Identification of a novel mutation in *CYP17A1* gene

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17α-hydroxylase/17,20-lyase deficiency (17OHD) is a rare autosomal recessive genetic disease that is characterized by low-renin hypertension, hypokalemia, and abnormal development of the genitalia. Mutations in the *CYP17A1* gene account for this disease. We aim to investigate the *CYP17A1* mutation and analyze its possible influence on phenotype in a Chinese patient with 17OHD. Steroid hormones were assayed. The 8 exons of the *CYP17A1* gene were amplified and directly sequenced. Wild-type and mutant CYP17A1 cDNA were cloned into pcDNA3.1 expression vectors and transfected into 293T cells. Finally, 17-hydroxylase and 17,20-lyase activity were detected by using progesterone and 17-hydroxypregnenolone as the substrates. A novel missense mutation c.716 G>A located in exon 4 that changed the amino acid from arginine to glutamine (R239Q) was discovered in the patient. Steric model analysis of CYP17A1 showed that R239Q changed the local structure and the electrostatic potential. Functional study indicated that the R239Q mutant caused the complete loss of both 17α-hydroxylase and 17,20-lyase activities. Our study expanded the CYP17A1 mutation spectrum. With a functional study, we confirmed that the novel mutation caused the complete loss of both 17α-hydroxylase and 17,20-lyase activities. (Translational Research 2013;161:44–49)

Abbreviations: ACTH = adrenocorticotropic hormone; CAH = congenital adrenal hyperplasia; DHEA = dehydroepiandrosterone; P = progesterone; PCR = polymerase chain reaction; RIA = radioimmunoassay; 17OHD = 17α-hydroxylase/17,20-lyase deficiency; 17OHP = 17-hydroxyprogesterone; 17OHPreg = 17-hydroxypregnenolone

17α-hydroxylase/17,20-lyase deficiency (17OHD) is a rare type of congenital adrenal hyperplasia (CAH) that accounts for only 1% of all CAH cases.[1] The human microsomal enzyme P450c17 (17α-hydroxylase/17,20-lyase) is one of the key enzymes in adrenal steroid hormone synthesis. This enzyme possesses the activity of both hydroxylase and lyase, with the former catalyzing pregnenolone or progesterone (P) into the cortisol

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AT A GLANCE COMMENTARY

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Background

17OHD is a rare type of CAH that accounts for only 1% of all CAH cases. Mutations in the *CYP17A1* gene account for this disease. We investigated the CYP17A1 mutation and analyzed its possible influence on phenotype in a Chinese patient with 17OHD.

Translational Significance

Our work expanded the CYP17A1 mutation spectrum. With the functional study, we ascertained the correlation between genotype and clinical phenotype of a patient with 17OHD.

precursors 17-hydroxypregnenolone (17OHPreg) or 17-hydroxyprogesterone (17OHP) and the latter cleaving the 17 and 20 carbon chains to produce estrogen and adrenal androgen precursors, mainly dehydroepiandrosterone (DHEA). The P450c17 enzyme is encoded by the *CYP17A1* gene, which consists of 8 exons[2] and maps to chromosome 10q24.3.[3] This gene is primarily expressed in the adrenal glands and gonads.[4] When the hydroxylation and cleavage functions are impaired, steroid hormone synthesis in the zona fasciculata and adrenal reticularis can be affected. In addition, the marked decrease of cortisol reactivity increased the secretion of adrenocorticotropic hormone (ACTH) and caused bilateral adrenal hyperplasia. However, the substrate of the enzyme accumulates, resulting in low-renin hypertension and hypokalemia. The deficiency of gonadal hormones, such as estrogen and testosterone, induces primary amenorrhea and hypogonadism in female subjects and pseudohermaphroditism in male subjects. Moreover, sexual infantilism occurs in both genetic sexes. At present, more than 80 different mutations have been reported since Biglieri et al[5] reported the first patient in 1966. These mutations, including missense, deletion, insertion, and splice site mutations, in the *CYP17A1* gene cause combined or isolated 17OHD.[6,7]

In this study, we discovered a compound heterozygous mutation including a previously reported c.985_987 del/ins AA in exon 6 and a novel missense mutation R239Q in exon 4 of the *CYP17A1* gene in a patient with 17OHD. A functional experiment also was performed to investigate the mechanism of the disease-causing effect of this novel mutation.

PATIENTS AND METHODS

Subjects. A 30-year-old woman presented to the hospital with pain in the abdomen. Approximately 6 years previously, she underwent breast augmentation surgery for breast dysplasia. She presented with primary amenorrhea, and when she was aged 18 years, she underwent estrogen and progestin sequential therapy. However, after 2 years on the treatment, she experienced menstruation and withdrew herself from the drug. Her karyotype was 46,XX. A physical examination showed a height of 168 cm, blood pressure of 190/125 mm Hg, and infantile female external genitalia with the absence of both pubic and axillary hair. She exhibited a decreased level of serum potassium (2.4 mmol/L, 3.5–5.1 mmol/L). The patient also exhibited an increased level of P and decreased levels of E2 and DHEA. Plasma hormone analysis revealed that the levels of follicle-stimulating hormone, T, luteinizing hormone, and prolactin were within the normal range (Table I). Her supine aldosterone and renin were 406.4 pg/mL (29.4–161.5 pg/mL) and 0.46 ng/mL/h (0.5–1.9 ng/mL/h), respectively, whereas her orthostatic aldosterone and renin were 407.36 pg/mL (38.1–313.3 pg/mL) and 0.74 ng/mL/h (1.9–6 ng/mL/h), respectively. Her 24-hour urinary 17-hydroxycorticosteroid excretion was 3.4 mg (2–8 mg), and her 24-hour urinary 17-ketocorticosteroid level was 11.8 mg (6–14 mg). The measurement of the basal and ACTH-stimulated steroids revealed slightly elevated levels of cortisol and 17OHP with a poor response to ACTH stimulation (Table I). The adrenal computed tomography scan indicated multibilateral adrenal nodules, and the ultrasonograph revealed a small uterus. The patient was diagnosed with CAH and 17OHD. After the administration of dexamethasone (0.4 mg every night) for 10 days, her P and cortisol (3.9 nmol/L) levels decreased significantly. Her blood pressure (130/80 mm Hg) was within the normal range.

Hormone assays. Cortisol, T, DHEA, 17OHP, P, and ACTH concentrations were measured as previously described.[8] A commercial radioimmunoassay (RIA) kit (DiaSorin Ltd, Wokingham, UK) was used to measure the cortisol concentration. T, DHEA, 17-OHP, and ACTH concentrations were measured using an antibody-coated tube RIA kit (DSL-8600; Diagnostic Systems Laboratories Inc, Webster, TX). The serum P level was measured with the automated Abbott Architect I2000SR System (Abbott Laboratories, Abbott Park, Ill).

DNA extraction and mutation analysis. Genomic DNA of the patient and her parents was extracted from peripheral blood leukocytes using a FUJIFILM QuickGene-610L system (Fujifilm Life Science,