CLINICAL GENETICS An International Journal of Genetics, Molecular and Personalized Medicine

Clin Genet 2014
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CLINICAL GENETICS
doi: 10.1111/cge.12388

Short Report

Hypotrichosis-lymphedema-telangiectasia-renal defect associated with a truncating mutation in the SOX18 gene

Moalem S., Brouillard P., Kuypers D., Legius E., Harvey E., Taylor G., Francois M., Vikkula M., Chitayat D. Hypotrichosis-lymphedema-telangiectasia-renal defect associated with a truncating mutation in the SOX18 gene.
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SOX18 mutations in humans are associated with both recessive and dominant hypotrichosis–lymphedema–telangiectasia syndrome (HLTS). We report two families with affected children carrying a *SOX18* mutation: a living patient and his stillborn brother from Canada and a Belgian patient. The two living patients were diagnosed with HLTS and DNA analysis for the *SOX18* gene showed that both had the identical heterozygous C > A transversion, resulting in a pre-mature truncation of the protein, lacking the transactivation domain. Both living patients developed renal failure with severe hypertension in childhood for which both underwent renal transplantation. To our best knowledge this is the first report of renal failure associated with heterozygous mutations in the *SOX18* gene. We conclude that this specific mutation results in a new, autosomal dominant condition and propose the acronym HLT-renal defect syndrome for HLTRS.

Conflict of interest

The authors declare that they have no conflict of interest.

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Key words: HLTRS – HLTS – lymphatics – lymphedema – mosaicism – renal failure – SOX18 gene

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Received 14 January 2014, revised and accepted for publication 20 March 2014

Over the past few years, many of the molecular underpinnings of the formation of the lymphatic architecture and system have started to be elucidated (1). The mechanisms responsible for the molecular control of the lymphatic endothelial cell fate include key transcription factors: *SRY*-related HMG box-containing protein 18 (*SOX18*) (2), *COUP-TFII*, and prospero-related homeobox 1 (*PROX1*) (3).

SOX18 is the human homolog of the gene mutated in the spontaneous mutant mouse strains *Ragged* for which four different mutations in *Sox18* were identified: *Ra*, Ra^{J}, Rag^{l} and Ra^{Op}. SOX18 has been shown to directly induce *Prox1* gene expression to initiate lymphangiogenesis (1, 2, 4). The mutant form of SOX18 can also interfere with the transcriptional activity of SOX-F family members including SOX7, SOX17 and SOX18 (5). The transcription factor *SOX18* was shown to play a role in the development of hair, blood vessels and lymphatic vessels and when mutated, results in hereditary lymphedema, with unique clinical association of hypotrichosis, lymphedema, and telangiectasia (6). Heterozygous (dominant) or homozygous (recessive) mutations in *SOX18* were originally described in three families with hypotrichosis–lymphedema–telangiectasia syndrome (HLTS) and included one of the two cases reported here (7). We report two unrelated patients with identical *de novo* heterozygous C > A mutation in the SOX18 gene with HTLS who developed renal failure requiring renal transplantation.

Case reports

Family I

This family was first reported by Irrthum et al. (7) and the parents were healthy and non-consanguineous. The proband's older brother was the product of their first pregnancy. The pregnancy was complicated with non-immune hydrops fetalis and resulted in an intrauterine fetal death at 30 weeks gestation. The autopsy showed pericardial and pleural effusions and generalized vascular congestion with pulmonary lymphangiectasia. The proband was the result of the couple's second pregnancy and the pregnancy with him was uncomplicated. He had large bilateral hydroceles that required surgical repair at 3 months of age, and scrotal telangiectasia (7). At 6 months of age, he experienced progressive hair loss, resulting in alopecia universalis by the age of two. As an infant and toddler, he experienced multiple episodes of facial, peripheral and pulmonary edema and recurrent epistaxis. He was originally diagnosed with HLTS.

Subsequently to the original report by Irrthum et al. (7), at 5 years of age, he presented with renal failure and severe hypertension (207/155 mmHg). Renal biopsy including electron microscopy (EM) showed a chronic microangiopathy involving the glomerular and extra-glomerular vasculature. On EM the podocytes showed microvillus hyperplasia and effacement of foot processes. He required almost a year of peritoneal dialysis but recovered sufficient renal function to discontinue dialysis for 9 years. During that time, he had a slow progressive deterioration in renal function requiring renal transplantation at age 14. Renal allograft function has been stable since transplantation and he is on enalapril (ACE-I). His facial features were dysmorphic (Fig. 1a) with puffy eyelids, broad nasal root and tip, full lips and prognathism. The parents of the proband went on to have a healthy daughter.

Family II

This case was originally described by Proesmans et al. (8). The proband was born to a healthy and non-consanguineous couple who had a healthy older child. The proband was born with hydrocele surgically corrected at 2.5 years. He was first referred at 9 years of age with a 2 year history of sparse hairs, absent eyebrows and eyelashes, cutaneous, nasal and gingival telangiectasia, low subcutaneous fat and normo-complementaemic membranoproliferative glomerulonephritis (MPGN) (8). Repeat renal biopsy showed glomerular hypercellularity and mesangial expansion with prominent glomerular basement membranes (Fig. 2). Of note, the histological characteristics of a MPGN can also be found in many cases of thrombotic microangiopathy, the renal histopathological findings in the proband in family I. Thus, both cases are compatible with a MPGN type of glomerulonephritis against the background of a thrombotic microangiopathy. He had facial dysmorphism with an ovale face, full lips, puffy eyelids, a long and narrow nose with a broad nasal root and tip and prognathism as well as facial oedema. Scalp hair electron

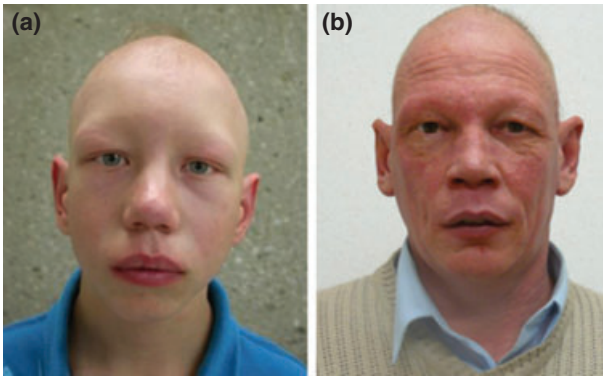
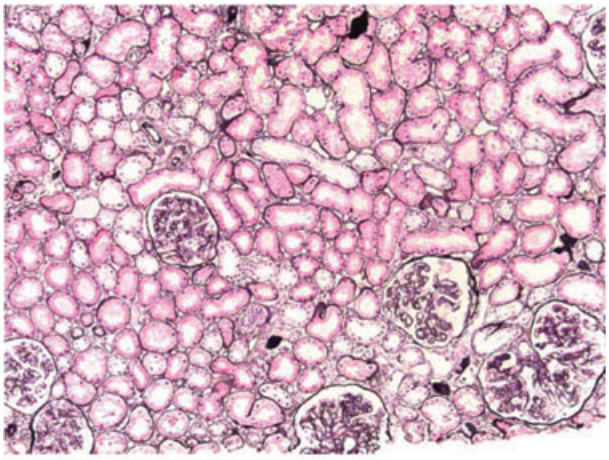


Fig. 1. (**a**) Index case of family I at 14 years of age. (**b**) Index case of family II at 38 years of age.

Panel A



Panel B

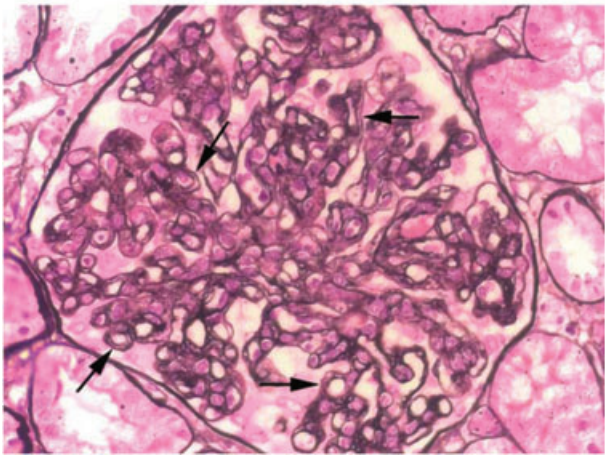


Fig. 2. Renal histology in proband 2. Panel A: (silvermethenamine staining, magnification ×100) glomeruli show hypercellularity and mesangial expansion with prominent glomerular basement membranes. Panel B: (silvermethenamine staining, magnification ×400) glomerulus with diffuse mesangial hypercellularity and a thickened capillary wall with cell interposition (arrows).

microscopic examination was normal and brain computed tomography (CT) scan showed calcified choroid plexus and abdominal ultrasound showed renal artery arteriosclerosis reminiscent of pre-mature aging. He did not have peripheral lymphedema. At age 10 he underwent abdominal surgery for ileocolic invagination. He had learning difficulties and at the age of 18 years, he developed progressive chronic renal failure with proteinuria and hypertension. Renal biopsy revealed MPGN and at the age of 25 he required peritoneal dialysis.

Ophthalmological examination revealed a right-sided naevus (2–3 mm) and a few central drusen. He had a successful renal allograft from a live donor at the age of 27. Since transplantation the patient has suffered from recurrent epistaxis and a unilateral idiopathic peripheral facial nerve palsy (*Bell's palsy*), which resolved completely with corticosteroid treatment. Renal allograft function has been normal and stable since transplantation and he

	Father		Child		Mother	
Marker	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D3S1358	16	17	16	17	16	17
TH01	8	9.3	6	9.3	6	7
D21S11	27	30	30	31	29	31
D18S51	13	15	11	13	11	?
Penta_E	13	21	13	21	7	13
D5S818	11		9	11	9	12
D13S317	9	14	13	14	12	13
D7S820	7	9	8	9	7	8
D16S539	11		11		11	
CSF1PO	10	11	10	12	10	12
Penta_D	?	12	10	12	10	?
AMEL	X	Y	X	Y	X	
vWA	16	20	16	20	19	20
D8S1179	13	16	13	16	12	13
TPOX	8	9	8	10	10	11
FGA	25		21	25	20	21

Fig. 3. Verification of the parentality in family II using the Powerplex 16 HS kit from Promega, Madison, WI.

is not on ACE-I or ARB therapy. A recent brain magnetic resonance imaging (MRI) was suggestive of right-sided hippocampal sclerosis and his electrocardiogram (EEG) was normal. He was also diagnosed with facial basal cell carcinoma, which was resected. At 38 years of age, the patient looks older than his age with a reddish facial skin complexion and alopecia universalis (Fig. 1b). He is on immunosuppressive medication and a statin.

Genetic analysis

The *SOX18* gene was sequenced on blood-derived genomic DNA of the two index cases and the stillborn brother in family I and all were found to have the same heterozygous nucleotide change ($c.720C>A$). This mutation results in the creation of a termination codon instead of the cysteine at position 240 (p.C240*). This residue is located in the second exon of *SOX18*, corresponding to the transactivation domain of the transcription factor [Fig. 4 in (7)]. Parental mutation analysis in both families showed no detectable mutation in the *SOX18* gene (Figs 3 and 4). Parentality was ascertained in both families [Fig. 3 and (7)]. To exclude the possibility of combined mutations in our patients, we sequenced the two exons of *SOX17* in all samples of both families and no mutation was detected.

Discussion

Over the past few years, many of the molecular mechanisms that underpin the formation of the lymphatic vascular tree and function have started to be elucidated (1). The *SOX18* gene has a major role in the formation of blood and lymphatic vessels and mutations in this gene are known to results in HLTS. However, renal defects have never been reported in patients with this condition who had other mutations in the gene (6) nor in the *Ragged* or the *Sox18* knockout mice (9). In contrast,

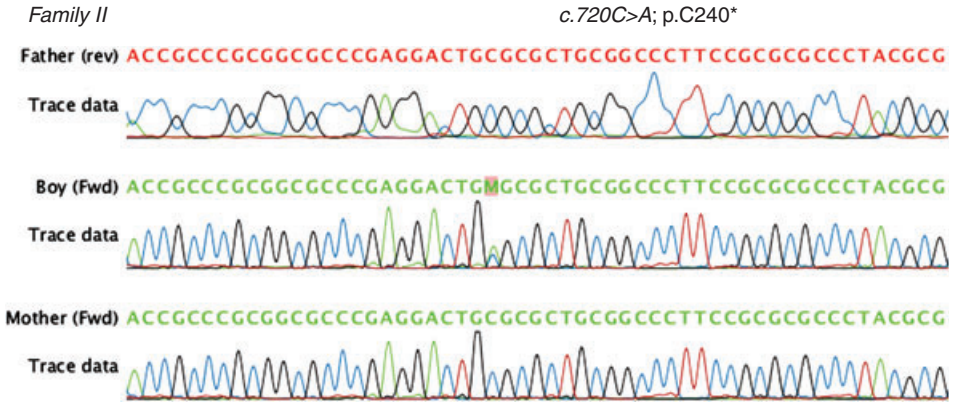


Fig. 4. Sequencing of the mutation in individuals of family II.

the double-knockouts *Sox17*$^{+/-}$; *Sox18*$^{-/-}$ present with reduced neovasculature in the liver and kidneys (10). To our knowledge this is the first report of two patients with HLTS associated with progressive, severe renal dysfunction which required transplantation with both patients being heterozygous for the *c.720C* > A mutation in the *SOX18* gene which resulted in a pre-mature truncation of the protein at the 240th amino acid (p.C240*) instead of the usual 384 residues. This mutation differs from the two other reported (p.A104P and p.W95R) which were associated with an autosomal recessive mode of inheritance and homozygosity because of parental consanguinity.

In contrast, the mutation reported in our patients was because of the same *de novo* mutation for which both of the patients were heterozygous. Interestingly, the same mutation was also found in the deceased fetus in family I. The presence of the mutation in both affected offspring in family I suggests gonadal mosaicism in one of the parents.

In family II, the older sib male was unaffected. In both families, the parentality was ascertained. We believe that this specific mutation is acting in a dominant-negative manner with a clearly distinct genotype/phenotype correlation.

So far, there are only two other reports that describe association of telangiectasia and glomerulonephritis (11, 12). A mutation in the *SOX18* gene may be the reason for the association in these cases.

Interestingly, *Sox18*$^{-/-}$ does not have a similar phenotype in the mice who present with only mild coat defect (5). In contrast, renal alterations were visible in *Sox17*/*Sox18* double heterozygotes (10). Moreover, mutations in *SOX17* gene in humans were also found to cause congenital renal anomalies and vesicoureteral reflux-1 (OMIM #19000) and 3 (OMIM #613674) (13). We screened *SOX17* in the patients and their parents and found no mutations. Thus, the role of the *SOX18* gene and the redundancy between the SOX proteins might differ between mice and man, as in the latter, a single truncated allele seems sufficient to display the renal phenotype. It is therefore very likely that the interaction with the *SOX17* gene is altered in our patients. *SOX18* might also play a role in the context of vascular development in the kidney through its interaction with other genes. For example, matrix metalloproteinase-7 (*MMP7*) has also been found to be a target of *SOX18* and the two genes are co-expressed in blood vessels of human skin (14). In contrast, MMP7 was overexpressed in a microarray gene expression analysis of patients with congenital renal dysplasia (15) characterized by disruption of normal renal development, cyst formation, impaired renal growth and reduced or absence of nephrons. It is thought that *MMP7* inhibits formation of branching structures in certain cells that are stimulated by bone morphogenetic protein-7 (*BMP7*), which is essential for normal kidney development. Thus, the renal phenotype seen in our patients could also result from altered MMP7 function, or a combination of it with altered SOX17 signaling. Thus, we believe that this specific mutation in both our patients might be acting in a dominant-negative manner with a clearly distinct genotype/phenotype correlation. This is the first report of renal failure associated with a heterozygous *SOX18* gene mutation, most probably because of abnormal glomerular lymph angiogenesis. Because it highlights are yet unreported renal manifestations associated with *SOX18* mutations, we propose to call the disease caused by mutations in the *SOX18* gene HLTRS, for hypotrichosis–lymphedema–telangiectasia-renal defect syndrome. In a clinical setting, this renal aspect linked to the *c.720C* > *A* mutation is very important as it leads to renal failure and the need for transplantation.

Acknowledgements

The authors' studies were partially funded by the Belgian Science Policy Office Interuniversity Attraction Poles (BELSPO-IAP) program through the project IAP P7/43-BeMGI: and the F.R.S.-FNRS (Fonds de la Recherche Scientifique), both to M. V.; and the Fédération Wallonie-Bruxelles, and the Lotterie nationale, Belgium, and the Walloon Excellence in Lifesciences & BIOtechnology (WELBIO). We would like to thank Prof. Dr Evelyne Lerut, Department of Pathology, University of Leuven, Belgium, for providing the microphotographs of the renal histology in family II.

References

1. Francois M, Koopman P, Beltrame M. SoxF genes: key players in the development of the cardio-vascular system. Int J Biochem Cell Biol 2010: 42: 445–8.

2. François M, Caprini A, Hosking B et al. Sox18 induces development of the lymphatic vasculature in mice. Nature 2008: 456: 643–7.
3. Wigle JT, Chowdhury K, Gruss P et al. Prox1 function is crucial for mouse lens-fibre elongation. Nat Genet 1999: 21: 318–22.
4. Downes M, Francois M, Ferguson C et al. Vascular defects in the mouse model of hypotrichosis-lymphedema-telangiectasia syndrome indicate a role for SOX18 in blood vessel maturation. Hum Mol Genet 2009: 18: 2839–50.
5. Pennisi D, Gardner J, Chambers D et al. Mutations in Sox18 underlie cardiovascular and hair follicle defects in ragged mice. Nat Genet 2000: 24: 434–7.
6. Brouillard P, Boon LM, Vikkula M. Genetics of lymphatic anomalies. J Clin Invest 124 (3): in press.
7. Irrthum A, Devriendt K, Chitayat D et al. Mutations in the transcription factor gene SOX18 underlie recessive and dominant forms of hypotrichosis-lymphedema-telangiectasia. Am J Hum Genet 2003: 72: 1470–8.
8. Proesmans W, Legius E, Van Herck K et al. Cutaneous telangiectasia, sparse hair and membranoproliferative glomerulonephritis. A new case of a newly recognized entity. Pediatr Nephrol 1989: 3: 162–5.
9. Matsui T, Kanai-Azuma M, Hara K et al. Redundant roles of Sox17 and Sox18 in postnatal angiogenesis in mice. J Cell Sci 2006: 119: 3513–26.
10. Alders M, Hogan BM, Gjini E et al. Mutations in CCBE1 cause generalized lymph vessel dysplasia in humans. Nat Genet 2009: 41: 1272–4.
11. Gupta IR, Tsai CH, Siegel-Bartelt J et al. Cutaneous telangiectasias, sparse hair, and type I membranoproliferative glomerulonephritis. Pediatr Nephrol 1999: 13: 129–31.
12. Sherwood MC, Pincott JR, Goodwin FJ et al. Dominantly inherited glomerulonephritis and an unusual skin disease. Arch Dis Child 1987: 62: 1278–80.
13. Gimelli S, Caridi G, Beri S et al. Mutations in SOX17 are associated with congenital anomalies of the kidney and the urinary tract. Hum Mutat 2010: 31: 1352–9.
14. Hoeth M, Niederleithner H, Hofer-Warbinek R et al. The transcription factor SOX18 regulates the expression of matrix metalloproteinase 7 and guidance molecules in human endothelial cells. PLoS One 2012: 7: e30982.
15. McGuire JK, Harju-Baker S, Rims C et al. Matrilysin (MMP-7) inhibition of BMP-7 induced renal tubular branching morphogenesis suggests a role in the pathogenesis of human renal dysplasia. J Histochem Cytochem 2012: 60: 243–53.