Journal of Steroid Biochemistry & Molecular Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

New, recurrent, and prevalent mutations: Clinical and molecular characterization of 26 Chinese patients with 17alpha-hydroxylase/17,20-lyase deficiency

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ARTICLE INFO

Article history:
Received 16 October 2014
Received in revised form 10 February 2015
Accepted 11 February 2015
Available online xxx

Keywords:
CYP17A1 gene
17Alpha-hydroxylase/17,20-lyase deficiency
Congenital adrenal hyperplasia
Mutation

ABSTRACT

Background: Combined 17alpha-hydroxylase/17,20-lyase deficiency (17OHD), caused by mutations in the *CYP17A1* gene, is a rare autosomal recessive form of congenital adrenal hyperplasia and characterized by hyporeninemic hypokalemic hypertension, primary amenorrhea and absence of secondary sexual characteristics.
Subjects and methods: Twenty six 17OHD subjects from 23 Chinese families were recruited. The *CYP17A1* gene was sequenced and 17alpha-hydroxylase/17,20-lyase enzymatic activities were assessed in vitro.
Results: Eight *CYP17A1* mutations were identified in 23 patients. Of eight mutations, c.985_987delinsAA/p.Y329Kfs and c.1460_1469del/p.D487_F489del mutations accounted for 60.8% (28/46) and 21.7% (10/46) of the mutant alleles, respectively. The enzymatic activities for both mutations were completely abolished. We also identified three novel mutations c.971_972insG/p.K325Afx, c.1464_1466delT/p.F489Sfx and c.1386G>T/p.R462S. The enzymatic activities for c.971_972insG/p.K325Afx and c.1464_1466delT/p.F489Sfx mutations were almost completely abolished, whereas the mutation c.1386G>T/p.R462S only resulted in partial reduction of 17alpha-hydroxylase (34.6%) and 17,20 lyase activities (27.0%), which is correlated with the partial 17OHD phenotype in this patient.
Conclusion: The c.985_987delinsAA/p.Y329Kfs and c.1460_1469del/p.D487_F489del mutations are prevalent in Chinese 17OHD patients. The genetic defects are well correlated with the phenotypes in both complete and partial forms of 17OHD.

1. Introduction

Congenital adrenal hyperplasia resulting from 17alpha-hydroxylase and 17,20-lyase deficiency (17OHD) is a rare autosomal recessive disease, caused by mutations in *CYP17A1* gene. 17OHD is classified primarily as complete or partial forms. The complete 17OHD is much more common than the partial form [1,2]. The complete loss of 17alpha-hydroxylase and 17,20-lyase activity results in a reduction of cortisol and sex hormones, and accumulation of progesterone and 11-deoxycorticosterone (DOC). The compensatory elevation of ACTH secretion leads to adrenal cortex hyperplasia and mineralocorticoid excess, which results in hypertension, hypokalemia and suppression of renin and aldosterone. The defect of sex hormone synthesis results in a female-appearing external genitalia and delayed pubertal development in both sexes. The clinical characteristics in partial 17OHD patients are different from those in complete ones by partial breast development and pubic hair, and oligomenorrhea or secondary amenorrhea in 46,XX patients and ambiguous genitalia in 46,XY patients due to partial deficiency of 17alpha-hydroxylase and 17,20-lyase activity [3].

The *CYP17A1* gene consists of eight exons [4] and is expressed in several steroidogenic tissues, including the adrenal cortex, ovary, and testis. Since the first mutation was identified in a patient with 17OHD in 1988 [4], more than 100 different mutations have been

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http://dx.doi.org/10.1016/j.jsbmb.2015.02.007
0960-0760/

reported. Those mutations result in either combined or isolated 17 alpha-hydroxylase/17,20-lyase enzyme deficiencies [5]. Two large studies have been reported in different ethnical populations including Brazilian and Japanese cohorts. The founder effects were associated with the prevalent 17OHD in those two countries [6,7]. However, the relationship between the genotype and phenotype has not been well established in 17OHD patients [7–9].

In our current study, we reported the clinical characteristics and analyzed the *CYP17A1* mutations in the second largest cohort of 17OHD patients from a single country. We also explored the relationship of genotype and phenotype in partial and complete 17OHD patients.

2. Subject and methods

2.1. Subject

26 subjects were recruited from 23 kindreds, including 10 46, XX females and 16 46,XY males. All patients presented with phenotypical female. Two families had consanguineous marriage. Blood pressure was measured using sphygmomanometer at the seated position for at least three separate occasions. The diagnosis for all the patients was based on the clinical manifestations and serum hormone assays. All clinical features are summarized in Table 1. The study protocol was approved by the Ethical Committee of Ruijin Hospital. In addition, the written informed consent was obtained from each patient.

2.2. Serum hormone measurement

Blood samples were collected in the morning after overnight fasting and immediately centrifuged at 4 °C. Serum progesterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), testosterone, and renin were analyzed using chemiluminescence. ACTH, cortisol and 17-hydroxyprogesterone (17-OHP) concentrations were determined by radioimmunoassay (RIA).

Table 1
Clinical and hormonal characteristics of patients with partial and complete 17OHD.

Items	Patient 17	46,XX $n=9$	46,XY $n=16$	Normal range
Age (years)	38	21.1 ± 5.9	20.8 ± 6.4	
Karyotype	46,XX	46,XX	46,XY	
Primary amenorrhea	None	7	9	
Height (cm)	162	163.6 ± 9.8	164.6 ± 8.1	
Weight (cm)	56	51.4 ± 10.1	51.9 ± 9.3	
Systolic (mmHg)	130	160.5 ± 29.3	150.1 ± 15.6	
Diastolic (mmHg)	90	108.3 ± 10.4	96.5 ± 29.3	
Breast Tanner stage	3	1	1	
Pubic Tanner stage	2	1	1	
LH (IU/L)	11.5	32.6 ± 19.1	37.5.3 ± 11.2	1.2–12.5
FSH (IU/L)	7.0	58.7 ± 19.7	71.61 ± 21.8	3.2–10
P (nmol/L)	26.7	17.8 ± 10.8	15.6 ± 13.9	0.60–4.7 (F) 0.7–4.3 (M)
17-OHP (nmol/L)	0.5	0.36 ± 0.12	0.2 ± 0.1	1.7–9.4
T (nmol/L)	0.69	0.35 ± 0.33	0.69 ± 0.35	0.52–3.64 (F) 5.7–29.1 (M)
E_2 (pmol/L)	172.5	71.9 ± 46.6	81.8 ± 63.9	44.0–176.2 (F)
K+(mmol/L)	2.0	2.5 ± 0.4	3.0 ± 0.7	3.5–5.5
ACTH (pmol/L)	35.88	55.4 ± 37.6	62.8 ± 47.6	2.64–17.16
Cortisol 08:00 h (nmol/L)	188.0	27.8 ± 18.5	22.1 ± 18.9	187.7–608.1
PRA(ug/L h)	0.01	0.05 ± 0.01	0.04 ± 0.02	0.1–0.5

Abbreviations: F: female; M: male.

2.3. DNA preparation, PCR, and sequencing

Genomic DNA was isolated from peripheral leucocytes using a DNA extraction kit (QIAGEN, Mississauga, Ontario, Canada). Eight exons and flanking introns of the human *CYP17A1* gene were amplified by PCR and sequenced as described previously [10]. The PCR products were purified using a gel extraction kit (QIAGEN, Mississauga, Ontario, Canada) and sequenced in both sense and antisense directions on an ABI 700 sequencer (Applied Biosystems PerkinElmer, Foster City, CA).

2.4. Site-directed mutagenesis

The FLAG-tagged wild-type *CYP17A1* expression vector (pCMV-Tag2A-*CYP17A1*) was kindly provided by Dr. Jie Qiao, Department of Endocrinology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine and used as the template for construction of the c.971_972insG, c.1464_1466delT, and c.1386G>T vectors. These *CYP17A1* mutant constructs were generated by using QuikChange II site-directed mutagenesis kit (Stratagene, La Jolla, CA). The primers were designed as follows: c.971_972insG, forward, 5′-TGCACAATCCTCAGGTGGAAGAAGAAG-CTCTAC-3′; reverse, 5′-GTAGAGCTTCTTCTTCCACCTGAG-GATTGTGCA-3″; c.1464_1466delT, forward, 5′-TCTTTCTGATC-GACTCTTCAAAGTGAAGATCA-AG-3′; reverse, 5′-CTTGATCTTCACTTTGAAGAGTCGATCAGAAAGA-3′; c.1386G>T, forward, 5′GCCTGGCTGCTGCAGAGTTTCGACCTGGAGGTGCC-3′; reverse-5′-GGCACCTCCAGGTCGAAACTCTGCAGCAGCCAGGC-3′. Positive clones were picked and sequenced to confirm the site-directed mutations.

2.5. Enzymatic activity assay

The wild type and mutant pCMV-Tag2A-C*YP17A1* plasmids were purified using an EndoFree plasmid maxi kit (QIAGEN). HEK-293T cells were cultured in 24-well plates and transfected with 0.8 μg of each pCMV-Tag2A-C*YP17A1* constructs using Lipofectamine 2000 reagents (Invitrogen, USA). After 40 h of transfection, various concentrations of progesterone and 17-hydroxypregnenolone (0.25, 0.5, 1.0, 2.0 μmol/L) were added and incubated for another 6 h. The culture medium was collected and frozen at −20 °C until assayed. 17-hydroxyprogesterone and DHEA in medium were using radioimmunoassay (Beckman, USA) and chemiluminescence immunoassay (DRG, USA), respectively. The measurements were carried out in triplicates and each experiment was repeated three times.

2.6. Western blots

The FLAG-tagged c.971_972insG, c.1464_1466delT and c.1386G>T constructs were expressed in HEK-293T cells. The cells were lysed in radioimmunoprecipitation (RIPA) buffer containing 50 mM Tris–HCl, 150 mM NaCl, 5 mM $Mgcl_2$, 2 mM EDTA, 1 mM NaF, 1% NP40 and 0.1% SDS. Western blotting was performed using antibodies against Flag antibody (Cell signaling, USA). The primary antibody against the FLAG octapeptide motif was mouse, and the secondary antibody was anti-mouse.

3. Results

3.1. Clinical characteristics and serum hormone concentrations

Twenty-five subjects (nine 46,XX and sixteen 46,XY) from twenty two kindreds presented with infantile female genitalia, absence of breast development and primary amenorrhea. All the patients but two presented with severe hypertension and hypokalemia. The age

Please cite this article in press as: M. Zhang, et al., New, recurrent, and prevalent mutations: Clinical and molecular characterization of 26 Chinese patients with 17alpha-hydroxylase/17,20-lyase deficiency, J. Steroid Biochem. Mol. Biol. (2015), http://dx.doi.org/10.1016/j.jsbmb.2015.02.007