Case Report

THE H SYNDROME: MOLECULAR DIAGNOSIS USING NEXT-GENERATION SEQUENCING

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ABSTRACT

Objective: H syndrome is a monogenic systemic inherited form of histiocytosis, with characteristic cutaneous findings accompanying systemic manifestations. The major common endocrine manifestations include hypogonadism, short stature, and diabetes mellitus with characteristic genodermatosis and lead to the diagnosis. Here, we report a rare case of H-syndrome, an autosomal recessive non-autoimmune disorder in a 19-year-old woman who presented with short stature, diabetes mellitus, and hypogonadism associated with characteristic hyperpigmentation and hypertrichosis. The molecular diagnosis was established utilizing next-generation sequencing (NGS) technology.

Methods: We describe the clinical spectrum of H syndrome with endocrine and non endocrine multisystem involvement. The solute carrier family 29 (nucleoside transporters), member 3 (*SLC29A3*) gene was screened for molecular diagnosis utilizing NGS based mutational analysis.

Results: H syndrome is caused by a mutation in the *SLC29A3* gene, which encodes human equilibrative nucleoside transporter 3. A 19-year-old woman was diagnosed to have diabetes mellitus at the age of 6 years. Her clinical phenotype included short stature, hypogonadotropic hypogonadism, hearing loss, hypothyroidism, heart involvement, and hyperpigmentation with hypertrichosis. Her erythrocyte sedimentation rate and C-reactive protein levels were elevated. A clinical diagnosis of H syndrome was considered, and utilizing an NGS-based approach, we identified a reported homozygous missense mutation (c.400C>T, p.Arg134Cys) in the *SLC29A3* gene, which was confirmed by Sanger sequencing.

Conclusion: The characteristic pigmentary hypertrichosis and elevated inflammatory markers differentiate H syndrome from mitochondrial disorders and Turners syndrome with similar endocrine manifestations. With its multiplexing option, NGS offers a rapid and robust platform for molecular diagnosis at an affordable cost. **(AACE Clinical Case Rep. 2016;2:e65-e69)**

Abbreviations:
hENT3 = human equilibrative nucleoside transporter 3; **NGS** = next-generation sequencing; **SLC29A3** = solute carrier family 29 (nucleoside transporter), member 3

INTRODUCTION

H syndrome is a monogenic autosomal recessive disorder characterized by little or no evidence of autoimmunity. Mutated proteins are involved in the regulation of inflammation. This rare genodermatosis caused by a mutation in the solute carrier family 29 (nucleoside transporters) member 3 (*SLC29A3*) gene, which encodes the human equilibrative nucleoside transporter 3 (hENT3). This report covers the case of a 19-year-old woman with H syndrome

Submitted for publication April 5, 2015
Accepted for publication April 29, 2015
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DOI:10.4158/EP15762.CR

See accompanying article, p. e80.

due to an *SLC29A3* mutation identified utilizing a next-generation sequencing (NGS) approach.

CASE REPORT

A 19-year-old woman who was born to nonconsanguineous parents was diagnosed to have diabetes mellitus at the age of 6 years, when she had presented with polyuria. She had ketosis at onset and was on premixed isophane insulin from the time of diagnosis, at a dosage of 1 unit/kg. Her glycated hemoglobin was 45 mmol/mol (6.3%), and she had frequent episodes of hypoglycemia. There was no history of abdominal pain or steatorrhea. She was born at term, with a birth weight of 2.7 kg, had no perinatal problems, and had normal developmental milestones. Short stature was noticed from the age of 6 years. She progressively developed hyperpigmented lesions over the lower limbs and abdomen, with hypertrichosis predominantly over the lower limbs. Hearing impairment (sensoryneural loss) was detected at 9 years. Evaluation for primary amenorrhea with a lack of secondary sexual characters was conducted at the age of 17 years. Primary hypothyroidism was diagnosed at that time, with elevated thyroid-stimulating hormone (TSH) level, and levothyroxine supplementation was started.

On examination, the patient had hypertelorism and short stature, with a height of 134 cm (<third centile), which corresponds to a height age of 10 years (midparental target height of 154 cm). There were no midline facial defects. Cutaneous features included symmetrical large hyperpigmented indurated plaques with hypertrichosis over the lower limbs, with characteristic sparing of the buttocks and knees (Fig. 1). There were additional hyperpigmented patches over the abdomen (Fig. 2). Other features included ichthyosis with nonpitting pedal edema and arthrogryposis at the ankles. Tanner's staging of the breasts was prepubertal (stage 1), with stage 2 pubic hair. There were no stigmata of Turner's syndrome. The blood pressure was normal, with no radio-femoral delay. There was an ejection systolic murmur in the aortic area. She had hepatomegaly 5 cm below the costal margin. Visual acuity and optic fundus examination were normal. The bone age was 15 years; her insulin-like growth factor 1 level corrected for bone age was 48.5 ng/mL (normal, 127 to 424 ng/mL). Magnetic resonance imaging of the brain was normal. The erythrocyte sedimentation rate and C-reactive protein levels were elevated (120 mm/hour [normal, <20 mm/hour] and 56.1 ng/mL [normal, <6 ng/mL], respectively). Antinuclear, glutamic acid decarboxylase and thyroid peroxidise antibodies were negative. Audiogram confirmed bilateral sensorineural hearing loss. An echocardiogram revealed an abicuspid aortic valve. Histologic examination of the skin lesions demonstrated thickening in the dermis due to fibrosis and a cellular infiltrate composed of small- to medium-sized histiocytes, lymphocytes, and plasma cells. Her parents and their siblings did not have any of the above-mentioned features.

A number of features observed in this patient are associated with Turner's syndrome and mitochondrial disorders; in particular, short stature, delayed puberty, diabetes, and sensorineural hearing loss. The presence of hypogonadotropic hypogonadism with the pigmentary hypertrichosis is contrary to a diagnosis of Turner's syndrome, which is characterized by hypergonadotropic hypogonadism. A clinical diagnosis of H syndrome was considered based on the clinical spectrum of manifestations which included *h*yperglycemia, reduced *h*eight (short stature), *h*ypogonadotropic *h*ypogonadism, *h*earing loss (sensorineural), *h*eart involvement, *h*epatomegaly, and the characteristic *h*yperpigmentation with *h*ypertrichosis.

After the confirmation of a normal karyotype, the *SLC29A3* gene was screened for hENT3 mutations. A novel polymerase chain reaction (PCR) coupled with the NGS strategy for mutational screening of the *SLC29A3* gene was developed. The coding region of the 6 exons, intron/exon boundaries, and 5' and 3' untranslated regions of the *SLC29A3* gene were amplified using 7 pairs of primers designed using Primer3 software. Following PCR-based target enrichment and library preparation, NGS was performed using an Ion Torrent personal genome machine (PGM) using 314 chips and an Ion PGM™ 200 sequencing kit (Ion Torrent, Life Technologies). Data analysis was performed using Ion Torrent suite software and DNA star software. Using this approach, we identified a homozygous missense mutation (c.400C>T, p.Arg134Cys) in the *SLC29A3* gene, encoding hENT3 (Fig. 3 *A*), establishing the diagnosis of H syndrome. The gene mutation was further confirmed by Sanger sequencing (Fig. 3 *B*).

The patient was managed with a basal-bolus insulin regimen (0.9 units/kg) with the insulin analogues aspart and glargine. Her levothyroxine dosage was increased to 100 μg once daily in view of persistently elevated TSH. Growth hormone replacement therapy was declined by the parents, and she was started on estrogen supplements for the development of secondary sexual characters. After 16 months of follow-up, the patient's height has remained static and thyroid functions are normal.

DISCUSSION

H syndrome is a monogenic autosomal recessive autoimmune syndrome. This multisystem disorder is caused by mutations in the *SLC29A3* gene, which encodes nucleoside transporter hENT3 (1). Patients with this syndrome were initially described in families of Arab and Bulgarian origin having consanguineous parents (1,2). A few patients have been reported from India (3,4).

The H syndrome is characterized by major clinical findings of pigmentary hypertrichosis, hyperglycemia (non-autoimmune diabetes mellitus), hepatosplenomegaly,

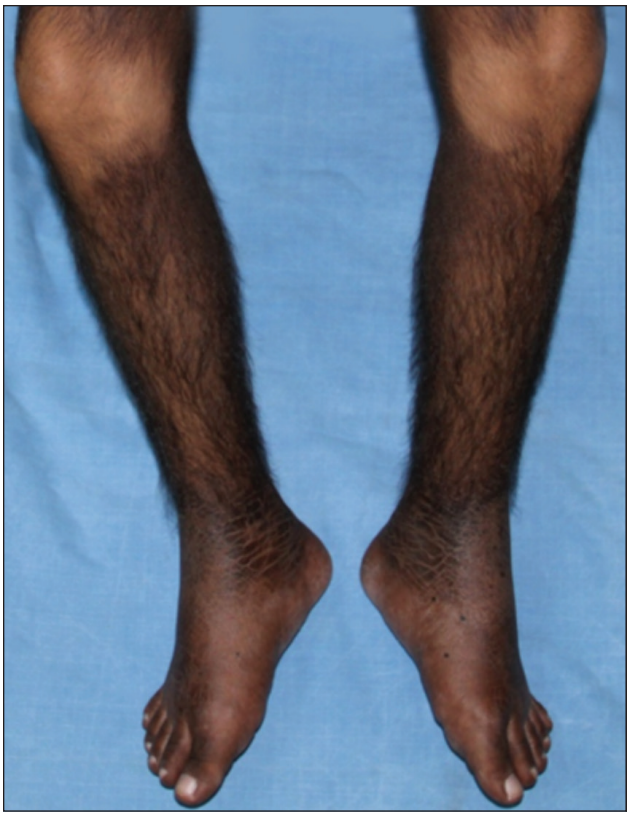

Fig. 1. Characteristic pigmentary hypertrichosis involving lower limbs.

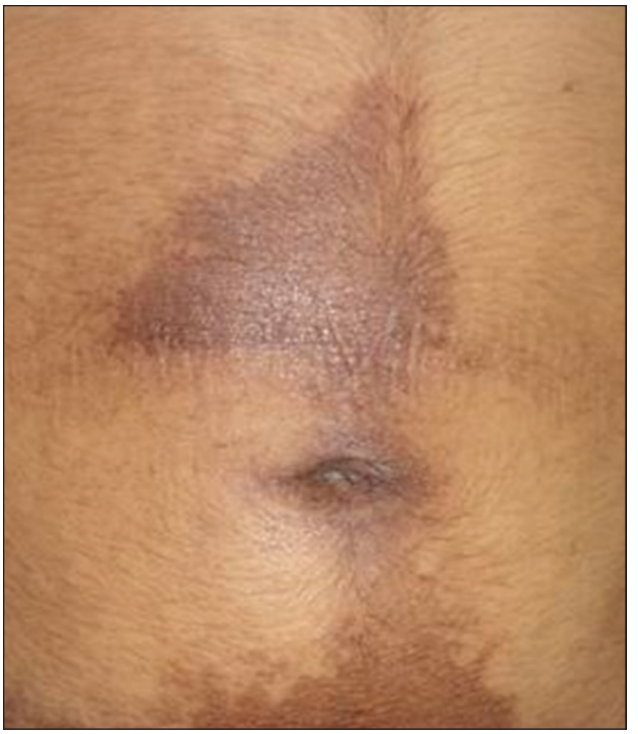

Fig. 2. Hyperpigmented patches over the abdomen.

heart anomalies, sensorineural hearing loss, hypogonadotropic hypogonadism, and growth hormone deficiency, manifesting with short stature (1). Male subjects have been reported to have scrotal masses, gynecomastia, and azoospermia (3). Other features that are described include varicose veins and joint deformities (hallux valgus and fixed flexion contractures of interphalangeal joints). The most common clinical features (>45% of patients) are hyperpigmentation, phalangeal flexion contractures, hearing loss, and short stature. Insulin-dependent diabetes mellitus and lymphadenopathy are found in around 20% of these patients (4). These patients develop progressive cutaneous hyperpigmented, hypertrichotic, and indurated plaques over the lower limbs and lower abdomen during the first or second decade of life, which is the hallmark for the diagnosis. Histopathologic examination of the involved skin is characterized by inflammation, with the basal layer showing seborrheic keratosis-like acanthosis, infiltration of histiocytes, and a perivascular mononuclear infiltrate, with plasma cells and mast cells throughout the dermis and subcutaneous fat (1-4).

The features of H syndrome are classically seen in patients with Turner's syndrome and those with mitochondrial disorders, including the endocrine abnormalities (short stature, hypogonadism and hyperglycemia) and sensorineuronal hearing loss. A normal karyotype and absence of elevated follicle-stimulating hormone essentially rules out Turner's syndrome. Cutaneous involvement is rarely described in those with mitochondrial disorders, thus making it the characteristic feature of H syndrome. The other possible differential diagnoses include Winchester syndrome and morphea (3,4). In addition to the clinical and pathologic features described previously, our patient presented with some unique features, including ichthyosis, clinodactyly, arthrogryposis of the ankle, and hypothyroidism.

Genetic analysis in this subject revealed a reported homozygous missense mutation, p.Arg134Cys, in the *SLC29A3* gene (3). Mutations in the *SLC29A3* gene were initially described to present as 2 allelic disorders designated diabetes-type pigmented hypertrichosis with insulin-dependent diabetes mellitus and deafness-type H syndrome (5). However, our patient and many other case series (6) report coexistent deafness and diabetes mellitus, contrary to the concept of discrete allelic disorders. Recently, 5 cases from the Asian Indian population have been reported, with insulin-dependent diabetes in 2 cases and 4 cases involving sensorineuronal hearing loss (6,7).

Homozygosity mapping in families has helped in the detection of mutations in the *SLC29A3* gene, which encodes the equilibrative nucleoside transporter hENT3. Though the precise hENT3 cellular protein sublocalization has not been determined, the protein has been suggested to be a late endosomal or lysosomal transporter that exports nucleosides from the lysosomal interior (8). hENT3 is a 475–amino acid protein that is a pH-dependent trans-

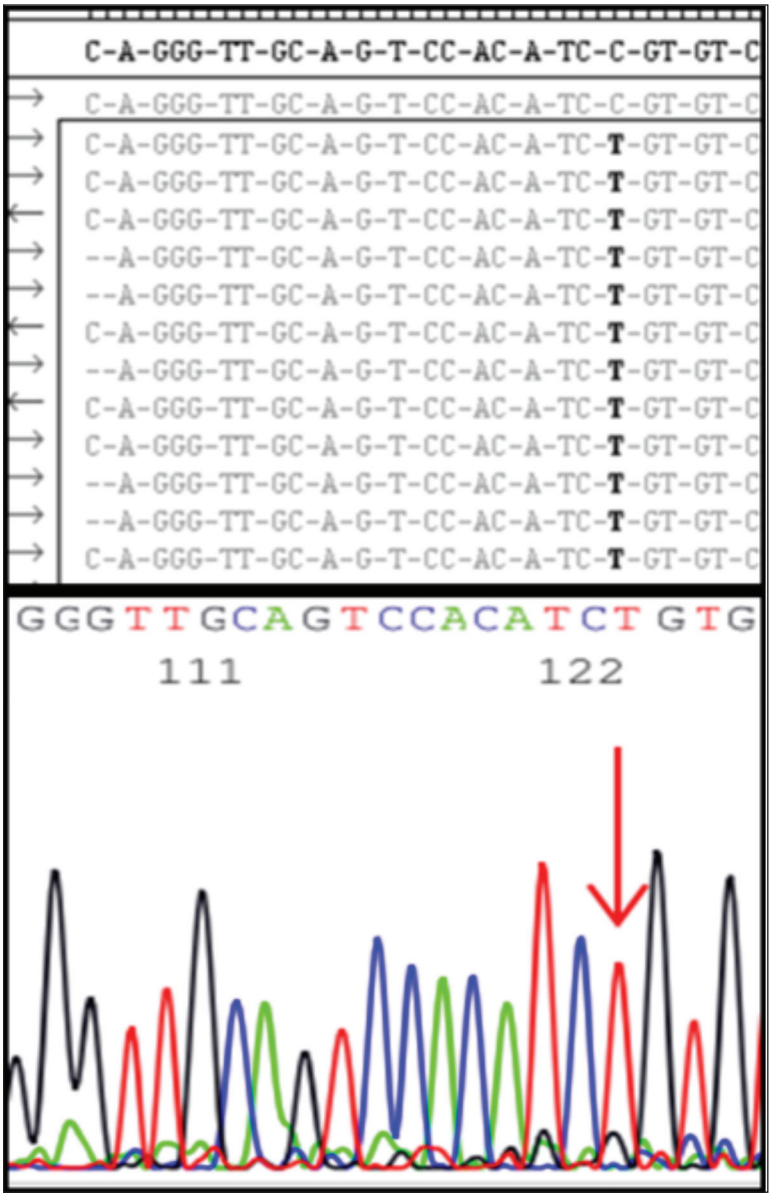

Fig. 3. (*A*) Homozygous mutation of *SLC29A3* gene (p. Arg134Cys) detected by NGS. (*B*) Phrenogram showing a missense mutation (c.400C>T p.R134C) in the *SLC29A3* gene.

porter of nucleobases across lysosomal membranes (5,9). Mutations in the *Drosophila* ortholog are believed to affect the insulin signalling pathway and hence result in hyperglycemia (5). A mitochondrial respiratory chain defect may account for some features (10). However, further research is required to delineate the exact role of hENT3 protein in the pathogenesis of this disorder. Mutations in the *SLC29A3* gene have also been found in Rosai-Dorfman disease and Faisalabad histiocytosis, which are associated with immunophenotypic similarities such as positivity for CD68, CD34, and factor XIIIa (11). Till date, around 20 different mutations have been identified in *SLC29A3*, with the majority of them located in exon 6 (3,4).

There has been an increased awareness of this syndrome, and more than 100 cases have been described. From India, 10 cases of H syndrome have been reported; however, only a few of these cases had a confirmed genetic diagnosis (6,7).

CONCLUSION

The characteristic pigmentary hypertrichosis and elevated inflammatory markers in H syndrome are the key differentiating features from both Turner's syndrome and other mitochondrial disorders. Genetic testing not only establishes the diagnosis, it also aids in future counsel-

ing. As shown in this study, the flexibility offered by NGS semiconductor chip sequencing (314 chip >20 Mb of data) with multiplexing capability that uses an inexpensive barcoding system (index up to 96 libraries) provides an affordable alternative genetic screening option suitable even for single-gene disorders.

DISCLOSURE

The authors have no multiplicity of interest to disclose.

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