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Hypoparathyroidism and central diabetes insipidus: in search of the link

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Abstract

Two siblings (a 15-year-old boy and an 11-year-old girl) who presented with hypocalcemic seizure at the age of 2 years and 2 months (boy) and 2 years and 4 months (girl) were diagnosed with hypoparathyroidism. At the age of 3 years, the girl developed central diabetes insipidus with good response to desmopressin acetate treatment. The family history was unremarkable, and there was no consanguinity between the parents. The father is of Iraqi/Egyptian Jewish origin and the mother is of Iranian/Romanian Jewish origin. Sequence analysis of the candidate genes for isolated hypoparathyroidism encoding calcium-sensing receptor, parathyroid hormone, and glial cells missing homolog B did not reveal any mutations. Whole-exome sequencing identified a homozygous mutation in the autoimmune regulatory gene (*AIRE*), c.374A>G;p.Y85C, characteristic for Jewish Iranians with autoimmune polyendocrine syndrome type 1 (APS1), which was confirmed by the Sanger sequencing. Antibodies against the adrenal, pancreatic islet cell, ovary, thyroid, pituitary, celiac, and parietal cell were negative in both siblings, while anti-diuretic hormone antibodies were positive only in the girl. No other symptoms or signs of APS1 developed during all the years of follow-up.

Conclusion—APS1 should be part of the differential diagnosis in children presenting with isolated hypoparathyroidism or hypoparathyroidism with central diabetes insipidus (CDI). These cases show that the *AIRE* mutation characteristic of Iranian Jews can also be found in non-Iranian Jews.

Keywords

Hypoparathyroidism; Central diabetes insipidus; Autoimmune polyendocrine syndrome type 1 (APS1)

Introduction

Hypoparathyroidism is a rare condition in the pediatric population. It is characterized by hypocalcemia, hyperphosphatemia, and inappropriately low or undetectable serum PTH concentration. Most cases of nonsurgical forms of hypoparathyroidism are sporadic, but familial forms with different modes of inheritance have been described, including syndromic and isolated forms [24, 26]. Familial isolated hypoparathyroidism, either congenital or later-onset, may be transmitted as an autosomal dominant (AD), autosomal recessive (AR), or X-linked (XL) recessive trait. The most common cause for AD hypoparathyroidism is activating mutations in the calcium-sensing receptor (*CaSR*) gene, typically characterized as hypercalciuric hypocalcemia [2, 8]. Recently, gain-of-function mutations in *GNA11* encoding the alpha subunit of the G protein G11 were described as another cause for AD

hypoparathyroidism [15, 18, 20]. AD or AR forms of hypoparathyroidism can be caused by rare mutations in the genes, including *preproPTH* [21] or glial cells missing B (*GCMB* or *GCM2*) [6, 17]. XL hypoparathyroidism can be caused by mutations in the *SOX3* gene [6]. Another cause of familial hypoparathyroidism inherited as an AR trait is autoimmune polyendocrine syndrome type 1 (APS1; OMIM #240300). The disease is caused by a homozygous mutation in the *AIRE* gene mapped to chromosome 21q22.1. More than 70 mutations in the *AIRE* gene have been reported [11]. Hypoparathyroidism in this condition is usually preceded by mucocutaneous candidiasis [19, 22].

We describe two siblings diagnosed with hypoparathyroidism during infancy. The older boy has isolated hypoparathyroidism, while the younger girl additionally developed central diabetes insipidus (CDI) 1 year after her initial presentation. Molecular analysis and identification of the causative gene mutation are presented.

Case reports

Patients' background

Patient 1 is a 15-year-old boy who presented with hypocalcemic seizure at the age of 2 years and 2 months. The initial laboratory evaluation was consistent with hypoparathyroidism. He also had mild hypomagnesemia. Treatment with calcium carbonate, alpha-D3, and magnesium citrate was initiated. Calcium levels were kept around 8 mg/dl.

Patient 2, an 11-year-old girl and a younger sister of patient 1, presented with hypocalcemic seizure at the age of 2 years and 4 months. At the age of 3 years, she developed polyuria and polydipsia while her calcium level was 8.1 mg/dl, and she was diagnosed as having CDI by a water deprivation test, with good response to desmopressin acetate (DDAVP) treatment. A brain magnetic resonance imaging (MRI) study revealed an absent posterior bright spot, consistent with the diagnosis of CDI.

No other pathological clinical manifestations or laboratory alterations were observed for either sibling during follow-up. The results of annual renal sonographic studies were normal.

The family history was unremarkable; there was no consanguinity between the parents, and each parent had normal calcium levels. The father is of Iraqi/Egyptian Jewish origin and the mother is of Iranian/Romanian Jewish origin.

Methods

Genetic testing methodology

The patients' parents gave written informed consent for this study, which was approved by the institutional review board.

Genomic DNA was isolated from blood samples obtained from all family members. Molecular analysis of the genes encoding *CaSR*, *PTH*, and *GCMB* genes were performed by Sanger sequencing the exons and exon-intron boundaries. Whole-exome analyses were performed at the Broad Institute, Cambridge, MA, using the Agilent SureSelect Human All Exon Kit v2 followed by massively parallel sequencing using an Illumina HiSeq Sequencer.

Data processing and variant calling were done as described elsewhere [18]. Identified mutations were confirmed by Sanger sequencing of PCR-amplified genomic DNA from the patients, their parents, and their younger healthy sibling (1.5 years old at the time of this investigation). The PCR amplification products were directly sequenced using BigDye 3.1 Terminator chemistry (Applied Biosystems) and separated on an ABI 3500 genetic analyzer (Applied Biosystems, Foster City, CA).

Anti-adrenal and anti-ovary antibodies (adrenal cortex autoantibodies and steroid-producing cell autoantibodies, respectively) were measured by indirect immunofluorescence on cryostat sections of monkey adrenal glands and ovaries (Euroimmune, Lübeck, Germany). Antibodies to hypothalamic cells were measured by a simple indirect immunofluorescence method on cryostat sections of young baboon hypothalamus and retested by four-layer double immunofluorescence as previously described [7]. Antibodies to the pituitary autoantigen tudor domain containing protein 6, which had been detected in sera of APS1 patients but not in control subjects, were also measured as previously described [3].

Results

Mutation screenings of the *CASR*, *PTH*, and *GCMB* genes failed to identify any deleterious disease-causing mutations. Whole-exome analyses identified a homozygous mutation in the *AIRE* gene, c.374A>G;p.Y85C, which has been described as characteristic for Iranian Jewish individuals with autoimmune polyendocrine syndrome type 1 (APS1) [5]. Sanger sequencing of PCR-amplified *AIRE* gene confirmed a homozygous *AIRE* mutation in the affected siblings. The parents were heterozygous carriers, and their younger healthy sibling was homozygous for the wild-type allele.

The girl had a positive antibody to hypothalamic cells (1:64 dilution), while her brother had a negative result (1:8 dilution) [7]. Both siblings with hypoparathyroidism had negative results for anti-pituitary antibodies. Annual screening during all the years of follow-up for thyroid functions and antibodies (anti-thyroid peroxidase and anti-thyroglobulin), celiac disease, pernicious anemia (anti-parietal antibodies and vitamin B12 levels), adrenal insufficiency (plasma renin, ACTH, and ACTH-stimulated cortisol levels), and adrenal and ovary antibodies were all normal (Table 1). Both affected siblings had normal growth and pubertal development, and there were no signs of mucocutaneous candidiasis.

Discussion

APS1, also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a rare AR disease caused by mutations in the *AIRE* gene located on chromosome 21q22.3. APS1 usually develops during childhood and typically includes the classic triad of mucocutaneous candidiasis, hypoparathyroidism, and adrenocortical failure [3, 5, 22, 29]. Other autoimmune diseases may appear concomitantly, such as type 1 diabetes, premature ovarian failure, celiac disease, autoimmune hepatitis, pernicious anemia, and Hashimoto thyroiditis. The worldwide prevalence of APS1 is very low, but the estimated prevalence is 1/9,000, 1/14,400, and 1/25,000 inhabitants among the Iranian Jewish, Finnish, and Sardinian communities, respectively [4, 19, 29]. Unlike Finland and Sardinia where

chronic mucocutaneous candidiasis is the first manifestation of the disease, often occurring before the age of 5 years [4], the first manifestation among affected Iranian Jews is hypoparathyroidism which can be the only manifestation of the disease for many years [29], as was the case in the older sibling of the two described above. The mechanism of hypoparathyroidism in patients with APS1 may be due to the presence of autoantibodies directed to CaSR [9, 13] or to the parathyroid protein NALP5 [1, 27].

Autoimmune CDI, such as that found in the younger sibling described above, is a very rare manifestation of APS1. None of the studies on the three large cohorts from Finland [22], Sardinia [19], and Israel [29] reported any cases of coexisting CDI.

No anti-pituitary antibodies were detected in the sera of our two patients. Antibodies against AVP-producing cells were positive in the girl with CDI and negative in the boy who did not have CDI [7]. Scherbaum et al. [25] studied 39 subjects with CDI and found autoimmune etiology in 13 (33 %), of whom only one had APS1. In addition, 8 of these 13 subjects (62 %) were positive for AVP-cell antibodies, as was discovered in our patient. Two other pediatric cases of APS1 with CDI have been described in the literature [12, 16].

APS1 in Israel has been described solely in Iranian Jews. They all have the same mutation in the *AIRE* gene, c.374A>G;p.Y85C, due to a founder effect. To the best of our knowledge, we describe the first discovery of APS1 in children of combined and not solely Iranian Jewish ancestry. We assume that the paternal mutation originated from the Iraqi side. This can be explained by the geographical proximity of these two countries. Indeed, there are genetic disorders characteristic to Iranian Jews, such as corticosterone methyl oxydase II deficiency, and to Iraqi Jews, such as familial Mediterranean fever [10] with a unique founder mutation, and there are some genetic disorders common to both communities, such as growth hormone insensitivity syndrome (Laron dwarfism) [14] and beta thalassemia (for a complete list, see the table in reference [28]).

Of note, while DNA samples were processed for the molecular analysis of the *AIRE* gene, whole-exome analysis detected the classical Iranian mutation causing APS1. This proves the efficiency of this relatively new method in finding the different genetic bases of similar phenotypes. Whole-exome sequencing provides coverage of more than 95 % of the exons, which contains 85 % of disease-causing mutations in Mendelian disorders and many disease-predisposing single-nucleotide polymorphisms throughout the genome, becoming a major tool for detection of genetic cause of different medical conditions [23]. To the best of our knowledge, this is first case of APS1 diagnosed by whole-exome analysis.

In conclusion, APS1 should be part of the differential diagnosis in children presenting with isolated hypoparathyroidism. It is likely that the *AIRE* mutation can be found in Iraqi Jews most probably due to the geographical proximity to Iran.

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Table 1

Autoimmunity investigation

Antibodies	Results
Adrenal ^a	Negative
Pancreatic islet cell (IAA, anti-GAD, ICA) ^a	Negative
Ovary ^a	Negative
Thyroid (TPO, thyroglobulin) ^a	Negative
Celiac screen ^a	Negative
Parietal cell ^a	Negative
Pituitary	Negative
Hypothalamus	1:64 in the girl with CDI (positive)
	1:8 in the boy (negative)

^aTested annually for 13 years in patient 1 and for 9 years in patient 2