

Original Article

Clinical characteristics and mutation analysis of two Chinese children with 17 α -hydroxylase/17,20-lyase deficiency

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Abstract: Combined with the literature, recognize the clinical features and molecular genetic mechanism of the disease. 17 α -hydroxylase/17,20-lyase deficiency, a rare form of congenital adrenal hyperplasia, is caused by mutations in the cytochrome P450c17 gene (CYP17A1), and characterized by hypertension, hypokalemia, female sexual infantilism or male pseudohermaphroditism. We presented the clinical and biochemical characterization in two patients (a 13 year-old girl (46, XX) with hypokalemia and lack of pubertal development, a 11 year-old girl (46, XY) with female external genitalia and severe hypertension). CYP17A1 mutations were detected by PCR and direct DNA sequencing in patients and their parents. A homozygous mutation c.985_987delTACinsAA (p.Y329KfsX418) in Exon 6 was found in patient 1, and a homozygous deletion mutation c.1459_1467delGACTCTTTC (p.Asp487_Phe489del) in exon 8 in patient 2. The patients manifested with hypertension, hypokalemia, sexual infantilism should be suspected of having 17 α -hydroxylase/17,20-lyase deficiency. Definite diagnosis is depended on mutation analysis. Hydrocortisone treatment in time is crucial to prevent severe hypertension and hypokalemia.

Keywords: 17 α -hydroxylase/17,20-lyase deficiency

Introduction

Deficiency in cytochrome p450c17 (MIM 202110) is an extremely rare form of congenital adrenal hyperplasia (CAH) [1]. The encoding gene of P450c17, cytochrome P450c17 gene (CYP17A1), is located on chromosome 10q24-q25, consisting of eight exons, and mainly expressed in the adrenal glands and gonads [2]. The mutations of CYP17A1 may lead to complete combined 17 α -hydroxylase/17,20-lyase deficiency or isolated 17,20-lyase deficiency [3]. It is characterized by variable degree of hypertension, hypokalemia, female sexual infantilism or male pseudohermaphroditism [4]. The diagnosis of 17 α -hydroxylase/17,20-lyase deficiency is depended on clinical, biochemical, and molecular features. However, clinical and biochemical presentations are variable, mainly based on the activities of these enzymes. Therefore, mutation analysis is critical for definite diagnosis. We herein investigated the clinical and biochemical features of two

patients with 17 α -hydroxylase/17,20-lyase deficiency, and made a confirmative diagnosis by mutation analysis of CYP17A1.

Materials and methods

Subjects

We reviewed clinical data of two patients admitted to our hospital. The patients were clinically diagnosed as 17 α -hydroxylase/17,20-lyase deficiency according by clinical and biochemical findings. Genomic DNA was extracted from peripheral blood leukocytes using by TIANamp Bood DNA Kit (Tiangen Biotech, Beijing, China). All the exons of CYP17A1 were amplified by polymerase chain reaction (PCR) with subsequent directing sequencing. This study was approved by the Nanjing Children's Hospital Affiliated to Nanjing Medical University. All of the patients and their parents were informed and signed informed consents.

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Table 1. The clinical, biochemical, hormonal and mutation findings in the patients

	Patient 1	Patient 2
Age at diagnosis (yr)	13	11
Height (cm)/Weight (kg)	155/44	145/45
Bone age (yr)	9	7
Blood pressure (mmHg)	148/102	188/109
Breast stage (breast/pubertal hair)	B1P1	B1P1
Karyotype	46, XX	46, XY
Na/K (mmol/L)	144/1.38	142/3.48
ACTH (8:00AM, 0-46 pg/mL)	51.18	56.70
LH (2.4-12.6 mIU/mL)	65.38	28.71
FSH (3.5-12.5 mIU/mL)	70.72	91.59
Estradiol (46-607 pmol/mL)	74.46	47.65
17OHP (4.5-11.9 ng/mL)	0.1	1.0
PRA (0.93-6.56 ng/ml/h)	0.37	0.04
Ang-II (25.3-145.3 pg/mL)	63.57	51.10
Aldosterone (48.5-123.5 pg/mL)	135.86	91.95
<i>CYP17A1</i> mutation	p.Y329 fs	p.D487_F489 del

ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; FSH, follicular stimulating hormone; 17OHP, 17 α -hydroxyprogesterone; PRA, plasma renin activity; Ang-II, Angiotensin II.

Results

Case presentations

Patient 1 was a 13-year-old Chinese girl, who presented with a progressively limb weakness, accompanied with neck pain and dizziness for one week. She was admitted to our hospital for acute unconsciousness for 3 hours, accompanied with dyspnea and gaitism. At physical examination she was a prepubertal girl with normal infantile female external genitalia but with blind vagina. Blood pressure was 148/102 mmHg. Muscle power of double upper limbs and double lower limbs was grade 3 and 2, respectively, with low level of serum potassium, 1.38 mmol/L. Serum sodium, blood urea nitrogen, creatinine, and blood PH were normal. X-ray examination of her bones revealed bone age retardation. Echocardiography was normal. A small uterus and absence of annexes were revealed by ultrasound imaging. Computed tomography scan showed enlargement of bilateral adrenal glands.

Her karyotype was 46, XX. Serum LH and FSH levels were high. She had low level of androgen and normal estradiol. Basal plasma renin activity was low, with elevated aldosterone and

ACTH. She had low-normal basal level of cortisol, which did not rise after ACTH stimulation. Progesterone was high and 17OH-progesterone was low. The clinical and laboratory findings of patient 1 agreed with the diagnosis of 17 α -hydroxylase/17,20-lyase deficiency. During therapy with hydrocortisone, serum level of potassium and blood pressure were normalized. **Tables 1** and **2** summarizes the clinical, biochemical and hormonal findings of patient.

Patient 2 was a 11-year-old Chinese girl who was presented with hypertension and admitted to our hospital for difficulty in control of hypertension with antihypertensive therapy. Physical examination showed hypertension (188//109 mmHg), Tanner stage I breasts, female external genitalia, and absence of both pubic and axillary hair with a blind vagina. Her weight and height was 45 kg and 145 cm, respectively, with bone age retardation. Gonads were not

found in bilateral inguinal canals and the pelvic. The level of serum potassium was normal (3.48 mmol/L). Serum sodium, blood urea nitrogen, creatinine, and blood PH were normal. Echocardiography showed mild mitral, tricuspid regurgitation. Renal arteriography was normal.

The karyotype was 46, XY. She has high level of LH and FSH, with low testosterone and estradiol. Progesterone was high and 17OH-progesterone was low. Basal level of cortisol and was low. There was no response of cortisol and androgen to ACTH stimulation. Renin activity was suppressed. The plasma concentration of aldosterone was slightly elevated. On hydrocortisone therapy, the blood pressure was maintained at 140-158/74-89 mmHg. **Tables 1** and **2** summarizes the clinical, biochemical and hormonal findings of patient.

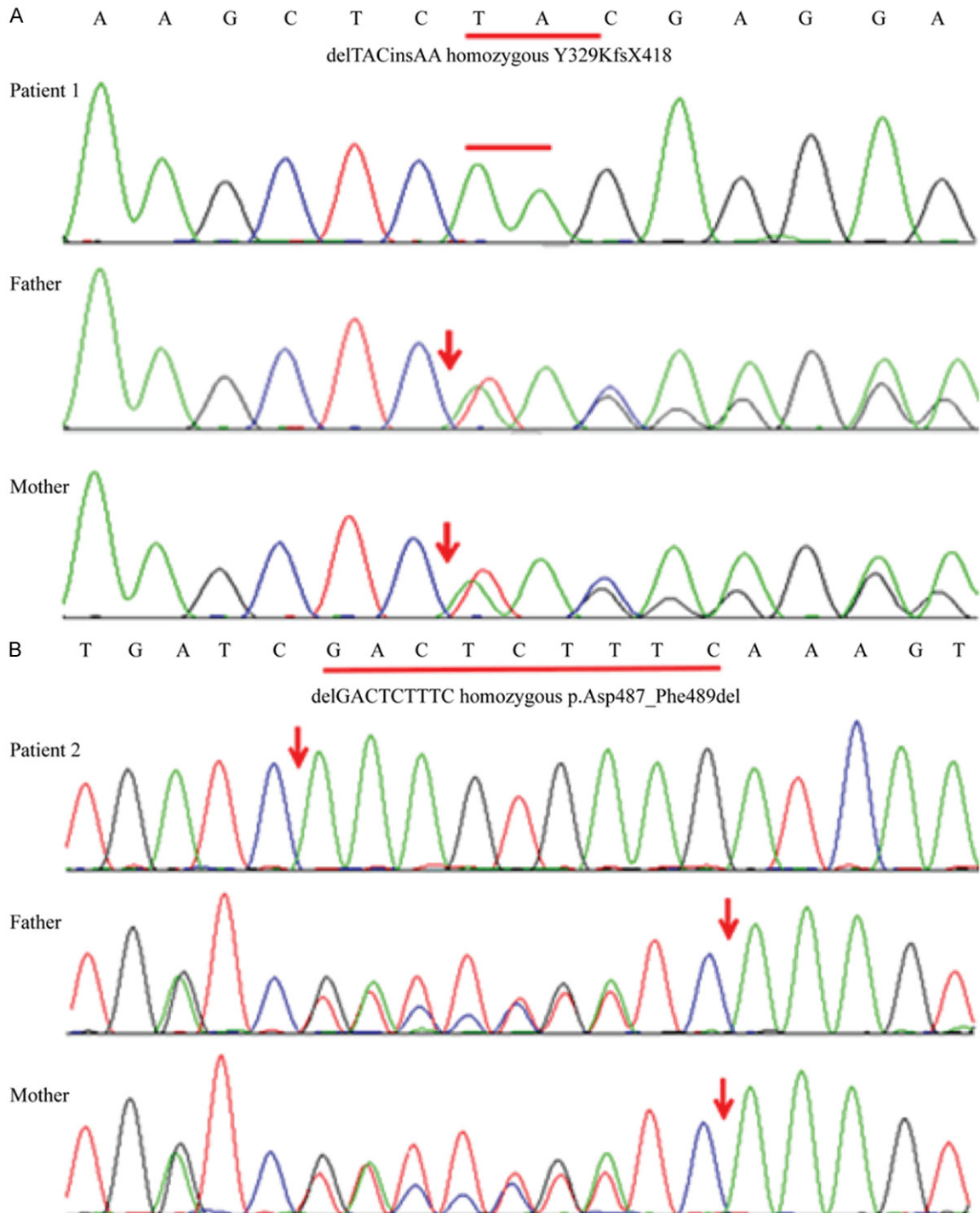
Mutation analysis of *CYP17A1* gene

Direct sequencing analysis of the *CYP17A1* gene showed that patient 1 carried a homozygous state in exon 6 (c.985_987delTACinsAA, p.Y329KfsX418) (**Figure 1**). Both her parents had been identified as heterozygous carriers of this mutation (**Figure 1**). Patient 2 was found to be a homozygous deletion mutation p.Asp487_

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Table 2. Plasma steroids before and after ACTH stimulation

Plasma steroids	Patient 1			Patient 2		
	0'	30'	60'	0'	30'	60'
Cortisol (171-536 nmol/L)	46.92	106.7	111.4	62.15	102.8	105.5
Progesterone (0.6-4.7 nmol/L)	17.52	28.52	30.09	25.8	40.53	42.36
Testosterone (0.42-38.5 nmol/L)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1



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Figure 1. Results of sequence analysis of genomic DNA from the patients and their parents. A: Homozygous for p.Y329KfsX418 (c.985_987delTACinsAA) in exon 6 identified from patient 1. Both her parents had been identified as heterozygous carriers of this mutation. B: Homozygosity of p.Asp487_Phe489del (c.1459_1467delGACTCTTTC) in exon 8 identified from patient 2. The parents were heterozygous carriers of this mutation.

Phe489del (c.1459_1467delGACTCTTTC) in exon 8 (**Figure 1**).

Discussion

The first clinical and biochemical description of 17 α -hydroxylase deficiency by Biglieri in 1966 [5]. Until now, a total of over 150 17 α -hydroxylase deficiency cases were reported. The microsomal enzyme P450c17 catalyzes two distinct reactions in the steroid pathway: 17 α -hydroxylation of steroid and the subsequent cleavage of C17-20 carbon bond, which are essential for the production of glucocorticoids and sex hormones [6]. Loss of P450c17 enzyme impairs cortisol production, and thus in turn increases ACTH secretion. ACTH drives compensatory overproduction of corticosterone and deoxycorticosterone. The weak glucocorticoids and significant mineralocorticoid action results in subclinical hypocorticalism, but severe hypertension and hypokalemia. By contrast, lyase deficiency causes a lack of sex hormones leading to absent or incomplete pubertal development in both sexes and 46, XY disorder of sex development (DSD) in male [7, 8]. CYP17A1 is the single coding gene of enzyme P450c17. Up to date, there are more than 90 mutations have been identified, including missense and nonsense mutations, insertions, deletions, and splice site variants (HGMD, <http://www.hgmd.org/>).

In our study, the clinical and laboratory findings of the patients agreed with the diagnosis of 17 α -hydroxylase/17,20-lyase deficiency. Patient 1 was found to be a homozygous mutation p.Y329KfsX418 in exon 6, which has been reported in Chinese patients with heterozygous or homozygous status [9]. Patient 2 was found to be a homozygous deletion mutation p.Asp487_Phe489del in exon 8, reported in 17 α -hydroxylase/17,20-lyase deficiency by Lam *et al.* [10]. The p.Y329KfsX418 and p.Asp487_Phe489del are proved lack both 17 α -hydroxylase and 17,20-lyase activity and the two most common mutations of the CYP17A1 gene in China, which are presumed to be caused by the founder effect in Asian populations [11-14]. Patient 2 (46, XY) was raised as a girl with

external genitalia, a blind vagina pouch and without the uterus and fallopian tubes. Some 46, XY male patients have ambiguous external genitalia or male pseudohermaphroditism in other studies. There is considerable heterogeneity in the clinical and biochemical features of 17 α -hydroxylase and 17,20-lyase deficiency. The age of onset and severity of hypertension, normotensive or normokalemic, and the aldosterone production rate do not seem to be consistent, even in those with same mutation [15]. In addition to CYP17A1, many modifying factors, such as other genes and environmental factors determine clinical manifestation [1].

Our patients both exhibited increased blood pressure at teenage, whereas several other cases of 17 α -hydroxylase and 17,20-lyase deficiency showed hypertension in infancy or in their thirties [16]. During the treatment of hydrocortisone, hypertension can be normalized in the majority of patients. In our case, the blood pressure of patient 2 failed to the normal level at the initial therapy of hydrocortisone. This phenomenon might be due to the cardiovascular impairment associated with prolonged high blood pressure [17]. Thus, in this situation, hydrocortisone and antihypertensives combination therapy is needed to control the blood pressure. Hypoaldosteronism has been observed in the majority of patients with 17 α -hydroxylase and 17,20-lyase deficiency. This was explained by suppressed renin-angiotensin system and hypokalemia due to accumulated deoxycorticosterone and corticosterone. However, in the present study, normal or high serum aldosterone was observed in our cases and some other patients in previous report [18]. Further studies are necessary to clarify this phenomenon.

In the reported cases, the majority of them were admitted for complaint of lack of puberty, and found to bear hypertension, and or hypokalemia by examination. 46, XY individuals with the hallmark of DSD seemed to be noticed early, but easily are misdiagnosed as androgen insensitivity syndrome (AIS) before hypertension and hypokalemia occur. 46, XY individuals with complete external genitalia usually are

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missed diagnosis and raised as girls. 46, XX individuals, especially the isolated 17,20-lyase deficiency with the normal blood pressure, in whom the diagnosis seems to be infrequently made, usually are misdiagnosed as gonadal dysgenesis, such as Turner syndrome. So, it is necessary to include the rare 17 α -hydroxylase/17,20-lyase deficiency in the differential diagnosis of 46, XY DSD and delayed puberty in girls because a missed diagnosis can lead to severe hypertension and reproductive dysfunction. Any patient presenting with hypertension, hypokalemia and sexual infantilism should be suspected of having 17 α -hydroxylase/17,20-lyase deficiency. CYP17A1 mutation analysis might be useful for accurate genetic diagnosis and early treatment. It's essential to allow induction of puberty at the appropriate time and to prevent complications resulting from uncontrolled hypertension. Hypertension and hypokalemia respond to hydrocortisone treatment. Hydrocortisone decreases mineralocorticoid production induced by the high ACTH. After identifying the phenotypic and psychosexual status of the patients, a sex steroid appropriate for the patient's phenotypic sex is started at the expected time of puberty. Prophylactic gonadectomy is recommended for genotypic males to prevent malignant change. Low-dose testosterone may be added for sexual hair development.

In summary, we have presented the clinical, metabolic and genetic findings in two additional patients with complete combined 17 α -hydroxylase/17,20-lyase deficiency. 17 α -hydroxylase/17,20-lyase deficiency should be suspected in any phenotype female patients manifested with hypertension, hypokalemia, metabolic alkalosis or sexual infantilism. As in the present study, mutation analysis can verify the diagnosis. Hydrocortisone treatment as early as possible is crucial to prevent severe hypertension and hypokalemia.

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Disclosure conflicts of interest

None.

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