Abstract. In the present study, we report the mutation of the 3β-hydroxysteroid dehydrogenase (3β-HSD) gene in a family suffering from adrenocortical insufficiency. The index patient was clinically diagnosed with adrenocortical insufficiency. Peripheral venous blood (5 ml) was collected from the proband and 5 members of his family, and genomic DNA was extracted. Exons 1, 2, 3 and 4 of the 3β-HSD gene and their flanking sequences were amplified by polymerase chain reaction (PCR). Some of the family members were examined by amplifying only exon 4. The PCR products were then purified and sequenced. The C to T homozygous mutation at nucleotide 1088 and C to G homozygous mutation at nucleotide 1132 within exon 4 of the 3β-HSD gene were found in the family members with abnormal phenotype. In the family members with normal phenotype, heterozygous mutations at the sites mentioned above were identified in the parents and Aunt 1, but not in Aunt 2 of the proband. In conclusion, a family with 3β-HSD deficiency was identified in the present study. The cause of the disease in the studied family appears to be two novel homozygous mutations in the 3β-HSD gene.

Introduction

Congenital 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency is an autosomal recessive hereditary disease and one of the causes of congenital adrenal hyperplasia (1,2). In humans, 3β-HSD has two isozymes that can catalyze the oxidative conversion of ∆5 into a ∆4 steroid hormone. Type I and II isozymes have a homology of 93.5% (3,4). Congenital 3β-HSD deficiency can be classified as classical or non-classical, according to clinical manifestations. Classic 3β-HSD deficiency is caused by a mutation in the Type II 3β-HSD gene, and patients with this type of deficit have different degrees of salt-wasting, possible pseudohermaphroditism in males, and normal sexual differentiation or mild masculinization in females (5,6). The salt-wasting type of 3β-HSD deficiency is usually diagnosed in newborns, whereas the non-salt-wasting type is diagnosed prior to adolescence (7,8).

Although clinical reports are available on 3β-HSD deficiency cases or 3β-HSD deficiency in families in foreign countries (Fig. 1) (9), there is currently no report on the mutation of the 3β-HSD gene in a Chinese family with adrenocortical deficiency. In this study, clinical analysis and molecular genetic research were carried out on a patient suffering from 3β-HSD deficiency and his family.

Materials and methods

Materials. The patient (male, 17 years old) was hospitalized because of generalized pigmentation for 17 years, and fever and convulsions for 4 days. His brown yellow generalized pigmentation was observed at birth but was not paid attention to because no abnormalities were found in the patient then in terms of sucking, breathing or sleeping. At age 3, the patient was found by his parents to be suffering from shortness of breath after activities, and he presented to the Southern Anhui Affiliated Hospital. He was diagnosed with adrenal insufficiency and was administered prednisone with an initial dose of 15 mg/d. Administration of the medication was continued at a dose of 5 mg/d for 3 months. Family members said the disease was somewhat relieved, thus other drugs were not used after the 3-month period of time. One week prior to admission, the patient demonstrated the following symptoms: fever, convulsions and trismus without any oral discharge. However, the duration was unknown. He was considered to be a possible case of encephalitis at a local hospital and was given mannitol for dehydration and appropriate electrolytes. He was also diagnosed with hepatic and renal dysfunction. No abnormalities were found by head computed tomography (CT).
Blood gas analysis indicated he had Type I respiratory failure and a mask ventilator was used to facilitate his breathing. The patient visited our hospital because his disease was not significantly relieved. Chest scan and B-ultrasound showed increased markings in bilateral lungs and no abnormalities in the liver, gallbladder, pancreas, spleen, bilateral kidneys and adrenal glands. The patient was diagnosed with adrenal insufficiency and admitted to our hospital. His family history is as follows: the patient's paternal grandmother and maternal grandmother are cousins, his healthy parents' marriage is consanguineous, and the patient's sister also had pigmentation. The following were the results of the physical examination: height, 173 cm; poor nutrition; weight, 62.5 kg; generalized pigmentation, especially in the abdomen and waist; obvious oral mucosa; and gingival pigmentation. This study was conducted in accordance with the declaration of Helsinki and with the approval of the Ethics Committee of the Kunshan People's Hospital Affiliated to the Jiangsu University. Written informed consent was obtained from the participant.

**Hormone determination.** Enzyme immunoassay (Serono Company, Switzerland) was conducted to assay follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol and testosterone. Radioimmunoassay was conducted to assay cortisol and aldosterone (DiaSorin Corporation, Stillwater, MN, USA). An immunoradiometric assay was conducted to assay adrenocortical hormone (DSL Systems Laboratories, Webster, TX, USA).

**PCR amplification.** Venous whole blood (5 ml) was collected from the patient, his parents, sister, 2 aunts and 3 other healthy physical examinees. EDTA was used for blood anti-coagulation, and an icebox was used to transport the samples. Genomic DNA was extracted using the routine phenol-chloroform method and was stored in a refrigerator (-20°C). Exons 1, 2, 3 and 4 of the 3β-HSD gene of the proband were analyzed to locate mutation sites, as well as exon 4 of the 3β-HSD gene of the other family members and the healthy physical examinees. The primers used for PCR are shown in Table I. The primers were synthesized by the Shanghai Saibaisheng Gene Technology Co., Ltd. (Shanghai, China). The primers were diluted to 10 pmol/µl and stored at -20°C.

The PCR reaction system contained genomic DNA, 150 ng; upstream primer, 0.625 µl; downstream primer, 0.625 µl; 10X HotStar buffer, 2.5 µl; dNTP, 0.5 µl; and HotStar DNA polymerase, 0.125 µl. Then, ddH₂O was added until the total volume was 25 µl. The PCR reaction conditions were F1/R1, F4/R4, F5/R5: 95°C for 10 min, 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and 40 cycles of 72°C for 10 min; F2/R2, F3/R3: 95°C for 10 min, 94°C for 45 sec, 62°C for 45 sec, 72°C for 1 min and 40 cycles of 72°C for 10 min. PCR products were assayed using 1.5% agarose gel electrophoresis, and then dyed with ethidium bromide. Bands were observed under ultraviolet light.

**Sequencing.** The PCR products were bidirectionally sequenced by the Shanghai Invitrogen Biotechnology Co., Ltd., and all amplified fragments were assayed through bi-directional sequencing.

**Results**

**Laboratory index.** Routine blood analysis revealed that RBC was 3.67x10¹²/l, Hb was 101 g/l, N% was 59.4% and platelets were 82x10⁹/l. Routine urinalysis showed the following results: occult blood, -; ketones, +; glucose, -; and protein, +. Routine fecal analysis was normal. Liver function showed that ALT was 64 U/l, AST was 197 U/l and albumin was 24 g/l. Renal function revealed serum creatinine to be 203 µmol/l. Endocrine hormone analysis showed the following results:
and 3β-HSD deficiencies decrease only the synthesis of cortisol and aldosterone, but increase the synthesis of sex hormones, which can lead to spurious sexual precocity in males and masculinization. CYP18 and 18-oxidase deficiencies decrease only the level of aldosterone and do not affect the levels of cortisol and sex hormones.

The physiological role of 3β-HSD is to catalyze the dehydrogenation of 3β-hydroxy steroids, of pregnenolone to generate progesterone, of 17α-hydroxyprogrenenolone to generate 17α-hydroxyprogesterone and of dehydroepiandrosterone to generate Δ4-androstenedione. The 3β-HSD deficiency can lead to the accumulation of pregnenolone, 17α-hydroxy pregnenolone and dehydroepiandrosterone (DHEA), and can decrease the synthesis of cortisol, aldosterone and testosterone (11-16). Mineralocorticosteroid and glucocorticosteroid deficiencies lead to clinical symptoms, such as salt loss, chronic adrenal dysfunction and mild masculinization (17) as fetal adrenal glands secrete excessive DHEA, and some DHEA can be translated into the testosterone through extra-adrenal paths. In some female patients manifesting masculinization, the level of 17-OHP is high due to the active effect of extra-adrenal 3β-HSD. If enzyme deficiencies affect the adrenal and sex glands, male embryos are not likely to secrete enough testosterone, which would result in incomplete masculinization at birth, with hypospadias, cryptoorchidism and even male pseudohermaphroditism (3,18). In this report, the cortisol level of the proband was 0.86 µg/dl, which was below the normal range of 6.2-19.4 µg/dl. The patient's ACTH level was 331 pg/ml, whereas the normal level is less than 46 pg/ml. The testosterone level of the proband was 2.53 nmol/l, which was significantly below the normal range of 9.9-27.8 nmol/l. His progesterone level was 0.39 nmol/l, which was slightly below the normal range of 0.4-3.1 nmol/l. The levels of cortisol, testosterone and progesterone in the patient were lower than normal, thus he was considered to have congenital adrenal hyperplasia caused by 3β-HSD.

3β-HSD deficiency is an autosomal recessive, inherited disease caused by 3β-HSD gene mutation (19-22). In addition to clinical diagnosis, genetic diagnosis is also important in ascertaining the existence of 3β-HSD deficiency. In this report, all the amplified products were analyzed by direct sequencing. Four exons of the 3β-HSD gene and their flanking sequences were amplified and sequenced. The method employed was simple and convenient. Although a small number of DNA sequences showed impure peaks, the results were reliable as bi-directional sequencing was performed. By comparing the sequencing results of the family members mentioned in this
study with the sequences of corresponding normal exons of the 3β-HSD gene (GenBank Accession No. G1184396), we
discovered the following: the family members with abnormal
phenotype had a transition mutation (C/T) at nucleotide 1088
and a transversion mutation (C/G) at nucleotide 1132 in exon 4
of the 3β-HSD gene; both mutations were homozygous.
The sequencing of family members with normal phenotype
revealed that the father, mother and Aunt 1 had heterozygous
mutations, whereas Aunt 2 did not have mutations at the
2 sites. The mutation discovered in this family has not yet been
reported in China or abroad.

In this report, the family members with abnormal pheno-
type had homozygous mutations at the 2 sites mentioned above.
The parents and Aunt 1 also had heterozygous mutations at the
2 sites. In addition, genomic DNA was extracted from the
venous blood of 3 healthy physical examinees, and exon 4 of
their 3β-HSD gene was also amplified. One of the examinees
had heterozygous mutations at the 2 sites, in contrast to the
other 2 examinees. Therefore, the mutations in the 2 sites may
be linked mutations.

Nucleotide 1088 in exon 4 of the 3β-HSD gene had transition
mutations (C/T) while nucleotide 1132 had trans-
versional mutations (C/G). The two mutations were located at
the 3’ end of exon 4, an untranslated region, suggesting that the
activity of 3β-HSD was possibly decreased by the regulation
of translation or transcription. The mechanism by which such
new mutations induce a decrease in the activity of 3β-HSD
needs to be further studied.

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