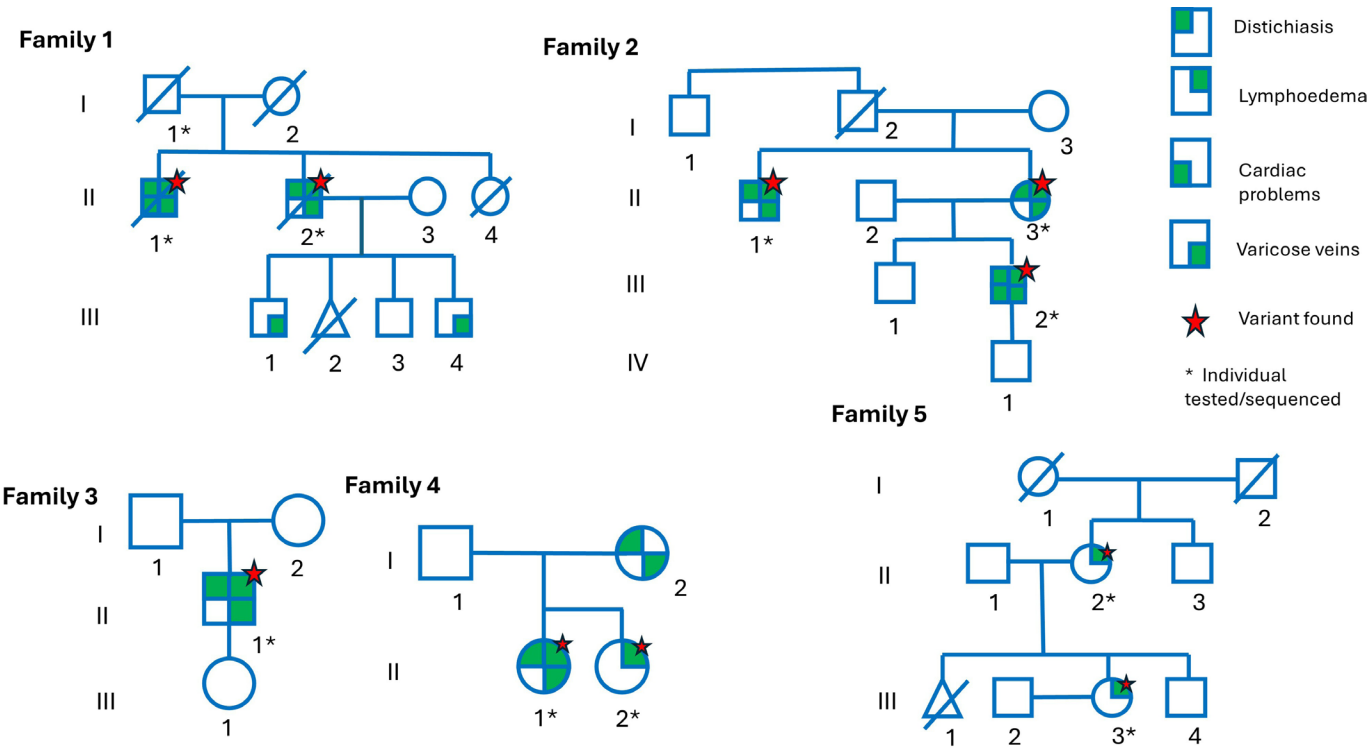


Figure 1 Pedigrees for families 1–5. Pedigrees for the five families we have presented with structural variants (SVs). Affected individuals are indicated with filled circles or squares. Patients sequenced/tested are shown by (*), with the red star indicating a heterozygous SV was found. Triangles denote first trimester miscarriages. A diagonal line through a square or circle indicates the individual has deceased.

4 (II.1 and II.2; not tested in I.2) using Thermo Fisher Scientific HT-CMA 96.r3 array with median resolution of 74 kb and 125 kb for losses and gains, respectively. The in situ hybridisation, array-CGH and SNP array assays were undertaken by National Health Service diagnostic laboratories.

In family 5, whole-exome sequencing (WES) was conducted as a duo-analysis on DNA from the affected mother (II.2) and daughter (III.3) using the Twist Human Core Exome kit (Twist Bioscience, San Francisco, California, USA) and NovaSeq 6000 sequencing (Illumina, San Diego, California, USA) to a median depth of >80X with 98% bases covered >20X. The WES dataset was analysed using VarSeq software (Golden Helix) and did not reveal any single nucleotides of interest in the applied in silico gene panel. Copy number variant (CNV) detection was carried out using the VarSeq build in CNV analysis tool, which indicated a heterozygous deletion downstream of *FOXC2*. The deletion was subsequently confirmed via array-CGH (400K oligoarray, Agilent SurePrint G3 design ID: 021850).

UCSC LiftOver tools[17] were used for transferring coordinates between builds where required. All variants are reported in GRCh38.

DECIPHER

We identified deletions reported in DECIPHER[18] (V.11.27) within the region chr16:86568841-88427540, <1.5 Mb in size. Variants classified as benign, or that started or ended outside of the region, were excluded. Individuals where another variant was classified as likely pathogenic were excluded. Those individuals who carried out the original analysis and collection of the DECIPHER data bear no responsibility for the further analysis or interpretation of the data.

Annotation and plots

All genomics locations are reported in build GRCh38. The genomic location of genes was extracted using Ensembl Genes 113 via BioMart.[19] GnomAD SVs and CNVs were downloaded for the region chr16:86469286-87559171 using gnomAD SVs and CNVs V.4.1.0.[20] Correlations between size and allele frequency were calculated in R (V.4.4.1) using a Spearman's rank correlation test. Promoter capture Hi-C data were obtained using the three-dimensional (3D) Genome Browser,[21] for endothelial precursor cells,[22] extending the default search window ±800 kb around the *FOXC2* gene, and extracting only interactions for the queried gene. TADs were identified from data obtained using the 3D Genome Browser selecting human umbilical vein endothelial cell (HUVEC) tissue with 10 kb resolution.[23] Lymphatic endothelial cells (LEC) cis-regulatory elements (CREs) and transcription factor PROX1 binding data were downloaded from Pirri *et al*[24] and Kazenwadel *et al*.[25]

Plots were produced in R using the Plotgardener package.[26]

Patient and public involvement

While patients were not involved in the design, conduct, reporting or dissemination plans of this study, our research was motivated by patients' requests for genetic diagnoses within clinics. Patients will be notified of clinically relevant findings where appropriate.

RESULTS

Family 1

This family was previously reported by Brice *et al*.[1] Two brothers presented with bilateral lower limb lymphoedema and distichiasis (figure 1, table 1). II.1 had extra eyelashes diagnosed in early childhood and required electrolysis to remove them. His lymphoedema presented at the age of 19 years. He later developed bilateral lower limb varicose veins. In addition, he had a congenital tricuspid valve abnormality, Raynaud's disease, early onset of bilateral cataracts, scoliosis and supernumerary teeth. He died of pulmonary fibrosis at the age of 63 years.

II.2 was born with congenital ptosis of the left eyelid and bilateral hearing loss. He had distichiasis diagnosed in early childhood and a left divergent squint. He presented with bilateral lower limb lymphoedema at the age of 23 years with varicose veins, which were stripped at the age of 25 years. He also developed scoliosis. His wife miscarried at 6 months' gestation; the baby was reported as having a 'cystic hygroma'. Two of their sons (III.1 and III.4) have early onset varicose veins and are at risk, so they will be offered testing.

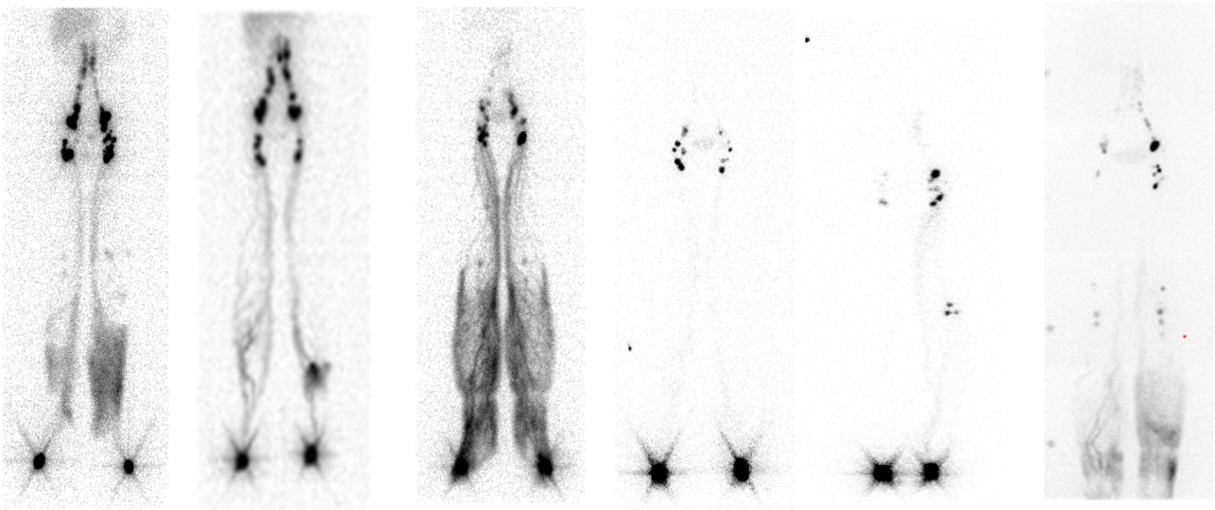
Their sister (II.4) died in the neonatal period—the cause was not known. Their mother (I.2) was also reported as having bilateral lower limb lymphoedema, although distichiasis was not reported.

Genome sequencing performed by Genomics England identified a heterozygous 1.6 Mb inversion mapping 271 kb distal to *FOXC2* (table 1, figure 3, chr16:86841016-88427540); present in both brothers (mother not sequenced) but absent from the unaffected father. The inversion was not previously detected via karyotype analysis due to its size being beyond the resolution of this technique. Confirmation of the inversion was attempted with FISH studies but, due to the close proximity of the inversion breakpoints, the FISH probes used in the analysis did not resolve in metaphase and confirmation was not possible. However, the inversion was successfully verified using Nanopore sequencing (online supplemental figure 1). It should be noted that the inversion fully incorporates 14 genes (*ZCCHC14*, *JPH3*, *LINC02181*, *SLC7A5*, *KLHDC4*, *ZCCHC14-DT*, *MIR6775*, *C16orf95*, *CA5A*, *LINC02182*, *BANP*, *C16orf95-DT*, *FBXO31*, *MAP1LC3B*) whose expression may also be disrupted, with the inversion end point predicted to fall in the third exon of *ZNF469* thereby also disrupting protein structure for this gene. Biallelic variation in ZNF469 has been previously associated with various disorders of the eye including Ehlers-Danlos syndrome and keratoconus but there were no features of

Table 1 Details of 16q structural variants in families with LDS

Family	Type	Location (GRCh38)	Size (kb)	Distance to 3' *FOXC2* (kb)	Lower limb lymphoscintigraphy scan	Vein scans
1	INV	chr16:86841016-88427540	1587	271	II.2: hyperplasia of the lymphatics in legs and thighs (images not available).	II.2: bilateral SV vein incompetence
2	ABT	chr16:86619099-chr5:1162909	N/A	49	II.3: dilated and tortuous lower limb lymphatic tracts with some superficial re-routing of tracer in the left lower limb. III.2: slightly tortuous lower limb lymphatic tracts with evidence of deep re-routing via the popliteal lymph nodes. Re-routing of tracer seen in both below-knee regions (creating an outline of the legs), likely due to lymphatic reflux as a result of lymphatic valve anomalies. The uptake of tracer in the groins is falsely elevated due to associated venous disease likely flushing the tracer through prematurely.	II.3: left lower limb venous incompetence confirmed III.2: right lower limb incompetent long SV
3	DEL*	chr16:86567588-86568736	1.1	Overlap 3' end *FOXC2*	II.1: dilated and tortuous lower limb lymphatic tracts with bilateral evidence of deep re-routing via the popliteal lymph nodes. Significant re-routing of tracer seen in the leg, likely due to lymphatic reflux. Quantification data confirm impaired lymphatic function in both lower limbs.	II.1: marked venous incompetence on right lower limb
4	DEL	chr16:86592947-87202476	610	23	II.1: reduced inguinal lymph node uptake visualised in both groins, and confirmed on quantification data, due to impaired lymphatic function. II.2: evidence of deep re-routing via the left popliteal lymph nodes. Asymmetrical reduced inguinal lymph node uptake visualised in both groins (right leg worse than left leg), and confirmed on quantification data, due to impaired lymphatic function.	II.1: bilateral long saphenous vein reflux was observed II.2: minor reflux bilaterally
5	DEL	chr16:86701917-88132633	1400	132	III.3: dilated and tortuous lymphatics with deep re-routing via the popliteal lymph nodes. There are signs of lymphatic reflux in the legs and reduced inguinal lymph node uptake bilaterally.	II.2: bilateral venous incompetence in superficial and deep veins III.3: bilateral long saphenous vein reflux

*Mosaic deletion. Distance to 3' UTR *FOXC2* defined based on GRCh38 coordinates of *FOXC2* transcript (ENST00000649859.1, including UTRs) from Gencode V46: chr16:86566829-86569728. Lymphoscintigraphy images presented in figure 2 where available.
ABT, assumed balanced translocation; DEL, deletion; INV, inversion; LDS, lymphoedema distichiasis syndrome; N/A, not available; SV, saphenous vein; UTR, untranslated region.



Patient	FAM2 III.2		FAM2 II.3		FAM3 II.1		FAM4 II.1		FAM4 II.2		FAM5 III.3	
Lower limb	R	L	R	L	R	L	R	L	R	L	R	L
Retention in foot	47.9	39.4	66%	64.3%	46.8%	58.2%	93%	94%	98.8%	92.7%	80%	58%
Uptake in groin	24.8 %	21.1 %	15.9%	14.3%	5%	5.4%	1.5%	0.7%	0.3%	4%	6.5%	10.6%

Figure 2 Lymphoscintigraphy images for families (FAMs) 2–5. Anterior view of lower limb lymphoscintigraphy. Quantification figures 2 hours postinjection are provided (<12% uptake of tracer in the groin lymph nodes is evidence of impaired lymphatic function/lymphoedema). FAM 2 shows characteristic lymph scan findings of lymphoedema distichiasis syndrome (LDS), namely widened and tortuous lymphatics with an accumulation of tracer in the lower limbs, likely due to reflux of lymphatic fluid as a result of abnormal lymphatic valve development. The uptake of tracer in the groins is falsely elevated due to associated venous disease likely flushing the tracer through prematurely. FAM 3 II.1 has characteristic features of LDS with tortuous lymphatic tracts, lymphatic reflux and reduced inguinal node uptake of tracer. FAM 4 shows reduced inguinal lymph node uptake visualised in both groins and confirmed on quantification data, due to impaired lymphatic function. FAM 5 (III.3) shows tortuous lymphatics, reduced inguinal uptake and lymphatic reflux/dermal backflow in the lower limbs. All four FAMs with scans also have evidence of deep re-routing via the popliteal lymph nodes, likely due to re-routing of the tracer via the deep lymphatic system. Scan results are summarised in table 1. No imaging was available for FAM 1.

these conditions in this family.[27 28] The likely disruption of *FOXC2* regulation remains the most clinically relevant impact of this inversion.

Family 2

The proband of family 2 (III.2, table 1, figures 1 and 2) presented in early childhood with tetralogy of Fallot and distichiasis. He developed varicose veins at the age of 11 years and mild bilateral lower limb lymphoedema in his teens.

His mother (II.3) had mild bilateral lower limb lymphoedema (figure 2) with varicose veins since age 19 years, and childhood onset of distichiasis. The maternal uncle (II.1) had bilateral lower limb lymphoedema with varicose veins, which began at the age of 17 years. He also had asymptomatic distichiasis. The maternal grandfather (I.2) was also reported as having distichiasis and bilateral lower limb lymphoedema.

Initial karyotypic analysis of both proband and mother was reported as normal. Genome sequencing of the three family members identified a balanced translocation between the terminal region of the short arm of chromosome 5 and the long arm of chromosome 16, with the breakpoint mapping to band 16q24.1, 49 kb downstream of *FOXC2* (table 1, figure 3, chr16:86619099-chr5:1162909). Review of archived images of metaphase cells from the original karyotype analysis, carried out in both mother and son, detected the derivative chromosome 16 in both. The karyotype analyst stated that this was subtle and likely only detectable with the knowledge of the WGS findings given that the translocated chromosome 16 segment was only ~4 MB, which is generally considered beyond the resolution of karyotype analysis. The derivative chromosome 5 was not discernible by karyotype (online supplemental figure 2). FISH studies, using probes specific for the subtelomere regions of the short arm of chromosome 5 and the long arm of chromosome 16, were also carried out and confirmed the translocation of the terminal region of the long arm of chromosome 16 to the short arm of chromosome 5. The chromosome 5 short arm breakpoint, detected by WGS as being ~1 Mb from the terminal end, was distal to the subtelomeric probe used in the FISH analysis (Cytocell 5p subtel probe; 5ptel48) and therefore the

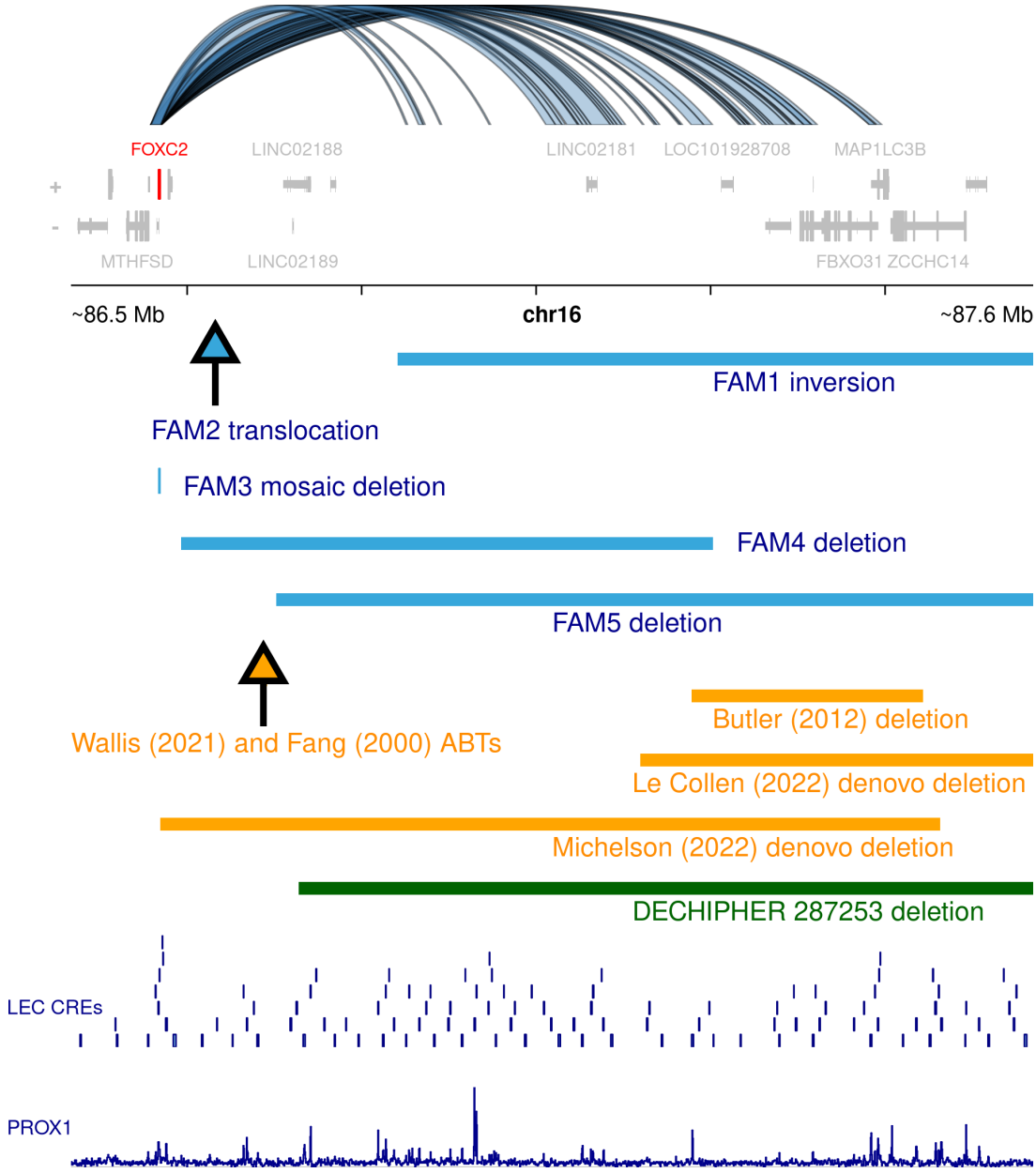


Figure 3 Location of structural variants (SVs) for the five families (FAMs) (and case studies from the literature). A regional plot of 16q24 details the genomic location of genes including *FOXC2* (highlighted in red). The uppermost track depicts promoter capture Hi-C interactions (arcs) between the *FOXC2* promoter and the downstream region in endothelial precursors.[22] FAM 1 carries a 1.6 Mb inversion mapping to chr16:86841016-88427540, 271 kb distal to *FOXC2* (full inversion not shown). FAM 2 carries a translocation (chr16:86619099-chr5:1162909) mapping 50 kb distal to *FOXC2*. FAM 3 carries a 1.1 kb de novo mosaic deletion (chr16:86567588-86568736) overlapping *FOXC2*. FAM 4 carries a 0.6 Mb deletion, 23.2 kb distal to *FOXC2* (chr16:86592947-87202476). FAM 5 carries a 1.4 Mb deletion, 132 kb distal to *FOXC2* (chr16:86701917-88132633). Additional cases identified through a literature review are also plotted, including: deletions in cases reported by Bulter *et al*,[5] Michelson *et al*[8] and Le Collen *et al*[6] (DECIPHER patient 400063), assumed balanced translocations reported by both Fang *et al*[3] and Wallis *et al*,[4] a DECIPHER variant for individual 287253 reported with lymphoedema. The lower tracks depict regulatory annotations including predicted cis-regulatory elements (CREs) and PROX1 transcription factor binding, both in lymphatic endothelial cells (LECs). ABT, assumed balanced translocation.

reciprocal translocation of telomeric chromosome 5 short arm material to the long arm of chromosome 16 was not detected by FISH.

Family 3

The proband of family 3 (II.1, table 1, figures 1–2) presented with bilateral lower limb lymphoedema with varicose veins onset aged 17 years following an episode of cellulitis after a road traffic accident. He reported extra in-growing eyelashes, which he regularly plucked. On examination, he had mild bilateral ptosis and distichiasis, with no family history of lymphoedema. Sanger

sequencing of *FOXC2* failed to identify a pathogenic variant.

An array-CGH analysis detected a 1.1 kb mosaic deletion on 16q24.1 resulting in an intragenic deletion of *FOXC2* (table 1, figure 3, chr16:86567588-86568736) estimated to be present in 60% of blood cells.

Family 4

The elder of two sisters (II.1, table 1, figures 1–2) presented with bilateral lower limb lymphoedema with varicose veins at the age of 16 years. On examination, she was found to have distichiasis.

Her sister (II.2) had bilateral lower limb lymphoedema (figure 2) with onset age 11 years. Their mother (I.2) suffered from varicose veins and had unilateral ptosis at birth. She had one single, aberrant eyelash. Sanger sequencing of *FOXC2* in the elder sister failed to identify a pathogenic variant. SNP array testing identified a 0.6 Mb deletion on 16q24.1-24.2, 23.2 kb distal to *FOXC2* in both sisters (table 1, figure 3, chr16:86592947-87202476). Their mother was not available for testing.

Family 5

The proband of family 5 (III.3, table 1, figures 1–2) had a history of perinatal asphyxia and mildly delayed psychomotor development. At 21 years of age, she had onset of bilateral lower limb lymphoedema. On examination, she had no dysmorphic features including normal eyelashes. While the vast majority of patients with LDS do have distichiasis, it is not fully penetrant, can be limited to a single aberrant eyelash and can be missed if examination is not conducted with a slit lamp.[1] Abdominal ultrasound revealed splenomegaly but normal kidneys.

Her mother (II.2) was born with a submucous cleft palate and had a mild learning disability. She had bilateral lower limb lymphoedema onset at 44 years of age. She also had ptosis but no distichiasis. Abdominal ultrasound revealed a misplaced atrophic left kidney. WES identified a heterozygous deletion located approximately 133 kb downstream of *FOXC2*, which was confirmed by array-CGH to be a 1.4 Mb deletion located approximately 132 kb downstream of *FOXC2* (table 1, figure 3, chr16:86701917-88132633).

Exploring the regulatory landscape and variation at 16q24

FOXC2 resides on the long arm of chromosome 16 at cytogenetic band 16q24.1. We sought to explore the regulatory landscape of this region through interrogation of publicly available resources (figure 3). Promoter capture Hi-C data in endothelial precursors identifies multiple contacts between the promoter of *FOXC2* and non-coding regions downstream of the gene. In agreement with this, we identify a TAD in Hi-C data in HUVEC, for the region chr16:85800000-87960000, encompassing *FOXC2* and an approximately 2 Mb region almost exclusively downstream of the gene. Other data, including Encyclopaedia of DNA Elements (ENCODE) candidate CREs and histone modifications (H3K27Ac), support this region as containing regulatory elements. Interestingly, focusing on the non-coding region shared between all families (chr16:87178148-87202447), we identify three potential enhancers in LECs, one with the transcription factor PROX1 bound (online supplemental figure 3).

We looked to explore observed structural variation within 16q24 in the general population. The gnomAD database of SVs identifies 401 deletions in the region (online supplemental figure 4), with an inverse correlation between variant size and frequency ($p<1\times10^{-7}$). While 94% of the region of interest is covered by at least one of these reported deletions, this drops to 8.1% when considering deletions that are more common in the general population (allele frequency >0.0001). Looking at the shared region discussed above, 29 deletions overlap this region. Five of these deletions are of modest size (>10 kb) but all are extremely rare (allele frequency <0.00003). Thus, while the region is not devoid of variation, no common (allele frequency >0.0001) large-scale deletions are observed.

Next, we interrogated DECIPHER and identified 28 individuals with deletions in this region of interest. The average size of deletion was 462 kb (52–1550 kb), with six reported as de novo. Of the 28 deletions, eight are reported as likely pathogenic for the phenotypes listed (two of these eight have no phenotypes provided) and an additional 6/28 have no listed phenotype. There were nine patients where the deletion fully encompasses *FOXC2*, none reported lymphoedema or distichiasis; however, eight out of nine of these individuals were last assessed as children and their full spectrum of phenotypes may therefore be incomplete. Additionally, no ptosis (which would typically be present from birth), and a single report of 'abnormal cardiovascular morphology' were recorded. Only two of the 28 patients with deletions in the region report lymphoedema, neither of which overlap *FOXC2* (figure 3: DECIPHER 287253, and DECIPHER 400063; both previously reported by Le Collen *et al*[6]). Half of patients report either intellectual disability or developmental delay, with other phenotypes reported in more than one patient including: obesity, hypotonia, hernias, hypertelorism, microcephaly/macrocephaly and seizure (none of these features are reported to be associated with LDS).

DISCUSSION

We add five families to the growing evidence of literature identifying SVs, likely impacting *FOXC2*, as a common mechanism for the development of LDS. We report one individual (family 3) with a 1.1 kb mosaic intragenic deletion of *FOXC2* likely producing a truncated protein product. However, for the four remaining cases, the SVs impact the non-coding portion of the genome downstream of *FOXC2*. These variants likely disrupt CREs key to FOXC2 expression, via deletion of these elements, or through translocation, resulting in aberrant TAD structure of the region. The SVs identified in patients with

LDS to date (online supplemental table 1) all impact either the coding portion of *FOXC2* or the region downstream of *FOXC2*, which, coupled with evidence from the ENCODE and promoter capture Hi-C experiments (figure 3), supports this as a region rich for regulatory elements for *FOXC2*.

We are not the first to identify non-coding SVs in this region in patients with LDS. Indeed, the first paper linking the *FOXC2* locus to LDS was based on a patient with a t(Y;16)(q12;q24.3) translocation, in the vicinity of, but not overlapping, *FOXC2*.[3 29] A number of additional cases have since been reported, sparking an exploration of the range of phenotypic associations observed with variation in this region.[6 8] These include deletions at 16q24.2 associated with intellectual disability, autism spectrum disorder, seizures, delayed speech, brain malformations and congenital renal disease.[30] However, inconsistent reporting of lymphoedema in patients with 16q24.1-q24.2 deletions harbouring *FOXC2* presents a complex picture. Literature reviews by both Le Collen *et al*[6] and Michelson *et al*[8] detail many patients with deletions within 16q24, of which only three are reported with lymphoedema. None of these three deletions encompasses the *FOXC2* coding region itself. Our own interrogation of SVs reported in the DECIPHER database demonstrates a similar picture, where none of the nine patients with deletions encompassing *FOXC2* are reported with lymphoedema, while two of 28 patients with SVs overlapping our reported cases (but without *FOXC2* deleted) report lymphoedema. Furthermore, combining evidence from the cases presented here, DECIPHER, and previous reports in literature suggest the possibility of a critical enhancer region in the vicinity of chr16:87178000-87202000.[3–6 8]

The lack of reported distichiasis or lymphatic phenotypes in individuals with whole gene deletion of *FOXC2* demands additional investigation. While the young age of these individuals cannot preclude the possibility of lymphatic dysfunction developing later in life, the lack of distichiasis, ptosis or any other features associated with LDS, typically presenting from an early age, may challenge the long-held view of haploinsufficiency as the basis for *FOXC2*-related LDS. Furthermore, as a single exon gene, a number of studies have now demonstrated that nonsense and frameshift variants likely avoid nonsense mediated decay, producing truncated protein products that sometimes bind DNA and cause protein nuclear aggregation impacting on cell viability.[31 32] However, *FOXC2* haploinsufficient mice do display abnormal lymphatics, and while oedema is rarely reported, imaging demonstrates a generalised hyperplasia in 92% of heterozygotes,[33] as well as bilateral distichiasis in all cases.[33 34] Therefore, we may postulate the hypothesis that, while disruption of the protein product or dysregulation of the protein may cause LDS, deletion of the entire gene does not necessarily produce a severe lymphatic phenotype.

In conclusion, it has previously been reported that 97% of patients with LDS have an identifiable pathogenic variant in *FOXC2*,[1] and by adding additional cases with non-coding SVs distal to *FOXC2*, we strengthen the case for *FOXC2* as a truly monogenic cause of LDS. The fact that these variants are undetectable by panel testing supports the recommendation for high-resolution array-CGH or genome sequencing in those patients with LDS but without a genetic diagnosis. Deletions impacting the entirety of *FOXC2* should be interpreted with caution. Further research is needed to unravel the likely complex regulatory framework of *FOXC2*. Such studies would enhance our understanding of the region and potentially lead to the discovery of additional clinically significant variants for patients with LDS.

Author affiliations
[1]School of Health & Medical Sciences, City St George's University of London, London, UK
[2]Translational Gastroenterology Unit, John Radcliffe Hospital, Oxford, UK
[3]Centre for Human Genetics, University of Oxford, Oxford, UK
[4]NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK
[5]Department of Genetics, Synnovis, Guy's and St Thomas' NHS Foundation Trust, London, UK
[6]Department of Genetics, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark
[7]Department of Dermatology and Copenhagen Wound Healing Center, Bispebjerg Hospital, Copenhagen, Denmark
[8]Department of Dermatology and Lymphovascular Medicine, St George's University Hospitals NHS Foundation Trust, London, UK
[9]Institute of Biomedical and Clinical Science Medical School, University of Exeter, Exeter, UK
[10]South West Thames Regional Genetic Services, London, UK

Acknowledgements The authors would like to acknowledge the child LIMPRINT[35] study focusing on children and young people with lymphoedema.

Contributors SED, ATP and SM conceptualised the research; SED, RM, JY, BS-G, MD and ATP investigated and analysed the data; AJN, HHU, EF, AD and HGK undertook variant validation; CVN, KG and SM performed patient recruitment and clinical assessment; SM-A, PO and ATP were involved in funding acquisition and supervision of the research. SED, RM and SM drafted the publication. All authors reviewed the manuscript. SED is the guarantor of this work and accepts full responsibility for the integrity of the data and the accuracy of the analysis.

Funding This research was made possible through access to data in the National Genomic Research Library, which is managed by Genomics England Limited (a wholly owned company of the Department of Health and Social Care). The National Genomic Research Library holds data provided by patients and collected by the NHS as part of their care and data collected as part of their participation in research. The National Genomic Research Library is funded by the National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council have also funded research infrastructure. This study makes use of data generated by the DECIPHER community. A full list of centres who contributed to the generation of the data is available from https://deciphergenomics.org/about/stats and via email from contact@deciphergenomics.org. DECIPHER is hosted by EMBL-EBI and funding for the DECIPHER project was provided by the Wellcome Trust (WT223718/Z/21/Z). The work was supported by research grants from the British Heart Foundation (FS/PhD/23/29393) to RM. KG, SED, MS, SM-A and PO were supported by the MRC (MR/Y013786/1) and BHF (SP/J/24/285005). ATP, HHU and JY were supported by the Oxford NIHR Biomedical Research Centre. AJN was/is supported by the Girdlers' New Zealand Health Research Council Fellowship (24/389) and OHSRC Research Grant 1459.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study was approved by the South West London Research Ethics Committee (REC Ref: 05/Q0803/267) for data analysis. Ethics approval for the 100kGP study was granted by the Cambridge South REC (14/EE/1112). The

LIMPRINT study was approved by the Danish Ethics Committee (j.nr: 2007-58-0015). Participants provided informed consent before taking part in the study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available. Genome sequencing data for families 1 and 2 are held in the National Genomic Research Library V.5.1, Genomics England. doi:10.6084/m9.figshare.4530893/7. 2020. Other data are available on reasonable request.

ORCID iD

Sara E Dobbins https://orcid.org/0000-0001-5320-3467

REFERENCES

1 Brice G, Mansour S, Bell R, *et al*. Analysis of the phenotypic abnormalities in lymphoedema-distichiasis syndrome in 74 patients with FOXC2 mutations or linkage to 16q24. *J Med Genet* 2002;39:478–83.
2 Sholto-Douglas-Vernon C, Bell R, Brice G, *et al*. Lymphoedema-distichiasis and FOXC2: unreported mutations, de novo mutation estimate, families without coding mutations. *Hum Genet* 2005;117:238–42.
3 Fang J, Dagenais SL, Erickson RP, *et al*. Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. *Am J Hum Genet* 2000;67:1382–8.
4 Wallis M, Pope-Couston R, Mansour J, *et al*. Lymphedema distichiasis syndrome may be caused by FOXC2 promoter-enhancer dissociation and disruption of a topological associated domain. *Am J Med Genet A* 2021;185:150–6.
5 Butler MG, Dagenais SL, Garcia-Perez JL, *et al*. Microcephaly, intellectual impairment, bilateral vesicoureteral reflux, distichiasis, and glomuvenous malformations associated with a 16q24.3 contiguous gene deletion and a Glomulin mutation. *Am J Med Genet A* 2012;158A:839–49.
6 Le Collen L, Delemer B, Spodenkiewicz M, *et al*. Compound genetic etiology in a patient with a syndrome including diabetes, intellectual deficiency and distichiasis. *Orphanet J Rare Dis* 2022;17:86.
7 Witte MH, Erickson RP, Khalil M, *et al*. Lymphedema-distichiasis syndrome without FOXC2 mutation: evidence for chromosome 16 duplication upstream of FOXC2. *Lymphology* 2009;42:152–60.
8 Michelson M, Lidzbarsky G, Nishri D, *et al*. Microdeletion of 16q24.1-q24.2-A unique etiology of Lymphedema-Distichiasis syndrome and neurodevelopmental disorder. *Am J Med Genet A* 2022;188:1990–6.
9 Gagnier JJ, Kienle G, Altman DG, *et al*. The CARE guidelines: consensus-based clinical case reporting guideline development. *BMJ Case Rep* 2013;2013:bcr2013201554.
10 Sarica M, Gordon K, van Zanten M, *et al*. Lymphoscintigraphic Abnormalities Associated with Milroy Disease and Lymphedema-Distichiasis Syndrome. *Lymphat Res Biol* 2019;17:610–9.
11 Weissleder H, Weissleder R. Lymphedema: evaluation of qualitative and quantitative lymphoscintigraphy in 238 patients. *Radiology* 1988;167:729–35.
12 The national genomic research library v5.1. Genomics England; 2020.
13 Roller E, Ivakhno S, Lee S, *et al*. Canvas: versatile and scalable detection of copy number variants. *Bioinformatics* 2016;32:2375–7.
14 Chen X, Schulz-Trieglaff O, Shaw R, *et al*. Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. *Bioinformatics* 2016;32:1220–2.
15 Yu J, Szabo A, Pagnamenta AT, *et al*. SVRare: discovering disease-causing structural variants in the 100k genomes project. *medRxiv* [Preprint] 2022.
16 Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform* 2013;14:178–92.
17 Hinrichs AS, Karolchik D, Baertsch R, *et al*. The UCSC Genome Browser Database: update 2006. *Nucleic Acids Res* 2006;34:D590–8.
18 Firth HV, Richards SM, Bevan AP, *et al*. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. *Am J Hum Genet* 2009;84:524–33.
19 Durinck S, Moreau Y, Kasprzyk A, *et al*. BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. *Bioinformatics* 2005;21:3439–40.
20 Chen S, Francioli LC, Goodrich JK, *et al*. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature New Biol* 2024;625:92–100.
21 Wang Y, Song F, Zhang B, *et al*. The 3D Genome Browser: a web-based browser for visualizing 3D genome organization and long-range chromatin interactions. *Genome Biol* 2018;19:151.
22 Javierre BM, Burren OS, Wilder SP, *et al*. Lineage-Specific Genome Architecture Links Enhancers and Non-coding Disease Variants to Target Gene Promoters. *Cell* 2016;167:1369–84.
23 Rao SSP, Huntley MH, Durand NC, *et al*. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 2014;159:1665–80.
24 Pirri D, Maude H, Stroobants A, *et al*. Loss of erg function causes lymphatic vessel malformations and primary lymphoedema. *Cardiovascular Medicine* [Preprint] 2025.
25 Kazenwadel J, Venugopal P, Oszmiana A, *et al*. A Prox1 enhancer represses haematopoiesis in the lymphatic vasculature. *Nature New Biol* 2023;614:343–8.
26 Kramer NE, Davis ES, Wenger CD, *et al*. Plotgardener: cultivating precise multi-panel figures in R. *Bioinformatics* 2022;38:2042–5.
27 Rohrbach M, Spencer HL, Porter LF, *et al*. ZNF469 frequently mutated in the brittle cornea syndrome (BCS) is a single exon gene possibly regulating the expression of several extracellular matrix components. *Mol Genet Metab* 2013;109:289–95.
28 Fransen E, Valgaeren H, Janssens K, *et al*. Resequencing of candidate genes for Keratoconus reveals a role for Ehlers-Danlos Syndrome genes. *Eur J Hum Genet* 2021;29:1745–55.
29 Erickson RP, Hudgins L, Stone JF, *et al*. A “balanced” Y;16 translocation associated with Turner-like neonatal lymphedema suggests the location of a potential anti-Turner gene on the Y chromosome. *Cytogenet Cell Genet* 1995;71:163–7.
30 Handrigan GR, Chitayat D, Lionel AC, *et al*. Deletions in 16q24.2 are associated with autism spectrum disorder, intellectual disability and congenital renal malformation. *J Med Genet* 2013;50:163–73.
31 Tavian D, Missaglia S, Maltese PE, *et al*. FOXC2 disease-mutations identified in lymphedema-distichiasis patients cause both loss and gain of protein function. *Oncotarget* 2016;7:54228–39.
32 Tavian D, Missaglia S, Michelini S, *et al*. *FOXC2* Disease Mutations Identified in Lymphedema Distichiasis Patients Impair Transcriptional Activity and Cell Proliferation. *Int J Mol Sci* 2020;21:5112.
33 Kriederman BM, Myloyde TL, Witte MH, *et al*. FOXC2 haploinsufficient mice are a model for human autosomal dominant lymphedema-distichiasis syndrome. *Hum Mol Genet* 2003;12:1179–85.
34 Iida K, Koseki H, Kakinuma H, *et al*. Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis. *Development* 1997;124:4627–38.
35 Moffatt C, Keeley V, Quere I. The Concept of Chronic Edema-A Neglected Public Health Issue and an International Response: The LIMPRINT Study. *Lymphat Res Biol* 2019;17:121–6.