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A Case of Congenital Adrenal Hypoplasia Caused By the Deletion of DAX-1 Gene on X Chromosome

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Abstract We report a case of congenital adrenal hypoplasia who presented with primary adrenal insufficiency in infancy. Fluorescent in situ hybridization identified a deletion of DAX-1 gene on his X chromosome. He improved dramatically after the hydrocortisone, fludrocortisone and sodium chloride treatment.

Key words Congenital adrenal hypoplasia; DAX-1 gene

Introduction

Congenital adrenal hypoplasia (AHC) is a rare cause of adrenal insufficiency during early infancy. AHC is inherited in an X-linked or autosomal recessive fashion. The X-linked form is caused by a mutation or deletion of the DAX-1 gene on the X chromosome. It is usually associated with hypogonadotropic hypogonadism and hearing impairment in adolescence. It also forms part of a contiguous chromosome deletion syndrome, including glycerol kinase deficiency and Duchenne muscular dystrophy. We presented a Chinese patient with congenital adrenal hypoplasia and deletion of DAX-1 gene. Early detection and treatment of this condition will improve the clinical outcome.

Case history

An 11-day-old baby boy presented with poor feeding, repeated vomiting and increased skin pigmentation. He was born at 38 weeks gestation by caesarian section for breech presentation. His birth weight was 2815 grams. Parents had no consanguinity and there was no family history of endocrine or renal diseases. His heart rate was 150 beats/minute, respiratory rate 50 breaths/minute and blood pressure 72/47 mmHg. On physical examination, diffuse increased skin pigmentation was noted. The stretched penis was 2.5 cm. Left testicle (2 ml) was palpable in scrotum. Right testicle was not palpable and a reducible hernia was noticed in the right inguinal area. He was not dehydrated and the body temperature was normal. On auscultation the chest was clear with no added sounds. The liver was within the normal limits and the spleen was not palpable. Cardiovascular, abdominal and neurological examinations were unremarkable. His head circumference was at the 10th percentile, weight was at the 3rd percentile, and length was at the 3rd percentile.

The initial laboratory tests showed sodium 121 mmol/L, potassium 8.8 mmol/L, blood urea nitrogen 2 mmol/L and creatinine 88 umol/L. His white cell count was 16.4×10^9/L with 21% neutrophils, 64% lymphocytes, 8% monocytes and 0% basophils. His haemoglobin was 14.8 g/dL and his platelet count was 349×10^9/L. Spot glucose was 6.5 mmol/L. Serum albumin, aspirate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, calcium, phosphate, bilirubin and arterial blood gases were normal. Chest and abdominal X-rays were unremarkable.

Further endocrine investigations were as follows: basal cortisol level, 155 nmol/L; adrenocorticotropic hormone (ACTH) level, >341 pmol/L (normal 2-11.4); 17 alpha

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hydroxyprogesterone (17-OHP), 3.9 nmol/L (normal 0.9-6.6); testosterone, 3.3 nmol/L (normal adult male 8.4-28.7); luteinizing hormone (LH), 2.0 IU/L (normal 2-12); follicle stimulating hormone (FSH), 4.5 IU/L (normal 1.8-8); dehydroepiandrosterone-sulphate (DHEAS), <0.1 umol/L (normal <4); aldosterone, < 45 pmol/L (normal 111-860), renin level, >5.5 ng/ml/hr (normal supine 0.51-2.64, normal erect 0.97-4.18); triglycerides, 0.8 mmol/L (normal 0.5-2.2) and very long chain fatty acids (VLCFA) concentration was normal. After ACTH stimulation, maximum serum cortisol level was 161 nmol/L and maximum serum 17-OHP level was 6.2 nmol/L. Urine steroids chromatography (data not shown) excluded defects in steroid biosynthesis associated with salt-losing congenital adrenal hyperplasia. Adrenal CT scan was normal with no haemorrhage, calcification or other focal lesions. It did not show the characteristic features of congenital adrenal hypoplasia, such as lack of definitive zone of adrenal cortex and vacuolated cells. A blood sample (5 ml whole blood in sodium heparin) of this patient was sent to Kleberg Cytogenetics Laboratory, Baylor College of Medicine, Houston. Fluorescent in situ hybridization (FISH) analysis, using AHC FISH probe, was performed on metaphase chromosomes of 20 cells in this patient. The absence of hybridization signals indicated a deletion in the adrenal hypoplasia congenital critical region on the X chromosome, consistent with the clinical diagnosis of AHC. However, FISH analysis was not done on his mother at risk of being a carrier.

The diagnosis was AHC. Contiguous deletion syndrome occurring in combination with glycerol kinase deficiency (GKD) and Duchenne muscular dystrophy (DMD) was excluded in our patient by normal levels of creatine kinase and triglycerides.

Hydrocortisone, fludrocortisone and sodium chloride supplement were initiated. His potassium dropped to 4.2 mmol/L and sodium increased to 138 mmol/L four days after the replacement. His urine output and blood pressure were stable. Over the course of 7 weeks in the hospital, sodium and potassium were maintained in the normal ranges. He had no vomiting, tolerated feeding and steady weight gain. Hyperpigmentation gradually disappeared. At the time of discharge on hospital day 50, his sodium was 141 mmol/L and potassium was 6.4 mmol/L.

Discussion

The clinical and biochemical findings of this infant directed our investigations to adrenal insufficiency with salt losing crisis. Other clinical features of combined glucocorticoid and mineralocorticoid deficiencies including dehydration, hypotension, convulsion, hypoglycaemia and metabolic acidosis were fortunately not present in this infant. The diagnosis of primary adrenal insufficiency was further supported by an elevation of ACTH concentration and suboptimal cortisol response level after ACTH stimulation.

The causes of primary adrenal insufficiency in infants can be divided in congenital and acquired forms (Table 1). Infants with *pseudohypoaldosteronism* have a similar clinical presentation of salt-wasting syndrome but the plasma and urine aldosterone levels are very high. Infants with this disorder usually do not respond to steroid administration. *Congenital adrenal hyperplasia* (CAH), caused by the salt-losing form 21-hydroxylase deficiency (21-OHD), should be considered in the differential diagnosis of AHC. Serum concentration of 17-OHP is elevated in 21-OHD, but normal or low in AHC. Thus normal basal 17-OHP and blunted 17-OHP response after ACTH stimulation in this infant support the diagnosis of AHC. Patients with *ACTH unresponsiveness* usually present in childhood with increasing skin pigmentation and hypoglycaemia but without symptoms of mineralocorticoid deficiency. Therefore plasma renin and aldosterone concentrations should be normal. Another rare cause of adrenal failure is *adrenoleukodystrophy* but it does not present in the neonatal period with salt-losing crisis. The plasma level of saturated unbranched very long chain fatty acids should be elevated in this disease. CT scan of the adrenal glands was performed to look for other uncommon causes of primary adrenal insufficiency in newborns, such as *adrenal haemorrhage, calcifications and focal lesions.*

Congenital adrenal hypoplasia is a developmental disorder of the adrenal gland inherited in an X-linked recessive manner. It is a rare disease with an incidence of 1 in 12500 births. The genetic locus for AHC has been mapped to Xp21.3, which is the dosage sensitive sex reversal locus, important in sex determination. Peter et al studied 18 AHC boys from 16 families. Fifteen patients were available for molecular analysis of the DAX-1 gene. He found that 6 patients had gene deletions and 7 had point mutations in the DAX-1 gene.[1] The gene deletion can be identified by fluorescent in situ hybridization studies using DAX-1 probe. AHC may be part of a contiguous gene deletion syndrome that includes GKD and DMD. GKD is diagnosed by the measurement of the serum concentration of triglycerides and urine glycerol. DMD is suspected if

Table 1 Causes of primary adrenal insufficiency[6]

Congenital	Acquired
Congenital adrenal hyperplasia (CAH)	Autoimmune Isolated Type I PGA Type II PGA
Congenital adrenal hypoplasia Miniature Cytomegalic 1. X-linked: DAX-1 deficiency 2. Associated with hypogonadotropic hypogonadism, Duchenne muscular dystrophy, glycerol kinase deficiency	Infectious Tuberculosis, coccidioidomycosis, histoplasmosis, torulosis, etc. Meningococcemia HIV associated
ACTH unresponsiveness Isolated Associated with alacrima and achalasia (triple A syndrome)	Infiltrative diseases Hemochromatosis, amyloidosis, sarcoidosis, metastatic malignancy Trauma Tumour Drugs 1. Steroid synthesis inhibitors 2. Glucocorticoid antagonist 3. Steroid catabolism enhancers
Aldosterone deficiency ↑ Aldosterone (P-450) ↓ Aldosterone = pseudohypoaldosteronism	
Adrenoleukodystrophy	
Wolman disease (lysosomal acid lipase deficiency)	

PGA=polyglandular autoimmune syndromes; ACTH=adrenocorticotropic hormone

the serum concentration of creatine phosphokinase is increased; the diagnosis is confirmed by molecular genetic testing of the DMD gene or dystrophin testing on a muscle biopsy. Point mutations in the DAX-1 gene also occur in patients with AHC, which can be detected by DNA sequencing.

Patients with infantile-onset acute adrenal insufficiency present at an average age of 3 weeks (range 1 week-3 years).[1] The initial clinical presentations are vomiting, feeding difficulty, dehydration, and shock caused by a salt-wasting episode. Hypoglycaemia, frequently presenting with seizures, may be the first symptom of AHC but is relatively uncommon. Adrenal insufficiency is rapidly lethal as a result of hyperkalaemia, acidosis, hypoglycaemia, and shock if the condition is not treated appropriately. Delay in treatment can cause neurodevelopmental abnormalities as sequels of shock and hypoglycaemia. On the contrary, adequate glucocorticoids and mineralocorticoids replacement would improve the clinical outcome. Their dosages must be increased with stress, such as trauma, surgery and intercurrent illnesses. On the other hand chronic steroid treatment can cause growth retardation. Therefore the treatment should be optimised to allow normal linear growth but avoid adrenal insufficiency. However the lack of biochemical markers in this condition causes difficulties in monitoring.

A small number of patients with AHC have delayed presentation after infancy, usually between the ages of 2 to 9 years. Therefore children with AHC may erroneously diagnosed to have acquired adrenal failure, such as autoimmune causes. Thus Loke suggested screening the DAX-1 gene for mutation in all patients presenting with primary adrenal insufficiency in order to establish an early diagnosis of AHC.[2]

Isolated hypogonadotropic hypogonadism (HHG) is also a feature of AHC. It remains unclear whether HHG associated with DAX-1 mutations results from hypothalamic or pituitary dysfunction. Recent study suggests that they may both be involved, consistent with the expression of DAX-1 in the hypothalamus and the

pituitary gland.[3] It can cause cryptorchidism and delay in puberty (onset after 14 years of age) in affected males. Without testosterone treatment, secondary sexual characteristics do not appear but affected males are still infertile despite treatment with exogenous gonadotropin therapy or pulsatile GnRH. Recently Tabarin reported a 28-year-old male presenting with a milder form of HHG. He had sufficient testosterone concentration to allow significant masculinization to occur during puberty and he was able to have sexual activity. However semen analysis revealed severe oligospermia, which did not improve after gonadotropin therapy, suggesting concomitant primary testicular abnormality.[4] This raises the possibility that the DAX-1 gene defect may affect spermatogenesis directly, indicating that the impaired spermatogenesis results in mechanisms other than HHG.

Progressive high frequency hearing impairment is reported to be associated with AHC. It usually starts at about 14 years of age. The hearing loss progresses with age, from high frequency to low frequency.[5] Therefore patients with this syndrome should be examined for hearing loss.

Carrier females may occasionally have symptoms of adrenal insufficiency or hypogonadotropic hypogonadism.

Conclusion

Clinicians should be aware that adrenal insufficiency may have a subtle or a life-threatening presentation. It is important to consider the possibility of AHC in infants who have primary adrenal insufficiency. This patient illustrates that early recognition and treatment will improve the clinical outcome. Molecular analysis of DAX-1 gene not only enable us to diagnose this important condition that has prognostic and counseling implications but also alert us that hypogonadotropic hypogonadism would occur in patients with DAX-1 mutation at the expected time of puberty, and hearing impairment in patients with DAX-1 deletion. Long term monitoring of this patient is essential but it would be difficult because of the lack of objective biochemical markers.

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