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

Jia-Wei Liu¹, Nuo Si², Lian-Qing Wang⁵, Ti Shen⁵, Xue-Jun Zeng⁴, Xue Zhang², Dong-Lai Ma¹

¹Department of Dermatology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China
²Department of Medical Genetics, McKusick-Zhang Center for Genetic Medicine and State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100005, China
³Department of Internal Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China

Jia-Wei Liu and Nuo Si contributed equally to this work.

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). 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. SLC29A3 encodes the human equilibrative nucleoside transporter 3 (hENT3), which is highly conserved and contains 11 transmembrane domains (TMDs). The disease was first reported by Molho-Pessach et al. in 2008. 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. The majority of patients are of Arab origin. Here, we report on the clinical and molecular data of a new patient from China.

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.

Address for correspondence: Dr. Dong-Lai Ma, Department of Dermatology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China E-Mail: mdonglai@sohu.com
All 6 exons of SLC29A3 were amplified by polymerase chain reaction (PCR) from the genomic DNA using Primex LA Taq (Takara Biotechnology Co., Dalian, China) and previously reported primers.[2] The amplified PCR products were directly sequenced on an ABI Prism 3730x1 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'-TCTGCTCTCTGTCCCCAGT-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.[3] 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 hypertrichrosis on his thighs [Figure 1a], knee [Figure 1b], buttocks [Figure 1c], back and lower abdomen. After puberty, he developed dysuria, gynecomastia, no spermatorrhoea 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.[12] 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.\[^{10}\] 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 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.\[^{14}\] H syndrome is reported to have emperipolesis with sinus histiocytosis with massive lymphadenopathy.\[^{16}\] 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.\[^{15}\] 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 10\(^{th}\) and delete 11\(^{th}\) 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.
ACKNOWLEDGMENTS
We acknowledge the family members involved in this study.

REFERENCES
1. Molho-Pessach V, Agha Z, Aamar S, Glaser B, Dovinov V, Hiller N, et al. The H syndrome: A genodermatosis characterized by indurated, hyperpigmented, and hypertrophic skin with systemic manifestations. J Am Acad Dermatol 2008;59:79-85.
2. Molho-Pessach V, Lerer I, Abellovich D, Agha Z, Abu Libdeh A, Broshilova V, et al. The H syndrome is caused by mutations in the nucleoside transporter hENT3. Am J Hum Genet 2008;83:529-34.
3. Baldwin SA, Beal PR, Yao SY, King AE, Cass CE, Young JD. The equilibrative nucleoside transporter family, SLC29. Pflugers Arch 2004;447:735-43.
4. Molho-Pessach V, Ramot Y, Camille F, Dovinov V, Babay S, Luis SJ, et al. H syndrome: The first 79 patients. J Am Acad Dermatol 2014;70:80-8.
5. Campeau PM, Lu JT, Sule G, Jiang MM, Bae Y, Madan S, et al. Whole-exome sequencing identifies mutations in the nucleoside transporter gene SLC29A3 in dysosteosclerosis, a form of osteopetrosis. Hum Mol Genet 2012;21:4904-9.
6. Al-Haggar M, Salem N, Wahba Y, Ahmad N, Jonard L, Abdel-Hady D, et al. Novel homozygous SLC29A3 mutations among two unrelated Egyptian families with spectral features of H-syndrome. Pediatr Diabetes 2014; doi: 10.1111/pedi.12160.
7. Molho-Pessach V, Mechoulam H, Siam R, Babay S, Ramot Y, Zlotogorski A. Ophthalmologic findings in H syndrome: A unique diagnostic clue. Ophthalmic Genet 2014 [Epub ahead of print].
8. Ma DL, Liu JW, Fang K. The H syndrome (in Chinese). Chin J Dermatol 2012;45:693-6.
9. Farooq M, Moustafa RM, Fujimoto A, Fujikawa H, Abbas O, Kibbi AG, et al. Identification of two novel mutations in SLC29A3 encoding an equilibrative nucleoside transporter (hENT3) in two distinct Syrian families with H syndrome: Expression studies of SLC29A3 (hENT3) in human skin. Dermatology 2012;224:277-84.
10. Morgan NV, Morris MR, Cangul H, Gleeson D, Straatman-Iwanowska A, Davies N, et al. Mutations in SLC29A3, encoding an equilibrative nucleoside transporter ENT3, cause a familial histiocytosis syndrome (Faisalabad histiocytosis) and familial Rosai-Dorfman disease. PLoS Genet 2010;6:e1000833.
11. Baldwin SA, Yao SY, Hyde RJ, Ng AM, Foppolo S, Barnes K, et al. Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. J Biol Chem 2005;280:15880-7.
12. Kang N, Jun AH, Bhatia YD, Kannan N, Unadkat JD, Govindarajan R. Human equilibrative nucleoside transporter-3 (hENT3) spectrum disorder mutations impair nucleoside transport, protein localization, and stability. J Biol Chem 2010;285:28343-52.
13. Hsu CL, Lin W, Seshasayee D, Chen YH, Ding X, Lin Z, et al. Equilibrative nucleoside transporter 3 deficiency perturbs lysosome function and macrophage homeostasis. Science 2012;335:89-92.
14. Melti I, Lambot K, Jonard L, Coulouigner V, Quartier P, Neven B, et al. Mutation in the SLC29A3 gene: A new cause of a monogenic, autoimmune condition. Pediatrics 2013;131:e1308-13.
15. Cliffe ST, Kramer JM, Hassain K, Robben JH, de Jong EK, de Brouwer AP, et al. SLC29A3 gene is mutated in pigmentary hypertrichosis with insulin-dependent diabetes mellitus syndrome and interacts with the insulin signaling pathway. Hum Mol Genet 2009;18:2257-65.
16. Rossbach HC, Dalence C, Wynn T, Tebbi C. Faisalabad histiocytosis mimics Rosai-Dorfman disease: Brothers with lymphadenopathy, intrauterine fractures, short stature, and sensorineural deafness. Pediatr Blood Cancer 2006;47:629-32.
17. Avitan-Hersh E, Mandel H, Indelman M, Bar-Joseph G, Zlotogorski A, Bergman R. A case of H syndrome showing immunophenotype similarities to Rosai-Dorfman disease. Am J Dermatopathol 2011;33:47-51.
18. Zheng M, Bi R, Li W, Landeck L, Chen QJ, Lao LM, et al. Generalized pure cutaneous Rosai-Dorfman disease: A link between inflammation and cancer not associated with mitochondrial DNA and SLC29A3 gene mutation? Discov Med 2013;16:193-200.
19. Jonard L, Coulouigner V, Pierrot S, Louha M, Gherbi S, Denoyelle F, et al. Progressive hearing loss associated with a unique cervical node due to a homozygous SLC29A3 mutation: A very mild phenotype. Eur J Med Genet 2012;55:56-8.
20. de Jesus J, Imane Z, Senée V, Romero S, Guillausseau PJ, Balafrej A, et al. SLC29A3 mutation in a patient with syndromic diabetes with features of pigmented hypertrophic dermatisis with insulin-dependent diabetes, H syndrome and Faisalabad histiocytosis. Diabetes Metab 2013;39:281-5.

Received: 30-11-2014
Edited by: Li-Shao Guo
How to cite this article: Liu JW, Si N, Wang LQ, Shen T, Zeng XJ, Zhang X, Ma DL. Identification of a Novel Mutation in Solute Carrier Family 29, Member 3 in a Chinese Patient with H Syndrome. Chin Med J 2015;128:1336-9.

Source of Support: This work was supported by a grant from the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT) (LRT1006 to Xue Zhang). Conflict of Interest: None declared.