CASE REPORT

Corticotroph adenoma of the pituitary in a patient with X-linked adrenal hypoplasia congenita due to a novel mutation of the DAX-1 gene

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Abstract

Objective: Mutations in the DAX-1 gene result in X-linked congenital adrenal hypoplasia. The classic clinical presentation is primary adrenal insufficiency in early life and hypogonadotropic hypogonadism at the time of expected puberty, but recent data have expanded the phenotypic spectrum of DAX-1 mutations. We report the occurrence of an ACTH-secreting adenoma in a patient with X-linked congenital adrenal hypoplasia.

Design and methods: Detailed clinical, radiological and pathological investigation of the pituitary adenoma. Genomic analysis of the DAX-1 gene in the patient and his mother.

Results: In this patient, primary adrenal failure had been diagnosed at 3 years of age and, despite replacement therapy, at 30 years of age progressive pigmentation developed and impairment of the visual field followed. ACTH was 24 980 pg/ml and nuclear magnetic resonance disclosed a huge pituitary adenoma. Three transsphenoidal operations and radiotherapy were necessary to remove the tumor mass and control ACTH secretion. Histologically, the adenoma was composed of chromophobic and basophilic neoplastic cells with positive immunostaining for ACTH. Moreover, a novel mutation was found both in the patient and his mother: a 4 bp insertion (AGCG) at nucleotide 259, in exon 1 resulting in a frame shift and premature termination.

Conclusions: This case suggests that in adrenal hypoplasia congenita the development of a pituitary adenoma should be considered when a sudden rise of ACTH occurs despite adequate steroid substitution.


Introduction

X-linked adrenal hypoplasia congenita (AHC, OMIM:300200) is characterized by primary adrenal insufficiency and hypogonadotropic hypogonadism. It is caused by mutations of the DAX-1 gene which controls the development of the adrenal and the hypothalamic-pituitary–gonadal axis (1, 2).

So-called feedback pituitary adenomas are tumors developing in the setting of the hyperplasia of pituitary cells secondary to untreated primary thyroid, gonadal and adrenal failure (3–5). In these cases the differential diagnosis between hyperplasia and adenoma may be difficult in clinical practice but true adrenocorticotropic hormone (ACTH)-secreting adenomas have occasionally been reported in Addison’s disease (6–9).

We report an ACTH-secreting adenoma occurring in a patient with X-linked AHC. This is the first case of association between pituitary adenoma and AHC, furthermore, the patient was found to carry a novel mutation of the DAX-1 gene.

Case report

A 33-year-old man was admitted for a symptomatic pituitary mass. His parents were alive and in good health, as were his 51-year-old sister and his 20-year-old nephew.

At the age of 3 years, primary adrenal failure was diagnosed during hospitalization for loss of consciousness. From that time sole treatment had been with cortone acetate, the daily dose ranging from 25 to 50 mg. The diagnosis was confirmed at the age of 9 years (urinary 17-hydroxycorticosteroids, <0.5 mg/24 h; 17-ketosteroids, 1.63 mg/24 h; serum dihydroepiandrosterone-sulfate (DHEA-S), 0.12 μg/ml). At the same age he was
found to have primary hypothyroidism (total thyroxine, 5.37 μg/100 ml) which was treated with thyroid extracts. Delayed puberty was diagnosed at the age of 17 years: testosterone was 0.26 ng/ml, basal luteinizing hormone (LH) was 0.55 mIU/ml and follicle-stimulating hormone (FSH) 0.78 mIU/ml, with a peak after gonadotropin-releasing hormone (GnRH) infusion of 0.92 and 1.23 mIU/ml respectively. Skull X-rays were negative. For a short period he was treated with human chorionic gonadotropin (HCG) and testosterone undecanoate and thereafter daily nasal puffs of gonadorelin. An adrenal crisis occurred at the age of 29 years during an infection of the upper respiratory airways.

Since the age of 30 years, he has developed progressive skin pigmentation and overweight. At the age of 33 years bilateral superior quadrantopsia occurred. A computed tomography scan revealed a large pituitary mass. On admission, his height was 187 cm and weight 108 kg (body mass index (BMI) 30.9) with abdominal distribution of fat. Only scanty pubic hair (P2) was present and the penis was short with slight hypospadias. Testicular volume was 3 ml bilaterally. Hyper-pigmentation of the skin and buccal mucosa was easily evident. Therapy at that time was cortone acetate 25 t.i.d. (thrice in die), Tiroide Vister (extracts of thyroid), and nasal gonadorelin. Routine blood tests were normal. Serum testosterone was 0.2 ng/ml, basal FSH and LH were both 0.1 mIU/ml with no response to 100 μg i.v. of GnRH. Serum-free thyroxine was 0.61 ng/100 ml and thyroid-stimulating hormone (TSH) 16.3 mIU/l. Antibodies against thyroid peroxidase, thyroglobulin and adrenals were negative. Serum prolactin was 19 ng/ml, growth hormone (GH; mean of four 30 minutes samples) was less than 0.1 ng/ml and insulin-like growth factor-I (IGF-I) was 103 ng/ml (normal values 110–465). Alpha-subunits were 0.3 IU/l (normal values 0.1–0.5). Despite replacement therapy, serum ACTH concentration was 24 980 pg/ml. After withdrawal of cortone for 24 h, serum cortisol was less than 0.2 μg/100 ml, DHEA-S less than 300 ng/ml, 17-hydroxyprogesterone less than 0.1 ng/ml and urinary free cortisol was 30 μg/24 h. Magnetic resonance (MR) scan showed a 3 × 2.5 cm pituitary lesion with suprasellar extension as well as invasion of the right cavernous sinus. The lesion was hypointense on T1-weighted sequences, and markedly non-homogeneous after gadolinium administration (Fig. 1). On transsphenoidal approach, because the tumor was firm and bleeding only partial removal was possible. The patient was discharged and prescribed cortone acetate 50 mg daily divided in three doses, fludrocortisone 50 μg daily, L-thyroxine 100 μg daily and testosterone enantate 250 mg every 3 weeks. Five months after surgery, his visual field had

![Figure 1](https://www.eje-online.org)
improved but there was no fading of skin pigmentation. MR imaging (MRI) showed a large residual tumor in the suprasellar region and in the right cavernous sinus. ACTH was 2180 pg/ml at 0800 h, 602 pg/ml at 2300 h and suppressed to 276 pg/ml after 1 mg of dexamethasone at midnight (for comparison, in two patients with genetically confirmed X-linked adrenal hyperplasia and normal nuclear MR (NMR) pituitary imaging, we found that 1 mg dexamethasone overnight suppressed ACTH from 1250 and 545 to 13 and 5 pg/ml respectively). Two additional transsphenoidal operations were performed to remove the residual tumor. After the first operation, morning ACTH level was 1660 pg/ml but fell to 150 pg/ml after the second, pigmentation also disappeared. The patient was given conventional external radiotherapy, 4800 cGy, to treat a small residue in the cavernous sinus. Two years later morning ACTH concentration is now 19 pg/ml and pituitary NMR is unchanged.

**Pathological findings**

Histologically, the primary tumor was composed of chromophobie and basophilic neoplastic cells showing a diffuse pattern of growth. Mitoses were absent. The majority of neoplastic cells were periodic acid-Schiff (PAS) positive and expressed ACTH. No other pituitary hormone was seen. The tissue from the second operation contained bands of dense and focally hyalinized collagen and several psammomatous calcifications, which were most likely due to previous surgery. There was no normal adenohypophysis in all samples examined.

**Genomic analysis**

Using standard methods DNA was extracted from the blood leucocytes of both the patient and his mother. The DAX-1 gene was PCR amplified using specific primers and the PCR products were directly sequenced according to methods previously described (1). A 4 bp insertion (AGCG) at nucleotide 259, in exon 1, resulting in a frame shift and premature termination, was identified in both the patient and his mother. No other mutations were detected in the coding region.

**Discussion**

We have reported the first case of a patient with X-linked adrenal hypoplasia congenita who developed an ACTH-secreting invasive macroadenoma of the pituitary gland three decades after the diagnosis of primary adrenal failure. The patient had a previously unreported 4 bp insertion at exon 1 of the DAX-1 gene.

Our patient developed an ACTH adenoma despite glucocorticoid replacement. After the first operation, he was treated with cortone 50 daily, but this dose failed to fade skin pigmentation or reduce the size of the lesion. ACTH did not suppress after dexamethasone, and control of ACTH was only achieved after three operations and radiotherapy. Histologically, the adenoma was indistinguishable from other ACTH adenomas and there was no normal gland in surgical samples to demonstrate an accompanying hyperplasia.

In untreated primary adrenal failure the pituitary gland shows diffuse or nodular hyperplasia, tumorlets and true microadenomas (4). In a few instances, hyperplasia may mimic a tumor causing sellar enlargement and symptoms due to mass effect (10–15). Hyperplasia differs from an adenoma in that it reduces or even disappears with substitution therapy although a few patients may have incomplete response to conventional replacement therapy because of the short half-life of hydrocortisone and cortone acetate or inter-individual variations of absorption and metabolism of these drugs (16, 17). True ACTH adenomas occur more rarely in patients with Addison’s disease, Dexter et al. (6) and Jara-Albarran et al. (7) described two Addisonian patients with marked pigmentation, high serum levels without circadian rhythm of ACTH-melanocyte-stimulating hormone (MSH); exogenous glucocorticoids caused clinical signs of hypercortisolism without suppressing ACTH. Krautli et al. (8) reported two historically proven ACTH adenomas in patients with Addison’s disease, one of which had a symptomatic pituitary mass extending to the sphenoidal sinus. Another ACTH-secreting adenoma has been documented in a patient with generalized glucocorticoid resistance (9), giving further support to the role of glucocorticoid feedback on corticotroph proliferation and adenoma formation.

The patient reported here was found to carry a 4 bp insertion at nucleotide 259 of the DAX-1 gene, causing a frame shift that probably prevented synthesis of a functional protein.

The DAX-1 gene is composed of two exons separated by a 3.4 kb intron and encodes a 470-amino-acid protein that belongs to the orphan nuclear receptor superfamily. The NH2 terminus consists of three 66–69 repeats and is devoid of the typical zinc finger DNA binding motif. The COOH-terminus (273–470) is very similar to the ligand-binding domain (LBD) of nuclear receptors, but no specific ligand has so far been identified for this protein. The encoded protein binds to DNA hairpin structures (18) and also to RNA (19).

DAX-1 is essential for the normal development of the steroidogenic axis and sex determination. It has been shown to repress transcription of several genes expressed in the adrenal cortex and hypothalamic-pituitary–gonadal axis. In particular, DAX-1 interacts with the co-repressors N-CoR (20) and alien (21) and inhibits the transcriptional effects of steroidogenic factor-1 (SF-1) through a specific transcriptional silencing domain within the carboxyterminus (20, 22). All
DAX-1 missense mutations identified in patients with AHC alter the protein C-terminus and impair its transcriptional repressor activity.

Patients with mutation of the DAX-1 gene present primary adrenal insufficiency in early infancy or childhood and hypothalamic hypogonadism during puberty. Phenotype is not correlated with genotype (23, 24) and different genetic and environmental factors may contribute to the ‘non-classic’ phenotypes reported in recent years. Adrenal failure may be transient (25), asymptomatic (26) or manifest in adulthood (27). In addition, isolated hypothalamic hypogonadism has been reported in a female patient (28) although, in the absence of adrenal insufficiency, DAX-1 mutations are an uncommon cause of hypogonadotropic hypogonadism or pubertal delay (29).

The role of DAX-1 in the pathogenesis of pituitary adenomas has not been established. In humans, DAX-1 is expressed at the hypothalamus and pituitary gland (30, 31) where it regulates the transcription of LH-beta subunit, the activation of the inhibin alpha promoter and down-regulates transcriptional activity mediated by androgen and estrogen receptors (32, 33). No data are available on DAX-1 in normal ACTH-secreting cells and DAX-1 is not expressed in the AtT-20 cell line, derived from corticotropes (34). In human pituitary adenomas DAX-1 mRNA has been found in tumors of the gonadotropic lineage and in somatotropic adenomas that co-secreted LH (12). In tumors of the gonadotropic lineage and in somatotropic adenomas that co-secreted LH (12) and in prolactinomas (35, 36). No data are available about expression of DAX-1 in ACTH-secreting adenomas.

In summary, we have described a pituitary ACTH adenoma in a patient with X-linked AHC due to a novel mutation of DAX-1. The reported case suggests that these patients should be carefully monitored for direct and indirect signs of pituitary adenoma, especially when a sudden rise of ACTH levels occurs despite adequate steroid substitution.

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