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A novel *SOX18* mutation uncovered in Jordanian patient with hypotrichosis–lymphedema–telangiectasia syndrome by Whole Exome Sequencing

Fatma Bastaki [a], Madiha Mohamed [a], Pratibha Nair [b], Fatima Saif [a], Nafisa Tawfiq [a], Mahmoud Taleb Al-Ali [b], Oliver Brandau [c], Abdul Rezzak Hamzeh [b, *]

[a] *Pediatric Department, Latifa Hospital, Dubai Health Authority, Dubai, United Arab Emirates*
[b] *Centre for Arab Genomic Studies, Dubai, United Arab Emirates*
[c] *Centogene AG, Schillingallee 68, Rostock 18057, Germany*

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ABSTRACT

The *SOX18* gene encodes a transcription factor that plays a notable role in certain developmental contexts such as lymphangiogenesis, hair follicle development and vasculogenesis. *SOX18* mutations are linked to recessive and dominant hypotrichosis–lymphedema–telangiectasia syndrome (HLTS). In this study we report on a novel heterozygous mutation in *SOX18* in a Jordanian patient suffering from HLTS that was revealed by Whole Exome Sequencing. In this case, a frameshift caused by 14-nucleotide duplication in *SOX18* appeared *de novo* resulting in a premature translational stop at the N-terminal region of the central trans-activation domain. Here we present the clinical manifestations of the above mentioned molecular lesion in the light of what is known from published *SOX18* mutations.

1. Introduction

Hypotrichosis–Lymphedema–Telangiectasia syndrome (HLTS) combines features that represent failure of proper vascularization, angiogenesis and hair formation. Dysfunctional development of blood vessels results in cutaneous telangiectasias and dilations of superficial vasculature, while disturbances in the maintenance of the lymphatic system are manifested by lymphedemas in the lower limbs and eyelids. The third constituent of this syndrome involves defects in hair follicle development, leading to progressive scalp hair loss and absence of eyebrows and eyelashes. Irrthum et al. [1] established the link between these symptoms and mutations in a member of the SOX (Sry-related HMG box) family; namely *SOX18*. Animal studies have indicated that *SOX18* plays a major role in lymphangiogenesis and angiogenesis [2,3], and in cardiovascular development and hair follicle formation [4]. To date, four mutations in this gene have been identified in HLTS patients from five different families (Table 1). Although the cardinal features of this syndrome remain the same, recent reports have shown the development of renal failure or aortic dilatation in some of the patients [5,6].

SOX18 belongs to the SOX-F group, and it encodes a transcriptional activator that plays important roles in the development of hair, lymphatic and blood vessels. Human SOX18 contains a HMG-type DNA-binding domain and two trans-activation domains (TAD) [7,8]. The above mentioned roles of *SOX18* clearly explain the phenotypic gamut that characterizes HLTS. Furthermore, there seems to be two types of *SOX18* variants at the heart of causality of HLTS; missense mutations affecting the HMG box and nonsense mutations leading to truncated SOX18 moieties that lack the trans-activation function. The latter class of mutations follows a dominant mode of inheritance, while the former class is generally recessive [1].

Here we report a case of HLTS in a Jordanian patient that is caused by a novel heterozygous mutation in *SOX18*. The mutation, which appeared *de novo* in the patient, involves 14-nucleotide duplication causing a frameshift and a premature translational stop at the N-terminal region of the central TAD. This study describes the full clinical consequences of this mutation along with a contextual analysis of the underlying molecular lesion that was

* Corresponding author. Centre for Arab Genomic Studies, P.O. Box 22252, Dubai, United Arab Emirates.
E-mail address: Abdul.hamzeh@hmaward.org.ae (A.R. Hamzeh).

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Table 1
Phenotypic features and their variation among *SOX18* mutations implicated in HLTS.

Family	Mutation	Protein change	Ethnicity	Phenotype	Reference	Inheritance
1	c.310G>C	p.Ala104Pro	Belgian	hypotrichosis, lymphedema (legs)	Irrthum et al., 2003	Autosomal recessive
2	c.283T>A	p.Trp95Arg	Turkish	hyportichosis, lymphedema (legs), telangiectasia	Irrthum et al., 2003	Autosomal recessive
3	c. 720C>A	p.Cys240Ter	Not reported	hypotrichosis, generalized lymphedema, telangiectasia, mild eczema, renal failure, hypertension	Irrthum et al., 2003; Moalem et al., 2015	AD, de novo
4	c. 720C>A	p.Cys240Ter	Belgian	hypotrichosis, telangiectasia, renal failure, peculiar facies	Moalem et al., 2015	AD, de novo
5	c.481C>T	p.Gln161Ter	Mixed European/ French Canadian	hypotrichosis, telangiectasia, livedoid erythema , early onset and progressive aortic dilatation	Wünnemann et al., 2015	AD, de novo
6	c.492_505dup	p.(Glu169Glyfs*14) official HGVS naming given by Mutalyzer (https://mutalyzer.nl/)	Jordanian	total alopecia, lymphedema, telangiectasia, distinct craniofacial features,	This report	AD, de novo

uncovered by Whole Exome Sequencing (WES) of *SOX18*.

2. Methodology

2.1. Exome capture and sequencing

Upon informed consent, peripheral blood samples were collected from the patient and his parents. Thereafter, DNA was extracted from blood samples according to standard protocols. Amplicon library construction, exome capture, sequencing, and standard data analysis for affected child and his parents was performed by Centogene (Rostock, Germany). First, approximately 37 Mb (214,405 exons) of the Consensus Coding Sequences (CCS) were enriched from fragmented genomic DNA by >340,000 probes designed against the human genome. The kit used for exome capturing is Nextera Rapid Capture Exome (Illumina) and the sample was processed on the NextSeq Platform (Illumina). An overall high coverage depth (>100X) was generated and >90% of all amplicons were covered at least by 20X.

For the medical evaluation, all disease causing variants reported in HGMD®, in ClinVar or in CentoMD® as well as all variants with minor allele frequency (MAF) of less than 1% in ExAc database were considered. Variants that possibly impair the protein sequence, i.e. disruption of conserved splice sites, missense, nonsense, read-throughs, or small insertions/deletions, were prioritized. All relevant inheritance patterns are considered.

The clinically relevant variant identified by NGS was validated by Sanger sequencing in the patient and his parents to confirm if it is a true positive. Therefore, the *SOX18* gene was analyzed by PCR and sequencing of both DNA strands of the entire coding region and the highly conserved exon/intron splice junctions. SOX18 NM_018419.2 was used as a reference.

2.2. Bioinformatic analysis

The functional consequences of the variant were obtained using SIFT Indel [9], which is available at http://sift-dna.org/www/SIFT_indels2.html. The latter algorithm predicts the effects of indels at 84% accuracy, it is an extension of the SIFT (Sorting Intolerant From Tolerant) algorithm, which predicts the effect of amino acid substitutions [10].

3. Results and discussion

The male patient was born at term via normal vaginal delivery. Initial antenatal ultrasound scan showed ascites with mild pericardial effusion, and chylothorax. After medically intervening to resolve the latter symptoms, ultrasound showed no ascites, pericardial/pleural effusion or bilateral hydrocele. Normal results were obtained regarding the umbilical artery and Middle Cerebral Arterial Doppler. Amniocentesis had been performed and the karyotype was normal. Additionally, extensive investigations were done, including virological and Kleihauer–Betke tests, only with negative results. Postnatal examination revealed no edema, or ascites, and the child was hemodynamically stable.

At two days of age, the child was referred to a dermatologist due to his alopecia and abnormal skin color, the initial diagnosis was xerosis cutis, and cutis marmorata.

His developmental history was normal. At 11-months of age, he was able to stand without support, crawl, hold and reach towards objects, demonstrate a pincer grasp, babble, respond to his name, demonstrate stranger anxiety, and laugh loudly. On examination, he was an alert and active boy. The following vital signs were reported; temperature: 36.2 °C, heart rate: 128/min, respiratory rate: 30/min and blood pressure: 125/67 mmHg. As for growth parameters, height: 78 cm (50th–75th centile), head circumference: 44.5 cm (just below 3rd centile), and weight: 10.8 kg (50th centile). He had distinct craniofacial features, including microcephaly, periorbital swelling, red thick everted lips, as well as absence of eyebrows and eyelashes (Fig. 1). Teeth appeared normal, and so were the results of cardiovascular, chest, abdomen, genitalia, and central nervous system examinations, except for a small left reducible inguinal hernia. Skin exam showed alopecia totalis, smooth skin, scar on the vertex, multiple hemangiomas of different sizes; on the scalp, nape, over the left eyelid, on the back and on the scrotal skin. Toe nails of the right foot were hypoplastic (Fig. 1, B). Additionally, acrocyanosis was noted but later it resolved spontaneously without treatment. The child had normal ophthalmological and auditory test results and his skeletal, cerebral and renal ultrasound screens were normal.

The parents were non-consanguineous and healthy. There was no known family history of hydrops or of any dermatological condition. One of the patient's paternal uncles died of a febrile illness at 2-years of age, while two of his paternal cousins had Down syndrome.

To uncover the underlying molecular cause of the phenotype displayed by the patient, Whole Exome Sequencing was performed for all available family members (index patient and parents). Consequently, a heterozygous 14bp-duplication was detected in the SRY (sex determining region Y)-box 18 (*SOX18*) gene. Upon checking the status of *SOX18* in the parents, it was concluded that the mutation appeared *de novo* in the child (Fig. 2, upper panel), as neither of parents harbored a mutated *SOX18*. This duplication was deemed to be “damaging” because it affects exon 2 of *SOX18* (c.492_505dup), which creates a premature stop codon as predicted by the SIFT Indel algorithm. For the latter, we used the query phrase: “20, 62680168, 62680168,-1,GCCCGGCGGCTGGA”, which contained the chromosomal coordinates of the indel, as per the genome assembly GRCh37. The predicted outcome indicated that the resulting SOX18 matches the wild-type protein in its N-

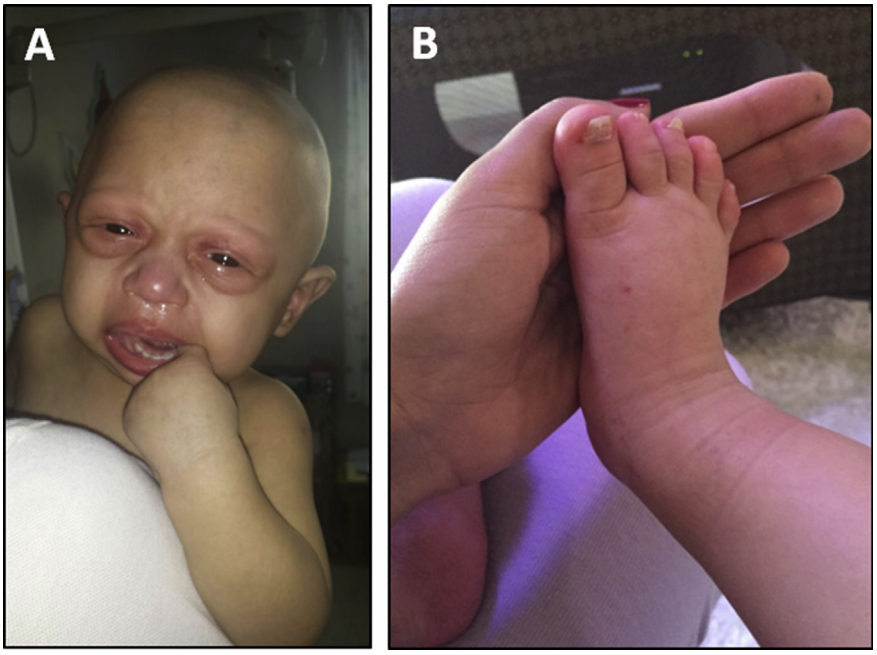

Fig. 1. The facial features of the Jordanian patient with clear periorbital edema, hemangioma in the left eyelid as well as alopecia totalis (A). The foot with hypoplastic toenails is shown in (B).

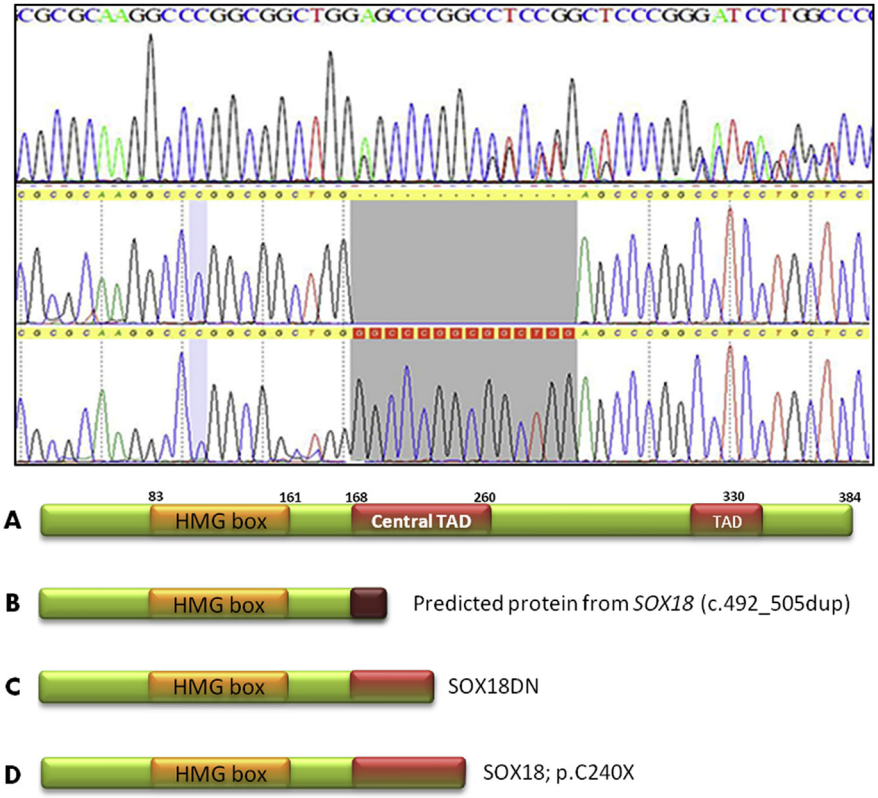

Fig. 2. Upper panel; sequence chromatograms from the patient, showing the heterozygous insertion with the typical electropherogram pattern. Below, split sequences providing the inserted nucleotides in the variant allele (SeqPilot, JSI)., Lower panel; schematic representations of the SOX18 protein with; A) wild type SOX18 with its three key domains, B) the protein resulting from SOX18 (c.492_505dup) as predicted the SIFT Indel algorithm with the aberrant terminal amino acids depicted in a darker shade, C) the dominant negative construct (SOX18DN) by Milivojevic et al. [11] and D) the truncated protein resulting from the mutation (p.C240X).

terminus till the residue 168 at the start of the central transactivation domain, then a 13-aa mismatch follows, after that it ends with a premature stop (Fig. 2, B). This mutation is not described in the Exome Aggregation Consortium, Exome

Sequencing Project, 1000 Genomes Browser or in Centogene's mutation/variation database (CentoMD®).

By linking the clinical combination of total alopecia, multiple hemangiomas and lymphedema to the heterozygous mutation in *SOX18*, it is possible to attribute the causality of HLTS in our patient to the above-mentioned novel *SOX18* variant. Analyzing the consequences of the duplication using SIFT Indel indicates that the frameshift leads to a prematurely truncated protein that is not preempted by Nonsense Mediated Decay. The resulting protein clearly resembles a dominant negative construct of human SOX18 protein that was expressed and analyzed functionally by Milivojevic et al. [11]. This dominant negative construct (Fig. 2, C), named SOX18DN encodes a truncated SOX18 protein that lacks part of the central trans-activation domain along and the entire second (C-terminal) trans-activation domain. Using *in vitro* assays, it was possible to demonstrate the dominant negative characteristics of SOX18DN, as compared to the potent trans-activation effects of the wild-type protein. Previous reports were published about a dominant negative mutation in *SOX18* in HLTS patients, who were heterozygous for this mutation (p.C240X) that resulted in SOX18 lacking its C-terminal trans-activation domain [1,5] (Fig. 2, D). Many studies in model organisms provided evidence of functional redundancy between members of SOX-F in the context of postnatal angiogenesis. SOX18 along with other members of the SOX-F group, namely; SOX7 and SOX17, seem to have overlapping functions in regulating vascularization and postnatal angiogenesis. It is due to this redundancy that null mutations in *SOX18* can be compensated for by other SOX-F members [12]. However, this redundancy may be overcome by dominant negative *SOX18* when present in a heterozygous state.

Although lymphedema, telangiectasia and alopecia remain the main features of HLTS, recent reports have shown additional clinical abnormalities in patients (Table 1), particularly so in the case of mutations that result in truncated SOX18 proteins. The dominant negative mutation described above has been shown to be linked to renal failure, while the other known termination mutation was found to be associated with cardiac defects. In this case, the patient showed no aortic dilatation and his renal function appeared to be normal (creatinine: 0.2 mg/dL). Although our patient showed normal renal parameters, we are aware that in both the cases mentioned above, the renal phenotype developed only later in childhood. In conclusion, this study reveals a novel dominant negative *SOX18* mutation linked to HLTS, which has a very limited number of reported causal molecular lesions worldwide, hence the high significance of this report.

Conflict of interest

Authors have no conflict of interest to declare.

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