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Clinical Research

Aortic Dilatation Associated With a De Novo Mutation in the *SOX18* Gene: Expanding the Clinical Spectrum of Hypotrichosis-Lymphedema-Telangiectasia Syndrome

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ABSTRACT

Background: We report a 13-year-old female patient followed since birth for multiple rare congenital defects, including hypotrichosis, telangiectasia, and severe dilatation of the ascending aorta.

Methods: Comprehensive phenotype assessment throughout childhood included repeated echocardiographic measurements, evaluation of renal function, and immunohistochemical analysis of skin biopsy samples. Whole-exome sequencing was performed for the patient and both unaffected parents.

Results: We identified a novel de novo mutation in the transcription factor *SOX18* (c.481C>T:p.Gln161*) in the patient, which was absent in all unaffected family members. Echocardiography revealed early onset and progressive dilatation of the ascending aorta. Skin biopsy results confirmed the defects of the blood vasculature in the presence

RÉSUMÉ

Introduction : Nous rapportons le cas d'une patiente de 13 ans suivie depuis la naissance pour de multiples anomalies congénitales rares, dont l'hypotrichose, la télangiectasie et la dilatation grave de l'aorte ascendante.

Méthodes : L'évaluation exhaustive du phénotype tout au long de son enfance comprenait des mesures échocardiographiques répétées, l'évaluation du fonctionnement rénal et l'analyse immunohistochimique des échantillons de peau obtenus par biopsie. Le séquençage de l'exome entier a été réalisé chez la patiente et les deux parents non affectés.

Résultats : Nous avons trouvé chez la patiente une nouvelle mutation de novo dans le facteur de transcription *SOX18* (c.481C>T:p.Gln161*), qui était absente chez tous les membres de la famille non affectés.

The circulatory system is among the first organs to develop in the course of embryogenesis and has to maintain integrity throughout development. Alterations of vascular function can lead to a heterogeneous group of malformations ranging from localized edemas to systemic vascular defects.[1] Genetic factors are involved in a number of rare syndromes with a vascular phenotype associated with dysregulation in regulatory or transcriptional pathways.[2,3] We present a case of an adolescent female patient with the co-occurrence of multiple congenital anomalies, including a severe defect of the blood vasculature. The diagnosis based solely on clinical characteristics remained elusive despite extensive clinical investigations. Several of the patient's symptoms are in line with the rare conditions Adams-Oliver (OMIM No. 100300, No. 614219, No. 614814, No. 615297, and No. 616028) and hypotrichosis-lymphedema-telangiectasia syndrome (HLTS, OMIM No. 607823).[4,5] Because of the absence of lymphedema, HLTS was initially not considered as a differential diagnosis. Both conditions are associated with mutations in genes that play an important role

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of intact lymphatic vessels. Assessment of renal function did not show any signs of renal problems or renal failure in the patient.

Conclusions: The genetic finding of a pathogenic *SOX18* mutation enabled the diagnosis of the rare hypotrichosis-lymphedema-telangiectasia syndrome in our patient. The identification of a novel stop gain mutation in the *SOX18* gene in association with dilatation of the aorta highlights the importance of this gene during the development of the circulatory system. Our study highlights the importance of whole-exome sequencing in the rapid identification of genes and gene mutations involved in rare conditions and thus expanding the knowledge and spectrum of clinical manifestations associated with them.

L'échocardiographie a révélé l'apparition précoce et la dilatation progressive de l'aorte ascendante. Les résultats de la biopsie de peau ont confirmé les anomalies de la vasculature sanguine en présence de vaisseaux lymphatiques intacts. L'évaluation de la fonction rénale n'a montré aucun signe de problèmes rénaux ou d'insuffisance rénale chez la patiente.

Conclusions : La découverte génétique de la mutation pathogène *SOX18* a permis le diagnostic du syndrome rare d'hypotrichose-lymphœdème-télangiectasie chez notre patiente. L'identification d'une nouvelle mutation non-sens dans le gène *SOX18* en association avec la dilatation de l'aorte souligne l'importance de ce gène durant le développement du système circulatoire. Notre étude souligne l'importance du séquençage de l'exome entier pour rapidement identifier les gènes et les mutations génétiques impliqués dans les affections rares et, par conséquent, élargir les connaissances et l'éventail des manifestations cliniques qui y sont associées.

in vasculogenesis.[6,7] Although mutations in genes linked to the Notch signalling cascade (*NOTCH1, EOGT, RBPJ*) are associated with Adams-Oliver syndrome,[8-10] HLTS is caused by mutations in the transcription factor gene *SOX18* acting upstream of the Notch pathway. The close resemblance of developmental malformations observed in the 2 syndromes highlights the involvement of SoxF and Notch signalling in functionally related vascular pathways. Recent findings support a combinatorial role of *RBPJ*, a Notch signalling mediator, and Notch binding with SoxF factors in the course of arterial development.[11] Despite increasing evidence on the important role of the SoxF-Notch signalling axis for vascular development and identity, our understanding of the specific events in vasculogenesis at an early embryonic stage that result in vascular defects are far from complete. However, a better understanding of how these factors influence vascular development is crucial when providing an accurate diagnosis for patients with syndromes that have a vascular component. Recent whole-exome sequencing studies have provided an unbiased view of the genetic determinants contributing to the heterogeneous group of diseases with congenital vascular phenotypes.[12] We performed whole-exome sequencing in our patient and her unaffected parents to identify the underlying genetic component of her syndrome, which includes severe vascular dysfunction of the ascending aorta. Integration of the genetic analysis in combination with the clinical assessment of the patient revealed a novel form of the rare HLT syndrome.

Case Presentation

A 13-year-old female patient presented at Sainte-Justine University Hospital Center in Montreal with a lifelong history of multiple rare congenital defects. The patient was born to a 30-year old-mother of mixed European descent and a 33-year-old father of French Canadian descent and has a healthy brother. The patient was delivered by cesarian section at term without undue complications. The couple is healthy, nonconsanguineous, and without a family history of disease associated with the observed congenital defects. At birth, the patient presented with diffuse striking livedo reticularis over the extremities, as well as dysplastic toenails and cutis marmorata. Arterial hypertension developed when the patient was 18 months old and was treated with a variety of medications, including propranolol, calcium-blocking agents and irbesartan, an angiotensin II receptor antagonist. She had sparse hair since birth, with an absence of eyelashes and eyebrows (Fig. 1A). Reticular telangiectatic patches on her face, limbs, and torso (Fig. 1, B and C) were apparent, with bluish discoloration of her toes (Fig. 1D). Skin biopsy specimens of livedoid erythema were collected and assessed for vascular and lymphatic function. Histopathologic analysis showed the typical appearance of malformed telangiectatic small vasculature (Fig. 1E). Immunohistochemical examination of skin biopsy samples confirmed the severe dilatation of the small blood vessels (Fig. 1F) and an intact lymphatic vascular network (Fig. 1G). Severe dilatation of the ascending aorta was diagnosed at the age of 2 years with a z score > 3 (Fig. 2).[13] Vascular function declined, and repeated echocardiographic measurements until the age of 13 years showed a progression of the dilatation of the ascending aorta with a z score of 4 in the absence of ventricular hypertrophy (Fig. 2). Renal function was normal based on angiography performed at the age of 3 years and remained intact throughout her life as indicated by repeated measurements of glomerular filtration rate (GFR) based on blood creatinine levels.

Complications included anemia of unknown origin, possibly secondary to frequent epistaxis caused by telangiectasia in the nasal mucosa and subsequent iron deficiency, without signs of a coagulation defect. Routine computed tomographic neuroimaging at 18 months of age revealed calcifications in the brain with unknown origin (Supplemental Fig. S1). Furthermore, at 4 years of age, an asymptomatic carcinoid tumor was discovered on histologic examination of surgical material after an appendectomy (Supplemental Fig. S2). Karyotype analysis was normal, and no large-scale chromosomal abnormalities were identified. Taken together, the comprehensive clinical examination of the patient remained elusive despite an overlap of phenotypic features with the rare genetic Adams-Oliver syndrome and HLTS. The latter was not considered as a differential diagnosis because of the absence of lymphedema in our patient.

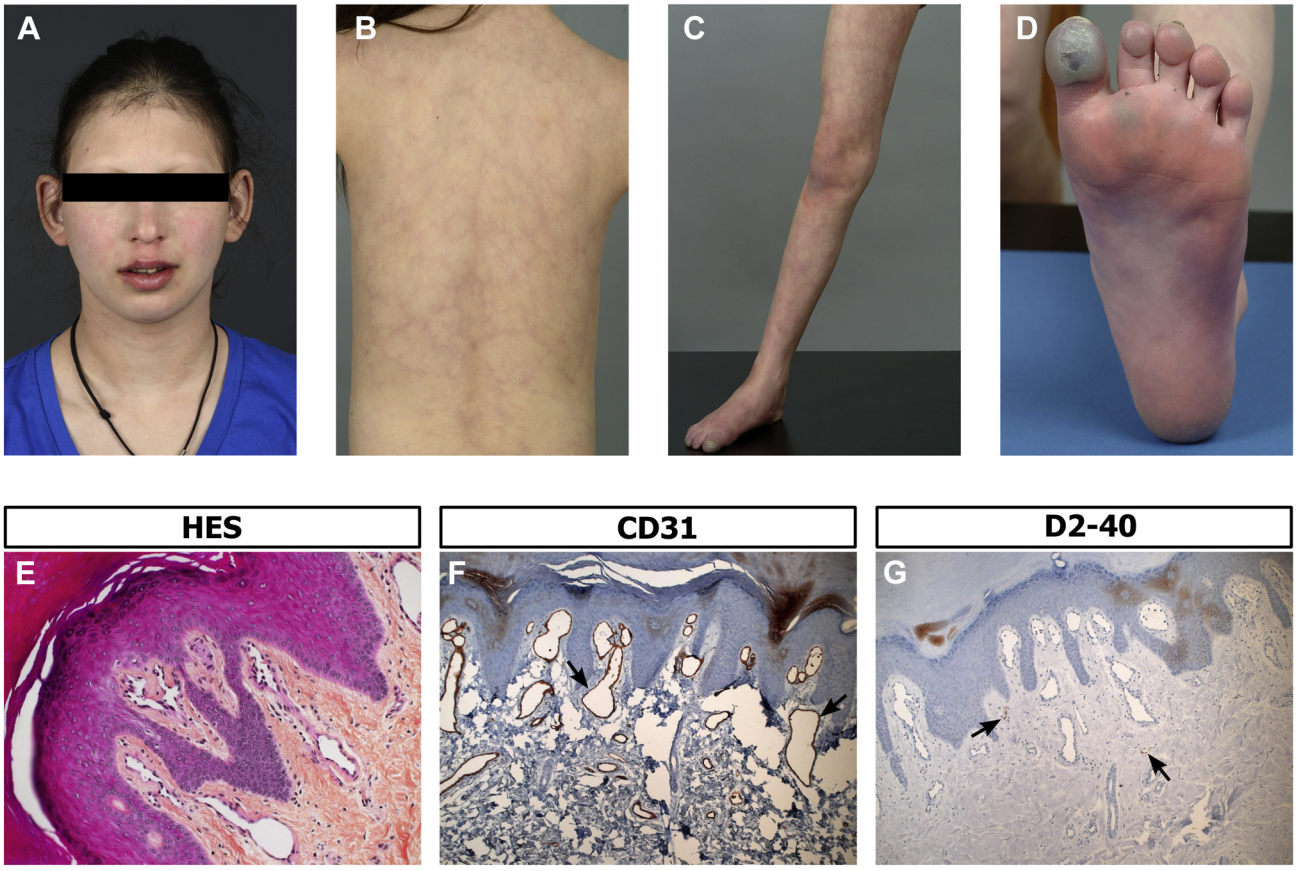

Figure 1. Patient characteristics of hypotrichosis-lymphedema-telangiectasia syndrome. (**A**) Hypotrichosis is demonstrated by the absence of eyebrows and eyelashes and sparse hair. (**B**, **C**) Telangiectatic networks are apparent on the back and the extremities. (**D**) Bleeding from cracks that develop in callused skin in the great toes are associated with dilatation of blood vessels. (**E**) Skin biopsy results highlight capillary vascular malformations with increased and dilated capillaries in the papillary dermis stained with hematoxylin-eosin-safranin (HES) (×200). (**F**) The capillaries express vascular endothelial marker CD31 (×100) (**black arrows**). (**G**) D2-40–stained section revealing lymphatic vessels of normal appearance (×100) (**black arrows**).

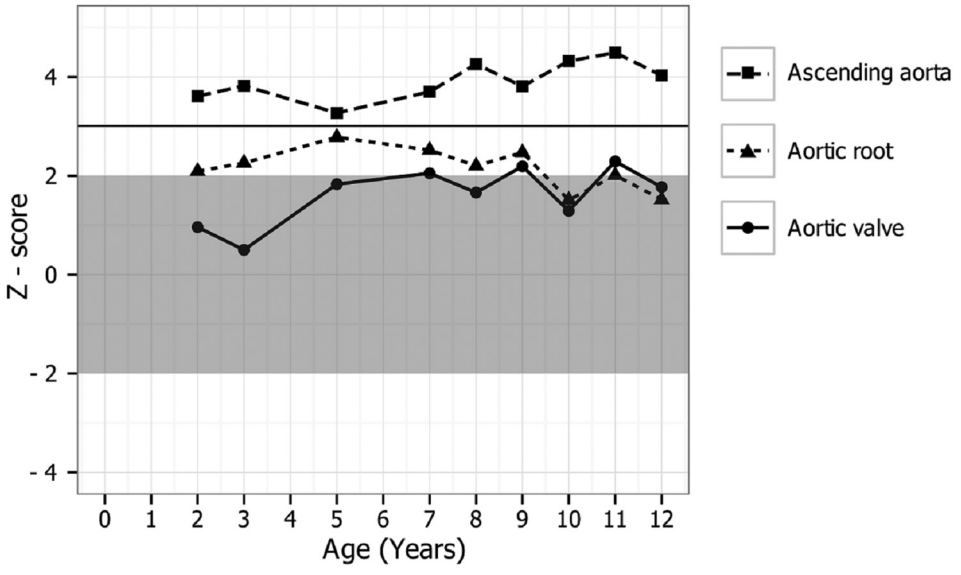

Figure 2. Progression of aortic dilatation. Echocardiographic measurements of the ascending aorta, the aortic root, and aortic valve are displayed as *z* scores.[13] **Grey area** depicts 95% of the area under the curve following the normal distribution of *z* scores among the general population. *z* scores > a threshold of 3 for the ascending aorta (**highlighted by solid black line**) indicate severe dilatation.

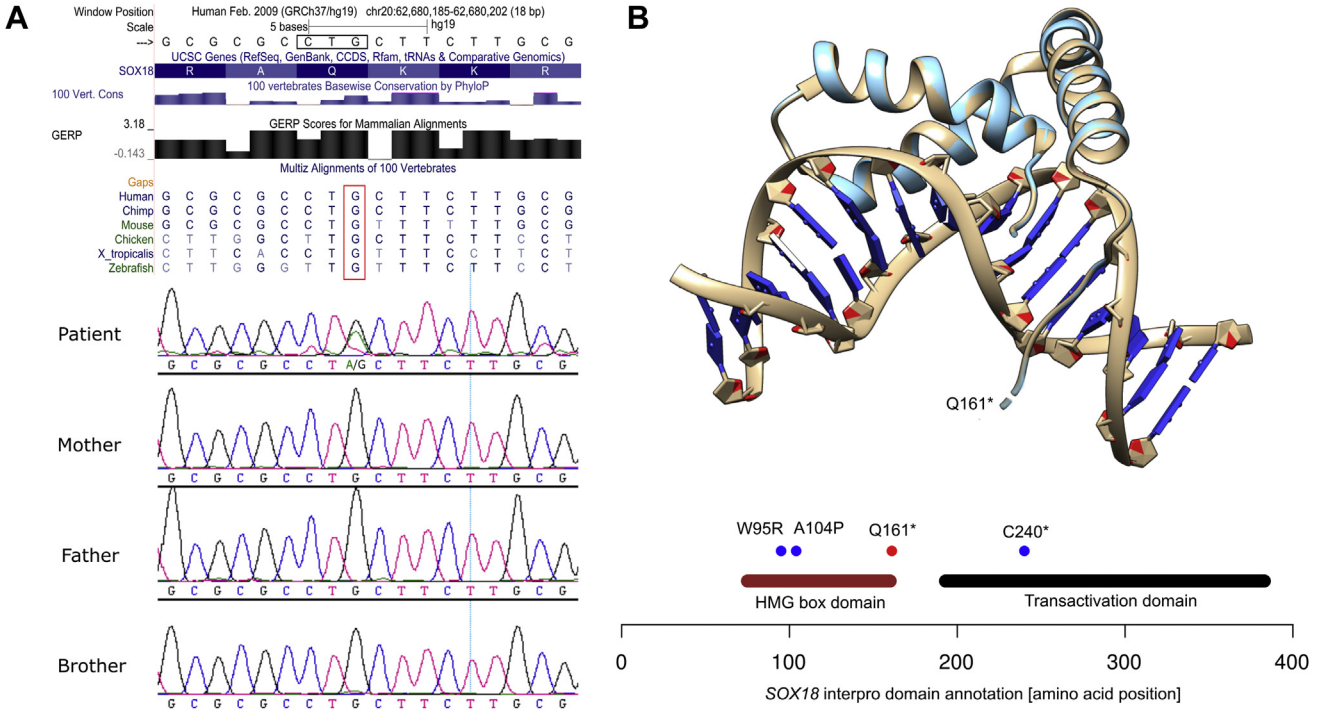

Figure 3. DNA and protein position of the novel *SOX18* de novo mutation. (**A**) (**Top**) Graphic representation of the novel *SOX18* mutation position in the UCSC browser (http://genome.ucsc.edu),[14] showing high base-pair conservation at the location of the mutation. (**Bottom**) Sanger sequencing chromatograms for the patient and the healthy parents and brother, highlighting heterozygosity for the stop gain mutation in the patient (only forward sequencing shown). Absence of the mutation in the parents and the brother shows that it is de novo. (**B**) Protein structure and DNA-binding prediction of *SOX18*. The location of the novel variant (**red**) is shown relative to previously reported hypotrichosis-lymphedema-telangiectasia—renal defect syndrome—causing mutations (**blue**). The SOX18 Q161* mutation is located in close proximity after the HMG box domain according to InterPro annotation, truncating the protein of its transactivation domain.[15] The DNA-binding domain is not affected by the mutation based on structure prediction of SOX18 and overlapping homology mapping to the SOX17 DNA binding domain for which crystallography is available (PDB entry:4a3n).[16]

Methods

Study subject

Written informed consent was obtained from the patient and her family. The study was approved by the Ethical Committee of the CHU Sainte-Justine, Montreal, Quebec, Canada.

Whole-exome sequencing analysis

Genomic DNA was extracted from peripheral blood using the QIAGEN Gentra Puregene blood kit (QIAGEN, Toronto, Canada). Standard library preparation protocols were used for the Agilent SureSelect 50 Mb exome enrichment kit (version 4), with subsequent 100—base pair, paired-end sequencing on the Illumina HiSeq 2000 platform (Illumina, San Diego, CA). Post—quality control reads were aligned to the reference human genome version 19 using Burrows-Wheeler Aligner and variants were called using SAMtools, version 0.1.13 utilities.[17,18] Only variants (single-nucleotide variants and small insertion-deletions [indels]) defined by a stringent Phred-like genotype quality cut-off > 20, which reflects 99% base calling accuracy in SAMtools, were kept for downstream analysis. This analysis was performed under the assumption of a de novo or recessive homozygous mutation, which is rare (< 1% minor allele frequency) or absent in the general population. We ranked variants for validation based on genotyping quality scores > 100 obtained through the SAMtools package. To obtain a tractable set of disease-causative candidate mutations, variants were correlated with dbSNP 138 (Database of Single Nucleotide Polymorphisms, National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID: 138); 1000 Genomes Project database (http://www.1000genomes.org; The 1000 Genomes Project Consortium),[19] and an in-house database of normal germ-line variants at CHU Sainte Justine Research Center that contains data from 360 French Canadian controls. Mutations were annotated with reference to National Center for Biotechnology Informatics genome assembly NCBI build 37 (GRCh37).

Copy number variation analysis

High-resolution G-banding on chromosome spreads of the affected patient was performed as previously described. Three separate bioinformatic tools were used to call copy number variation (CNV) from whole-exome sequencing data (CNVkit, ExCopyDepth, XHMM).[20-22] Genomic regions predicted to contain CNVs were subsequently correlated for any overlap with a list of genes known to be associated with developmental disorders from the DECIPHER project.[23]

Confirmational Sanger sequencing

We performed polymerase chain reaction (PCR)-based bidirectional Sanger sequencing using BigDye Terminator,

version 3.1 (Life Technologies, Grand Island, NY) on an ABI 3130xL genetic analyzer (Applied Biosystems/Life Technologies, Grand Island, NY) to confirm the *SOX18* mutation detected by whole-exome sequencing. PCR for the patient, both parents, and the healthy brother were set up with the following primer pairs: SOX18_F (GACGGCGTTCCACTCACT) and SOX18_R (CTCCAGGCCGTCCAGAGG) amplifying the part of the coding region of *SOX18* containing the identified stop gain mutation. Electropherograms were aligned to the GRCh37 reference sequence of *SOX18* and analyzed using SeqMan Pro, which is part of the Lasergene suite (DNASTAR, Madison, WI).

Histologic Examination

Tissue was fixed in 10% formaldehyde and incorporated into paraffin blocks. Paraffin-embedded sections, 4 μm in thickness, were stained with hematoxylin-eosin-safranin (HES), and immunohistochemistry was performed using a Dako autostainer (Dako North America, Carpinteria, CA) with Dako antibodies against endothelial cell marker CD31 and lymphatic cell marker D2-40.

Results

Whole-exome sequencing analysis

Whole-exome capture and sequencing was performed on genomic DNA of the patient and both unaffected parents. Exome-sequencing data was analyzed under the hypothesis of a rare genetic disorder caused by a germ line mutation, because of the absence of a family history of disease. Variant calls from exome data were first filtered to identify rare coding variants that are present in the patient but absent in either parent (de novo) (Supplemental Table S1). Only 1 de novo variant met the criteria of a rare disease-causing variant, after excluding regions with segmental duplications and synonymous variants. This is in line with previous estimations of functionally important de novo variants in the human genome.[24,25] The identified stop gain mutation is located in the coding region of the SRY (sex determining region Y)-box 18 gene (*SOX18*). The mutation NM_018419.2:c.481C>T (ClinVar accession: SCV000189850) changes the highly conserved glutamine at position 161 to a premature stop codon (p.Gln161*) (Fig. 3A). The variant is absent in public exome data sets (Exome Aggregation Consortium [ExAC], Cambridge, MA [http://exac.broadinstitute.org] [March 2015]) and an in-house repository holding 360 whole exomes from French Canadian controls. Bidirectional Sanger sequencing validated the mutation in the patient and its absence in the parents as well as in the healthy brother (Fig. 3A). To exclude additional rare variants or missing sequence information in known candidate genes of Adams-Oliver syndrome, we calculated mean read coverage for the genes *NOTCH1*, *ARHGAP31*, *DOCK6*, *EOGT*, and *RBPJ* and manually checked for variation in the exome data. All exons of the known candidate genes were highly covered, with a mean coverage of > 30 reads per base per exon, and no additional rare potentially deleterious variants were identified. Mutations in *SOX18* are known to cause the rare HLTS syndrome, which overlaps with symptoms observed in our patient, including hypotrichosis and telangiectasia. Vascular anomalies reported in HLTS have been linked to the regulatory effect of *SOX18* during microvascular maturation.[26] Based on in silico predictions and domain analysis, the stop gain mutation observed in our patient truncates the protein of its transactivation domain while keeping the DNA-binding domain (HMG box) intact (Fig. 3B). CNV analysis was performed on whole-exome data, using 3 separate tools to identify potential cofounding CNVs. The analysis did not reveal any de novo CNVs overlapping the critical *SOX18* interval or other genes associated with developmental disorders or vascular syndromes.

Discussion

The diagnosis of complex and heterogeneous congenital defects based solely on clinical investigations is challenging and might be unsuccessful. We highlight how the integration of whole-exome sequencing data can guide the diagnosis for a patient with incomplete penetrance of a complex clinical phenotype. We identified a de novo stop gain mutation in the transcription factor gene *SOX18* in a patient clinically suspected to have Adams-Oliver syndrome. Mutations in *SOX18* are known to cause the rare developmental disorder HLTS, which affects hair follicles, lymphatic vasculature, and small blood vessels. HLTS was initially described in 2003 by Irrthum et al.,[5] who reported 5 individuals with either recessive or dominant *SOX18* mutations. The phenotypic spectrum of HLTS is highly variable among the small number of patients identified in the literature. Hypotrichosis and telangiectasia have been reported for all HLTS patients. However, the manifestation and onset of lymphedema in HLTS is highly variable (Supplemental Table S2). The presence of hypotrichosis and telangiectasia, 2 of the 3 key features of HLTS, was crucial for the clinical diagnosis in our patient. The absence of lymphedema might be explained because of the following observations. First, a variable onset of lymphatic defects in patients with HLTS has been reported in the past. Second, environmental factors and differences in genetic backgrounds are likely to translate into different outcomes during vascular development. Treatment with high doses of the nonspecific β-blocker propranolol throughout childhood might also have had an effect in preserving lymphatic function. Propranolol has been shown to affect the expression of vascular endothelial growth factor (VEGF) levels as well as promote lymphatic vessel growth, and is 1 of the treatment options for pediatric lymphangioma.[27-29] Vascular endothelial growth factor D *(VEGFD)* has recently been shown to directly affect *SOX18* transcriptional activity in vascular endothelial cells.[6] Therefore, early treatment with propranolol might have contributed to an intact lymphatic system by modulation of VEGF levels and subsequent decreased transcriptional activity of SOX18 in the blood vasculature. Besides the role in lymphatic vessel development, studies using a naturally occurring *Sox18* mutant mouse strain (ragged-opossum Ra[Op]), have implicated *Sox18* in the blood vascular anomalies observed in HLTS.[26] Intriguingly, defects of the major vessels are observed in double knockout mouse models of *Sox18/Vegfd* but not in single knockouts for *Sox18*.[6] For this reason, it is tempting to provide a novel link between the dysfunction of *SOX18* and the severe aortic dilatation observed

in our patient. Other factors, including hypertension, are less likely to play an important role in causing the disease. Most large-scale epidemiologic studies have reported only a weak effect of blood pressure on aortic diameter, which is lost when adjusting for age and sex.[30] Even when considering the different subtypes of hypertension, age and body size rather than blood pressure account for differences in aortic root size.[31] In contrast, heredity explains a substantial proportion of variance in aortic root diameter, pointing to a genetic cause for aortic dilatation, as reported in the Strong Heart Study.[32] Furthermore, dilatation of the aorta is often observed in patients with familial genetic conditions, including Marfan syndrome and bicuspid aortic valve.[33,34] The severe aortic dilatation observed in our patient at 2 years of age points to a strong genetic effect that is likely driven by *SOX18*.

The asymptomatic carcinoid tumors in the appendix and cerebral calcifications found in our patient have not been previously associated with HLTS. However, they might present a coincidental finding because of other contributors and the more common nature of these observations. Furthermore, in a recent study by Moalem et al.,[35] 2 unrelated patients with renal failure requiring renal transplantation were reported to carry the same stop gain mutation in *SOX18* (NM_018419.2:c.720C>A:p.Cys240*).[35] Thus, the authors expanded the *SOX18*-associated HLTS phenotypes and proposed the term "hypotrichosis-lymphedema-telangiectasia–renal defect syndrome" (HLTRS) as a novel and distinctive autosomal dominant condition. The variant identified by Moalem et al. and the mutation observed in our patient both truncate the transactivation domain of SOX18 interfering with the transcriptional activation of downstream targets. Despite similar functional consequences at a molecular level, the phenotypic spectrum of *SOX18* mutations remains highly variable. Renal angiography and assessment of GFR levels in our patient throughout childhood showed no signs of renal problems or renal failure. This suggests different functional consequences of deleterious mutations in *SOX18* during the course of early vascular development. Homozygous missense or heterozygous dominant nonsense mutations can result in different phenotypic outcomes as a result of transcriptional dysregulation of downstream targets in the SoxF-Notch signalling cascade. Further genetic studies are required to elucidate the specific role of the different *SOX18* mutations during vasculogenesis and their effects in disease progression and outcome.

In summary, our patient shows distinct phenotypic manifestations that have not been observed in previously reported HLTS and HLTRS cases. The identification of an early onset of severe dilatation of the aorta underlines the important role of *SOX18* in the development of the circulatory system.

Conclusions

This is, to our knowledge, the first report linking aortic dilatation with mutations in *SOX18*. The identification of *SOX18* as the underlying genetic disease driver in our patient enables precise genetic counselling and case-specific stratification for renal and lymphatic complications in HLTS. Furthermore, the integration of whole-exome sequencing provides a valuable tool for the rapid identification of candidate genes in rare developmental syndromes with a cardiovascular component.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at http://dx.doi.org/10.1016/j.cjca.2015.04.004.