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The diagnosis of adrenal insufficiency in a patient with Allgrove syndrome and a novel mutation in the ALADIN gene

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Abstract

Allgrove syndrome is a genetic disorder inherited in an autosomal recessive pattern and characterized by a triad of adrenal insufficiency, achalasia, and alacrima. The gene affected by the mutation in patients with Allgrove syndrome is termed either AAAS or ALADIN (alacrima/achalasia/adrenal insufficiency/neurologic disorder). Adrenal insufficiency in patients with this disorder may develop as late as the third decade of life.

We describe a 24-year-old female with Allgrove syndrome, in whom initial testing with 250 μg corticotropin (ACTH) stimulation test performed on 3 occasions produced normal serum cortisol values and results of the 1-μg ACTH stimulation tests performed on 6 occasions were conflicting. Insulin-induced hypoglycemia produced a nadir serum glucose value of 36 mg/dL without adequate serum cortisol stimulation, confirming presence of adrenal insufficiency. Gene sequencing identified 2 mutations in the triple A gene: an IVSC14 +1 G to A mutation, which has been previously reported, and a novel R155P exon 6 mutation.

We conclude that a novel R155P mutation in the ALADIN gene is associated with Allgrove syndrome and that insulin-induced hypoglycemia, rather than ACTH stimulation tests, should be used for accurate diagnosis of adrenal insufficiency in this disorder.

1. Introduction

In 1978 Allgrove et al [1] described 2 pairs of siblings with the combination of the symptoms of adrenal insufficiency, achalasia, and alacrima. This triad of symptoms became known as Allgrove syndrome or "triple A syndrome" [2].

Patients with Allgrove syndrome usually present with hypoglycemia due to adrenal insufficiency in the first decade of life. In some patients, however, alacrima or hypolacrima may precede adrenal insufficiency. Alacrima-achalasia syndrome without adrenal insufficiency has been described and is thought to represent the delayed penetrance of adrenal insufficiency or its inadequate diagnosis. In addition, progressive and disabling neurologic manifestations due to central, peripheral, or autonomic nervous system involvement may be present [2].

Allgrove syndrome is inherited in an autosomal recessive pattern. Genetic linkage analysis has revealed a locus on chromosome 12q13 commonly affected by mutations in the individuals with Allgrove syndrome [3]. Subsequently, the gene termed either AAAS or ALADIN (for alacrima/achalasia/adrenal insufficiency/neurologic disorder) and encoding a protein of 547 amino acids [4,5] was identified as the site of mutations in Allgrove syndrome. The AAAS gene product belongs to the WD-repeat family of functionally diverse regulatory proteins. High expression of this protein is seen in the adrenal gland, gastrointestinal tract, and brain-the organs in which the main pathologic manifestations of Allgrove syndrome occur. Homozygous and compound heterozygous mutations in the AAAS gene have been identified in a number of families of diverse ethnic backgrounds affected by Allgrove syndrome [4-7].

As mentioned above, adrenal insufficiency in patients with triple A syndrome usually begins in childhood but it may become manifested in as late as the third decade of life [8]. Therefore, monitoring the hypothalamic-pituitary-adrenal

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axis in these patients to make a prompt diagnosis and to administer the necessary replacement therapy is essential. Selecting the most reliable test for this purpose is important.

We describe a patient with Allgrove syndrome in whom diagnosis of adrenal insufficiency could not be established using corticotropin (ACTH) stimulation tests. Insulin-induced hypoglycemia testing was required to establish presence of adrenal insufficiency with certainty. Sequencing of the ALADIN gene in this patient identified 2 compound heterozygous mutations, one of them novel and the other previously reported.

2. Case report

A 24-year-old Puerto Rican woman was referred for evaluation of adrenal function. She was diagnosed with Allgrove syndrome manifested by peripheral neuropathy, alacrima, and achalasia at the age of 11 years.

She was a product of nonconsanguineous marriage and was born at term without complications. She started walking at 14 months of age, but her gait was unsteady. Speech appeared at 24 months but remained slurred. Mental development was normal. Menarche occurred at the age of 15 years and menstrual periods have remained regular.

Achalasia was manifested by difficulty in swallowing and required repeated esophageal dilatations. Alacrima initially presented with decreased mucosal secretions resulting in chronic sinusitis. She was also suffering from a slowly progressive demyelinating polyneuropathy, involving sensory and motor nerves, and from orthostatic symptoms thought to be secondary to autonomic neuropathy. She had no history of severe fatigue, diarrhea, or weight loss.

On physical examination, an elongated face and a narrow upper lip characteristic of Allgrove syndrome were noted. The weight was 68 kg and the height was 164 cm. Her blood pressure in supine position was 145/90 mm Hg and heart rate was 80 beats per minute. She had an orthostatic change of 30/30 mm Hg in her blood pressure and of 30 beats per minute in her heart rate. Hyperpigmentation over the knuckles of the hands and the base of toenails was present. Flat intonation of her speech with hyperresonance suggested the abnormal function of cranial nerves IX and X. A marked distal muscular wasting, particularly in the lower extremities, was present. Peripheral sensations of vibration and temperature were reduced. Deep tendon reflexes were hyperactive in arms and knees but ankle jerks were absent. Her gait was very stiff and she had to use cane for support.

3. Methods

3.1. 250-μg ACTH stimulation test

Serum cortisol concentration was measured immediately before and 60 minutes after intravenous injection of 250 μg (85 nmol, or 40 IU) of cosyntropin (Cortrosyn, Organon, West Orange, NJ).

3.2. 1-μg ACTH stimulation test

Serum cortisol concentration was measured before and 15, 30, and 60 minutes after injection of 1μg cosyntropin. The solution of cosyntropin was prepared by hospital pharmacists. One milliliter of the diluent was injected into the vial of 250-μg cosyntropin (Cortrosyn, Organon) to produce a 250 μg/mL solution and shaken thoroughly. Subsequently, by using a 1-mL tuberculin syringe, 0.2 mL (ie, 50 μg cosyntropin) was withdrawn and injected into a vial containing 24.8 mL of sterile normal saline solution to produce a 2 μg/mL solution. After shaking thoroughly, 0.5 mL (1.0 μg cosyntropin) of the solution was injected immediately intravenously using a tuberculin syringe. On all occasions, test was performed between 9:30 and 11:00 AM by one physician.

3.3. Insulin-induced hypoglycemia test

The patient was admitted to the intensive care unit after an 8-hour fast. A 22-gauge angiocatheter was inserted into the right forearm. In the supine position, 0.10 U/kg (7 units) of regular insulin (Humulin R, Eli Lilly and Company, Indianapolis, Ind) was administered intravenously through the angiocath. Blood pressure, capillary blood glucose (Accu-chek Inform glucose meter, Roche Diagnostics Corporation, Indianapolis, Ind), and symptoms were monitored throughout the test. Serum for measurements of glucose and cortisol concentrations and plasma for measurements of ACTH concentrations were obtained at time 0 and every 15 minutes until the hypoglycemic target of serum glucose of 40 mg/dL was reached.

3.4. Glucose and hormone assays

Serum levels of glucose and cortisol were determined at the Beth Israel Medical Center laboratory using colorimetry (Roche Diagnostics Corporation) and chemiluminescence (Advia Centaur System, Bayer Corporation, Tarrytown, NY), respectively. Plasma levels of ACTH were measured either by immunochemiluminometric assay or radioimmunoassay, serum aldosterone by radioimmunoassay, and plasma renin concentrations by immunochemiluminescence assay (Quest Diagnostics, Nichols Institute, San Juan Capistrano, Calif, or Chantilly, Va).

3.5. Gene sequencing

DNA was extracted from blood samples obtained from the patient and both affected and unaffected individuals from affected families. The exons 2 to 15 and flanking intronic regions of the triple A gene were amplified by the polymerase chain reaction. The intronic regions between exons 4 and 5, 10 and 11, 12 and 13, and 14 and 15 were small enough to be amplified together. Polymerase chain reaction was carried out in a total volume of 50 μL, which contained 20 ng of DNA, 0.2 mM dNTPs, 1 unit TaqGold polymerase, 1.5 mM $MgCl_2$, 75 mM Tris-HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, and 50 pmol of each

primer. Polymerase chain reaction was performed on a Perkin Elmer 9700 thermal Cycler (Perkin Elmer, Applied Biosystems, Foster City, Calif). Polymerase chain reaction fragments were evaluated on a 1% agarose gel. The polymerase chain reaction products were purified using a Qiaquick purification kit (Qiagen, Hilden, Germany) and resuspended in 50 μL of deionized water. For each exon 100 ng of amplified product was sequenced using forward and reverse primers and BigDye Terminator cycle sequencing kit (Perkin Elmer, Boston, Mass). Sequencing was performed on an ABI377 automated sequencer; alignment and analysis were carried out with Sequence Navigator (Perkin Elmer).

4. Results

Endocrinologic evaluation revealed normal thyroid function and normal serum prolactin concentration. The 250-μg ACTH stimulation test was performed on 3 occasions with normal results (peak stimulated serum cortisol concentrations of 29 μg/dL [800 nmol/L], 23 μg/dL [634 nmol/L], and 29 μg/dL [800 nmol/L]) (Fig. 1A).

Because 250-μg stimulation test may not be sensitive enough to detect adrenal insufficiency, we performed 1-μg ACTH stimulation test on 6 occasions. On 4 occasions the test was consistent with adrenal insufficiency (peak stimulated serum cortisol concentration less than 18 μg/dL [497 nmol/L]) whereas it was normal on 2 occasions (peak stimulated serum cortisol concentration of 30 μg/dL [828 nmol/L] and 36 μg/dL [994 nmol/L]) (Fig. 1B). To exclude technical problems with the 1-μg stimulation test, we performed the test on 3 normal volunteers. Mean serum cortisol concentrations were 15 $\pm$ 2 μg/dL (414 nmol/L) at 0 minutes, 25 $\pm$ 3.7 μg/dL (690 nmol/L) at 15 minutes, and 26 $\pm$ 1.1 μg/dL (718 nmol/L) at 30 minutes, producing normal results in each case (Fig. 1B).

Serum ACTH levels were obtained on 7 occasions. They ranged between 16 and 37 pg/mL, with a mean value of 25 $\pm$ 7 pg/mL; all serum ACTH concentration measurements were within the reference range.

Because of the conflicting results of the ACTH stimulation tests in the patient we performed the definitive insulin-induced hypoglycemia test. Forty-four minutes after receiving intravenous insulin the patient complained of hunger, weakness, fatigue, and had difficulty counting from 10 to 1; administration of intravenous glucose terminated the test. The patient's serum glucose reached a nadir of 36 mg/dL (2 mmol/L) without adequate cortisol production (peak serum cortisol concentration of 16.1 μg/dL [444 nmol/L]) in spite of serum ACTH concentration rising to a peak of 161 pg/mL (35.4 pmol/L) (Table 1).

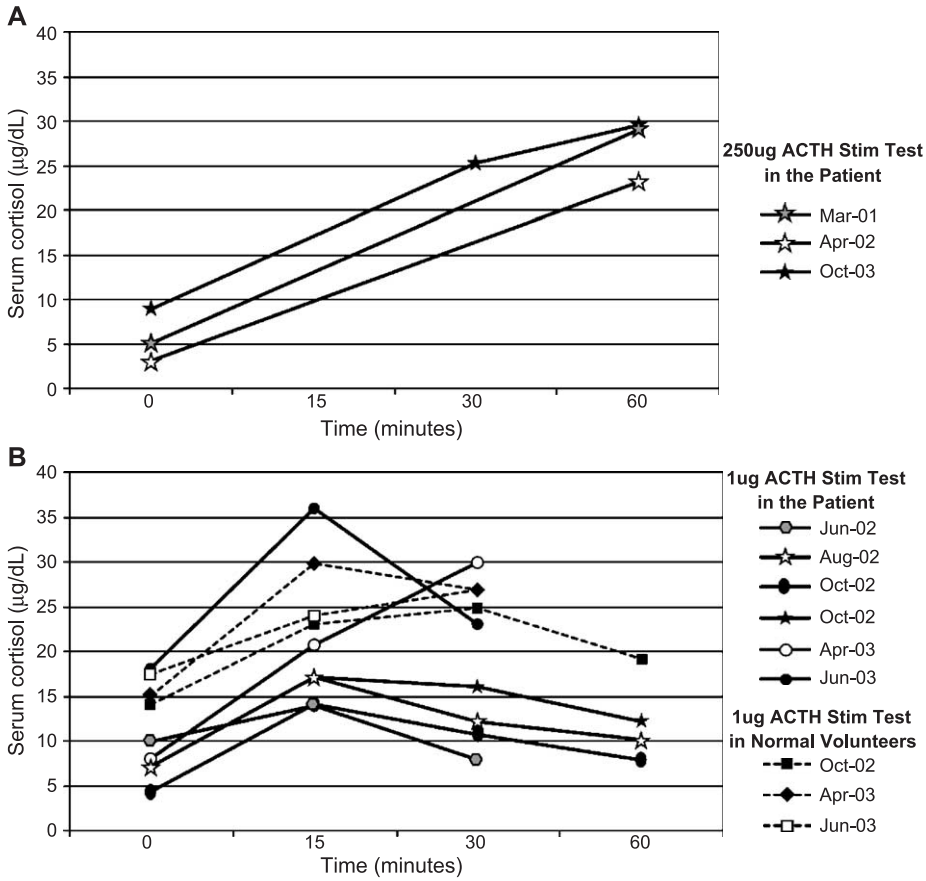

Fig. 1. A, Serum cortisol in the patient during 250-μg ACTH stimulation test. To convert serum cortisol to nanomoles per liter, multiply by 27.6. B, Serum cortisol in the patient and in normal volunteers during 1-μg ACTH stimulation test.

Table 1
Results of the insulin-induced hypoglycemia test[a]

Time (min)	Blood pressure (mm Hg)	Capillary blood glucose (mg/dL)	Serum glucose (mg/dL)	Cortisol (ug/dL)	ACTH (pg/mL)	Symptoms
0	142/90	83	78	7.5	22	None
10	133/78	78	48	5.3	25	None
30	131/75	39	36	7.0	19	Hungry
40	113/86	41	39	9.6	16	Hungry, tired
44	108/66	50	44	16.1	161	Hungry, tired, weak–test terminated
85	120/89		110	14.8	44	

To convert glucose to millimoles per liter, multiply by 0.056.
To convert serum cortisol to nanomoles per liter, multiply by 27.6.
To convert plasma ACTH to picomoles per liter, multiply by 0.22.
[a] Seven units of regular insulin (Eli Lilly and Company) was administered intravenously at 0 minutes.

Aldosterone response to 2-hour upright position was blunted (serum aldosterone concentration of 4 ng/dL in supine position and 5 ng/dL in upright position), whereas plasma renin concentrations increased from 9 to 18 μU/mL, respectively. Similarly, aldosterone did not respond to endogenous ACTH rise from 22 to 161 pg/mL induced by hypoglycemia, as serum aldosterone concentration was 4 ng/dL at baseline and 3 ng/dL at the conclusion of insulin-induced hypoglycemia test.

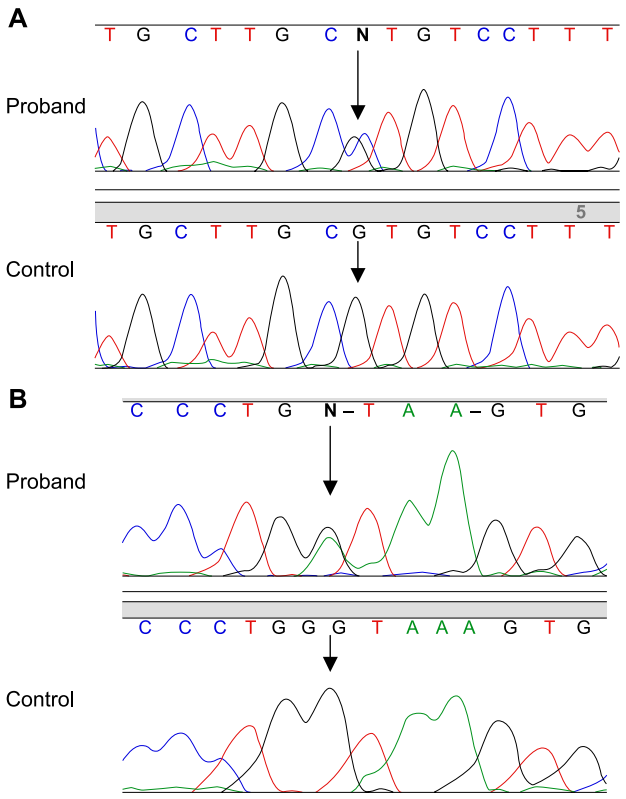

Fig. 2. A, DNA sequencing electropherogram depicting the R155P mutation. The arrow indicates the mutation in the proband, which is a G to C heterozygous change. In the control, the arrow indicates the normal nucleotide. B, DNA sequencing electropherogram depicting the IVS14 +1 mutation. The arrow indicates the mutation in the proband, which is a G to A heterozygous change. In the control, the arrow indicates the normal nucleotide.

Two mutations in the triple A gene were identified. They were an IVSC14 +1 G to A mutation, which has been previously reported in Hispanic patients with Allgrove syndrome [8] and an exon 6 mutation (R155P) not previously reported (Fig. 2). We also identified an exon 2 silent intronic change, likely a polymorphism. The exon 6 and exon 2 changes were not present in 40 control blood samples.

5. Therapeutic response

The patient was initiated on therapy with oral hydrocortisone, 10 mg in the morning and 5 mg in the afternoon. Several weeks after the initiation of therapy, the patient reported decreased fatigue and significant improvement in orthostatic symptoms. This improvement was sufficient to enable her to begin an exercise regimen. Orthostatic changes in blood pressure and pulse rate were no longer present on physical examination. She gained about 10 lb of weight. The dose of hydrocortisone was decreased 5 months after initiation of treatment to 7.5 mg in the morning and 5 mg in the afternoon without reoccurrence of symptoms. Mineralocorticoid replacement (fludrocortisone, 0.3 mg/d initially and then 0.2 mg/d) that had been started for postural symptoms by the patient's neurologist was discontinued after initiation of hydrocortisone therapy because of supine hypertension. The patient remains free of symptoms on hydrocortisone replacement.

6. Discussion

Our patient has 2 compound heterozygous mutations in the ALADIN gene consistent with the recessive inheritance present in Allgrove syndrome. The first mutation is the heterozygous IVSC14 +1 G to A change which has been reported previously in Algerian and Hispanic patients and, therefore, is highly likely to be pathogenic [5,7]. This splice site mutation produces various abnormal messenger RNA

transcripts and leads to premature translation termination upstream from exon 16 [5].

The second mutation is the heterozygous exon 6, R155P change not previously reported. It results in changes of amino acid sequence and is not present in controls. The mutation is located in the first WD repeat of the protein. The amino acid change that this mutation produces may disrupt the β-propeller structure of the protein, leading to a conformational change of the translated protein and impairing putative protein-protein interactions [4]. For these reasons and also because most individuals with Allgrove syndrome have 2 mutations in the ALADIN gene, the newly reported exon 6, R155P mutation in our patient is likely to be pathogenic.

Certain correlations between genotype and phenotype in individuals with Allgrove syndrome have been reported, although such studies are small and affected by a significant heterogeneity in clinical features as well as genetic characteristics [6,7]. For example, the IVSC14 +1 G to A mutation was present in all Puerto Rican patients in one case series and was associated with adrenal abnormalities in all patients [7]. We are unable to conclude whether the new mutation (R155P) in our patient is responsible for the difficulties that we encountered in diagnosing her adrenal insufficiency. Further genotype-phenotype association studies as well as formal functional studies will be required to better characterize individuals with this mutation.

Adrenal insufficiency in patients with Allgrove syndrome usually is not present at birth but may manifest itself as a progressive disorder of isolated glucocorticoid deficiency diagnosed at a variable time from birth until adult age. Postmortem adrenal histology in few cases demonstrated atrophy of zona fasciculata and reticularis with a relative preservation of the zona glomerulosa, suggestive of an isolated abnormality in glucocorticoid function [9]. Abnormalities in adrenal androgen production, characterized by low serum dehydroepiandrosterone sulfate (DHEA-S) concentration and blunted DHEA response to ACTH stimulation, however, also have been reported [10].

Metyrapone test can be inconvenient and dangerous in patients with Allgrove syndrome because it may precipitate adrenal crisis in patients with compensated adrenal insufficiency [11]. Insulin-induced hypoglycemia is a "gold standard" test; however, it is time-consuming, inconvenient, and contraindicated in some patients with history of seizure disorders or ischemic heart disease [9]. For these reasons, ACTH stimulation test has largely substituted the above tests for diagnosing primary and secondary adrenal insufficiency and for monitoring adrenal function in patients with Allgrove syndrome.

In the initial version of this test, an 8-hour infusion of purified ACTH extracted from animal pituitaries was used on 3 consequent days and urine steroid metabolites were measured to assess response [12,13]. The short ACTH stimulation test, using 250 μg of ACTH, was introduced after the synthesis of ACTH [1-24] and the development of plasma cortisol assay methods. From the early studies it became clear that cortisol responses to 4 or 100 μg of ACTH infusion or to a single intramuscular injection of 250 μg of ACTH were similar [14]. The 250-μg ACTH stimulation test became a standard method for diagnosing adrenal insufficiency because of its convenience.

The 250-μg ACTH stimulation test, however, was found to be inferior to insulin-induced hypoglycemia [15] or metyrapone test [14] in diagnosing adrenal insufficiency because of its low sensitivity. Injection of 250 μg ACTH produces supraphysiologic plasma ACTH concentration that may overcome and mask subtle abnormalities of hypothalamic-pituitary-adrenal axis or adrenal gland ACTH-insensitivity. Mean plasma ACTH concentrations in stressful conditions (such as severe sepsis or multiple trauma) are similar to plasma ACTH concentrations after the injection of 1μg of ACTH or insulin-induced hypoglycemia and are almost 10-fold lower than the mean plasma ACTH concentrations achieved after injection of 250 μg of ACTH [17-19].

Infusion of 0.02 μg/kg of ACTH results in serum cortisol and plasma ACTH concentrations comparable to those found in insulin-induced hypoglycemia [20]. Therefore, 1-μg ACTH stimulation test has been developed [21]. At this time there is no agreement on the diagnostic criteria for the 1-μg stimulation test. In addition, technical problems in administering 1 μg of ACTH and the need to use intravenous route for administration make it less favorable in daily practice. Discrepancies between 1- and 250-μg ACTH stimulation tests are common [17,22-27].

In our patient, who had a high pretest probability for adrenal insufficiency, 1-μg ACTH stimulation test demonstrated subnormal response of cortisol on 4 out of 6 occasions while the 250-μg ACTH stimulation test response was consistently normal. Thus, neither of these tests was reliable to diagnose adrenal insufficiency with confidence and insulin-induced hypoglycemia was required to establish the diagnosis of adrenal insufficiency with certainty.

Because it is impossible to conclude that inability to establish the diagnosis of adrenal insufficiency using ACTH stimulation tests in Allgrove syndrome is limited to patients with the 2 mutations described in this paper, we would like to propose the following approach for evaluation of all patients with Allgrove syndrome for presence of adrenal insufficiency. Diagnostic evaluation could begin with 250-μg ACTH stimulation test. If there is inadequate rise in serum cortisol concentration, then the diagnosis of adrenal insufficiency is proven and no further testing is necessary. However, if the response to 250 μg ACTH is normal, one should proceed directly to the insulin-induced hypoglycemia test. The accurate diagnosis of adrenal insufficiency in patients with Allgrove syndrome is important because they may need either daily or intermittent (during periods of stress) glucocorticoid replacement therapy.

We conclude that a novel R155P mutation in the ALADIN gene is associated with Allgrove syndrome and

that insulin-induced hypoglycemia, rather than ACTH stimulation tests, should be used for a definitive diagnosis of adrenal insufficiency in this disorder.

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