Original Article

Identification of a Novel Mutation in Solute Carrier Family 29, Member 3 in a Chinese Patient with H Syndrome

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Abstract

Background: H syndrome (OMIM 612391) is a recently described autosomal recessive genodermatosis characterized by indurated hyperpigmented and hypertrichotic skin, as well as other systemic manifestations. Most of the cases occurred in the Middle East areas or nearby countries such as Spain or India. The syndrome is caused by mutations in solute carrier family 29, member 3 (*SLC29A3*), the gene encoding equilibrative nucleoside transporter 3. The aim of this study was to identify pathogenic *SLC29A3* mutations in a Chinese patient clinically diagnosed with H syndrome.

Methods: Peripheral blood samples were collected from the patient and his parents. Genomic DNA was isolated by the standard method. All six *SLC29A3* exons and their flanking intronic sequences were polymerase chain reaction (PCR)-amplified and the PCR products were subjected to direct sequencing.

Results: The patient, an 18-year-old man born to a nonconsanguineous Chinese couple, had more extensive cutaneous lesions, involving both buttocks and knee. In his genomic DNA, we identified a novel homozygous insertion-deletion, c. 1269_1270delinsA, in *SLC29A3*. Both of his parents were carriers of the mutation.

Conclusions: We have identified a pathogenic mutation in a Chinese patient with H syndrome.

Key words: China; H syndrome; Novel Mutation; The Solute Carrier Family 29, Member 3 Gene

Introduction

H syndrome (OMIM 612391) is an autosomal recessive genodermatosis characterized by progressive cutaneous hyperpigmentation, hypertrichosis, hepatosplenomegaly, heart anomalies, hypogonadism, short stature, hearing loss, hallux valgus, camptodactyly and occasionally insulin-dependent diabetes mellitus (IDDM).[1] The genetic analysis of patients with H syndrome revealed that homozygous mutations in the solute carrier family 29, member 3 (*SLC29A3*) gene on chromosome 10q22.1 are responsible for the phenotype.[2] *SLC29A3* encodes the human equilibrative nucleoside transporter 3 (hENT3), which is highly conserved and contains 11 transmembrane domains (TMDs).[3] The disease was first reported by Molho-Pessach *et al*. in 2008.[1] So far, 85 patients have been reported in the literature with the clinical phenotypes characteristic of this syndrome and a total of 20 mutations have been identified in *SLC29A3*.[4-7] The majority of patients are of Arab origin. There are also patients who from Spain, India and Japan. Here, we report on the clinical and molecular data of a new patient from China.

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Methods

Peripheral blood samples from the family members and 100 population-matched unrelated healthy control individuals were collected following informed consent and Institute Ethics Committee approval. Genomic DNA was isolated from peripheral blood leucocytes according to standard techniques.

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All 6 exons of *SLC29A3* were amplified by polymerase chain reaction (PCR) from the genomic DNA using Premix LA Taq (Takara Biotechnology Co., Dalian, China) and previously reported primers.[2] The amplified PCR products were directly sequenced on an ABI Prism 3730×l Automated Sequencer, using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). For restriction analysis, a 269-bp fragment containing exon 6 of the human *SLC29A3* gene was PCR-amplified using the forward primer 5'-CAAGGGTTCGGGCTCACTG-3' and the reverse primer 5'-TCTGCTCTCTGTCCCCAAGT-3'. The PCR products were subsequently digested with the BanII restriction enzyme (Takara Biotechnology Co., Dalian, China) and analyzed on agarose gel.

Results

Clinical features

We have reported the a Chinese patient with a clinical diagnosis of H syndrome.[8] The patient was an 18-year-old male born to a nonconsanguineous Chinese couple. The clinical diagnosis was made according to his typical clinical phenotype, laboratory test abnormalities, and histological changing.

The cutaneous lesion presented as symmetrical brown patches with tenderness, sclerodermoid induration and hypertrichosis on his thighs [Figure 1a], knee [Figure 1b], buttocks [Figure 1c], back and lower abdomen. After puberty, he developed dysuria, gynecomastia, no spermatorrhea and erectile dysfunction. At the age of 18 years, he was 145 cm tall. He also had snaggletooth, enlarged inguinal lymph nodes, hearing loss, hepatosplenomegaly, heart anomaly, flat feet, bilateral fixed hammertoe deformity, and bilateral camptodactyly of the proximal interphalangeal joints of his second and third toes. His intelligence was of the average level.

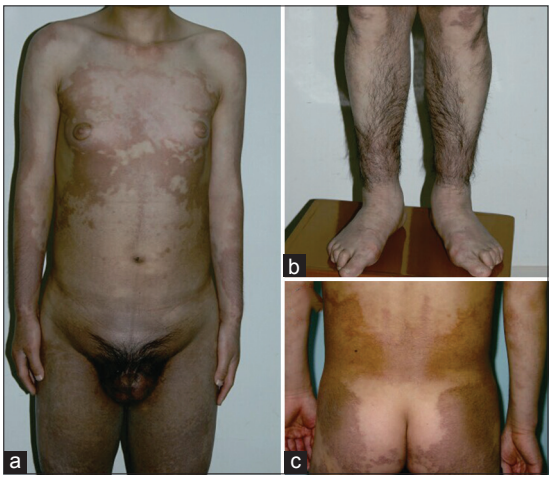

Figure 1: Clinical presentation in H syndrome patient. (a) Extensive hyperpigmentation and hypertrichosis on his trunk, extremities; subcutaneous firm masses in the scrotal sac, obscuring the penis; prominent gynecomastia; (b and c) Indurated, hyperpigmented and hypertrichotic skin on the buttock and knee.

The patient had abnormal inflammatory indicators and abnormal hormonal levels: Elevated erythrocyte sedimentation rate (42 mm/h), C-reactive protein (34.6 mg/L) and CH50 (68.1 U/ml); significantly decreased level of testosterone (5.1 nmol/L) and elevated level of estradiol (270.56 pmol/L).

Histopathology also showed a typical changing of H syndrome, such as widespread fibrosis and mononuclear infiltration. We did not find Psammoma body in the patient.

Prednisone and short-acting androgen partially relieve the symptoms. After a follow-up of 5 years, the lesion became more severe than his first visit.

Identification of a novel mutation in solute carrier family 29, member 3

Sequence analysis of all exons of *SLC29A3* in patient revealed a homozygous insertion-deletion (indel), c. 1269_1270delinsA, producing a frameshift and a premature termination codon (*p.Leu423Serfs*28*). This indel was predicted to disrupt the 10th and delete the 11th TMD of the *SLC29A3* protein. The father and mother were found to be heterozygous carriers of the indel [Figure 2a]. The indel is not reported in the public dbSNP (http://www.ncbi.nlm.nih.gov/snp/) and was not detected in chromosomes from 100 ethnically matched control individuals, suggesting that c. 1269_1270delinsA (*p.Leu423Serfs*28*) in *SLC29A3* is the pathogenic mutation underlying H syndrome in the Chinese patient.

Discussion

The skin lesion of H syndrome usually begins on lower limbs and generally extends to the whole body; however, knees and buttock were reported to be spared, which is considered to be a distinguishing feature of this disease.[9] In our case, patient has the typical clinical and histological features. The diagnosis for H syndrome is made with certainty. The result of gene analysis further affirmed our diagnosis. However, it is noteworthy that in our case the cutaneous lesion is more extended, involving the areas such as buttocks and knees that were previously considered to be uninvolved.

H syndrome is caused by mutations in the *SLC29A3* gene, which encodes the hENT3. hENT3 is a 475 amino acid protein with 11 TMDs. hENT3 belongs to a group of SLC transporters that are widely conserved in eukaryotes, the ENT or SLC29 family.[10] The four members of the ENT mediate passive sodium-independent transport of nucleosides and display a broad tissue distribution such as brain compartments, spinal cord, eyes, lungs, kidneys, placenta, pancreas, stomach, and liver.[11] Nucleoside transporters are essential for nucleotide synthesis by salvage pathways in cells that lack de novo synthetic pathways, such as histiocytes and monocytes.[10] hENT3 is also expressed in the endothelium of blood and lymphatic vessels in normal human skin, as well as histiocytes that

reside in the dermal sheath around the hair follicle.[12] Disorder mutations could impair nucleoside transport, protein localization, and stability of hENT3.[11] *SLC29A3* encodes a nucleoside transporter localized in lysosomes and is highly expressed in some white blood cells such as histiocytes and macrophages. Mice deficient in *SLC29A3* have significant lysosomal dysfunction in macrophages.[13] It is possible that mutations in the *SLC29A3* gene may induce an abnormal proliferation of histiocytes and thus lead to the immune response, resulting in skin sclerosis and hypertrichosis.[11] Germline mutations in *SLC29A3* have been reported in rare patients with a wide range of overlapping clinical features and inherited disorders including H syndrome, pigmented hypertrichosis with insulin-dependent diabetes (PHID), Faisalabad histiocytosis, sinus histiocytosis with massive lymphadenopathy, dysosteosclerosis (DSS) and monogenic autoinflammatory syndrome.[14] H syndrome is characterized by skin hyperpigmentation and hypertrichosis, hepatosplenomegaly, heart anomalies, hearing loss, hypogonadism and low height. PHID is mainly characterized by presence of IDDM and lack of sensorineural hearing loss.[15] Faisalabad histiocytosis presents with massive but painless lymphadenopathy. Lymph node histology reminiscent of sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease), but the presence of short stature, joint contractures, and sensorineural deafness distinguishes this disorder.[16] H syndrome is reported to have emperipolesis with sinus histiocytosis with massive lymphadenopathy.[17] DSS is the form of osteopetrosis distinguished by the presence of skin findings such as red-violet macular atrophy, platyspondyly and metaphyseal osteosclerosis with relative radiolucency of widened diaphyses.[5] The first four disorders were suggested to be grouped under the term histiocytosis–lymphadenopathy plus syndrome (OMIM #602782). There is some clinical overlap between PHID and H syndrome, Faisalabad histiocytosis and sinus histiocytosis with massive lymphadenopathy, and in some families both conditions manifest.[10,18] In addition, mildly affected individuals have also been described,[19] as well as a severe case presenting strikingly homologous with several syndromes.[20] Twenty mutations have been identified so far in the *SLC29A3* gene in affected individuals [Figure 2b]. However, the reasons for the pleiotropism and variability of *SLC29A3*-related diseases are not known. This indel (c. 1269_1270delinsA) changed leucine 423 to serine and was predicted to result in a frameshift and the generation of a stop codon at residue 451 of the protein, which will disrupt the 10th and delete 11th TMD of *SLC29A3*, leads to the dysfunction of the hENT3 and presents a series of clinical disorders. This mutation also affects the glycine at position 427, which is critical for nucleoside transport activity.[12] This may explain the relatively extended cutaneous lesion in our case.

In conclusion, the identification of the homozygous pathogenic indel confirmed the clinical diagnosis of H syndrome in our study. This is a Chinese mutation with H syndrome, extends the known geographical mutation distribution of the disease and expands the spectrum of *SLC29A3* mutations. The remarkable buttock and knee involvement of the patient add new features to the clinical spectrum. Knowledge of this disease is very limited, and precise pathogenesis of H syndrome has remained largely unknown. Future studies exploring the function of hENT3 will help in elucidating the pathophysiological basis for this disorder.

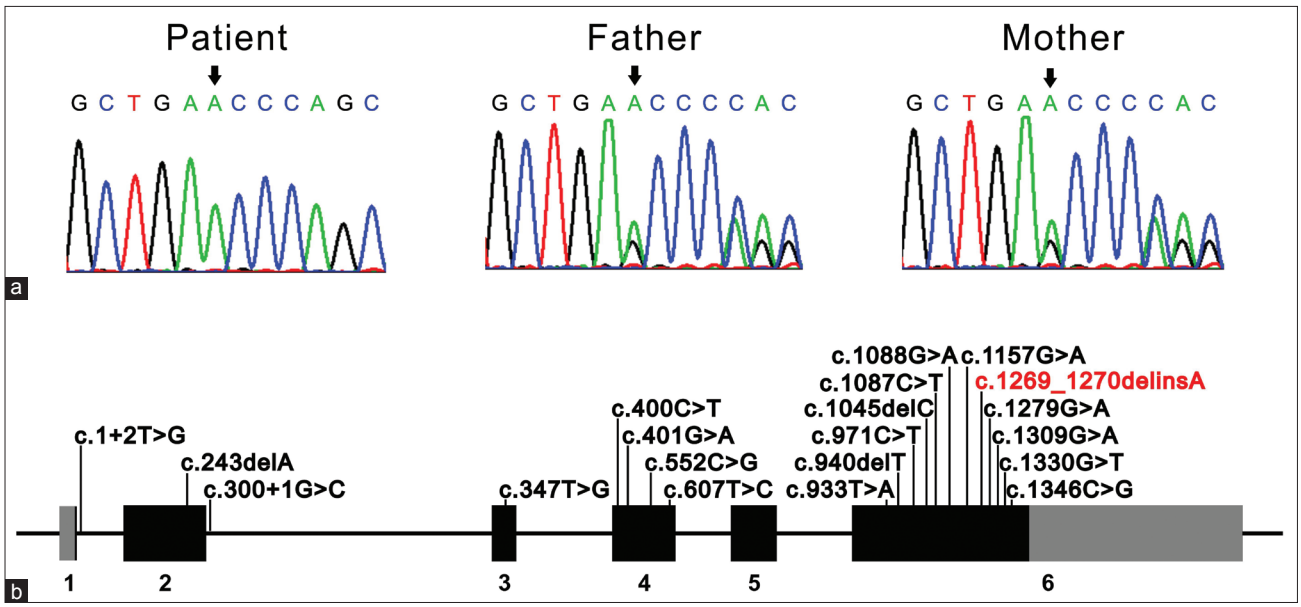

Figure 2: Identification of a novel mutation in the solute carrier family 29, member 3 (*SLC29A3*) gene. (a) Sequence analysis in patient and his parents; (b) Diagram of *SLC29A3*. The exons are represented by closed rectangles. 5'UTR and 3'UTR are represented by gray rectangles. Introns are represented by a straight line. Mutations are marked at the corresponding locations. The mutation identified in our patient is shown in red color.

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