Current Research in Translational Medicine 64 (2016) 65–68

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Short communication

Compound heterozygous *SLC29A3* mutation causes H syndrome in a Moroccan patient: A case report

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ARTICLE INFO

Article history:
Received 30 July 2015
Accepted 19 January 2016
Available online 1 June 2016

Keywords:
H syndrome
SLC29A3
Mutation
Direct sequencing
Morocco

ABSTRACT

H syndrome is an autosomal recessive syndrome, which affects the skin and some vital organs, it is caused by mutations in the *SLC29A3* gene, encoding the human equilibrative nucleoside transporter hENT3. This report describes a patient with typical features of H syndrome. Based on the patient's clinical features, *SLC29A3* was selected for molecular investigation. Through direct sequencing, a compound heterozygous alteration in the *SLC29A3* gene was found. The c.243delA frameshift mutation leading to a premature termination, resulting in a truncated protein, and a splice site mutation c.300+1G>C predicted to cause a splicing error. This contribution extends the clinical variability of compound heterozygous *SLC29A3* mutations resulting in an additional multisystemic manifestation of the clinical spectrum of *SLC29A3* disorders.

1. Introduction

H syndrome (OMIM: 612391) is an autosomal recessive genetic disorder characterized by progressive cutaneous hyperpigmentation, skin sclerosis, hepatosplenomegaly, hypertrichosis, hypogonadism, heart anomalies, low weight due to a short stature and sensorineural hearing loss [1]. This genodermatosis is caused by mutations in the *SLC29A3* gene encoding for the human equilibrative nucleoside transporter 3 (hENT3), a member of the equilibrative nucleoside transporter (ENT) family [2,3] that is proposed to act as a nucleoside transporter. hENT3 has been initially localized in the lysosomes [4], but recently it has been shown to be highly expressed in mitochondria [5].

Reports have shown that along with H syndrome, three others diseases are caused by recessively mutations in the *SLC29A3* gene: pigmented hypertrichosis with insulin-dependent diabetes mellitus syndrome (PHID), familial Rosai-Dorfman disease (RDD) and Faisalabad histiocytosis (FHC). This points out that these four syndromes can be considered as one disease with different phenotypic characteristics [6]. Recently, the term histiocytosis–lymphadenopathy plus syndrome [OMIM: 602782] was suggested to group these four disorders. In this study, a patient who had features compatible with H syndrome was reported, we presumed that a mutation in *SLC29A3* gene can cause the disease in this family, why this gene was selected to be analyzed.

2. Case report

In this report, we identified a non-consanguineous Moroccan family with H syndrome (Fig. 1A). This family has one affected individual, no history of similar problems was observed in his brother. This study was approved by the committee on research ethics of Pasteur Institute of Morocco. We acquired written informed consents from the family before including them in this study.

2.1. Clinical features

A 13-year-old female presented with history of symmetrical and bilateral hyperpigmentation, she was short for her age and had

Abbreviations: *SLC29A3*, solute carrier family 29 (nucleoside transporter), member 3; hENT3, human equilibrative nucleoside transporter 3; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; PHID, pigmented hypertrichosis and insulin-dependent diabetes mellitus; FHC, Faisalabad histiocytosis; RDD, Rosai-Dorfman disease.

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http://dx.doi.org/10.1016/j.retram.2016.01.008
2452-3186/

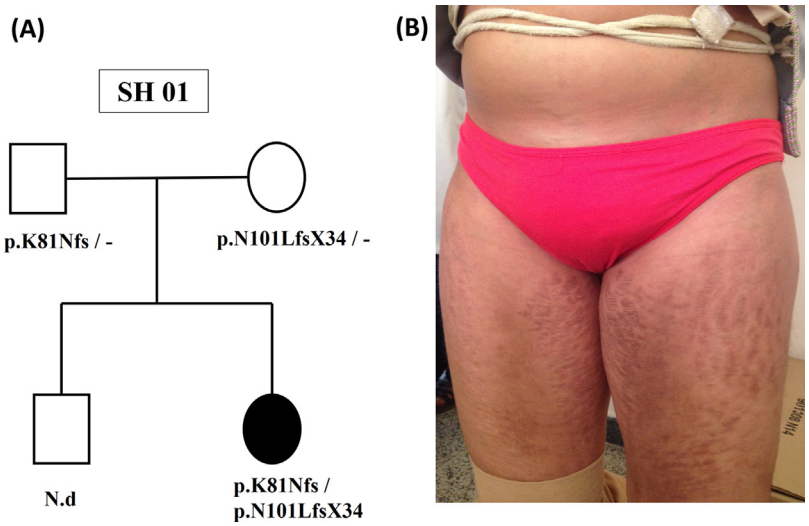

Fig. 1. A. Pedigree of the family presenting the segregation of the mutations., affected patient marked by black circle. B. Clinical manifestations of the patient, demonstrating extensive hyperpigmentation of the thighs and varicose veins.

a medical history of progressive hearing loss that she developed since the age of 4 years. At the age of 10 years, she had been diagnosed for skin sclerosis. Physical examination showed hard subcutaneous masses in the legs. She was treated for morphea, also known as localized scleroderma, and orally prednisolone was prescribed with a good evolution. Histology showed a perivascular inflammatory infiltrate composed of histiocytes and lymphocytes with thickened collagen bundles in hypodermis. Then, the patient started developing hyperpigmented patches over the thighs that later on progressed and reach the genital areas, she developed also varicose veins in the left inguinal area (Fig. 1B). At 12 years old, the patient had also renal problems, including urethral dilation and distension of calyces without individualized obstacle, later on, the patient underwent a left nephrectomy. She had a normal neurological development, her glucose level was normal. On examination, her face and limbs showed no abnormalities. The abdominal echography was normal. She did not have hepatosplenomegaly or heart anomalies. She was treated with colchicine and local corticotherapy.

2.2. Genetic screening

Using the phenol chloroform method, Genomic DNA was extracted from the blood from affected patient and from her parents [6]. Mutation screening was performed using direct DNA sequence analysis. All the 6 exons and exon-intron junctions of the *SLC29A3* gene were amplified using PCR (polymerase chain reaction). Direct sequencing of PCR products was performed with the ABI prism Big Dye Terminator cycle sequencing Ready Reaction kit V 3.1 (ABI Prism/Apllied Biosystems, Foster City, CA) and analyzed on an ABI Prism 3100 Genetic Analyser (Applied Biosystem).

Two heterozygous mutations were identified in exon 2 and intron 2, respectively (Fig. 2A). The c.243delA mutation leading to a premature termination, resulting in a truncated protein, and the splice site mutation c.300+1G>C predicted to cause a splicing error. The affected girl was compound heterozygous for these mutations. These two mutations co-segregated with the disease phenotype in s family using Sanger sequencing as previously described (Fig. 2B).

Four missense polymorphisms were identified in *SLC29A3* gene: p.Arg18Gly (rs2277257), p.Ser158Phe (rs780668), p.Val239Ile (rs2252996), and p.Ile326Val (rs2487068), located in exons 2, 4, 5 and 6, respectively. In addition, two synonymous variants were also found; p.Thr238 = (rs2252997) in exon 5 and p.Gly336 = (rs1084004) in exon 6. All these polymorphisms were found at the homozygous state except the p.Arg18Gly variant.

3. Discussion

In this report, we described a Moroccan family with H syndrome and found a compound heterozygous alteration in the *SLC29A3* gene (Fig. 1A). The splice site mutation c.300+1G>C (p.N101LfsX34), is predicted to abolish the splice donor site of exon 2, leading to a premature termination, resulting in a truncated protein. This mutation was previously reported in a Moroccan girl [7] and in two Egyptian siblings [8] with overlapping features of pigmented hypertrichotic dermatosis with insulin-dependent diabetes, Faisalabad histiocytosis and H syndrome. It's important to note that these three patients presented the most extreme clinical features identified to date in patients with *SLC29A3* mutations such as hyperpigmentation, insulin-dependent diabetes, cardiac anomalies, hearing loss, arthrogryposis and anaemia with erythroblastopenia. The idea supporting that this severe phenotype is correlated with the extreme N-terminal position of the mutation [7] cannot be controverted, since in this work the c.300+1G>C mutation was found at the heterozygous state, which may explained the fact that the affected patient presented features specific only to H syndrome. At the same position of the gene, the c.300+1G>A mutation was described in patients with Faisalabad histiocytosis [9,10], but this mutation has not been identified in the PHID or H syndromes. In addition, to the c.300+1G>C mutation, the affected girl in this family was also compound heterozygous for the c.243delA (p.K81Nfs). This frameshift deletion was reported for the first time in two Moroccan siblings at the homozygous state and responsible for a mild form of *SLC29A3* disorder [11]. This hypomorphic mutation decreased the level of the normally coding transcripts via nonsense-mediated mRNA decay (NMD), it instead allows the expression and function of a noncoding mRNA splice variant, result in a truncated protein that seems to show some functional activity which may explain the mild phenotype observed in the patients [11]. Interestingly, another male patient of Moroccan origin bearing the same mutation (c.243delA) presented features specific to H syndrome [12] as in the present study. This again confirmed that patients carrying the same mutation in *SLC29A3* gene may display