# Three cases of 3β-hydroxysteroid dehydrogenase deficiency: Clinical analysis

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## Abstract

**Background.** 3β-HSD deficiency is a rare type of congenital adrenal hyperplasia (CAH), which is caused by *HSD3B2* gene mutations.

**Objectives.** In order to improve the understanding and diagnosis of the disease, we analyzed and summarized the clinical characteristics, genetic variants and treatment for 3 children with 3β-HSD deficiency in this study.

**Materials and methods.** A summary of the clinical data, hormone levels (17-hydroxyprogesterone, adrenocorticotropic hormone, cortisol, testosterone, dehydroepiandrosterone, androstenedione, renin, and aldosterone), therapeutic drugs, and gene sequencing results from 3 3β-HSD deficiency patients was created.

**Results.** The 3 patients developed external genital abnormalities and adrenal insufficiency in infancy. Steroid hormone levels were consistent with 3β-hydroxysteroid dehydrogenase deficiency. Gene sequencing for the 3 patients detected complex heterozygous mutations in the *HSD3B2* gene, which confirmed the diagnosis of 3β-HSD deficiency type II. Among the mutation types, c.154_162delinsTCCTGTT and c.674T>A have not been reported in the literature. The 3 children were treated with glucocorticoid and mineralocorticoid replacement, which controlled the adrenal insufficiency satisfactorily. In 2 male patients, external genital dysplasia manifested as hypospadias and small penis. After long-acting testosterone intramuscular injection to increase the penis size, the hypospadias were repaired. Mild masculinization in the female patient resulted in skin pigmentation and clitoral hypertrophy; however, no surgical intervention was required.

**Conclusions.** The main clinical manifestations of 3β-HSD deficiency were adrenal insufficiency and sex hormone synthesis dysfunction. There was a strong phenotype correlation between the observed clinical manifestations in conjunction with steroid hormone levels and *HSD3B2* mutations. The novel mutations c.154_162delinsTCCTGTT and c.674T>A were classified as pathogenic variants. Adrenal cortical function control was satisfactory after hormone replacement therapy, and hypospadias and small penis were attenuated using testosterone replacement therapy during mini-puberty for optimal surgical outcome.

**Key words:** treatment, clinical features, 3β-hydroxysteroid dehydrogenase deficiency, *HSD3B2*

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# Background

There are 2 types of human 3β-hydroxysteroid dehydrogenase (3β-HSD), type I (3β-HSD1) and type II (3β-HSD2),[1] which have 93.6% homology and are encoded by the *HSD3B1* and *HSD3B2* genes, respectively. The 3β-HSD1 enzyme is mainly expressed in organs such as the placenta, breast, skin, and prostate. It catalyzes the production of different steroids at low substrate concentrations, which are necessary for the synthesis of placental progesterone during pregnancy.[2] The 3β-HSD2 enzyme is expressed in the adrenal gland and gonadal tissues.[3] It is the rate-limiting step in the synthesis of the adrenocortical and sex hormones. Their expression is regulated by negative feedback from downstream products such as sex hormones and cortisol. It mainly catalyzes the conversion of Δ5-3β-hydroxysteroids to Δ4-3β-ketosteroid.[4]

3β-HSD deficiency is a rare type of congenital adrenal hyperplasia (CAH), which is caused by *HSD3B2* gene mutations. The neonatal incidence is less than 1/1,000,000,[5] accounting for less than 5% of all CAH cases. The *HSD3B2* gene is located on chromosome 1 at 1p12 and is composed of 4 exons and 3 introns. The protein contains 371 amino acids and has 4 functional domains that include a cofactor binding domain, a ligand binding domain and 2 transmembrane domains.[6] *HSD3B2* gene mutations cause steroid hormone synthesis disorder, which eventually leads to decreased aldosterone, cortisol and sex hormone synthesis. The main clinical manifestations are adrenal insufficiency and sex hormone synthesis disorder. The severity of the clinical manifestations depends mainly on the remaining enzyme activity. Therefore, the disorder can be divided into classical and non-classical types according to enzyme activity levels and the degree of adrenal insufficiency. Children with classic 3β-HSD deficiency frequently have adrenal insufficiency, genital abnormalities in the neonatal period or infancy, and salt loss manifested through vomiting and dehydration. Typically, male genitalia have different degrees of incomplete masculinization while women may consistently be affected with mild masculinity. Therefore, the treatment of glucocorticoid combined with mineralocorticoid replacement therapy to improve adrenal function is recommended, and if necessary, surgical treatment may also be required.

Because 3β-HSD deficiency is a rare disease, there are a number of different types of mutation across different populations. More than 110 cases have been confirmed using genetic testing[7–9] and more than 50 types of *HSD3B2* gene mutations have been detected, including frameshift, missense, nonsense, splicing pathogenic variants, and deletions, among which missense variants are the most common type, while nonsense or frameshift pathogenic variants often lead to typical clinical phenotypes.[10] In order to improve the understanding and diagnosis of the disease, we analyzed and summarized the clinical characteristics, genetic variants and treatment for 3 children with 3β-HSD deficiency in this study.

# Materials and methods

## Subjects

During the period from January 2011 to March 2019, 3 children diagnosed with 3β-HSD deficiency were admitted to the Department of Endocrinology, Children's Hospital of Chongqing Medical University, China. Informed consent from the parents of each child was obtained and reviewed by the ethics committee of the Children's Hospital of Chongqing Medical University. Our study on humans also complies with the Helsinki Declaration.

The gender, age of onset, clinical history, family history, phenotypic features, and medication history was collected for the 3 patients.

## Determination of hormone levels

Peripheral blood was taken from the children and cortisol, testosterone (T), adrenocorticotrophin (ACTH), renin, and aldosterone levels were determined with immunoluminescence. Dehydroepiandrosterone (DHEA), androstenedione (AND) and 17-hydroxyprogesterone (17-OHP) levels were determined using a radioimmunoassay.

## ACTH excitatory test

A corticotropin (ACTH39 peptide 25 U = 0.25 mg) peptide was used in the excitatory test. Approximately 10 mL of normal saline was used to dilute the vein with slow intravenous injections for 5–10 min. Post-injections blood was collected at 0 min (directly afer the injection), 30 min, and 60 min to measure progesterone, cortisol, AND, DHEA, and 17-OHP levels.

## Gene sequencing analysis

Blood (treated with EDTA anticoagulation) taken from the children and their parents was collected and sent to the Beijing Jinzhun Medical Laboratory (Beijing, China), the Molecular Pathology Center of the Affiliated Hospital of the Air Force Aviation Medical Research Institute (Beijing, China) and the DeyiDongfang Conversion Medical Research Center (Beijing, China) for sequencing. After DNA extraction, polymerase chain reaction (PCR) using gene-specific primers for the 4 *HSD3B2* exons, exon intron junction and V-flanking region was performed. Amplicons were sequenced using the HiSeq2500 instrument (Illumina, San Diego, USA) and variants were confirmed with Sanger sequencing.

# Results

## Clinical data for Case 1

Case 1 is a male with a normal karyotype (46, XY), 5 years and 8 months old now. When he was at the age of 1 month and 13 days, his parents sought medical advice of an endocrinologist due to abnormalities in the external genitalia of their son (the penis was short with hypospadias), inability to gain body weight by 1 month of age and diarrhea for at least 3 days. Soon after birth, abnormal genitalia, low body weight and other symptoms were noted, such as poor suction and regurgitation of milk, diarrhea, irritability, convulsions, lack of sweating ability, poor response, short stature, as well as intellectual and developmental retardation. His mother has had 1 pregnancy before and that has delivered once (Tanner staging of her 1st child was G1P1). He was a full-term baby, breastfed, and his birth weight was 3.4 kg, with unknown birth length. Newborn screening showed abnormal results, namely, an elevated 17-OHP value. His parents were healthy, with no consanguinity, normal puberty development age, and negative family history of genetic metabolic disease and CAH. Physical examination at the age of 1 month and 13 days showed a body length of 52 cm, body weight of 3.5 kg, normal development, normal nourishment, no abnormal facial features, rough skin, lack of pigmentation of the lip, gum and breast, lack of hair, hemorrhoids, and normal heart, lungs and muscle tension in the limbs. The features of the external genitalia included dark-colored bilateral scrotum, palpable testis, and embedded penis of approx. 1.0 cm in length and 3.0 cm in circumference with hypospadias. The Tanner staging was G1PH1 at the age of 1 month and 13 days. Laboratory findings are shown in Table 1. Laboratory results at initial diagnosis showed elevated potassium, 17-OHP and dehydroepiandrosterone, and low levels of sodium, testosterone and cortisol.

## Clinical data for Case 2

Case 2 is a male (chromosome 46, XY), 3 years and 3 months old now. He was treated with hormone therapy for abnormal genitalia, poor suck and low weight after reaching 1 month of age. Shortly after birth, external genital malformations were observed. A variety of physical issues were present, namely, short penis with hypospadias, scrotal skin pigmentation, poor suction, low weight gain, irritability, convulsions, lack of sweating, and poor response. Blood gas analysis revealed abnormal potassium (high), sodium (low) and chlorine (low), with suspected CAH and treatment with 1.25 mg hydrocortisone 3 times per day and 50 μg of fluorohydrocortisone 2 times a day at 1 month of age. After treatment, weight gain was observed along with better suction and spit responses. He was a full-term baby, delivered by cesarean section, with birth weight of 3.4 kg and unknown birth length. At 7 months

**Table 1.** Laboratory tests of Case 1 during follow-up

| Age | ACTH [pg/mL] | 17-OHP [mmol/L] | Cortisol [nmol/L] | Testosterone [nmol/L] | DHEA [μmol/L] | AND [nmol/L] | Renin [μIU/mL] | Aldosterone [ng/dL] | Electrolyte [mmol/L] |
|---|---|---|---|---|---|---|---|---|---|
| 1 month and 13 days | 34 | >75.75 | – | 4.02 | 5.92 | >35 | 10.63 | 0.49 | $K^+$ 6.12<br>$Na^+$ 126.1 |
| 1 month and 27 days | <10 | 8.82 | – | <0.69 | 0.502 | 3.26 | – | – | normal |
| 3 months and 2 days | 131 | 12.78 | – | 2.96 | <0.407 | 1.07 | – | – | normal |
| 5 months and 12 days | 19 | <0.303 | – | <0.69 | <0.407 | <1.05 | – | – | normal |
| 27 months and 15 days | 12.4 | <0.303 | – | <0.69 | <0.407 | <1.05 | – | – | normal |
| 11 months and 3 days | 13.7 | 1.03 | – | <0.69 | <0.407 | <1.05 | – | – | normal |
| 1 year and 1 month | 12.7 | 3.5 | – | <0.69 | <0.407 | <1.05 | – | – | normal |
| 1 year and 4 months | 29.4 | <0.303 | 37.8 | <0.69 | <0.407 | <1.05 | – | – | normal |
| 1 year and 8 months | 31.9 | <0.303 | – | <0.69 | <0.407 | <1.05 | – | – | normal |
| 1 year and 11 months | 275 | 1.03 | <4.41 | 6.31 | <0.407 | <1.05 | 19.1 | 7.51 | normal |
| 2 years and 4 months | <10 | <0.303 | 147 | 1.98 | <0.41 | <1.05 | 30.9 | 8.07 | normal |
| 2 years and 11 months | 339 | 18.7 | 50.3 | <0.69 | 3.12 | <1.05 | 26 | <0.97 | normal |
| 3 years and 6 months | 220 | 11.26 | <27.6 | <0.69 | 6.08 | <1.05 | 14.6 | <0.97 | normal |
| 4 years | 101 | 3.79 | 38.4 | <0.69 | 2.77 | <1.05 | 94.7 | <0.97 | normal |
| 4 years and 7 months | 629 | 22.57 | 33.4 | <0.69 | 23.1 | 2.77 | 258.2 | 1.98 | normal |
| 4 years and 11 months | 93.3 | 1.47 | – | <0.69 | 3.61 | <1.05 | 4.8 | <0.97 | normal |
| 5 years and 2 months | 599 | 7.12 | <27.6 | <0.69 | 4.34 | <1.05 | 25.5 | <0.97 | normal |
| 5 years and 8 months | 264 | 6.55 | <27.6 | <0.69 | 10 | <1.05 | 251.1 | 3.88 | normal |

Normal values: K+: 3.5–5.5 mmol/L; Na+: 130–150 mmol/L; ACTH: <46 pg/mL; 17-OHP: <29.1 mmol/L; cortisol: 124–662 nmol/L; testosterone: <0.69 nmol/L; renin: 2.8–39.9 μIU/mL; aldosterone: <23.6 ng/dL; DHEA: <0.41 μmol/L.

of pregnancy, ultrasound examination revealed that the fetus had hypospadias and amniotic fluid chromosome karyotyping showed 46 XY. After birth, neonatal screening revealed an abnormal 17-OHP level (110 nmol/L). His parents were healthy, with normal puberty, no consanguinity and no family history of genetic metabolic disease (including CAH).

Upon physical examination at the time of initial diagnosis (2 months and 27 days of age), body length was 61 cm and body weight was 6 kg. The child showed normal development, good nutrition, no abnormal facial features, lack of pigmentation in the gums, areola and skin folds, hemorrhoids, and normal heart and lungs. The external genitalia had several features such as a short penis similar to a clitoris (about 1.2 cm long, 3.0 cm in circumference), a urethral opening in the base of the penis, scrotum hypertrophy with no skin pigmentation, and Tanner staging of G1PH1. The laboratory results are shown in Table 2. The 17-OHP and potassium levels were significantly elevated, but sodium remained low at 2 months and 27 days of age. Other laboratory findings at the time of initial diagnosis were abnormal, including high ACTH and renin levels; however, 17-OHP, dehydroepiandrosterone, aldosterone, and electrolyte levels remained normal. These laboratory results may be related to hormone treatments that were being administered to the patient. At 1 year and 8 months of age, the ACTH stimulation test was completed (Table 3). The cortisol level after the challenge was less than 500 nmol/L, suggesting a glucocorticoid deficiency. Progesterone, 17-OHP, androstenedione, and other byproducts remained low, which also suggested an adrenal cortical hormone synthesis disorder. The observed lesion was located at the adrenal gland and was consistent with 3β-HSD deficiency type II, but dehydroepiandrosterone was not elevated during the challenge, which may be explained by long-term hormone replacement therapy. During the follow-up period, 17-OHP, DHEA, ACTH, aldosterone, and electrolytes levels were normal after treatment.

## Clinical data for Case 3

This is a female child (46, XX) who is now 8 years and 2 months old. At 5 months and 8 days of age, she was examined by an endocrinologist due to skin pigmentation identified shortly after birth, intermittent vomiting, diarrhea, and developmental delay. This patient has bilateral clitoral hypertrophy. There was pigmentation of the labia, intermittent vomiting, diarrhea, growth delay, irritability, convulsions, lack of sweating, poor response, hemorrhoids, but normal stature and normal intelligence development. Electrolyte test results showed a concentration range of potassium from 7.26 mmol/L to 9.2 mmol/L and sodium from 96 mmol/L to 100 mmol/L. She was born at G2P1 (Tanner staging), full-term, with birth weight of 3.1 kg and unknown birth length; she was subject to artificial feeding after birth. The parents of the child are healthy, non-consanguineous, had normal puberty development, an abortion of the first child and negative family history of genetic any metabolic diseases, including CAH.

Upon physical examination at the time of initial diagnosis (age of 5 months), the patient had a body length of 59.8 cm and weight of 4.8 kg, and presented with developmental delay, malnutrition, abnormal skin and areola

**Table 2.** Laboratory tests of Case 2 during follow-up

| Age | ACTH [pg/mL] | 17-OHP [mmol/L] | Cortisol [nmol/L] | Testosterone [nmol/L] | DHEA [μmol/L] | AND [nmol/L] | Renin [μIU/mL] | Aldosterone [ng/dL] | Electrolyte [mmol/L] |
|---|---|---|---|---|---|---|---|---|---|
| Outside the hospital | – | 110 | – | – | – | – | – | – | $K^+$ 5.67<br>$Na^+$ 114.1 |
| 2 months and 27 days | 262 | 3.7 | 810 | 4.3 | <0.41 | 2.13 | 464 | <0.97 | normal |
| 3 months and 26 days | 42.9 | 3.78 | 81.3 | 17.8 | 0.497 | 5.39 | 88.5 | <0.97 | normal |
| 5 months and 27 days | <10 | <0.303 | 42.8 | <0.69 | <0.41 | <1.05 | 6.5 | <0.97 | normal |
| 9 months and 15 days | 73.6 | 0.45 | 30.3 | 17.8 | <0.41 | <1.05 | <0.5 | 1.02 | normal |
| 1 year and 4 months | 150 | 1.74 | 46.9 | 1.76 | <0.41 | <1.05 | 29.1 | 1.94 | normal |
| 1 year and 8 months | 126 | 1.97 | 63.2 | <0.69 | <0.41 | <1.05 | 77.3 | <0.97 | normal |
| 2 years and 3 months | 48.3 | 0.83 | 46.4 | <0.69 | <0.41 | <1.05 | 1.3 | 1.83 | normal |
| 2 years and 8 months | 157 | 2.66 | 67.9 | <0.69 | <0.41 | <1.05 | 77.3 | <0.97 | normal |

**Table 3.** ACTH stimulation test of Case 2

| Items | Base value | 30 min | 60 min | 90 min | 120 min |
|---|---|---|---|---|---|
| Cortisol | 370 | 284 | 284 | 250 | 170 |
| Progesterone | <0.64 | <0.64 | <0.64 | <0.64 | <0.64 |
| Dehydroepiandrosterone | <0.41 | <0.41 | <0.41 | <0.41 | <0.41 |
| Androstenedione | <1.05 | <1.05 | <1.05 | <1.05 | <1.05 |
| 17-hydroxyprogesterone | 1.61 | 1.93 | 2.1 | 1.96 | 1.59 |

pigmentation, lack of pigmentation in the oral mucosa, pigmentation in the vulva, and slightly enlarged clitoris.

The laboratory results are shown in Table 4. At the time of initial diagnosis, they showed high levels of potassium, ACTH, 17-OHP, and DHEA, but sodium levels were low and renin and aldosterone level remained normal. During the treatment period, ACTH and 17-OHP, AND, aldosterone, and electrolytes were normal. At the age of 8, her bone age is 6 years and 9 months, which is slightly behind normal.

## Gene sequencing results of the 3 cases

The gene sequencing results for *HSD3B2* for the 3 children are shown in Table 5 and Fig. 1. The *HSD3B2* gene screening results in Case 1 showed 2 heterozygous sequence changes, namely, c.154_162delinsTCCTGTT/p.Arg52Serfs*10 and c.1003C>T/p.Arg335Ter. The c.154_162delinsTCCTGTT pathogenic variant was inherited from the mother, indicating that bases between 154 and 162 were deleted, creating a frameshift mutation with a premature stop codon. In addition, the c.1003C>T nonsense pathogenic variant, which was predicted to result in premature termination, was not detected in the father. The *HSD3B2* gene screening results for Case 2 showed 2 missense heterozygous pathogenic variants, c.424G>A (p.E142K) change inherited from the father and c.674T>A (p.V225D) inherited from the mother. Moreover, *HSD3B2* gene screening of Case 3 showed 2 pathogenic variants, a missense c.776CcT (p.T259M) change inherited from the mother and a nonsense c.1003C>T (p.R335X) change expected to result in premature termination inherited from the father.

**Table 4.** Laboratory tests of Case 2 during follow-up

| Age | ACTH [pg/mL] | 17-OHP [mmol/L] | Cortisol [nmol/L] | Testosterone [nmol/L] | DHEA [µmol/L] | AND [nmol/L] | Renin [µIU/mL] | Aldosterone [ng/dL] | Electrolyte [mmol/L] |
|---|---|---|---|---|---|---|---|---|---|
| 5 months and 8 days | >1250 | >75.5 | 497 | 2.13 | >27.1 | >35 | 10.59 | 0.408 | $K^+$ 7.66<br>$Na^+$ 119.5 |
| 6 months and 18 days | <10 | 1.38 | – | <0.069 | – | – | 8.27 | 0.059 | normal |
| 7 months and 15 days | <10 | <0.303 | – | <0.069 | – | – | 0.27 | 0.058 | normal |
| 10 months and 29 days | 23.9 | – | – | <0.069 | – | – | 0.41 | 0.078 | normal |
| 1 year and 2 months | 14.5 | <0.303 | – | <0.069 | <0.407 | <1.05 | 0.46 | 0.08 | normal |
| 1 year and 6 months | 15.4 | <0.303 | 196 | <0.069 | <0.407 | <1.05 | 0.63 | 0.13 | $K^+$ 3.3 |
| 2 years and 2 months | 44.2 | <0.303 | – | <0.69 | – | – | 0.69 | 0.14 | normal |
| 2 years and 7 months | 142 | – | – | 1.07 | – | – | 0.62 | 0.13 | normal |
| 3 years and 2 months | <10 | <0.303 | – | <0.69 | <0.407 | <1.05 | – | – | normal |
| 3 years and 7 months | 261 | <0.303 | 32 | <0.69 | <0.407 | <1.05 | 0.57 | 0.13 | normal |
| 4 years and 1 month | 1067 | 3.96 | – | <0.69 | 0.738 | <1.05 | 2.09 | 0.15 | normal |
| 4 years and 6 months | 447 | 5.04 | 23.6 | <0.69 | 0.532 | <1.05 | 7.6 | <0.97 | normal |
| 5 years | 1250 | 9.01 | 24.6 | <0.69 | 3.61 | 2.58 | 14.5 | <0.97 | normal |
| 5 years and 7 months | 674 | 2.66 | 35 | <0.69 | 0.54 | <1.05 | 5.8 | 3.86 | normal |
| 6 years and 1 month | 643 | 18.37 | 67.6 | <0.69 | 5.84 | 5.18 | 170.2 | 3.33 | normal |
| 6 years and 5 months | 268 | <0.303 | – | <0.69 | <0.41 | <1.05 | 15.7 | 1.28 | normal |
| 6 years and 8 months | <10 | <0.303 | 118 | <0.69 | <0.41 | <1.05 | 12.6 | <0.97 | normal |
| 6 years and 11 months | 117 | 3.54 | <27.6 | <0.69 | 0.456 | <1.05 | 109.8 | 1.43 | normal |
| 7 years and 5 months | <10 | <0.303 | 60.7 | <0.69 | <0.41 | <1.05 | 8.1 | <0.97 | normal |
| 8 years | 55.2 | 0.48 | <27.6 | <0.69 | <0.41 | <1.05 | 61 | <0.97 | normal |

**Table 5.** *HSD3B2* gene results of 3 cases

| Cases | Mutation site | | Mutation type | Exon | Father | Mother |
|---|---|---|---|---|---|---|
| | Nucleotide change | Amino acid change | | | | |
| Case 1 | c.154_162delinsTCCTGTT | p.Arg52Serfs*10 | frameshift mutation | 3 | none | hybrid |
| | c.1003C>T | p.Arg335Ter | nonsense mutation | 4 | none | none |
| Case 2 | c.424G>A | P.E142K | missense mutation | 4 | hybrid | none |
| | c.674T>A | P.V225D | missense mutation | 4 | none | hybrid |
| Case 3 | c.776C>T | p.259, T>M | missense mutation | 4 | none | hybrid |
| | c.1003C>T | P.335, R>X(38) | nonsense mutation | 4 | hybrid | none |


Fig. 1. Gene mutation sites

## Adrenal cortical hormone replacement therapy

Case 1 was treated with hydrocortisone replacement therapy immediately after the initial diagnosis (Table 6). The highest dose of hydrocortisone was administered at the beginning during infancy but was gradually decreased with age. The dose at 2–5 years of age was 8–10 mg/m$^2$/day, and was slightly increased after 5 years of age. The dose of fludrocortisone was gradually increased within the first 3 months of life, but the maximum dose did not exceed 100 μg/day and was slowly decreased after 3 months of age. Adrenal insufficiency control was satisfactory during treatment as evidenced by lack of adrenal crisis, hypertension and normal electrolytes. Dehydroepiandrosterone sulfate (DHEAS) and other androgens were well-controlled. Bone age was not advanced and consistent with age. In infancy, the curve of children's height fluctuates greatly due to repeated pneumonia, diarrhea and other diseases. After 1 year and 8 months, the height of children grows along the P3 curve of children of the same age. At 4 years old and 4 months of age, the height curve significantly increased.

Case 2 was treated with hydrocortisone and fludrocortisone replacement therapy. The dose of the drug was adjusted after the initial diagnosis (Table 7). The dose of hydrocortisone fluctuated between 5 mg/m$^2$/day and 10 mg/m$^2$/day. The dose of fludrocortisone was gradually decreased with age. During treatment, adrenal insufficiency was controlled satisfactorily (no adrenal crisis, no hypertension, no bone advanced age and normal electrolytes), with normal physical development.

Case 3 was treated with hydrocortisone and fludrocortisone replacement therapy after the initial diagnosis (Table 8). The dose of hydrocortisone was highest at initial dose in infancy and gradually decreased with age. The dose at 3–6 years old was maintained at 10 mg/m$^2$/day, and the dose was slightly increased after reaching the age of 6. The dose of fludrocortisone was gradually reduced with age. During treatment, adrenal insufficiency was controlled satisfactorily. At 4 years and 1 month, there was an increase in blood pressure, which indicated that there may be mineralocorticoid overtreatment; after dose reduction, blood pressure returned to normal. Electrolytes were normal. At the age of 8, the age of the bones was 6 years and 9 months, slightly behind normal, suggesting that the treatment of sex hormone synthesis disorders may also be warranted.

Table 6. Physical examination and medication results of Case 1 during follow-up

| Age | Height/weight [cm/kg] | Blood pressure [mm Hg)] | Penis length* circumference [cm] | Testis [mL] | Hydrocortisone [mg/m$^2$/day] | Fluorocortisone [μg/day] | Bone age [years] |
|---|---|---|---|---|---|---|---|
| 1 month and 13 days | 52/3.5 | – | 1.0*3.0 | 1.5 | 33.7 | 50 | – |
| 1 month and 27 days | 58/4.0 | – | 1.0*3.0 | 1.5 | 31.25 | 75 | – |
| 3 months and 2 days | 59/5.0 | – | 1.5*3.0 | 2 | 27.27 | 100 | – |
| 5 months and 12 days | 65/7.0 | – | 2.0*3.0 | 2 | 21.74 | 75 | – |
| 7 months and 27 days | 65/7.5 | – | 2.0*3.0 | 2 | 20.69 | 75 | – |
| 11 months and 3 days | 67/8.0 | – | 2.0*3.0 | 2 | 15.79 | 75 | – |
| 1 year and 1 month | 70/8.5 | – | 2.0*3.5 | 2 | 15.09 | 75 | – |
| 1 year and 4 months | 70/8.5 | – | 2.0*3.5 | 2 | 12.58 | 60 | – |
| 1 year and 8 months | 78/10 | – | 2.0*4.0 | 2 | 10.18 | 50 | 1.4 |
| 1 year and 11 months | 80.5/11.5 | 96/56 | 3.5*4.0 | 2 | 12.12 | 50 | – |
| 2 years and 4 months | 88/13 | 100/50 | 3.5*4.0 | 2 | 9.01 | 50 | 3.2 |
| 2 years and 11 months | 91/13.5 | 98/53 | 4.0*4.5 | 2 | 8.73 | 50 | – |
| 3 years and 6 months | 93/15 | 100/60 | 4.0*4.5 | 2 | 9.28 | 33.3 | 5.3 |
| 4 years | 97/15 | 101/53 | 4.0*4.5 | 2 | 9.6 | 50 | 5.8 |
| 4 years and 7 months | 101/17 | 129/96 | 4.0*4.5 | 2 | 8.63 | 40 | – |
| 4 years and 11 months | 103.3/17 | 107/62 | 4.0*4.5 | 2 | 11.99 | 40 | – |
| 5 years and 2 months | 104.5/18 | 105/65 | 4.0*4.5 | 2 | 11.41 | 40 | 6.5 |
| 5 years and 8 months | 110.5/20 | 110/68 | 4.0*4.5 | 2 | 12.5 | 40 | 7.2 |

**Table 7.** Physical examination and medication results of Case 2 during follow-up

| Age | Height/weight [cm/kg] | Blood pressure [mm Hg] | Penis length* circumference [cm] | Testis [mL] | Hydrocortisone [mg/m$^2$/day] | Fluorocortisone [μg/day] | Bone age [years] |
|---|---|---|---|---|---|---|---|
| 2 months and 27 days | 61/6 | – | 1.2*3.0 | 2.5 | 9.68 | 66.67 | – |
| 3 months and 26 days | 64/7 | – | 1.5*3.0 | 2.5 | 8.7 | 75 | – |
| 5 months and 27 days | 68/8.7 | – | 2.0*3.5 | 2.5 | 7.42 | 60 | – |
| 9 months and 15 days | 74/11 | – | 3.5*4.5 | 3 | 6.19 | 50 | – |
| 1 year and 4 months | 83/13 | – | 4.0*4.5 | 3 | 5.41 | 50 | – |
| 1 year and 8 months | 88/13 | 102/73 | 4.0*4.5 | 3 | 9.01 | 50 | 2.5 |
| 2 years and 3 months | 94.5/14 | 100/73 | 4.0*4.5 | 3 | 8.47 | 33.3 | – |
| 2 years and 8 months | 99.5/15 | – | 4.0*4.5 | 3 | 9.33 | 40 | – |

**Table 8.** Physical examination and medication results of Case 3 during follow-up

| Age | Height [cm] | Blood pressure [mm Hg] | Penis length* circumference [cm] | Testis [mL] | Hydrocortisone [mg/m$^2$/day] | Fluorocortisone [μg/day] |
|---|---|---|---|---|---|---|
| 5 months and 8 days | 59.8 | 4.8 | – | 37.31 | 50 | – |
| 6 months and 18 days | 65 | 6.3 | 94/46 | 23.41 | 60 | – |
| 7 months and 15 days | 66 | 9.2 | 71/41 | 17.77 | 60 | – |
| 10 months and 29 days | 72 | 9 | 90/65 | 14.46 | 50 | 0.8 |
| 1 year and 2 months | 72.3 | 9.2 | 72/42 | 14.22 | 50 | – |
| 1 year and 6 months | 78 | 9.5 | 86/64 | 13.87 | 50 | – |
| 2 years and 4 months | 83 | 11 | 86/55 | 12.37 | 50 | 1.7 |
| 2 years and 7 months | 86 | 12 | 90/60 | 14.42 | 50 | – |
| 3 years and 2 months | 88 | 14 | 90/62 | 10.17 | 50 | – |
| 3 years and 7 months | 94.5 | 15 | 90/62 | 8.8 | 33.3 | – |
| 4 years and 1 month | | 15 | 140/106 | 9.6 | 33.3 | 3.9 |
| 4 years and 6 months | 102.5 | 16.5 | 101/55 | 8.86 | 33.3 | – |
| 5 years | | 17.5 | 106/48 | 8.42 | 33.3 | 5.2 |
| 5 years and 7 months | 109 | 19 | 104/54 | 9.8 | 16.7 | – |
| 6 years and 1 month | 113.5 | 20 | 93/60 | 9.38 | 16.7 | 6.6 |
| 6 years and 5 months | | 21 | – | 13.96 | 16.7 | – |
| 6 years and 8 months | 116 | 21.5 | 101/61 | 11.73 | 50 | – |
| 6 years and 8 months | 117 | 23 | 96/55 | 14.73 | 33.3 | 6.8 |
| 7 years and 5 months | 118.9 | 25 | 116/73 | 11.96 | 16.7 | – |
| 8 years | 122.5 | 27 | 109/54 | 11.16 | 16.7 | 6.8 |

## Masculine insufficiency treatment for 2 cases

### Case 1

Figure 2A shows the abnormal external genital manifestations of the child at the time of initial diagnosis. The child was given long-acting testosterone intramuscular injections at the age of 1 year and 9 months at a dose of 100 mg/m$^2$ once every 15 days, 4 times in total over 2 months. The penis size increased significantly at 1 year and 11 months (Fig. 2B); namely, the length was about 3.5 cm and the circumference was 4.0 cm. After topical application of dihydrotestosterone cream for 1 month, the drug was discontinued for 3 months. At the age of 2 years and 3 months, the patient underwent hypospadias repair at the local hospital. The postoperative penis (Fig. 2C,D) was about 4.0 cm long and 4.5 cm in circumference.

### Case 2

Figure 3A shows the external genital performance of the child at the time of initial diagnosis. At the age of 3 months, intramuscular injection of gonadotropin (1500 U) was administered once every 2 weeks for a total of 6 times over 3 months. The penis of the child was slightly enlarged (Fig. 3B) – about 2.0 cm long and 3.5 cm in circumference. At 6 months of age, long-acting testosterone began to be injected (intramuscularly) in a dose

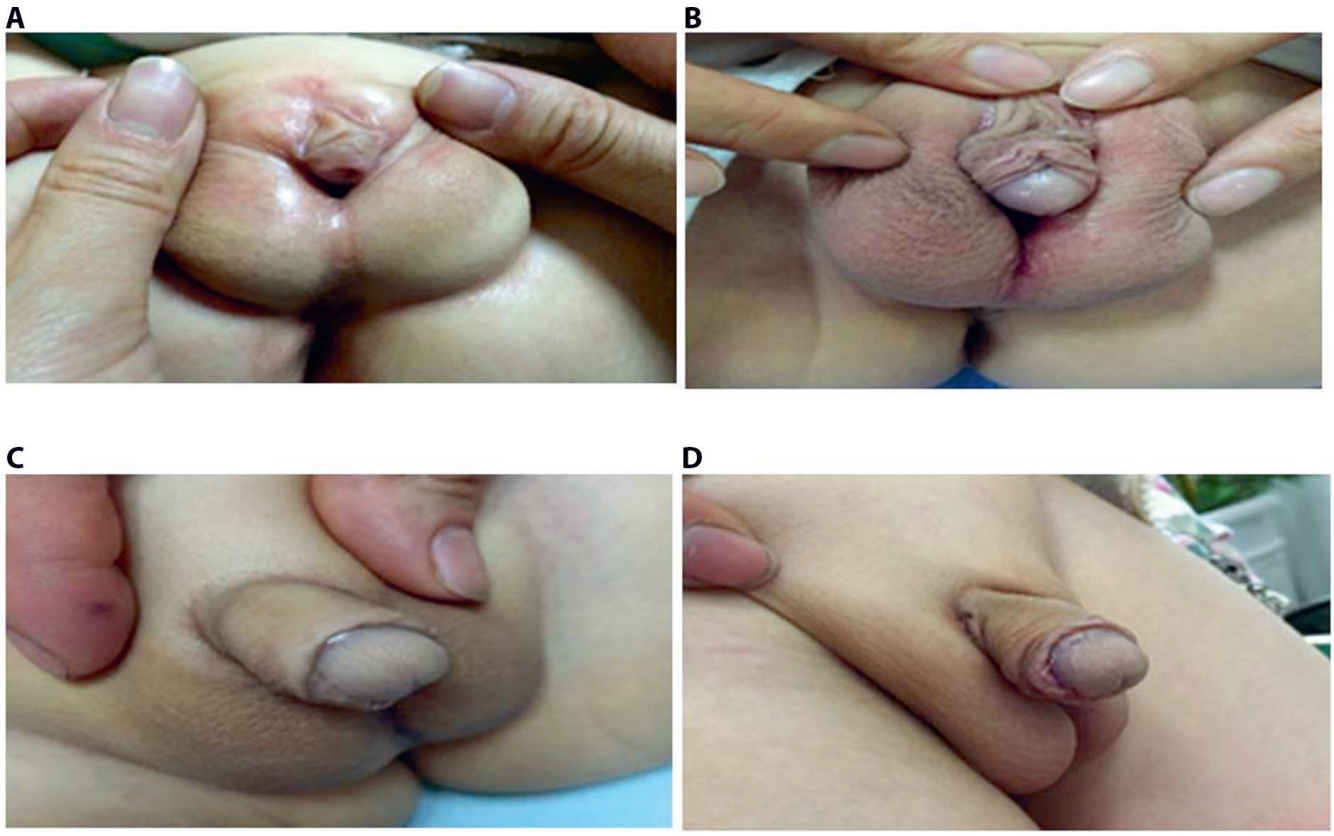


Fig. 2. External genitalia size of intramuscular long-acting testosterone (A), intramuscular long-acting testosterone 2 months later (B), 8 months after hypospadias repair (C) and 28 months after hypospadias repair (D) for Case 1

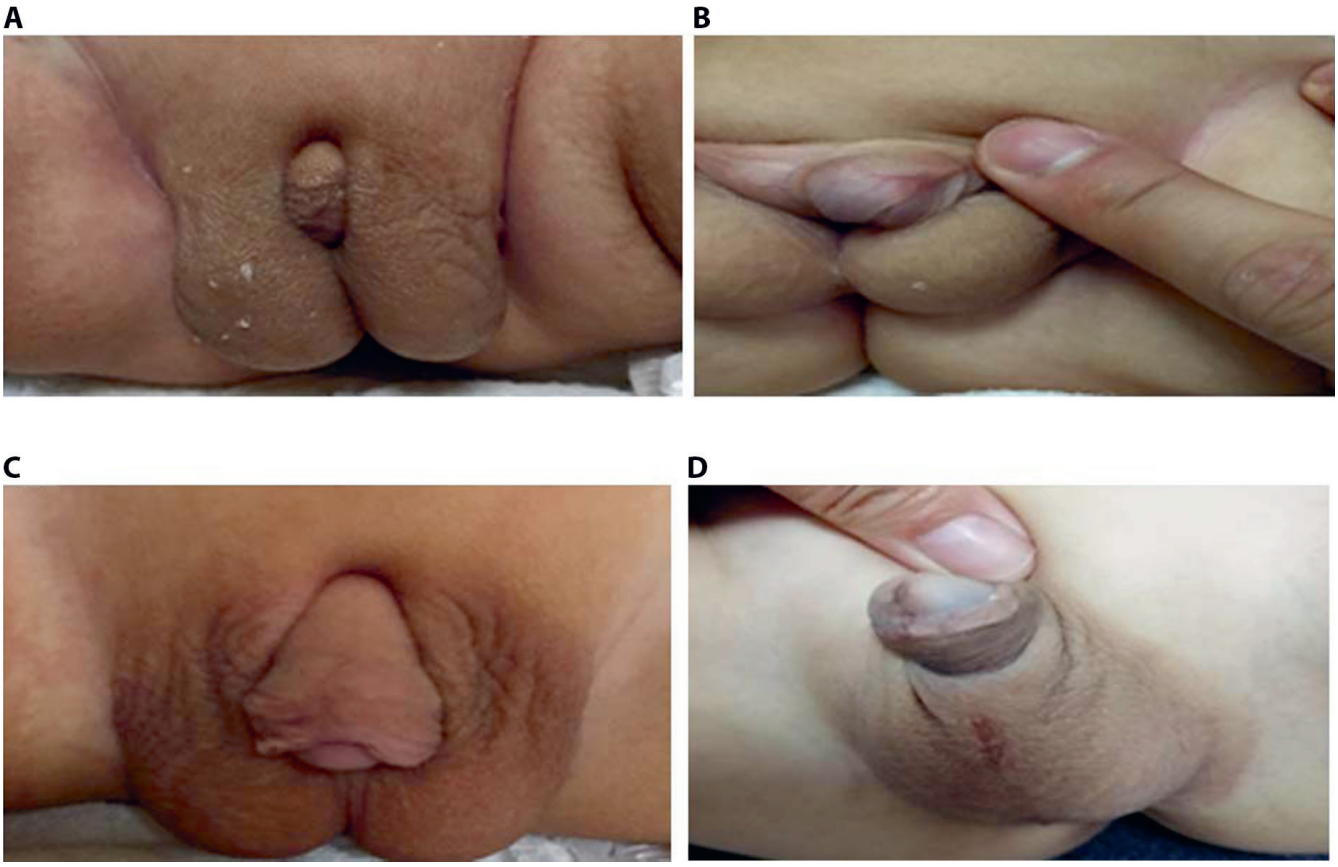


Fig. 3. External genitalia size of HCG and long-acting testosterone (A), intramuscular injection of HCG 6 times (B), intramuscular long-acting testosterone 3 months later (C) and 4 months after hypospadias repair (D) for Case 2

of 100 mg/m$^2$ once every 2 weeks. The course of treatment was 3 months and the size of the penis increased significantly (Fig. 3C) to a length of about 3.5 cm and circumference of 4.5 cm. At the age of 2 years and 10 months, the patient underwent hypospadias repair at the local hospital. After 4 months, the penis (Fig. 3D) was about 4.0 cm long and 4.5 cm in circumference.

### Case 3

Female child had mild masculinization of the external genitalia (slightly hypertrophied clitoris). However, the pigmentation of the vulva was not aggravated and the clitoris size did not increase progressively. No surgical correction was needed.

## Discussion

3β-HSD deficiency is mainly caused by mutations in the *HSD3B2* gene that cause adrenocortical hormone and sex hormone synthesis disorders.[11] Levels of pre-steroidal steroids, such as pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone, and androstenediol, were observed to be increased, while progesterone, 17-OHP, androstenedione, testosterone, and other downstream products were decreased, resulting in adrenal insufficiency and external genital abnormalities as clinical manifestations. In this paper, we reported 3 3β-HSD deficiency cases, including 2 male children and 1 female child, with the age of onset in infancy, who suffered from loss of salts caused by decreased aldosterone synthesis with clinical consequences – namely, spitting milk, diarrhea, slow weight gain, and high potassium and low sodium levels. All cases had reduced cortisol levels, which is known to promote melanocytes to stimulate hormones that lead to increased melanin synthesis, causing pigmentation in skin folds, areola, vulva, and other parts of the body. All of the 3 children had abnormal genital function caused by sexual steroid synthesis disorder. Among them, 2 male patients showed serious insuffciency in masculine features and the female child showed mild masculinity with heavy salt loss. Usually, 3β-HSD deficiency can cause levels of 17-OHP and testosterone and other downstream products to decrease, but in all 3 children, we observed an elevation of 17-OHP and testosterone, which was mainly due to a lack of 3β-HSD2 and 3β-HSD1 expression; the latter is known to convert 17-hydroxypregnenol to 17-OHP in extra-adrenal tissue to cause an increase in its levels. An increase in testosterone may be due to the conversion of excess dehydroepiandrosterone to testosterone by 3β-HSD1 in the periphery, or further conversion of dehydroepiandrosterone to testosterone by elevated 17-OHP level under the action of an enzyme such as 17,20 carbon lyase. All 3 children suffered from severe salt loss in early infanthood, as shown with laboratory tests. Dehydroepiandrosterone increased in Case 1 and Case 3 at diagnosis. On the basis of the observed clinical manifestations and laboratory results, it can be established that the 3 children had the classic manifestations of 3β-HSD deficiency.

The results of *HSD3B2* gene sequencing in the 3 children are shown in Table 5. All of the children had compound heterozygous mutations and the mutation types were different, including 1 nonsense mutation, 1 frameshift mutation and 3 missense changes. The frameshift mutation was located at exon 3 while the remaining mutations were located at exon 4. After reviewing the OMIM (Online Mendelian Inheritance in Man; www.omim.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and NCBI (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov) databases, it was found that the mutation sites c.1003C>T, c.424G>A and c.776C>T have been reported in the literature,[6,12,13] while c.154_162delinsTCCTGTT and c.674T>A are novel. According to a previous study, the c.424G>A and c.776C>T mutations result in undetectable enzyme activity in vitro, whereas the c.1003C>T mutation yielded an activity level of only 2% in vitro.[7] It has been demonstrated that more than 2% of residual enzyme activity may offer sufficient mineralocorticoid production to avoid severe salt loss.[8] Therefore, the genetic results of the 3 children explain the clinical manifestations as a classical type of 3β-HSD deficiency. Thus, there was a strong genotype–phenotype correlation in the 3 patients.

The gene sequencing results presented in Table 5 showed that the healthy father and healthy mother of Case 2 and Case 3 were carriers of these mutations of the *HSD3B2* gene. In addition, *HSD3B2* gene sequencing resulted in the identification of 2 compound heterozygous mutations consistent with an autosomal recessive form of 3β-HSD deficiency. Interestingly, the mutation was not detected in the father of Case 1 and was a de novo change. The c.154_162delinsTCCTGTT/p.Arg52Serfs*10 was a frameshift mutation that was predicted to end in an early termination of the peptide chain. Thus, this change is considered to be associated with a pathogenic variant. In Case 2, sequencing results suggested that there were 2 compound heterozygous mutations, c.424G>A (p.E142K) and c.674T>A (p.V225D). Segregation demonstrated that the 2 mutations were derived from each of the healthy parents, thus supporting an autosomal recessive mode of inheritance and providing more evidence for the pathogenicity of these 2 changes.

The standard treatment for 3β-HSD deficiency is hormone replacement therapy. The commonly used drugs in children are hydrocortisone and fludrocortisone. Garagorri et al.[14] showed that cortisol increased linearly with age beginning at 6 months of age, and that the concentration of basal cortisol in children did not change dramatically.[15] There were both similarities and differences in hydrocortisone treatment options for the 3 cases in this study. The similarities were that the dose of hydrocortisone in infancy decreased with age and was maintained

at 8–10 mg/m$^2$/day in early childhood, increased slightly before and after school age, and was maintained at about 10–12 mg/m$^2$/day thereafter. The differences included the initial doses in Case 1 and 3 being significantly higher than those in Case 2. Case 3 had significantly higher doses of hydrocortisone in infancy compared to Case 1, mainly due to the late treatment of Case 3.[16] The physiological secretion of endogenous cortisol in children is 6–8 mg/m$^2$/day, and pre-child cortisol levels fluctuate less. The dose of hydrocortisone in early childhood is maintained at slightly higher concentrations than physiological levels. A slight increase in levels before and after school age may be related to learning and environmental changes.

We gained important insight into disease treatment from the 3 cases studied here. Treatment is somewhat similar to the treatment for 21-hydroxylation deficiency. However, compared with 21-hydroxylation deficiency treatment, the hydrocortisone dosage for the 3 cases in our study was limited and androgen elevation was easier to control. It should be noted, though, that the patients could be prone to overtreatment. The dose of fludrocortisone is gradually decreased with age, and the maximum dosage should not exceed 100 μg/day. The dose should be adjusted according to blood pressure and electrolyte and renin activity during treatment to maintain plasma renin activity in the normal to medium range.[17]

Masculinization treatment is mainly aimed at treating male patients with a small penis and with hypospadias and other incomplete masculinization.[18] Genital abnormalities in 3β-HSD deficiency are caused by 3β-HSD2 activity being the highest during the critical period of external genital development before the 12$^{th}$ week of gestation during the fetal period.[19] Therefore, male children can be affected by impaired testosterone biosynthesis in the early fetal stage due to a lack of 3β-HSD2 in the testis, causing androgen deficiency leading to genital abnormalities, small penis, hypospadias, and severe external genitalia underdevelopment.[20] 3β-HSD1 activity is higher than 3β-HSD2, which is most active in the 3$^{rd}$ trimester of pregnancy, after genital development.[21] Although 3β-HSD1 mediates the conversion of excess dehydroepiandrosterone to testosterone, for a man with a 46 XY karyotype and 3β-HSD2 deficiency, androgen levels are not sufficient for the normal development of genitalia. For female children, the lack of testosterone in early pregnancy can degrade the Wolffian catheter, while the Müllerian catheter can develop into the fallopian tubes and uterus,[21] and 3β-HSD1 mediates the conversion of dehydroepiandrosterone to testosterone in the 3$^{rd}$ trimester. Elevated androgen levels can cause affected women to be mildly masculinized (with different degrees of clitoral enlargement, with or without labial fusion).[22] Treatment of hypospadias after the penis is enlarged is usually recommended for hypospadias from 6 months to 2 years of age, which is in the mini-puberty range. We reported Cases 1 and 2 at 1 year and 9 months and 6 months of age, respectively, treated with long-acting testosterone to increase penis size. Case 1 was treated with a combination of topical testosterone cream and penile lengthening, both of which resulted in satisfactory penis growth and excellent recovery after hypospadias repair. During follow-up for growth and development, 2 patients who were treated with long-acting testosterone had bone age consistent with their age, which indicates the safety of short-term testosterone therapy in patients younger than 2 years. The masculinization of Case 3 was mild, and there was no progress in masculinization with age, so no surgical intervention was needed.

## Conclusions

This study reported on 3 children with classic 3β-HSD deficiency. All infants had typical adrenal insufficiency and abnormal genital manifestations, along with abnormal steroid hormone levels and with compound mutations in *HSD3B2*. Specifically, the mutations were diverse and located in exons 3 and 4, with a strong genotype–phenotype correlation. The c.154_162delinsTCCTGTT and c.674T>A (p.V225D) mutations were novel changes and were classified as pathogenic. The treatment of the disease was mainly corticosteroid replacement, after which adrenal function was observed to normalize. For male children with hypospadias and small penis, in order to obtain the best surgical effect of hypospadias repair, testosterone use was recommended instead of interventions to increase penis size at adolescence. In addition, the 3 probands reported in this study did not enter puberty and are still being followed to evaluate hormone levels and puberty development performance.

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