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New compound heterozygous mutations of *p. Thr101Ilefs*2* and *p. Thr306Ale* in a child from a Chinese family with 17α-hydroxylase/17, 20-lyase deficiency

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ABSTRACT. We determined whether a child with 17α-hydroxylase/17, 20-lyase deficiency possessed the sex-determining region (*SRY*) gene, and examined the mutations present in the *CYP17A1* gene that led to 17α-hydroxylase/17, 20-lyase deficiency. In the child, karyotype analysis was performed and polymerase chain reaction analysis and electrophoretic techniques were used to identify the *SRY* gene. A total of 50 normal individuals were included as a control group. Polymerase chain reaction and DNA sequencing were used to identify *CYP17A1* gene mutations in all samples. The karyotype of the child was 46, XY, which was inconsistent with her social sex, *SRY* was positive, and a compound heterozygous mutation *p. Thr101Ilefs*2* in exon 2 and *p. Thr306Ale* in exon 5 were identified in the *CYP17A1* gene. These mutations were inherited from her parents. In the 20 normal individuals, these mutations were not identified. In the child, sex reversal may have been caused by *CYP17A1* mutations. The compound heterozygous

mutation of *p. Thr101Ilefs*2* and *p. Thr306Ale* is a new gene mutation of 17α-hydroxylase/17, 20-lyase deficiency.

Key words: 17α-Hydroxylase/17, 20-lyase deficiency; Mutations; *p. Thr306Ale*; *p. Thr101Ilefs*2*

INTRODUCTION

Congenital adrenal cortical hyperplasia (CAH), an autosomal recessive disease, is caused by mutations in the gene encoding cortical hormone synthase, and manifests as the dys-synthesis of adrenocorticosteroids. The incidence of CAH is approximately 1/5000 individuals. 17α-Hydroxylase/17, 20-lyase deficiency (17OHD) is a rare disease that accounts for only 1% of CAH cases (Yanase et al., 1991) and mainly manifests as hypokalemia, hypertension, and sexual development disorders (pseudohermaphroditism or progenital feminization in males, and maldevelopment of secondary sexual characteristics in females) (Kronenberg and Williams, 2008).

The *CYP17A1* gene encoding P450c7l enzyme is located on chromosome 10q24.3 (Fan et al., 1992) and contains 8 exons and 7 introns. The P450c17 enzyme has 2 types of catalytic activities, including 17α-hydroxylation for progesterone and enelone, and 17, 20 carbon chain cleavages for 17α-hydroxyprogesterone and 17α-hydroxypregnenolone. Thus far, more than 110 *CYP17A1* mutations have been documented in the Human Gene Mutation database (www.hgmd.cf.ac.uk/ac/gene.php?gene=*CYP17A1*). These mutations are located in the coding and non-coding regions of the *CYP17A1* gene and include base repeats, base deletions, missense mutations, nonsense mutations, and compound heterozygous mutations. These mutations affect P450c17 enzyme activity, leading to the dys-synthesis of 17α-hydroxylation hormone and C_{19}-steroid hormones (Chung et al., 1987). The degree of P450c17 enzyme inactivation is associated with the type of gene mutations and mutable sites in the *CYP17A1* gene.

The first female case with 17OHD was reported by Biglieri et al. (1966), and the first male case with 17OHD was reported by New (1970). After this, approximately 150 cases of 17OHD have been reported, but the cases with 17OHD reported from Asian countries, such as China, Korea, and Japan, are limited (Fardella et al., 1993; Yamaguchi et al., 1997; Suzuki et al., 1998; Lam et al., 2001; Hahm et al., 2003; Qiao et al., 2003). In this study, we report a new compound heterozygous mutation in the *CYP17A1* gene in a Chinese child with 17OHD.

MATERIAL AND METHODS

All study methods were approved by the Ethics Committee of People's Hospital of Zhengzhou University. All subjects gave written formal consent.

Subject and materials

A 15-year old child with female social sex was referred to hospital due to hypertension. The child was diagnosed with 17OHD by serological and imaging examinations. The child's clinical features included hypertension, hypokalemia, breast development in Tanner stage I, no pubic hair or armpit hair, young girl-type pudendum, 3-cm vagina with a caecum, and no uterus or ovaries.

The blood from the child's parents was collected for analysis, and 50 individuals without P450c17 enzyme deficiency were included as normal controls.

Karyotype analysis

Anticoagulated blood was placed in 1 mL heparin sodium, and then the blood was incubated in 1640 medium for 72 h. After collection, sections were prepared for G banding and karyotype analysis of the child.

DNA extraction

Anticoagulated blood was placed in 2 mL EDTA, and then DNA extraction was performed using the DNeasy Tissue Kit (Qiagen, Hilden, Germany).

Amplification by polymerase chain reaction

According to the gene sequences obtained from the National Center for Biotechnology Information, primers were designed using the Primer 5.0 software and synthesized by Sangon Biological Engineering Co., Ltd. (Shanghai, China) for amplification of exons in the *CYP17A1* gene (Table 1). The polymerase chain reaction system included 1 μL DNA, 1 μL upstream and downstream primers, 5 μL Taq buffer, 5 μL 25 mM $MgCl_2$, 0.5 μL 5 U/μL Taq enzyme, and 35.5 μL ddH_2O. The amplification conditions were as follows: 95°C for 3 min, 94°C for 30 s, 57°C for 35 s, and 72°C for 45 s for 35 cycles; 72°C for 8 min followed by storage at 4°C. The sex-determining region (*SRY*) primer was synthesized by Sangon Biological Engineering Co., Ltd. (Shanghai, China) for amplification of the *SRY* gene. Polymerase chain reaction products were subjected to 2% agarose gel electrophoresis. After ethidium bromide staining, the electrophoretic bands were observed under an ultraviolet lamp.

Table 1. Sequences of primers.

Exons	Sequences of primers (3'-5')	Size of fragments (bp)
1	ATCAACTGACCTCCCTTACCTAG TCTGAAGACCTGAACAATCCC	635
2-3	GGGACCAGAGGTGTAAGGGC AAGATGGGTCATTGCGGCT	783
4	GCCCTCCTCCCTTGTTTAG CCACCCTGCTCTTGTGATTA	354
5	TCAGGGACAGAAGTATGGCAG GGGTCAAAGCCAACTACTGC	439
6	AGGGACTGGACAGGCTCTT TTGATGGTTGACTGACTTTAGGT	396
7	ATGAGGCTGAGCAAGGAAG TGAGGGTGTCAACAGGTCC	309
8	CTCAACCAGGGCAGAACCA AGGTGCTCAATAAAATCGGTGT	599

Sequence analysis

Sequencing was performed by Sangon Biological Engineering Co., Ltd. Sequencing chromatograms were evaluated using the Chromas software (Technelysium, South Bris-

bane, Australia). Sequencing results were compared with GeneBank: NM_000102.3 using the DNAMAN software to determine the genotype of the samples.

RESULTS

Chromosome banding

G banding indicated that the subject had a karyotype of 46, XY (normal male karyotype, Figure 1), which was not consistent with the social sex.

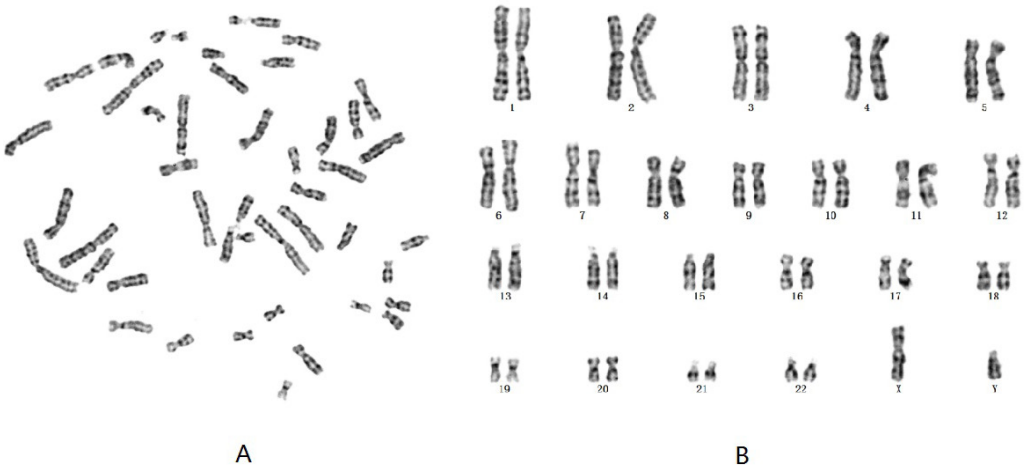

Figure 1. Karyogram of chromosome Giemsa banding in the child **A.** Karyogram; **B.** Rearranged karyogram.

Detection of *SRY*

SRY electrophoresis indicated positive amplification bands in the child and positive controls, but no amplification bands in the negative control (Figure 2). These results further confirmed that child's genetic sex was male.

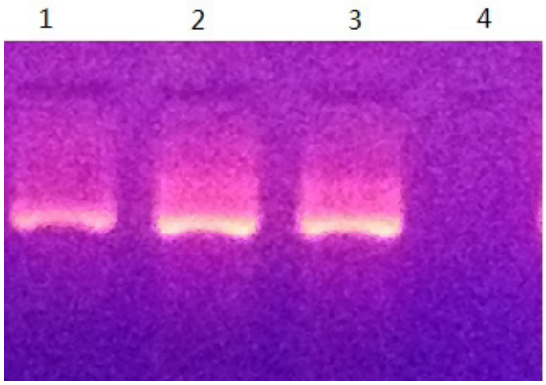

Figure 2. *SRY* electrophoresis. *Lane 1* = child (patient); *lanes 2* and *3* = positive controls; *lane 4* = negative control.

Detection of *CYP17A1* gene

All exons of *CYP17A1* showed amplification (Figure 3). Comparison of the *CYP17A1* exon sequences and NCBI sequences indicated the presence of a compound heterozygous mutation in the *CYP17A1* gene in the child. Because of the deletion of C from c.301-303 in exon 2, ACT was changed into AT, and then threonine (ACT) was changed into isoleucine (ATC) at amino acid 101. This led to a frame-shift mutation that introduced a premature termination codon (TAG, c.301_303ACT→AT) at amino acid 102, forming truncated protein (*p. Thr101Ilefs*2*). At codon 306 in exon 5, ACC was changed into GCC, coding for alanine rather than threonine (*p. Thr306Ale*). Sequencing indicated that the mutations p. T101I, 102X, and p. T306A in the child all were inherited from the child's parents and that the child's parents were heterozygous mutation carriers. In the 50 normal individuals, the mutations p. T101I, 102X, and p. T306A were not identified (Figure 4).

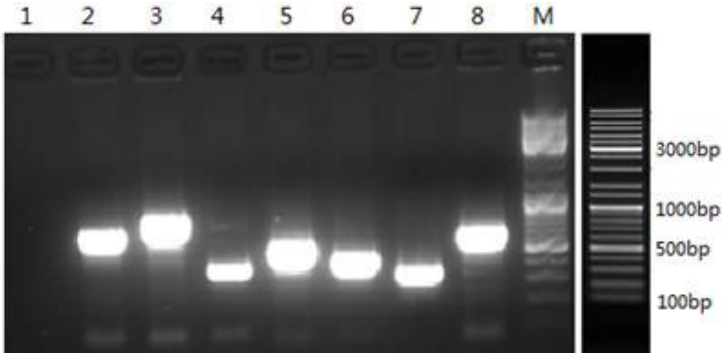

Figure 3. Electropherogram of exon amplification of *CYP17A1*. *Lane M*: marker; *lane 1*: Blank control; *lane 2*: Exon 1; *lane 3*: Exons 2 and 3; *lane 4*: Exon 4; *lane 5*: Exon 5; *lane 6*: Exon 6; *lane 7*: Exon 7; *lane 8*: Exon 8.

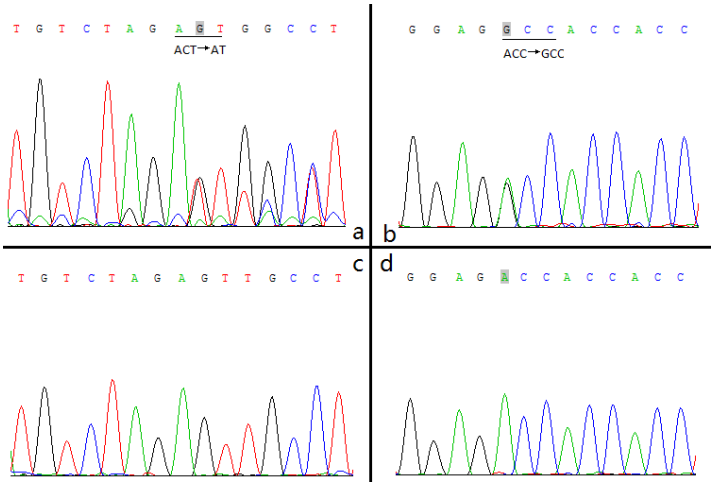

Figure 4. Results of *CYP17A1* gene sequencing a: Reverse sequencing of exon 2 in the child (patient); b: Forward sequencing of exon 5 in the child (patient); c: Reverse sequencing of exon 2 in normal control; d: Forward sequencing of exon 5 in normal control.

DISCUSSION

The incidence of secondary hypertension is higher in children than in adults. Secondary hypertension in children is mainly caused by adrenal gland diseases as well as cardiovascular disease, central nervous system disease, and endocrine disease (such as thyroid dysfunction and low rennin hypertension) (Wang et al., 2011). 17OHD, a rare disease, accounts for only 1% of CAH and is caused by *CYP17A1* gene mutation. The clinical manifestations are different in patients with17OHD from different families; even in siblings with the same mutations, clinical manifestations and biochemical indicators may be very different. This may be because of individual differences in synthetic abilities of enzymes and metabolic abilities of steroid hormones (New, 1970; Yanase et al., 1991). Hypertension is a typical clinical sign and can be observed early in some patients, identified by chance when they go to a hospital for other reasons or for health examination (Wang et al, 2011). Most patients are diagnosed with 17OHD because of the occurrence of abnormal sexual characteristics or hypertension symptoms during adolescence. In this study, the child was diagnosed with 17OHD because of hypertension.

The child in this study had the karyotype of 46, XY and was positive for the *SRY* gene, suggesting male genetic sex; however, the child exhibited young girl-type pudendum. This may be because during the 7th gestational week, the medulla of the genital ridge in the embryos with Y chromosome develops as seminiferous tubules, which form the testis; however, the deficiency in testosterone allows testicular development to remain in an early stage, affecting the formation of the penis and scrotum with young girl-type pudendum. The *CYP17* gene, which encodes 17α-hydroxylase/17, 20-lyase, is expressed in both adrenal glands and gonads (Chung et al., 1987), so the testis without normal function cannot secrete testosterone in patients with the male genotype. Because of the lack of androgen, the external genitalia develop as young girl-type pudendum in most patients with the karyotype of 46, XY. A few patients with male genotype have pseudohermaphroditism, which may be because enzyme activity varies according to different locus mutations in the *CYP17* gene and because of individual differences.

According to the structure model of P450c17 (*CYP17A1*) constructed by Auchus (2001), the 508-amino acid P450c17 protein contains 4 important structural domains, including a substrate-binding domain, catalytic activity area, heme-binding region, and redox-partner binding site (Auchus and Miller, 1999). In this study, because of the deletion of C from c.301-303 in exon 2, ACT was changed into AT, and threonine (ACT) was changed into isoleucine (ATC) at amino acid 101. This led to a frame-shift mutation that introduced a premature termination codon (TAG) at amino acid 102, forming a truncated protein (*p. Thr101Ilefs*2*). The number of amino acids was reduced from 508 to 101, leading to the deletion of protein domains, particularly the heme-binding region, which contains a highly conserved sequence at amino acids 435-455 and Cys442, which plays a key role in the activity of 17α-hydroxylase/17, 20-lyase. Therefore, the activity of 17α-hydroxylase/17, 20-lyase is severely affected. Currently, it has not been reported that the mutation *p. Thr101Ilefs*2* leads to 17OHD.

When the missense mutation c.916A>G was present in exon 5, ACC was changed to GCC at codon 306, and then threonine was changed to alanine (*p. Thr306Ale*). The threonine at amino acid 306 of P450c17 is a proton donor in catalytic activity, and is highly conserved in all cytochrome P450 enzymes (Poulos et al., 1987). Therefore, when the polar amino acid threonine is changed into the non-polar amino acid alanine, the alanine cannot provide a proton for the catalytic action. The mutation *p. Thr306Ale* reportedly decreased hydroxylation of P450c17 to 6% of wild-type levels, and the lyase activity of P450c17 was decreased to 29-

42% of wild-type levels (Lee-Robichaud et al, 1998).

In summary, both the *p. Thr101Ilefs*2* and *p. Thr306Ale* mutations can affect P450c17 enzyme activity. In this study, the child's parents were *p. Thr101Ilefs*2* and *p. Thr306Ale* heterozygous mutation carriers, so the function of the P45017 enzyme failed to be completely lost and they had no symptoms or signs of 17OHD. However, the child had the symptoms and signs of 17OHD because of the complete loss of P450 enzyme activity caused by the compound heterozygous mutation of *p. Thr101Ilefs*2* and *p. Thr306Ale*. The compound heterozygous mutation of *p. Thr101Ilefs*2* and *p. Thr306Ale* is a new gene mutation of 17OHD.

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