Hypospadias in a Male Patient with 21-hydroxylase Deficiency

KANAKO KOJIMA-ISHII^{*,***}, IKUMA FUJIWARA^{*}, NORIYUKI KATSUMATA^{**}, JUNKO KANNO^{*}, EISHIN OGAWA^{*} AND SHIGERU TSUCHIYA^{*}

^{*}Department of Pediatrics, Tohoku University Hospital, 1-1 Seiryō-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan

^{**}Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

^{***}Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Abstract. A 17-day-old Japanese boy was transferred to the hospital because of vomiting and impaired consciousness. His external genitalia was pigmented associated with small penis and penoscrotal hypospadias. He was diagnosed as suffering from adrenal deficiency according to severe electrolyte abnormality, moderate hypoglycemia, metabolic acidosis and extremely elevated 17-OHP and testosterone levels. He turned out to be a compound heterozygote of *CYP21A2* mutations by genetic analysis. Through endocrinological evaluation, he seemed to have normal hypophyseal function, intact testosterone production and appropriate 5- α -reductase-2 activity. Partial androgen insensitivity could not be ruled out by slight decrease of SHBG in hCG loading test, although mutation was not detected on androgen receptor gene. This is a rare case of a male patient with 21-hydroxylase deficiency accompanied by hypospadias. As the cause of hypospadias in this case has yet to be elucidated, further investigation and careful follow-up are required.

Key words: Congenital adrenal hyperplasia, Hypospadias, Androgen insensitivity

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TWENTY-ONE-HYDROXYLASE deficiency (21-OHD) is the most common cause of congenital adrenal hyperplasia (CAH), that is characterized by glucocorticoid and mineralcorticoid deficiency and androgen excess [1]. The endocrinological abnormality can cause ambiguous genitalia in girls, but usually not in boys.

Hypospadias, defined as incomplete fusion of the penile urethra, is one of the common congenital anomalies, which is estimated to occur 0.3 to 8 per 1000 male births [2]. Incomplete masculinization of external genitalia is thought to be a responsible mechanism in theory. For example, subnormal Leydig cell function in utero, a mild degree of androgen resistance, 5- α -reductase2 deficiency, or abnormality in the action of dihydrotestosterone (DHT) can cause hypospadias

[3]. In addition, several genes such as *SOX9* [4], *XH2* [5], *HOXA13* [6], *WT1* [7] and *CXorf6* [8] are reported to be associated with hypospadias. But, at present, the etiology can be elucidated in only 30% of cases even in severe forms of hypospadias [2].

Here, we report a boy with CAH and hypospadias, who turned out to have mutations on *CYP21A2* that caused 21-OHD. Since this is the first report of hypospadias in a male patient with 21-OHD, we describe the endocrinological and genetic examination of the patient with some discussion.

Case report

The patient was born at gestational age of 37 weeks and 6 days as the first child of healthy and non-consanguineous Japanese parents. The pregnancy was uneventful and the mother had not been treated with any drugs.

On the neonatal screening 7 days after the birth, his

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Correspondence to: Kanako KOJIMA-ISHII, Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Fig. 1. External genitalia of the patient. Although male-shaped, his penis was small and accompanied with penoscrotal hypospadias. Pigmentation is not apparent because this picture was taken after the treatment.

blood 17-hydroxyprogesterone (17-OHP) level was extremely high (>80 ng/ml), but close examination was not done because he was admitted to a the maternity hospital due to low birth weight (2004 g) and had no symptoms at that time. At the age of 17 days, he developed severe condition such as vomiting and impaired consciousness, and was referred to the Tohoku University Hospital.

On admission, he was severely dehydrated and blood pressure was unmeasurable. His small penis (length: 1 cm) with penoscrotal hypospadias was evident (Fig. 1), and his external genitalia was pigmented. There were palpable tissues in the scrotum suggesting testes.

Laboratory tests revealed hyponatremia (92.8 mEq/l), hyperkalemia (6.71 mEq/l), hypochloremia (69 mEq/l), mild hypoglycemia (48 mg/dl) and metabolic acidosis (pH 7.344, HCO_3^- 19.9 mmHg, B.E. -5.5 mmol/l). His serum 17-OHP level was extremely high [1308 ng/ml

(normal range, 0.1–3.7 ng/ml)]. Serum 17-hydroxyprogrenolone level was also elevated [139 ng/ml (normal range, 0.1–4.0 ng/ml)], but not as high as 17-OHP. In addition, his testosterone level turned out to be remarkably elevated [52 ng/ml (normal range of early infant, 0.59–4.08 ng/ml)]. His karyotype was normal male type of 46, XY.

Adrenal deficiency due to 21-OHD was strongly suspected because of his clinical symptoms, salt-wasting state, pigmentation and elevated 17-OHP. This diagnosis was later confirmed by genetic analysis later. We treated him with hydrocortisone, fludrocortisone and NaCl. After 1 month of treatment, his condition was improved and the serum 17-OHP and 17-hydroxyprogrenolone decreased to nearly normal levels, then he could discharge from the hospital.

When he was 1 year and 3 months old and in good condition with 17-OHP level of 0.1 ng/ml, we performed hCG loading test to evaluate his gonadal function. Basal values of LH and FSH were <0.5 IU/l and 0.41 IU/l, respectively, which were within normal pre-pubertal limits. HCG loading test resulted in normal testosterone response from <0.05 ng/ml to 5.627 ng/ml. Ratio of testosterone to DHT (T/DHT) after hCG loading was 7.6. After hCG loading, sex hormone-binding globulin (SHBG) decreased from 55.2 nmol/l to 48.1 nmol/l (13% decrease).

Currently the patient is 5 years old and in good health with normal 17-OHP. He has been in failure to thrive since infancy and his body height is below mean -3 SD though his neurological development is normal.

Genetic analysis

DNA extracted from the peripheral blood was used for genetic analysis both from the patient and the parents (for *CYP21A2* only). *CYP21A2* was screened by PCR-RFLP method for common mutations and when mutations were suspected, they were confirmed by direct sequencing. Other genes such as *HSD3B2*, androgen receptor gene (*AR*) and *CXorf6* were analyzed by direct sequencing of all the exons and exon-intron boundaries. Informed consent was obtained from the parents. Through the genetic analysis for CAH, he turned out to be a compound heterozygote of *CYP21A2* mutations. One mutation was Q318X + R356W inherited from his mother, and the other was an 8-base deletion in exon3 from his father. Both of

them were common mutations in 21-OHD [3]. Each of his parents was a heterozygote of the single mutation. No mutation was found on *HSD3B2* that codes for 3- β -hydroxysteroid dehydrogenase. In addition, no mutation was detected on *AR* or *CXorf6*.

Discussion

The patient we report here was diagnosed as 21-OHD because he was a compound heterozygote of two common mutations in *CYP21A2*. Although most endocrinological and clinical findings of the patient were compatible with the diagnosis, he had hypospadias that is unusual in 21-OHD. Hypospadias is commonly seen in male patients with 3 β -HSD deficiency [9], but no mutation was found in the coding regions of *HSD3B2* in the patient.

Hypospadias, defined as a midline fusion defect of the penile urethra, is one of the common congenital anomalies. It can be classified according to anatomical location of the urethral meatus [2]. Our patient had penoscrotal hypospadias, which is categorized as severe type. Even in severe forms, the etiology of hypospadias can be elucidated in only 30% of cases [2]. Theoretically, some defects in masculinization could be responsible for hypospadias. In this case, we checked for causes of masculinization defect leading to hypospadias. The basal values of LH and FSH were appropriate for his age which suggested normal hypothyseal function. HCG loading test confirmed intact testosterone biosynthesis in the testes that was compatible with the hyperandrogenemia in the neonatal period. T/DHT ratio after hCG loading is reported to be useful in the evaluation of 5- α -reductase-2 activity [10, 11]. In our patient, T/DHT ratio after hCG was 7.6, that was considered to be normal.

Giwerzman *et al.* reported a girl with mutations in both *CYP21A2* and *AR*, whose genital masculinization was mild [12]. The case implied the possibility that

androgen insensitivity due to *AR* mutation might modify genital phenotype derived from hyperandrogenism of CAH. Bertelloni *et al.* suggested that the SHBG changes during hCG test was useful to assess the response to androgen [13]. In the report, SHBG after hCG did not change ($1.7 \pm 12.7\%$) in androgen insensitivity patients, whereas SHBG was significantly decreased in other male pseudohermaphroditisms ($-23 \pm 19.6\%$) or controls ($-46.9 \pm 15.2\%$). The change of SHBG in our patient was rather small (-13%) compared to the reported controls, which suggested a possibility of partial androgen insensitivity in our patient, although no mutations were detected in *AR*.

Recently *CXorf6* was identified as one of the causative genes for hypospadias [8]. We have checked for the mutation of *CXorf6* in our patient, but no mutation was found. Furthermore, mutations in transcription factors such as *SOX9* [4], *XH2* [5], *HOXA13* [6] and *WT1* [7] can cause syndromes with hypospadias. But our patient does not have characteristic phenotypes of these syndromes.

Unknown environmental chemicals or variations of certain genes responsive to environmental agents might have a link to hypospadias of the patient, but we have no means to confirm this hypothesis at present.

In summary, we reported a rare case of a male patient with hypospadias despite androgen excess caused by 21-OHD. Although the etiology of his hypospadias has not been elucidated, the possibility of partial androgen insensitivity remains. Further etiological investigation and careful clinical observation are required.

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