Clinical Course of Patients with Nonclassical 21-hydroxylase Deficiency (21-OHD) Diagnosed in Infancy and Childhood

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Abstract. We report four cases of nonclassical 21-hydroxylase deficiency (21-OHD) diagnosed in neonate or early childhood. The four patients comprised a 6-year, 5-month-old male (case 1); a 3-year, 10-month-old female (case 2); a 13-year, 11-month-old female (case 3) and a 17-year, 1-month-old male (case 4). Cases 3 and 4 were siblings. None had any signs of virilization or salt wasting at birth. 21-OHD was diagnosed using ACTH loading test and other adrenal steroid evaluations. Mutations of the CYP21 gene were detected in all patients. Three patients (cases 1, 3 and 4) had positive results in neonatal mass screening. Cases 1 and 2 showed no apparent signs of virilization and were observed without conventional treatment. In cases 3 and 4, because of increased growth velocity and accelerated bone maturation, hydrocortisone administration was initiated from their late infantile period. In spite of hydrocortisone treatment, in case 4, the final height of 159.7 cm was less than his predicted final height. Besides he revealed adrenal insufficiency at the age of 9 years and 2 months old caused by viral infection. Hydrocortisone supplementation therapy may cause adrenal insufficiency in nonclassical patients due to suppression of the hypothalamus-pituitary-adrenal axis. The clinical courses in these cases were various, and it was difficult to predict the appearance of any symptoms of virilization. Careful observation is necessary.

Key words: Congenital adrenal hyperplasia (CAH), 21-hydroxylase deficiency (21-OHD) nonclassical type, Neonatal mass-screening, 17-hydroxyprogesterone (17-OHP)

CONGENITAL adrenal hyperplasia (CAH) [OMIM: 201910] is one of the most common autosomal recessive disorders in humans; 21-hydroxylase deficiency (21-OHD) accounts for 90 to 95% of all cases of CAH. Three subtypes of 21-OHD exist, which are based on the degree of clinical manifestation. First, the salt-wasting form is characterised by life-threatening crisis with salt loss. Second, the simple virilizing form is characterised by ambiguous genitalia in females and precocious puberty in both sexes. The salt-wasting and simple virilizing forms are also called classic type CAH. The third type is the nonclassical type [1–4]. This type of CAH due to 21-OHD was originally characterised 30 years ago [5]. Although both the nonclassical and the classical types of CAH show biochemical evidence of 21-OHD, their clinical features differ. Unlike patients with the classic type, patients with the nonclassical type do not show virilization at birth and begin to show signs of virilization, such as premature pubarche, hirsutism, acne and menstrual abnormality, in later childhood or puberty. Growth acceleration and final short stature are also clinical problems. Almost all cases of nonclassical 21-OHD are found in puberty or adulthood because of virilization or family history [1–3, 5–10].

In Japan, neonatal mass screening for 21-OHD has been performed since 1989, and some cases of nonclassical 21-OHD have been found in infancy [11, 12]. The clinical course of the nonclassical type is not well
documented. We have followed four nonclassical CAH cases diagnosed in infancy or early childhood. We treated two cases with hydrocortisone and followed them up until the end of their puberty. Here, we report the clinical courses of these four cases of nonclassical 21-OHD from infancy to date.

Patients

The characteristics of each patient are indicated in Table 1.

Case 1. This 6-year, 5-month-old male patient was a full-term infant with a birth weight of 3785 g. His first measured value of 17-hydroxyprogesterone (17-OHP) in dried blood on filter paper at the age of 5 days was 25.1 nmol/l. He was recalled for sampling and his second 17-OHP value was 33.8 nmol/l. He was referred to our hospital at the age of 32 days. He did not have any signs of 21-OHD, such as skin pigmentation, poor body weight gain or vomiting.

Case 2. This 3-year, 10-month-old female patient had a birth weight of 2890 g and a gestational age of 38 weeks. Her 17-OHP value was in the normal range at the time of the neonatal mass screening. Her mother was a patient with a simple virilizing form of 21-OHD who had been taking 30 mg of hydrocortisone per day during her pregnancy. The serum 17-OHP level of case 2 was therefore measured to validate the result of the mass screening. She was found to have a high 17-OHP value (52.1 nmol/l) at 33 days of age. At 3 months of age, she was admitted to our hospital for further investigation. She exhibited neither virilization nor pigmentation.

Case 3 and 4. Case 3 was a 13-year, 11-month-old female patient; her 17-OHP value in dried blood was 58.7 nmol/l at 5 days of age and 63.8 nmol/l at 12 days of age during newborn mass screening. Her external genitalia were neither virilized nor pigmented. Although her milk intake was fair, her body weight gain was not sufficient. She was admitted to our hospital for further investigation at 15 days of age.

Case 4, a 12-year, 9-month-old male patient, had a history of high 17-OHP values during CAH screening: 23.0 nmol/l at 5 days of age and 53.3 nmol/l at 17 days. He had not been examined further because he had no apparent symptoms suggesting 21-OHD. His height and weight velocity were normal, with no acceleration. He was also examined at 3-years, 4 months of age when his sister, case 3, was admitted. Their paternal aunt had been diagnosed as having 21-OHD and was treated with hydrocortisone.

Methods

17-OHP, 21-deoxycortisol and 17-hydroxypregnenolone were measured by our methods, with pretreatment of an LH-20 column for chromatographic separation [13]. Plasma ACTH and plasma renin activity and serum testosterone and androstenedione were measured with commercially available RIA kits. Urinary pregnanetriol was measured by gas chromatography.

Rapid ACTH loading test: To ascertain the diagnosis, a rapid ACTH loading test was performed for each subject. For the test, 250 micrograms of ACTH (Cortrosyn, Daiichi Pharmaceutical Co., Tokyo, Japan) per
square meter of body surface area was injected intravenously and blood was collected at 0, 60 and 120 min.

Circadian rhythm: Circadian variations in 17-OHP were examined by collecting capillary blood on filter paper at 06:00, 08:00, 14:00, 16:00, 22:00 and 24:00.

Gene analysis: The CYP21 gene was analysed using a PCR-RFLP method, as previously described. The amplified CYP21 gene was directly sequenced using the ABI PRISM 310 Genetic Analyser (Applied Biosystems) for samples whose mutations had not been detected by PCR-RFLP [14].

Bone age was measured by the Greulich-Pyle method.

Results

Endocrinology results for the four cases are presented in Tables 2 and 3. In all cases, serum electrolyte and blood glucose concentrations were within the normal range, and no laboratory findings suggested adrenal insufficiency (data not shown).

The basal serum concentration of 17-OHP in case 1 was slightly elevated. The stimulated serum 17-OHP concentration of 289.9 nmol/l confirmed the diagnosis of 21-OHD (Table 2). Regarding the values for the circadian rhythm of 17-OHP, the maximum level was 219.7 nmol/l (Table 3). Molecular analysis showed that case 1 had a P30L mutation in exon 1 and a splicing mutation in intron 2. We therefore diagnosed this patient as having 21-OHD.

Basal and stimulated concentrations of serum 17-OHP were 6.1 nmol/l and 260.0 nmol/l, respectively, in case 2 (Table 2). This intensive response in 17-OHP suggested a deficiency in 21-hydroxylase. The maximum level of 17-OHP on filter paper during circadian variations was 22.7 nmol/l (Table 3). Gene analysis showed that the patient had homozygous mutations of I236N, V237E and M239K in exon 6. He was thus diagnosed as having 21-OHD.

The basal serum 17-OHP concentration of 57.2 nmol/l was not sufficiently high to warrant diagnosis of 21-OHD in case 3; however, the 21-DOF concentration of 308.8 nmol/l strongly suggested 21-OHD (Table 2). The stimulated level of serum 17-OHP and the maximum level during the circadian rhythm study were also high: 91.3 nmol/l and 736.2 nmol/l, respectively (Table 3). Gene analysis showed mutations in exon 1, exon 4 and exon 8 (Fig. 1). We thus diagnosed this patient as having 21-OHD. Her brother, case 4, was also diagnosed as having 21-OHD based on his ACTH

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<th>Table 2. Endocrinology data for each patient at admission</th>
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<td>Case (age at examination) Case 1 (32 days) Case 2 (67 days) Case 3 (15 days) Case 4 (3 years, 3 months)</td>
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<tr>
<td>ACTH [pmol/L]</td>
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<td>PRA [ng/(L·s)]</td>
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<th>Table 3. Circadian Rhythm in 17-OHP (nmol/l)</th>
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loading test results. His normal rates of height, weight and bone maturation suggested that he was of nonclassical type. His genotype was the same as that of case 3; the results of gene analysis involving the parents are shown in Fig. 1.

The clinical course of each case is shown in Fig. 2.

Because case 1 did not show typical signs of 21-OHD when he was referred to us, such as skin pigmentation or salt loss, he has been carefully observed without treatment. As shown in Fig. 2a, his height gain does not seem to be accelerated at present. His bone maturation gradually accelerated, being 7 years, 0 months when he was 6 years, 0 months of age.

Case 2 did not present ambiguous genitalia, skin pigmentation or salt loss; therefore, we have followed her up without treatment. Both her height and body weight have ranged from –1 SD to –2 SD, and her bone age was 2 years, 8 months when she was 3 years, 0 months of age. Her serum concentrations of 17-OHP ranged between 1.70 and 91.1 nmol/l (Fig. 2b).

Because case 3 had no virilizing symptoms at birth and her genetically identical brother did not have androgen excess, the nonclassical 21-OHD was suspected. However, we started treatment with 12 mg of hydrocortisone per day because of poor weight gain. After her weight gain became sufficient, we gradually decreased the hydrocortisone dose and ceased the ther-

![Cross-sectional Growth Chart for Boy (0-18 years)](image1)

![Cross-sectional Growth Chart for Girl (0-18 years)](image2)
apy when she was 11 months old. Her growth velocity and bone maturation did not accelerate until 3 years of age. Hydrocortisone was reinitiated at a dose of 10 mg per day when she was 3 years, 8 months of age, because her growth velocity and bone age began to accelerate and her serum 17-OHP concentration remained markedly high. Despite this treatment, bone maturation was not suppressed and her bone age was 5 years, 8 months when she was 4 years, 6 months of age. The hydrocortisone dose was therefore increased to 15 and 18 mg per day. At 12 years, 2 months of age, her serum estradiol concentrations increased. At present, she is being treated with 25 mg of hydrocortisone and her height is 149.7 cm, which is her estimated final height (Fig. 2c). This is slightly less than the predicted target height (153.5 cm) calculated from the heights of her father and mother (162 cm and 158 cm, respectively).

Case 4 has been observed without treatment. His height was about –1 SD, and his bone age was 60–70% of his chronological age. From 4 years, 6 months of age, his growth velocity began to increase and his bone maturation accelerated. His height reached the mean, and the ratio of bone age to calendar age was nearly 100%. We initiated hydrocortisone therapy at 18 mg/day when he was 8 years, 5 months of age. Despite this treatment, his bone age acceleration was not sufficiently suppressed, and therefore, the hydrocortisone dose was increased to 28 mg/day. This increment transiently reduced the serum concentration of 17-OHP; however, bone age acceleration was not suppressed. Then, when he was 9 years, 10 months old, secondary sexual characteristics were observed and growth velocity increased considerably (Fig. 2d). His final height was 159.4 cm, which is less than the height initially estimated (166.5 cm) from the heights of his father and mother.

When he was 9 years, 2 months old, severe hyponatremia (Na = 123 mmol/l) and hyperkalemia
(K = 5.2 mmol/l) occurred after common cold signs, such as fever and headache. Disturbance of consciousness was also observed. After treatment with a sufficient amount of hydrocortisone, these signs and symptoms disappeared promptly with no sequelae. This clinical course and signs strongly suggest adrenal insufficiency as a result of suppression of the hypothalamus-pituitary-adrenal axis by hydrocortisone treatment.

**Discussion**

Nonclassical 21-OHD is a mild form of CAH that includes signs of postnatal androgen excess without any signs of glucocorticoid or mineralocorticoid deficiency [1, 5, 6]. Affected female patients are born without ambiguous external genitalia. Concentrations of 21-hydroxyprogesterone in nonclassical CAH are mildly elevated and are intermediate between those of heterozygote carriers and those of severely affected patients with the classic form of the disease. The diagnosis may be missed if only a baseline 17-OHP concentration is measured. The ACTH stimulating test is the most useful diagnostic test; patients with nonclassical 21-OHD have 17-OHP concentrations greater than 30 nmol/l at 60 min after injection of ACTH [6, 15, 16]. Therefore, neonatal mass screening is not sufficient for detecting nonclassical CAH, and most nonclassical patients are found after puberty because of symptoms of androgen excess or in the course of family studies [6].

Our patients were diagnosed as having 21-OHD during the neonatal period or in early childhood. In all cases, mutations in the CYP21 gene were detected, and concentrations of 17-OHP were elevated. Although it is difficult to distinguish the nonclassical type from the classical type during the neonatal period in males, we consider case 1 to have nonclassical 21-OHD. He did not show any signs of adrenal insufficiency or androgen excess during the neonatal period, but his peak level of 17-OHP in the ACTH stimulating test was moderately elevated. In case 2, neonatal mass screening was negative and we might have missed her diagnosis if the familial history was not available. Her endocrinology data also indicated that she had nonclassical 21-OHD. In gene analyses, a CYP 21 gene mutation was detected. In previous reports, the activity of 21-hydroxylase resulting from this mutation was estimated to be virtually abolished. However, she did not have any androgen excess, including ambiguous genitalia or adrenal insufficiency. Case 3 had no obvious genitalia but an elevated level of 17-OHP in the ACTH stimulating test and a mutation in the CYP21 gene. We observed case 4 without any treatment despite the result of the neonatal mass screening. He did not show any signs or symptoms of adrenal insufficiency or severe androgen excess until our examination. The mutation in the CYP21 gene was identical to that in his sister, case 3.

In these nonclassical patients, it is difficult to decide when to start treatment. For case 1, the endocrinology data and the mutation analysis indicated he had nonclassical 21-OHD. At the time of diagnosis, the patient did not show any adrenal insufficiency or androgen excess. Thus, we did not start treatment. He is exhibiting nearly normal growth with mild bone age acceleration. We will consider treatment if his predicted height becomes lower than his target height.

Case 4 did not display adrenal insufficiency until 21-OHD was diagnosed. Signs and symptoms indicating androgen excess, including bone age, were not observed. His mutation in the CYP 21A gene was the same as in his sister, case 3, who did not have any ambiguous genitalia at her birth. Thus, we observed him without any treatment.

Considering final height, it is difficult to initiate treatment in nonclassical 21-OHD patients appropriately, especially before the appearance of clinical signs. Regarding cases 3 and 4, they would have missed the opportunity of treatment had they not been followed up since the neonatal period or early childhood. It would have been very difficult to recognise the necessity of treatment, because their height remained below the mean despite their bone age advancement.

Because of precocious puberty and premature epiphyseal fusion, the final heights of nonclassical patients are below their predicted heights [15]. To prevent final short stature, prepubertal treatment with glucocorticoid and suppression of adrenal androgen levels is beneficial [17, 18]. In general, height prognosis for patients with precocious puberty depends on pubertal stage and bone age at the start of therapy. To prevent short stature, when growth spurts or bone age acceleration is apparent, treatment should be considered. However, it is impossible to predict the onset of virilization from endocrinology data and results of gene analysis; bone age and growth velocity should be
monitored prospectively. Despite careful observation and treatment induction without severe bone age acceleration, we could not prevent pubertal precocity in case 4. His final height was short of his genetically predicted height. It is noteworthy that some patients require treatment earlier in childhood than is described in other reports [17, 18].

The episode of adrenal insufficiency in case 4 indicates that we should also consider the possibility of adrenal crisis, even in nonclassical cases, when the patient is treated with hydrocortisone. To suppress growth spurts or bone age acceleration, the hydrocortisone requirement is larger than physiologic levels. This will lead to suppression of the hypothalamus-pituitary-adrenal axis. In physical stresses, such as fever, surgery and trauma, additional supplementation of hydrocortisone should be administered in nonclassical patients treated with hydrocortisone. These problems should be considered when therapy is initiated.

According to genotype, the predicted phenotype of case 2 is the more severe classic type [19, 20]. These discrepancies between genotype and phenotype are often observed in nonclassical 21-OHD [18], the precise reason for this has not been elucidated. Furthermore, the mass screening result in case 2 was negative. Although we could not completely eliminate the possibility that the hydrocortisone administered to her mother had some effect on her fetal adrenal function, the precise reason was not determined.

In the mass screening program for CAH in Japan, patients with 21-OHD are detected at a prevalence of 1 in 18,000 live births, whereas the recall rate for medical examination is about 1 in 3000 live births, on average. The prevalence of Japanese nonclassical patients detected via mass screening is low compared with that in Ashkenazi Jews. Clearly, not every nonclassical patient can be detected during mass screening. Nonclassical patients are among the recalled neonates and may be missed. To consider final heights, in some cases, the treatment should be started before signs of virilization are obvious, and it is quite difficult when the nonclassical patients are missed in neonatal mass screening. Although mass screening may not detect all nonclassical patients, it is a valuable opportunity for diagnosing nonclassical patients during the neonatal period. Thus, we consider it important that neonatal infants referred from mass screening be diagnosed adequately, and that 21-OHD neonates without typical symptoms be followed up carefully.

In conclusion, we have followed four cases with nonclassical 21-OHD diagnosed during the neonatal period or in early childhood, and we have observed them carefully without any treatment. We treated two of the cases with hydrocortisone because of increased growth velocity and acceleration of bone maturation. It is very difficult to decide when and in whom treatment should be initiated. Furthermore, the risk of adrenal insufficiency must be considered when treatment is started. In nonclassical 21-OHD patients, a precise examination at the time of diagnosis and careful observation throughout childhood are necessary.

References


