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Brief communication

Identification of a novel homozygous frameshift mutation in *SLC29A3* gene in a case with H syndrome from Iran

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ABSTRACT

H syndrome is a rare monogenic autosomal recessive disease with characteristic cutaneous findings and multisystem involvement. The aim of this study is to present an Iranian patient with H syndrome and to describe a novel frameshift mutation in *SLC29A3* gene. The patient was diagnosed with a few small areas of hyperpigmentation and accompanying hypertrichosis in the lumbar area of her back. Her clinical phenotypes included short stature, hepatosplenomegaly, facial widespread bilateral telangiectatic lesions, bilateral hypertrophy of the parotid gland, upper extremity flexion contracture, elevated inflammatory markers (ESR, CRP) and diabetes mellitus. The identification of a novel homozygous frameshift mutation (c.307_308delTT, p.F103Ter) in *SLC29A3 gene*, together with the characteristic clinical manifestations of H syndrome, provided accurate diagnosis for this patient.

1. Introduction

The H syndrome (OMIM #612,391) is an autosomal-recessive disorder, characterized by a wide range of clinical features including hyperpigmentation and hypertrichosis, hepatosplenomegaly, heart anomalies, hearing loss, hypogonadism, low height, and hyperglycemia [1,2]. This syndrome is caused by mutations (homozygous or compound heterozygous) in *SLC29A3* (Solute Carrier Family 29 Member 3) gene, located on chromosome 10q23 [3]. The wild type product of *SLC29A3* gene functions in nucleoside transport, a vital task for cells that are unable to produce nucleosides *de novo* [3]. Up to now, around 100 cases of H syndrome have been reported worldwide, with the majority of cases observed in the patients of Arab descent [4–6].

Here we report the first Iranian patient of non-Arab descent with H syndrome, to our best knowledge, in a family with non-consanguinity. In addition, we describe a novel homozygous frameshift mutation in *SLC29A3* gene [Fig. 1A].

2. Case report

In this study, we report a patient with clinical features compatible with H syndrome, born to a non-consanguineous family in Iran, with no family history of similar findings. The study was approved by the Research and Ethics Board of Qazvin Medical University and appropriate Informed consent was obtained from parents.

2.1. Clinical features

The proband is a 5-year old girl who is the first child of a non-consanguineous family. At about 6 months of age, a few small areas of

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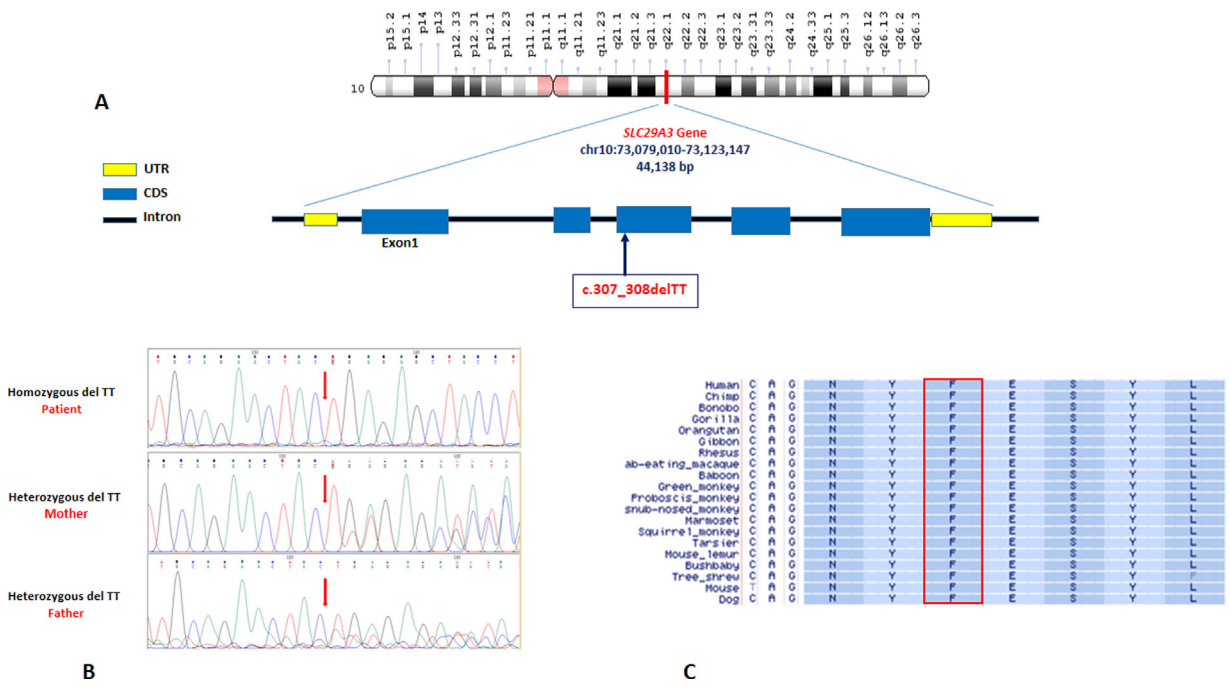

Fig. 1. (A) SLC29A3 gene structure and mutation position. (B) Electropherograms from Sanger confirmation in family members showing NM_001174098 (SLC29A3): c.307_308delTT; p.F103Ter. Heterozygous and homozygous sequence. (C) ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2) alignment/comparison of SLC29A3 across vertebrate species showing conservation at p.F103 (red rectangle) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

hyperpigmentation accompanied with hypertrichosis [Fig. 2A] in the lumbar area of her back were noted. These patches grew in size and number along with skin sclerosis, spreading to her anterior thighs as she grew older. After being off treatment for 4 years, she was referred to our center – Pediatric Hospital for the Sick Children, an academic center affiliated to Qazvin University of Medical Sciences in Qazvin, Iran – with recurrent febrile episodes. She was seen by a pediatric oncologist for potential hepatosplenomegaly, but her bone marrow biopsy provided normal results. She was then referred to a pediatric immunologist for immunological examinations. All immunoglobulin levels were normal except for significant increased IgG and IgA levels. Lastly she was seen by a dermatologist to have a skin biopsy taken from her skin lesions. Histopathological findings from the skin biopsy revealed perivascular infiltration of lymphocytes and mast cells with oval to spindle nuclei and granular cytoplasm, consistent with mastocytosis. Immunohistochemical staining showed diffuse staining of cells for CD117(C-Kit). At 4.5 years of age, she was referred to a pediatric gastroenterologist. Additional significant findings on physical examination included growth retardation, short stature, frontal bossing, prominent scalp veins, exophthalmos, dilated lateral scleral vessels, upper extremity flexion contracture, hallux valgus, and non-pitting edema in extremities. There were no midline facial defects. It is of interest that our case presents unique clinical features: facial widespread bilateral telangiectatic lesions as well as characteristic bilateral hypertrophy of the parotid gland [Fig. 2B]. To our knowledge, these features have not been previously reported in patients with H syndrome. There were no stigmata of Turners syndrome, as she demonstrated a normal neurological development. Visual acuity and optic fundus examination were normal. The patient exhibited normal hearing functions and the audiogram results confirmed normal bilateral sensorineural hearing. However, her parents started noticing that she was not responding properly as before. Additional diagnostic audiometry test revealed that she had developed bilateral hearing impairment, a common clinical finding in about half of the patients

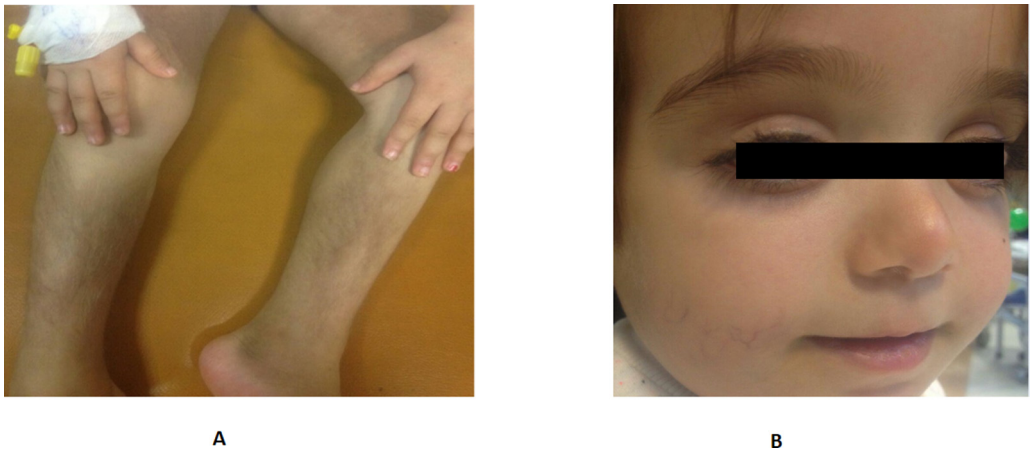

Fig. 2. (A) facial widespread bilateral telangiectatic lesions as well as characteristic bilateral hypertrophy of the parotid gland. (B) Hypertrichosis.

with H syndrome. Her height was below the third percentile for age and sex and the bone age was 3 years. Her blood pressure was normal with no radio femoral delay, and she had a normal echocardiography Computed Tomography (CT) with intravenous contrast of the abdomen and pelvis showed duplicated inferior vena cava. The patient had normal thyroid function test outcomes, and Follicle stimulating hormone and luteinizing hormone levels were also normal. She has been under insulin treatment for IDDM (insulin-dependent diabetes mellitus) and her diabetes has been under control. Magnetic resonance imaging of the brain was normal. Important laboratory findings included elevated total protein with diminished serum albumin, normal transaminases levels, mild microcytic anemia and elevation of erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) levels (85 mm /hour, [normal, <20 mm/hour] and 62 mg/dl [normal,<10 mg/dl], respectively). In addition, no immunologic abnormalities or immune-mediated manifestations were observed in this patient. Her parents did not have any of the above mentioned features.

The patient received systemic prednisolone (1 mg/kg twice daily) for 2 weeks. ESR and CRP decreased and recurrent febrile episodes stopped. Prednisolone was tapered over 2 months to 0.25 mg/kg/day and continued for 5 months. Symptoms and inflammatory markers remained improved on this low dose of prednisolone. Routine HbA1C test has been performed to control her diabetes which is usually within the normal range (below 6%). Subsequently, her height increased from below the 3rd percentile to the 3rd percentile by the age of 5-years. She has not required hospitalization for febrile episodes.

2.2. Genetic screening

DNA was extracted from peripheral blood leukocytes using a commercial kit (High Pure PCR Template Preparation, Roche). All *SLC29A3* exons and splice junctions were PCR-amplified using intronic primers. PCR products were purified and sequenced on ABI 3700 capillary sequencer (Applied Biosystems, Foster City, CA).

In this patient, a novel homozygous frameshift mutation (c.307_308delTT) in exon 3 of *SLC29A3* gene was found, changing phenylalanine 103 to stop codon (p.F103Ter). Segregation analysis revealed that both parents showed heterozygosity at the mutation position [Fig. 1B]. Detailed computational analysis of p.F103Ter mutation using prediction methods (PolyPhen-2, SIFT, MutationTaster) revealed it as disease-causing. The local NGS database, currently consisting of 1406 whole exome sequencing (WES) data from healthy controls, was investigated and no samples exhibited the identified novel mutation in the *SLC29A3* gene. Although the majority of reported mutations were found in the sixth exon of the *SLC29A3* gene, the novel mutation described in this report, reside in the third exon – a highly evolutionarily conserved position [Fig. 1C] related to the third transmembranous domain of the nucleoside transporter.

3. Discussion

Here, we describe an Iranian patient from non-Arab descent with H syndrome and report a homozygous alteration in the *SLC29A3* gene. This frameshift mutation in exon3 (c.307_308delTT, p.F103Ter), is changing Phenylalanine to the stop codon, resulting in a premature termination and a truncated protein. This variation – which has not been reported before – is predicted to be a disease-causing mutation based on computational analysis. Local database survey in a relatively large cohort of healthy controls (1406 individuals) revealed that none of the healthy individuals exhibited the identified novel mutation in the *SLC29A3* gene.

It has been relatively recently reported that mutations in *SLC29A3* gene are implicated in H syndrome along with two other diseases: pigmented hypertrichosis with insulin-dependent diabetes mellitus syndrome (PHID) and Faisalabad histiocytosis (FHC). H syndrome and PHID both exhibit the dermatological features of skin lesions [7]. Overlapping clinical features of these syndromes has led to the notion that these clinical manifestations should be grouped together as "SLC29A3 disorders" [8]. However, clinodactyly and deafness are two distinctive characteristics of H syndrome which are absent in PHID [8,9]. It is of interest that our case have unique clinical features: facial widespread bilateral telangiectatic lesions as well as characteristic bilateral hypertrophy of the parotid gland. To our knowledge, these features have not been previously reported in patients with H syndrome. Flexion contracture of the fingers was also evident in this patient. Sensorineural hearing loss is a common clinical finding in about half of the patients with H syndrome, and this patient has recently acquired bilateral hearing impairment.

There is no data assessing the correlations between the type of mutation or its position with the severity and spectrum of the clinical phenotype. As mentioned above, the frameshift mutation (c.307_308delTT, p.F103Ter) in *SLC29A3* gene has never been reported in H syndrome patients. Interestingly, while the majority of 20 different mutations that have been previously identified in the SLC29A3 gene are located in exon 6 [10], the mutation we discovered resides in exon 3. Furthermore, almost all the patients with H syndrome reported until now are teenagers or older, while the patient in our study is 5 years old, presumably suggesting a phenotype-genotype correlation in H syndrome and a possible explanation for the distinct clinical features observed in our patient.

Up to now, around 100 cases with H syndrome have been reported worldwide, with the majority being of Arab descent. Recently, a study reported a 17-year old Iranian patient with hyperpigmented, hypertrichosis and indurated seborrheic keratosis-like cutaneous patches and microphallus. Although the patient is Iranian, he is of Arab descent and the case in our study has Persian ancestry [11].

To sum up, this report described a novel case of H syndrome with unique clinical features caused by a novel homozygous frameshift mutation for the c.307_308delTT in *SLC29A3* gene. Additional reports from a large cohort of cases with SLC29A3 mutations are required to clarify certain phenotype–genotype correlation in SLC29A3-related disorders.

Disclosure of interest

The authors report no conflicts of interest.

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