Therapeutic needs from early childhood in four patients with 21-hydroxylase deficiency harboring the P30L mutation on one allele

Tomoyo Itonaga1, 2, Kazuhisa Akiba1, 3, and Yukihiro Hasegawa1

1 Division of Endocrinology and Metabolism, Tokyo Metropolitan Children’s Medical Center (TMCMC), Tokyo, Japan
2 Department of Pediatrics, Oita University Faculty of Medicine, Oita, Japan
3 Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan

Abstract. 21-hydroxylase deficiency (21-OHD) is the most common type of congenital adrenal hyperplasia. Phenotypically, 21-OHD can be divided into classical and non-classical (NC) forms. The genotype-phenotype correlation in 21-OHD is well established. The P30L mutation is usually associated with the NC form and common among Japanese patients with the NC form of 21-OHD. Herein, we report the clinical course of four patients with 21-OHD with the P30L mutation on one allele and loss-of-function variants on the other allele. Contrary to the findings of most previous studies, all patients were treated with hydrocortisone, and two required fludrocortisone therapy in early childhood. The management strategies for patients with 21-OHD, especially those with the P30L mutation on at least one allele, should be determined based on the clinical phenotype predicted by the CYP21A2 genotype and individual clinical symptoms and biochemical data.

Key words: 21-hydroxylase deficiency, congenital adrenal hyperplasia, P30L, non-classical phenotype, genotype-phenotype correlation

Introduction

21-hydroxylase deficiency (21-OHD), caused by mutations in CYP21A2, is the most common type of congenital adrenal hyperplasia (1, 2). Phenotypically, 21-OHD can be divided into classical and non-classical (NC) forms, with the classical form presenting as salt-wasting (SW) or simple-virilizing (SV) type 21-OHD. Female neonates with either of the classical types present with virilized external genitalia, whereas male and female neonates with NC form are asymptomatic.

The genotype-phenotype correlation in 21-OHD is well-established (3–12). The clinical phenotype correlates with the severity of the two allelic mutations and residual 21-hydroxylase activity. In vitro studies performed on a relatively limited number of mutations confirmed a rough correlation between disease severity and the degree of functional loss of 21-hydroxylase. Moreover, mutations resulting in complete inactivation of 21-hydroxylase (e.g., gene deletion/conversion, Δ8 bp, E6 cluster, F306+t, Q318X, and R356W) were associated with the SW phenotype. Mutations that reduced 21-hydroxylase activity to 2% (e.g., intron 2 splice site and I172N) were associated with the SV phenotype, whereas mutations, such as P30L, V281L, and P453S, which reduced its activity to 20–30%, 10%, and 75%, respectively, were found to result in the NC phenotype (7, 9).

The P30L mutation is usually classified in the NC form based on the presence of 20–30% residual 21-hydroxylase activity in vitro (6), and it is the most common mutation in Japanese patients with the NC form of 21-OHD (13). A divergence between genotype and phenotype has been observed (14–17) in some patients with this disorder, and a similar clinical spectrum of virilization and SW have been reported in patients with a heterozygous P30L mutation and a different mutation on the other allele (18).

All four patients with 21-OHD caused by the P30L mutation in the present study were treated with...
hydrocortisone, and two of these patients required treatment with fludrocortisone. The present study reported the clinical course of the four patients from infancy to date.

**Patients and Methods**

**Measurement of 17-hydroxyprogesterone (17-OHP) levels and criteria**

In Japan, blood samples for neonatal screening are collected between ages 4 and 7 d by a heel prick blotted onto a filter paper, and 17-OHP levels are measured using ELISA (Eiken Chemical Co., Ltd., Tokyo, Japan) after steroid extraction. The measured values are then doubled to match the serum levels. Patients with 5–20 ng/mL 17-OHP undergo a second 17-OHP level measurement. If the 17-OHP level is higher than 20 ng/mL or remains higher than normal on a third test, the patient is considered positive for 21-OHD. Patients with a positive result are referred to a pediatric endocrinologist for a more detailed endocrinological evaluation (19). At our hospital, serum 17-OHP levels were assessed using ELISA (IBL International Co., Toronto, Canada). In the present study, the biochemical abnormalities indicative of 21-OHD were basal serum 17-OHP level ≥2.0 ng/mL and peak serum 17-OHP level ≥10.0 ng/mL after ACTH stimulation test (dose of 250 µg/dose or 250 µg/m²) (20).

**Genotyping of CYP21A2**

According to standard procedures, CYP21A2 mutations were detected by Sanger sequencing, and its deletions, duplications, and large gene conversions were studied using multiple ligation probe amplification.

**Ethics**

This study was approved by our ethical committee of TMCMC (2020b-101).

**Case Report**

The characteristics of cases 1–4 are summarized in Table 1.

**Case 1**

The patient was a female born at 39 wk of gestation to healthy, nonconsanguineous parents. Her birth weight was 2,925 g. At birth, virilization of the external genitalia was observed. At 8 d of age, she presented with hyperkalemia (K 6.1 mEq/L), failure-to-thrive, and a second measurement showed its increase to 24.6 ng/mL. She was examined at the pediatric division of a regional hospital at the age of 30 d. Her body weight gain was satisfactory. The laboratory data showed that serum sodium, serum potassium, plasma ACTH, serum cortisol, serum DHEA-S, and serum testosterone levels were 140 mEq/L, 5.0 µg/dL, 5.0 µg/dL, 442 µg/dL, and 0.81 ng/mL, respectively. Due to the lack of clinical evidence of 21-OHD, she received no treatment. Genetic testing of CYP21A2 revealed a heterozygous, pathogenic variant of p.P30L and IVS2-13C>G. ACTH stimulation test performed at 60 min after loading. She was referred to our hospital at the age of 7 mo and hydrocortisone treatment was initiated. The attending physician reported mild clitoromegaly. Her growth was satisfactory (Fig. 1b). At her last visit (age 1 yr and 11 mo), she received only hydrocortisone treatment (5.3 mg/m²/d), and her clitoral length was 8 mm (reference < 5 mm).

**Cases 3 and 4**

The patients in Cases 3 and 4 were siblings born at term to healthy, nonconsanguineous parents. The patient in Case 3 was male, with a birth weight of 2,404 g. He was referred to our hospital because his 17-OHP level measured by DBS during neonatal screening at 6 d of age was 9.7 ng/mL. Laboratory data were normal except for elevated 17-OHP levels (13.4 ng/mL). His serum cortisol level using the ACTH stimulation test was 25.5 µg/dL (Table 2). Thereafter, he was placed under close observation without medication. At age 2 yr and 6 mo, the peak serum cortisol level on the stimulation test was low (14.6 µg/dL), and urine pregnanetriol level, one
of the indices of 21-OHD status in our hospital protocol, was 4.9 mg/m²/d (optimal range: 1.2–2.1 mg/m²/d) (22). Based on these data, treatment with hydrocortisone was initiated, although he had no symptoms of 21-OHD, such as accelerated growth velocity or bone maturation (Fig. 1c). Treatment with fludrocortisone was considered unnecessary because plasma renin activity was normal.

The patient in Case 4 was male, with a birth weight of 2,745 g. His first 17-OHP measurement using DBS at 4 d of age was 2.8 ng/mL. Laboratory data were unremarkable, and he had no signs of 21-OHD. After his first visit, his 17-OHP levels gradually increased to 13.0 ng/mL at 12 d of age and 51.4 ng/mL at 1 mo of age. At 6 mo of age, treatment with hydrocortisone and fludrocortisone was initiated due to hyperkalemia (5.6 mEq/L), elevated 17-OHP levels (140 ng/mL), high first morning urine pregnanetriol levels (7.8 mg/gCr; target value, 2.2–3.3 mg/gCr) (21), and increased plasma renin activity (16.8 ng/mL/h) were observed; however, no clinical symptoms were observed. His growth curve up to the age of 2 yr showed no growth acceleration or failure to thrive (Fig. 1d). Genetic testing of CYP21A2 revealed a pathogenic compound heterozygous variant of p.P30L and p.R356W.

**Discussion**

The patients with 21-OHD analyzed in the present study harbored a compound heterozygous mutation of P30L and loss-of-function mutations in CYP21A2. Although the patients were heterozygous for the P30L mutation, all of them required steroid treatment because of abnormal biochemical data from early childhood. In general, the NC forms of 21-OHD are distinguished by the absence of symptoms of adrenal insufficiency or excess androgen during the neonatal period. Based on this definition, Case 1 patient was diagnosed with the classical form of 21-OHD, whereas the other patients were diagnosed with the NC form.

To date, several studies have reported the classical form of 21-OHD associated with the P30L mutation. The simple virilization phenotype has been reported to be associated with some cases (23–25), and in a study conducted by New et al. with a cohort consisting of 1,507 families with 21-OHD, they reported that 23 of 74 patients harboring at least one allele with the P30L mutation showed the classical phenotype. Based on these findings, they suggested that P30L mutations could yield a wide variety of phenotypes other than the NC form. Similar phenotypic diversity was also observed in patients with intron 2 splice site and I172L mutations (16).

The precise etiology of the divergence between genotypes and phenotypes requires clarification. There are three following possibilities for this divergence: first, some phenotypic variations, such as SW and age at onset, are clearly dependent on the clinical course, such as whether screening for 17-OHP was performed, if the
Fig. 1. Clinical course of each patient. (a) Case 1, (b) Case 2, (c) Case 3, and (d) Case 4. Growth curves are based on a cross-sectional growth chart for Japanese children of both sexes. Open circles indicate bone age by the Greulich and Pyle atlas. In Case 1, bone age at 3 yr and 3 mo and 5 yr and 4 mo did not differ from the chorological age.
Fig. 1. continued.
patients had an affected sibling(s), and early initiation of steroid therapy. Second, the severity of mutations other than the P30L mutation on the other allele has a marked impact on the clinical phenotype. For example, when the P30L mutation is biallelic, the phenotype is likely to be NC. In contrast, if one of the mutations is nonfunctional, the phenotype is theoretically more severe. Thus, in patients who were compound heterozygous for the P30L mutation and other mutations, the clinical phenotype correlates with the average of the two theoretical enzyme activities inferred by the presence of the two mutations. Third, the phenotype likely depends on the activity of genes other than CYP21A2, such as genes that play a pivotal role in fetal sex development or sodium/potassium homeostasis. The length of CAG repeats in the AR modulating androgen activity may also be involved (26, 27).

The goal of treating childhood 21-OHD is to prevent adrenal crisis and virilization and to allow normal growth and development (20). The treatment strategy in the present study was also based on this concept; low cortisol levels after the stimulation test and high urine pregnanetriol levels were considered a sign of adrenocortical insufficiency and a risk factor for virilization and precocious puberty, respectively. In Case 1, treatment was started after the diagnosis of classical 21-OHD. In Cases 2 and 3, treatment was initiated because the peak cortisol levels were below 18 µg/dL and urine pregnanetriol levels were high. The clinical course in Case 4 was unique. The levels of 17-OHP determined using DBS were initially below the mass-screening cut-off value, but they increased gradually. Treatment was initiated because the patient presented with hyperkalemia and elevated urine pregnanetriol levels. As demonstrated in the four cases analyzed in the present study, the appropriate timing of steroid therapy should be decided based on clinical data rather than gene analysis findings. The clinical practice guidelines of the Endocrine Society suggest glucocorticoid treatment for the NC form only in children and adolescents with 21-OHD with abnormally early onset and rapid progression of pubarche or bone aging and adolescents with overt virilization (20). The treatment in the three NC cases in the current study began earlier than recommended in the guidelines mentioned above because we believe that follow-up biochemical data, especially the peak serum cortisol level determined using the ACTH stimulation test and urine pregnanetriol levels, are important to achieve the goal of treating childhood 21-OHD. Notably, the ACTH stimulation test has already been established as a diagnostic method for adrenal insufficiency (28), and increased urine pregnanetriol levels have been previously reported to be associated with symptoms of childhood 21-OHD, such as pubarche and growth acceleration (21).

Conclusion

The management of 21-OHD patients, especially those harboring the P30L mutation on at least one allele, should be decided based on clinical symptoms and biochemical data.

Conflict of Interests: The authors have no conflicts of interest.

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