Original research

Ureteropelvic junction obstruction with primary lymphoedema associated with *CELSR1* variants

Murat Alpaslan ,[1] Sandrine Mestré-Godin,[2,3] Aurélie Lay,[2] Guido Giacalone,[4] Raphaël Helaers,[1] Salma Adham ,[2,3] Hélène Kovacsik,[2] Sophie Guillemard,[5] Erick Mercier,[3,6] Laurence Boon,[1,7] Nicole Revencu,[7] Pascal Brouillard,[1] Isabelle Quere ,[2,3] Miikka Vikkula ,[1,8]

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For numbered affiliations see end of article.

Correspondence to
Professor Miikka Vikkula, Human Molecular Genetics, de Duve Institute, Brussels 1200, Belgium;
miikka.vikkula@uclouvain.be

MA and SM-G contributed equally.
IQ and MV contributed equally.

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ABSTRACT

Background Primary lymphoedema (PL) is a chronic, debilitating disease caused by developmental and functional defects of the lymphatic system. It is marked by an accumulation of interstitial fluid, fat and tissue fibrosis. There is no cure. More than 50 genes and genetic loci have been linked to PL. We sought to study systematically cell polarity signalling protein cadherin epidermal growth factor laminin G seven-pass G-type receptor 1 (*CELSR1*) variants linked to PL.

Methods We investigated 742 index patients from our PL cohort using exome sequencing.

Results We identified nine variants predicted to cause *CELSR1* loss of function. Four of them were tested for nonsense-mediated mRNA decay, but none was observed. Most of the truncated CELSR1 proteins would lack the transmembrane domain, if produced. The affected individuals had puberty/late-onset PL on lower extremities. The variants had a statistically significant difference in penetrance between female patients (87%) and male patients (20%). Eight variant carriers had a kidney anomaly, mostly in the form of ureteropelvic junction obstruction, which has not been associated with *CELSR1* before. *CELSR1* is located in the 22q13.3 deletion locus of the Phelan-McDermid syndrome. As variable renal defects are often seen in patients with the Phelan-McDermid syndrome, *CELSR1* may be the long-sought gene for the renal defects.

Conclusion PL associated with a renal anomaly suggests a *CELSR1*-related cause.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Heterozygous cell polarity signalling protein cadherin epidermal growth factor laminin G seven-pass G-type receptor 1 (*CELSR1*) nonsense variants suggested to give rise to primary lymphoedema in a sex-limited manner.

WHAT THIS STUDY ADDS

⇒ This study details the clinical phenotype of patients with *CELSR1* variants, identifies unrecognised association with renal defects and suggests the gene behind the renal problems in Phelan-McDermid syndrome.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ *CELSR1* should be included in routine genetic testing for patients with lymphoedema and/or renal anomalies as it may be the cause.

INTRODUCTION

Tissue homeostasis is accomplished by complex processes in which the lymphatic and the vascular systems play crucial roles. Defects in the development and/or the function of these structures can result in various diseases.[1 2] Lymphoedemas and vascular anomalies are among those.

Lymphatic capillaries collect body fluids from the extremities and around the intestine and transport them through collecting lymphatic vessels and lymphatic nodes back to blood circulation. Lymphoedema results from an accumulation of extracellular fluid, especially in extremities, causing chronic swelling. Lymphoedema can be primary (presumed to arise from genetic defects) or secondary (eg, following an infection or an invasive therapy).[1] Primary lymphoedema (PL (MIM: 153 100)) can be hereditary, visible at birth or puberty, or with late onset.[3] PL is often isolated (Milroy disease), but it can also be part of a syndrome (such as in lymphoedema–distichiasis, hypotrichosis–lymphoedema–telangiectasia or microcephaly–chorioretinopathy–lymphoedema–mental retardation syndrome).[1] There is no cure for PL. Management, including daily use of compressive garments, multilayer bandages, repetitive manual lymphatic drainages and, in selected cases, surgeries, is focused on alleviating the effects of lymphoedema and its progression. Relapse of the disease and infections are a common problem.[4] Therefore, a better understanding of the causes of PL should give a clearer basis for development of novel therapeutic approaches. Identification of the genes in which variants cause PL is a crucial step towards this goal.

Up to now, 32 confirmed genes or chromosomal loci, as well as 22 possible PL genes, in which variants are considered as potentially disease-causing, but await validation, have been found.[1 5] The function of many of the encoded proteins gears around the *VEGF-C/VEGFR-3*, *ANGPT2/TIE1-2* and *HGF-cMET* signalling axes.[1] Among these, the planar cell polarity signalling protein cadherin epidermal growth factor laminin G seven-pass G-type receptor 1 (CELSR1 (MIM: 604 523]) has been identified to be mutated in nine families (figure 1 and online

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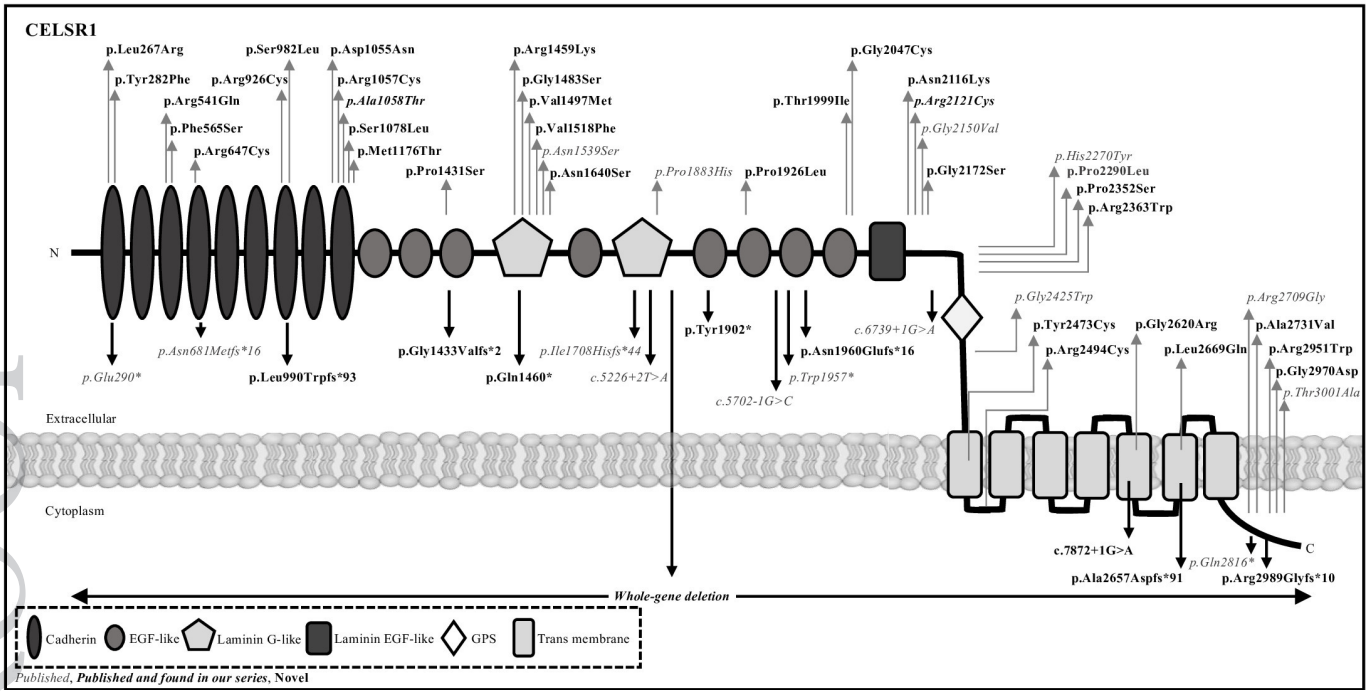


Figure 1 CELSR1 transmembrane protein with LoF and missense variants found in patients with PL. These variants have a frequency of <1% in the Exome Aggregation Consortium database and an allele count of <100 in the Genome Aggregation Database and were not detected in more than three distinct pathologies in our internal WES database. Top: missense (VUS) variants; bottom: LoF variants. Published variants in italic (Erickson *et al*,[6] Gonzalez-Garay *et al*,[7] Maltese *et al*,[8] Seo *et al*[9] and Xia *et al*[10]), variants from this study in bold. Three variants reported elsewhere and in our cohort in bold italic. EGF, epidermal growth factor; LoF, loss of function; PL, primary lymphoedema; VUS, variants of unknown significance; WES, whole-exome sequencing.

supplemental table 2).[6–10] Two of these reports suggested that *CELSR1* haploinsufficiency resulted in female-limited penetrance of PL.[6 8]

CELSR1 is a large transmembrane protein comprising nine cadherin domains, eight epidermal growth factor-like domains, two laminin G repeats, one laminin EGF-like domain and one G-protein coupled receptor proteolytic site in its extracellular part, as well as seven transmembrane domains followed by a short cytoplasmic tail (figure 1).[11] We investigated 742 patients with PL index by whole-exome sequencing (WES) to study systematically the frequency of *CELSR1* variants linked to PL. We also investigated the sex-limited penetrance of the disease in variant carriers and detailed clinical characteristics of the phenotype, including lymphoscintigraphy and lympho-MRI.

MATERIALS AND METHODS

Population of *CELSR1* variant carriers

Half of the patients in this cohort were collected by the Department of Vascular Medicine, University Hospital Centre of Montpellier, France, a national reference centre for the management of lymphoedema, and the other half by the Laboratory of Human Molecular Genetics, de Duve Institute, University of Louvain, Brussels, Belgium. The patients were referred to us by general practitioners, vascular physicians or other specialties for assessment and specific management. All patients for whom a *CELSR1* variant was identified were invited to be re-examined and to complete explorations if necessary. Venous Doppler ultrasound of the lower limbs, abdominal ultrasound, thoracoabdominal–pelvic lympho-MRI and lymphoscintigraphy of the lower limbs were performed for a large proportion of patients at the University Hospital Centre of Montpellier, France.

Sample collection

DNA from the patients were extracted with Wizard Genomic kit (Promega) or similar, and concentrations were measured using a Nanodrop 8000 (Thermo Scientific). Exome sequencing was performed by Macrogen on Illumina HiSeq or NovaSeq machines, using either Agilent SureSelect V.6 or V.7 or Twist Human-core Exome Capture kits. Fastq files were retrieved and the reads were aligned against human reference genome hg38 to generate *bam* files, subjected then to variant calling with the Genome Analysis Toolkit (Broad Institute). The data were imported in Highlander (http://sites.uclouvain.be/highlander/), a user-friendly tool developed in our laboratory, to visualise and filter variants annotated with a variety of prediction tools.

Variant calling and filtering

Filtering for possible disease-causing variants included variants predicted to be damaging by at least 5 prediction algorithms out of 20 used in Highlander (computed as *consensus prediction*), with read depth of a minimum of 10 at the variant position; with a frequency of <1% in the Exome Aggregation Consortium database (http://exac.broadinstitute.org) (the estimated prevalence of PL in the population is ~1.4‰); with an allele count of <100 in the Genome Aggregation Database (GnomAD, https://gnomad.broadinstitute.org); in the Regeneron Genetics Centre Mexico City Prospective Study (RGC-MCPS) variant browser (https://rgc-mcps.regeneron.com), as well as in the deCAF database (https://decaf.decode.com); and not detected in more than three distinct pathologies in our internal database (>3000 WES). CNVs were detected by using exome sequencing data and ExomeDepth V.1.1.10 in Highlander. Two default parameters were used for ExomeDepth; transition probability (0.0001) and expected CNV length (50 000 bp). Our clinical collaborator

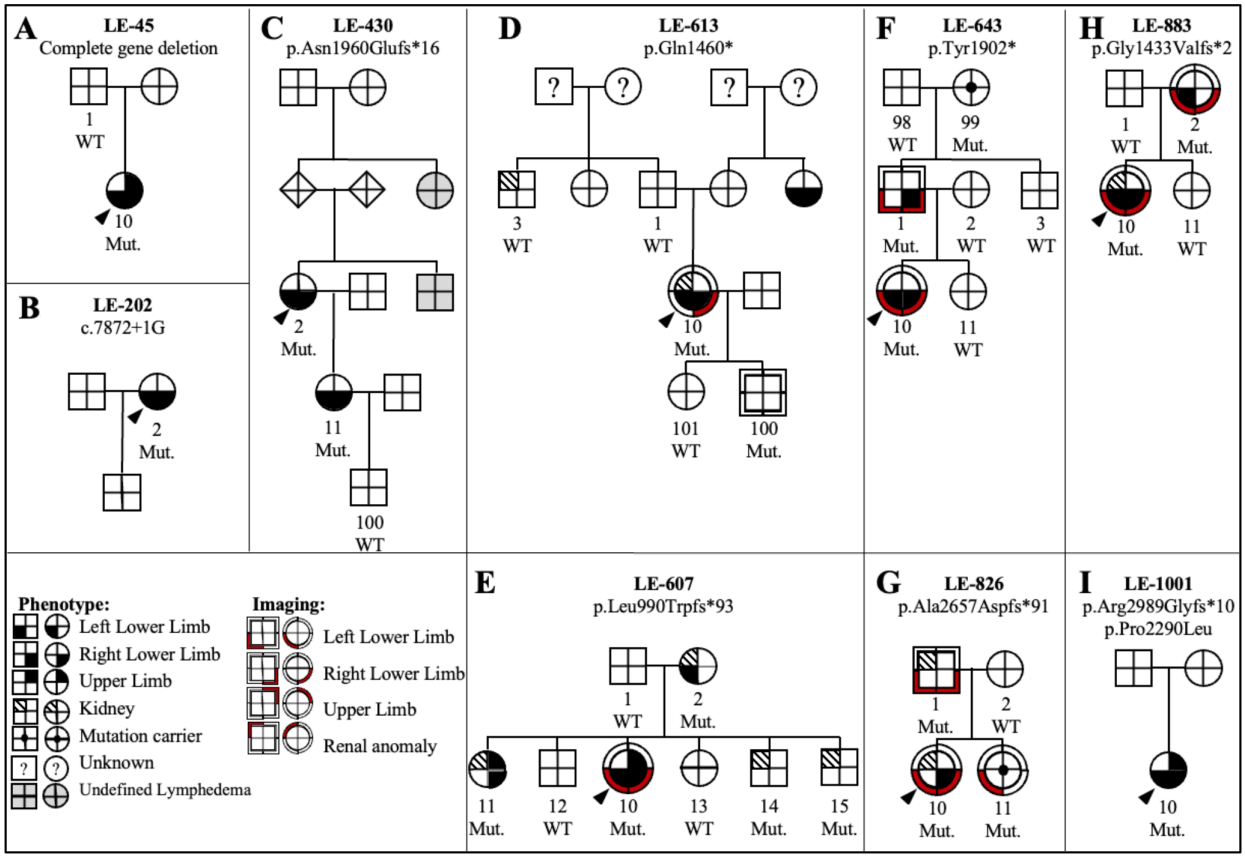


Figure 2 Nine PL families with *CELSR1* LoF variants. Autosomal dominant transmission of PL with incomplete penetrance. Only individuals with numbers were studied. Arrowheads indicate the probands; circles denote female; squares denote male; rhombuses denote unknown. Anomalies according to lymphoscintigraphy and MRI are indicated in the outer layer of symbols. LoF, loss of function; Mut, mutation carrier; PL, primary lymphoedema; WT, wild type.

(Cliniques Universitaires Saint-Luc) confirmed identified CNV by performing a fluorescence in situ hybridisation experiment.

Cosegregation analyses

Sanger sequencing was used to assess variant cosegregation in families. Primers were designed to amplify fragments of 250–500 bp around the variants (sequences available on request).

RT-PCR analyses

mRNA extraction was performed with TriPure Isolation reagent (Roche) according to the protocol supplied by the provider. Subsequently, cDNA was synthesised with RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific) using random hexamers. For PCR amplification of *CELSR1* fragments with a TAQ polymerase (QIAGEN), newly synthesised cDNAs and fresh dilutions of primers were used. Primers were designed to amplify fragments around the variants spanning over at least one exon–intron junction before and after the variant-containing exon (sequences available on request).

Lymphoscintigraphy and lympho-MRI for the *CELSR1* variant carriers

Lymphoscintigraphy is a standardised, minimally invasive and reproducible procedure allowing morphological and functional exploration of the lymphatic circulation.[12 13] It is performed after subcutaneous injection in the first interdigital space of a small quantity of 99 m Tc-nanocolloid, which travels in the lymphatic circulation, releasing gamma radiation detected by a gamma camera.[13] In collaboration with the nuclear medicine department of the ICM (Montpellier Regional Cancer Institute), we have been using for several years a locally instituted and reproducible stage classification called node deep stasis. It considers the lymph node impregnation at the root of the limb, the presence of deep lymph nodes and signs of stasis. 'N' takes into account the number and activity of nodes over time correlated to a semiquantitative index 'k' corresponding to the activity at 4 hours of the first radioactive node, divided by the activity of the same node at 45 min.

Lympho-MRI is an MRI performed without injection, with long acquisition times, allowing visualisation of deep lymphatic tracts and lymph nodes. Thoracic duct images are obtained using coronal heavy T2 three-dimensional volume sequence using no angulation. Z-axis coverage was from the lower neck to the diaphragm. A second acquisition is performed from the diaphragm to the femoral head to image the retroperitoneal lymphatics. Coronal maximum intensity projection reconstruction is performed for each acquisition. The images of the lymphatic system of the trunk were identified as hypoplastic, rich (high density of lymphatic vessels and lymph nodes compared with the general population), hyperplastic or normal in the absence of standardised classification of the results.

MRI and lymphoscintigraphy were used to evaluate the functionality of the pelvic and lumboaortic lymph nodes. Asymptomatic research participants had a significant number of functional nodes. This double evaluation allowed reassurance of the asymptomatic heterozygotes.

Table 1 Summary of the nine families with a loss-of-function *CELSR1* variant

Family	Family code	HGVS DNA	HGVS protein	Variant carriers		Renal anomaly in family
				Affected	Unaffected	
1	LE-45	CELSR1del (CNV 6.1 Mb)	p.0	1	0	–
2	LE-202	c.7872+1G>A	p.?	1	0	
3	LE-430	c.5877dup	p.Asn1960Glufs*16	2	0	
4	LE-607	c.2967del	p.Leu990Trpfs*93	3	2	+
5	LE-613	c.4378C>T	p.Gln1460*	1	1	+
6	LE-643	c.5706C>A	p.Tyr1902*	2	1	–
7	LE-826	c.7966_7969dup	p.Ala2657Aspfs*91	1	2	+
8	LE-883	c.4297_4298insTATGAGAGGCCACACTGTATGTGTGTCCTCCTGTATGAGAG	p.Gly1433Valfs*2	2	0	+
9	LE-1001	c.8961_8964G([6])	p.Arg2989Glyfs*10	1	0	

Background colour alternates per family. RefSeq: NM_014246.3. Blank cells denote unknown; '–' means not present; '+' means present.

RESULTS

CELSR1 loss-of-function (LoF) variants

We discovered altogether nine index patients with a heterozygous LoF *CELSR1* variant: two nonsense and five frameshift variants, one predicted splice site change (right after end of exon 26, in LE-202–2) and one complete *CELSR1* gene deletion, part of a larger 6.1 Mb deletion, on chromosome 22, encompassing altogether 136 genes (figures 1 and 2, table 1 and online supplemental table 1). They were all predicted to result in a premature stop codon. *CELSR1* is predicted to be LoF variant-intolerant in GnomAD, with a pLI score as 1. The nine index patients with these *CELSR1* disease-causing variants represented 1.21% of the index patients in our PL cohort.

The variants were all novel and not reported in any other disease nor in GnomAD, except for the *c.8961_8964G*[6] (p.Arg2989Glyfs*10) variant, once reported in GnomAD. Moreover, the variants were absent in RGC-MCPS and deCAF databases, except for *c.7872+1G>A* and *c.5706C>A* (p.Tyr1902*), which were both reported once in deCAF. Cosegregation analysis unravelled that four of the nine variants were inherited from three affected and one unaffected parent (figure 2). DNAs were unavailable for testing for the other ones.

CELSR1 mRNA carrying premature stop codons are stable

We were able to obtain lymphocytic RNA to assess the stability of the mutant *CELSR1* allele for four families with predicted LoF variants (LE-613, LE-643, LE-826 and LE-883). Sequencing of RT-PCR products encompassing the mutation site showed that both alleles were expressed at similar levels for the four mutants (online supplemental figure 1). As there is no induction of nonsense-mediated mRNA decay (NMD), the truncated proteins may be expressed; yet we cannot exclude that the protein products may be unstable and/or aberrantly folded. Six out of eight of the truncated proteins would lack the transmembrane domain of CELSR1, eventually leading to secretion of CELSR1 partial pieces of the extracellular domain.

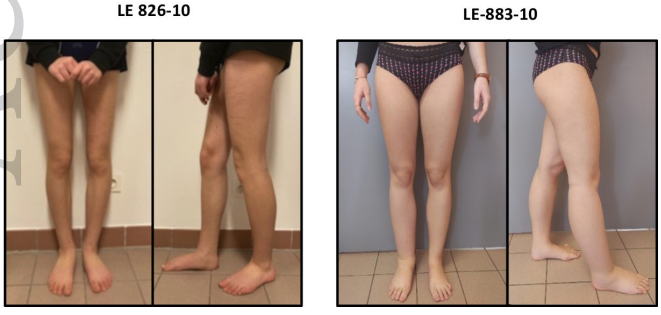


Figure 3 Images of two patients with primary lymphoedema with *CELSR1* disease-causing variant. LE-826–10, lymphoedema of the distal right lower limb; volume asymmetry of the right foot is discrete; LE-883–10, lymphoedema of the right lower limb with involvement of the toes, dorsum of the foot with accentuation of the folds at the instep. Increased volume of the right calf.

Differential penetrance of *CELSR1* LoF variants

We identified altogether 20 variant carriers (15 female patients and 5 male patients); 14/20 had PL, 3/20 defect/delay in lymphatic system without PL (LE-613–100, LE-826–1 and LE-826–11) and the three last ones were asymptomatic (LE-607–14, LE-607–15 and LE-643–99). Therefore, penetrance of *CELSR1* LoF variants in our cohort is 70%. Thirteen of the affected variant carriers were female and only one was male (online supplemental table 3) giving a penetrance of *CELSR1* LoF variants of 87% in female patients and 20% in male patients.

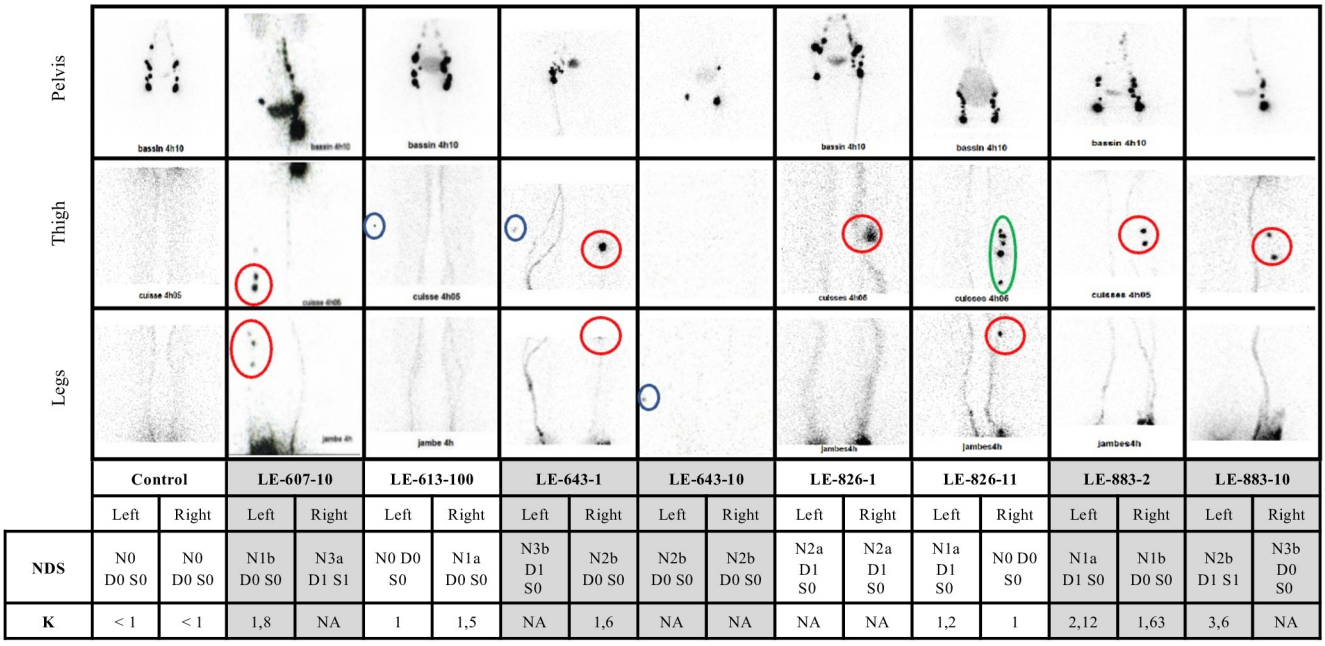
Clinical phenotype linked to *CELSR1* LoF variants

Clinical data of all the affected patients with PL in the nine families with a *CELSR1* LoF variant are summarised in table 1 and online supplemental table 1. Five of the patients had adolescent-onset (between the age of 10 and 20 according to WHO) PL (5/14, 36%), three as young children, two late-onset (before the age 27) PL, and only one had congenital PL (LE-613–10). For three of the patients, we do not have data on age of onset. Lymphoedema affected the lower extremities in all patients, either bilaterally (n=9) or unilaterally (n=5) (figure 3). Four patients (29%) had upper extremity lymphoedema as well (three right-sided and one bilateral). The phenotype of the single affected male patient did not differ from those of the affected female patients.

Lymphoedema was progressive in 1 patient (LE-607–11), extending from the feet towards the hips, stable in 11 patients and spontaneously disappeared in 2 patients (only right upper extremity PL disappeared for LE-607–10 and LE-607–11). There was a total of 10 episodes of cellulitis in the patients' history; only four patients had any. One patient had one episode (LE 613–10); another one had three episodes (LE 607–10); and a third patient had at least six episodes (LE 607–11). The last two from the same family refused explorations and an adapted management to prevent infectious events.

Clinical phenotype linked to the 22q13.3 deletion encompassing *CELSR1* and *SHANK3*

The female proband LE-45–10 was diagnosed with bilateral lower limb lymphoedema and right upper limb lymphoedema



	Control		LE-607-10		LE-613-100		LE-643-1		LE-643-10		LE-826-1		LE-826-11		LE-883-2		LE-883-10	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
NDS	N0 D0 S0	N0 D0 S0	N1b D0 S0	N3a D1 S1	N0 D0 S0	N1a D0 S0	N3b D1 S0	N2b D0 S0	N2b D0 S0	N2b D0 S0	N2a D1 S0	N2a D1 S0	N1a D1 S0	N0 D0 S0	N1a D1 S0	N1b D0 S0	N2b D1 S1	N3b D0 S0
K	< 1	< 1	1,8	NA	1	1,5	NA	1,6	NA	NA	NA	NA	1,2	1	2,12	1,63	3,6	NA

Figure 4 Lymphoscintigraphy images of eight patients with PL and one normal control. Five patients with PL are indicated with grey background; the other four are asymptomatic heterozygotes. Semiquantitative index 'K' corresponds to the activity at 4 hours of the first radioactive node divided by the activity of the same node at 45 min. D, deep relay; D0, absent; D1, present; N0, normal number at 45 min and decrease of activity between 45 min and 4 hours (k<1); N1, normal number at 45 min and increased activity between 45 min and 4 hours; N1a, moderate increase in activity (1<k<1.6); N1b, net increase in activity (1.6<k); N2, insufficient numbers at 45 min; N2a, normal number at 4 hours; N2b, insufficient number at 4 hours; N3, no nodes at 45 min; N3a, nodes at 4 hours; N3b, no lymph nodes at 4 hours; NA, not available; NDS, node deep stasis; S, stasis; S0, absent; S1, present; PL, primary lymphoedema.

during puberty (figure 2, table 1 and online supplemental table 1). She had also encephalopathy, macrocephaly, developmental delay and syndactyly. No data were available for other family members. The 6.1 Mb 22q13 deletion that was identified included several genes, among which included *SHANK3* (MIM: 606230). Such a deletion is associated with the Phelan-McDermid syndrome (MIM: 606232).

Kidney anomalies linked to *CELSR1* LoF variants

In four families, eight *CELSR1* variant carriers had a kidney abnormality (table 1, figure 2 and online supplemental table 1). It was an ureteropelvic junction obstruction (pyeloureteral junction syndrome (MIM: 143 400)) in five patients (LE-607–14, LE-607–15, LE-613–10, LE-826–1 and LE-883–10), renal atrophy with complete loss of renal function in two patients (right kidney in LE-607–2 and left kidney in LE-607–11) and several acute kidney infections in one patient (LE-826–10). All five female patients with a kidney disease had also lymphoedema, whereas the three male patients had only a renal defect. Moreover, one of the patients with a variant of unknown significance (VUS) (LE-626–10, p.Ser1078Leu) also had a right kidney dysplasia.

Lympho-MRI for the *CELSR1* variant carriers

The lymphatic system was normal for two patients and abnormal for eight patients (four in index cases (LE-607–10, LE-643–10, LE-826–10 and LE-883–10), one in symptomatic carriers (LE-883–10) and three asymptomatic heterozygotes (LE-613–100, LE-826–1 and LE-826–11)) (figure 4 and online supplemental table 1). Among all carriers, seven had rich pelvic lymphatic tissue (three men and four women) (LE-613–100, LE-643–1, LE-643–10, LE-826–1, LE-826–10, LE-883–2 and LE-883–10), and two of the patients had dilated thoracic duct (LE-613–100 and LE-826–1). In one carrier (LE-643–1), the thoracic duct was found hypoplastic and associated with a high density of lymphatic vessels and lymph nodes compared with the general population in laterotracheal, right cervical, retroperitoneal and right iliac axis areas. Two symptomatic carriers had normal deep lymphatic network MRIs (two female patients, LE-613–10 and LE-883–2; data not shown).

Lymphatic phenotype linked to *CELSR1* LoF variants

Lymphatic drainage was delayed or absent in both symptomatic and asymptomatic patients. This shows that at least one subclinical abnormality of more or less severe stage (or anatomical variation) is present in asymptomatic subjects. Dermal stasis was seen in three cases (LE-607–10, LE-826–10 and LE-883–10) (figure 4). Under the knee, lymphatic collateral trunks were frequent in LE-613–10, LE-613–100, LE-643–1, LE-826–1 and LE-883–2. Rerouting towards the deep lymphatic popliteal nodes was seen in seven patients (LE-607–10, LE-643–1, LE-826–10, LE-826–1, LE-826–11, LE-883–2 and LE-883–10) (figure 4). In these patients, lymphatic drainage takes place through the normal anatomical deep lymphatic channels in relay of the non-functional superficial lymphatic channels.

Lymphatic venous ultrasound for the *CELSR1* variant carriers

Venous Doppler ultrasound of the lower limbs was also performed for 11 patients (data not shown). Bilateral venous insufficiency was found as backflow in the great saphenous vein

in two female patients (LE-613–10 and LE-883–10); segmental truncal incontinence of the left great saphenous vein was found in a male patient (LE-643–1); and for three subjects, anatomical variations were noted. Extrafascial course of the great saphenous vein in the subgonal region was found in one female patient and in one male patient (LE-607–10 and LE-613–100), and collateral branches of the great saphenous vein were visible bilaterally in another female patient (LE-607–11).

Identification of *CELSR1* missense variants

In GnomAD, Z-score for *CELSR1* missense variants is 1.2, indicating some negative selection for such variants in the general population. In our cohort, we discovered 31 rare missense variants with a consensus score above 5 in 29 index patients: LE-498–10 and LE-555–10 had two missense variants each, while LE-1001–10 had one *CELSR1* missense variant and one *CELSR1* frameshift variant (figure 1, online supplemental figure 2 and online supplementary table 4). The majority were absent or very rare in deCAF, GnomAD and RGC-MCPS. All these missense variants were considered as VUSs. In nine families, additional members were available for cosegregation analysis, leading to a total of 41 subjects with 1 of these 30 VUSs (excluding LE-1001–10). Among those, five female and seven male heterozygotes did not have lymphoedema (online supplementary tables 3 and 4). If all these amino acid substitutions would be disease-causing variants, the penetrance would be 80% for female patients and 58.8% for male patients. In 12 out of 29 index patients, we detected VUSs in other lymphoedema genes, which could likewise be the cause of the disease or act together with the *CELSR1* VUS to cause the pathology (online supplementary table 4).

DISCUSSION

We identified nine index patients with a *CELSR1* LoF variant using WES. Cosegregation studies identified 11 other patients. Phenotypically, PL in patients with *CELSR1* LoF variants showed a variable age at onset, concentrated around puberty. All patients had lower-extremity lymphoedema, mostly bilateral (64%), although among those affected, four patients also had upper extremity PL, of which two resolved spontaneously.

Based on clinical examination, the penetrance was higher in women than in men. This underscores the results from nine reported families, accounting for 27 *CELSR1* LoF variant-carrier individuals, of which 7 had no clinical sign of lymphoedema (online supplementary tables 2 and 3).[6–10] When we combine our results with the literature, the difference in penetrance between female patients and male patients becomes statistically highly significant: female patients 91% vs male patients 23% (32/35 and 3/13 respectively, p=0.00001) with an overall penetrance of *CELSR1* LoF variants of 73% at the age of 25 (online supplementary table 3).

Subclinical anomalies of the lymphatic system, however, were detected in all variant carriers by lymphoscintigraphy. This suggests an abnormal lymphatic function, which could be a predisposing factor under environmental triggers. This might explain the late onset of clinical symptoms. Moreover, when sufficient numbers of nodes (>5 in the pelvis or lumboaortic region) were present, the subjects carrying a LoF variant were not or only slightly symptomatic. There is also a sex-linked lymphatic anomaly based on lympho-MRI in *CELSR1* variant carriers. The pelvic lymphatic tissue was rich, and deep lymphatic tracts were more dilated in all men, while they were normal in women.

Lymphatic stasis is not a marker of lymphoedema severity in the clinics. The variant present in the LE-643 family is associated with more severe changes in lymphoscintigraphy, but this is not the case clinically. Symptoms do not correlate with the lymphoscintigraphic stage. The index cases have lymphoedemas that remain moderate by volume compared with other PLs, although the lymphoscintigraphy images resemble that of a severe lymphoedema for a few patients (n=5/14) without any drainage (unilateral or bilateral). Further studies will be necessary to clarify these differences, but it can be hypothesised that the abnormality of lymphatic tissue placement at the level of the pelvic and lumboaortic lymph nodes leads to lymphatic dysfunction. It is also necessary to take into account the hormonal influence because of the female predominance, with symptoms that begin at the time of the first pubertal signs.

The *CELSR1* variants likely cause LoF. We showed that the mutant mRNAs for four variants leading to premature stop codons do not undergo NMD (online supplementary figure 1). As these variants are located at different positions in the *CELSR1* gene, hence in different exons, it could be the same for the other non-tested LoF variants in our series, as well as the previously published variants. Thus, they could result in truncated proteins, which, stable, would be truncated at various positions, varying from very short amino-terminal polypeptides (p.Leu990Trpfs*93) to an almost complete extracellular domain (figure 1). As one patient had a deletion of the whole gene, haploinsufficiency is the likely disease-causing mechanism. This is underscored by the pLI=1 for *CELSR1* (refractory to LoF variants). However, we cannot exclude that some of the truncating variants could have a dominant-negative effect, and thus there could be further genotype–phenotype correlations.

Three of the nine index patients with a LoF variant had a second variant affecting another PL gene, which could contribute to the phenotype (online supplementary table 1). Patient LE-1001–10 harboured a *CELSR1* missense variant on top of the frameshift variant (online supplementary tables 1 and 4). We were not able to define whether the two changes were allelic or not, but as this patient is severely affected with four-limb PL and venous insufficiency, both variants might have an impact on CELSR1 function. LE-202–2 carries an additional heterozygous missense variant in *PIEZO1* (MIM: 611184) (online supplementary table 1). Recessive variants in this gene can lead to (1) generalised lymphatic dysplasia (MIM: 616 843), a rare form of a lymphoedema which affects all body parts uniformly, associated with intestinal lymphangiectasia, pleural effusions and/or chylothorax; or (2) non-immune hydrops fetalis.[14] In this patient, lymphoedema was limited to the lower extremities without other signs or symptoms. Thus, it remains elusive as to whether the *PIEZO1* variant is pathogenic or not. Finally, LE-613–10 and LE-613–100 harboured a *SOS1* (MIM: 182530) variant located in a canonical splice site, which could affect mRNA splicing, resulting in NMD or altered protein structure. Gain-of-function variants in *SOS1* are associated with Noonan syndrome (MIM: 610733). The variant is reported 37 times in GnomAD and is classified as VUS in ClinVar and by others.[15] The absence of signs of Noonan syndrome in the two patients tends to confirm the non-pathogenicity of this *SOS1* variant.

The 31 rare missense variants (VUSs) that we identified (online supplementary table 4) might also alter CELSR1 function, leading to lymphoedema. Like some of the patients with a *CELSR1* LoF variant, the patients with a rare *CELSR1* missense

variant (41%) could also carry a VUS in another PL gene. Those can either be the main cause of the disease, or both variants could contribute to the pathogenicity. All the VUSs require in vitro and/or in vivo functional studies to uncover their potential pathogenicity.

Patient LE-45–10 had a 22q13.31–33 deletion that includes *CELSR1* and *SHANK3*, a gene associated with Phelan-McDermid syndrome.[16 17] This syndrome is characterised by developmental delay, autism or autistic-like behaviour. Between 10% and 29% of the individuals with this syndrome linked to 22q13.3 deletion develop lymphoedema, which correlates with the size of the chromosomal deletion.[10 18 19] Moreover, these patients usually develop puberty or adult-onset lymphoedema, which fits with the age-at-onset of *CELSR1*-related PL in our series. The data suggest that lymphoedema in the Phelan-McDermid syndrome may be caused by CELSR1 LoF.

We discovered eight *CELSR1* disease-causing variant carriers (including family members without signs of lymphoedema) to have a developmental kidney anomaly (table 1, figure 2 and online supplemental table 1). Interestingly, a recent study reported that up to 40% of individuals with Phelan-McDermid syndrome have a variable developmental kidney disorder.[19] They are seen in patients with a 22q13.3 deletion, rather than in those with a *SHANK3* point mutation. Four candidate genes commonly deleted with *SHANK3* in these patients, including *CELSR1*, were proposed for the renal phenotypes. Our study suggests that *CELSR1* may be the gene predisposing to renal anomalies and PL. Therefore, it seems essential to assess for kidney abnormalities and dysfunction in all patients with lymphoedema with a *CELSR1* disease-causing variant, including patients with Phelan-McDermid syndrome due to a 22q13.3 deletion. Indeed, patients with this contiguous gene deletion syndrome can have various renal anomalies, including agenesis/hypoplasia, kidney cysts, hydronephrosis, vesicoureteral reflux, kidney dysplasia and pyelectasis.[19] Association of any of these symptoms with PL may suggest a *CELSR1*-related cause.

In conclusion, our results confirm that *CELSR1* LoF variants are associated with autosomal dominant, puberty-onset, bilateral lower limb lymphoedema with variable expressivity and incomplete penetrance. Our family studies reinforce previous findings of gender-related penetrance of *CELSR1*-PL. The missense variants found require further assessment, especially via functional studies. Moreover, we identified renal anomalies as a novel symptom caused by variants in *CELSR1*. Thus, this gene should be included in routine genetic testing not only for patients with PL (especially if puberty/late-onset) but also for patients with kidney abnormalities. In addition, special attention should be paid to study kidney anomalies in individuals with a genetic testing result showing a *CELSR1* LoF variant.

Author affiliations
[1]Human Molecular Genetics, de Duve Institute, Brussels, Belgium
[2]Department of vascular medicine, CHU Montpellier, Montpellier, France
[3]IDESP - Institut Desbrest d'Épidémiologie et de Santé Publique, Montpellier, France
[4]Department of Lymphatic Surgery, VASCERN PPL European Reference Centre, General Hospital Sint-Maarten, Mechelen, Belgium
[5]Department of Nuclear Medicine, Montpellier Regional Cancer Institute, Montpellier, France
[6]Hematology Laboratory, University Hospital Centre Nimes, Nimes, France
[7]Center for Vascular Anomalies, Division of Plastic Surgery, VASCERN VASCA European Reference Centre, Cliniques universitaires Saint-Luc, Brussels, Belgium
[8]WELBIO, WEL Research Institute, Wavre, Belgium

Twitter Miikka Vikkula @MiikkaVikkula

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ORCID iDs
Murat Alpaslan http://orcid.org/0000-0002-6684-341X
Salma Adham http://orcid.org/0000-0003-2955-0922
Isabelle Quere http://orcid.org/0000-0002-1492-9764
Miikka Vikkula http://orcid.org/0000-0002-6236-338X

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