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Case Report

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

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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

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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/m^2) (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) and failure-to-thrive. At 4 d of age, dried blood spotting (DBS) on filter paper revealed elevated 17-OHP levels (10.4 ng/mL). Based on these findings, 21-OHD was diagnosed, and treatment with hydrocortisone, fludrocortisone, and sodium chloride supplements was immediately initiated. She was discharged at 36 d of age. Genetic testing of *CYP21A2* revealed a heterozygous pathogenic variant of p.P30L and gene deletion. Sodium chloride was discontinued at 9 mo of age. She presented with breast development, pubic hair development, and menarche at age 8 yr and 11 mo, 10 yr and 4 mo, and 11 yr and 0 mo, respectively. Bone age was advanced (13 yr and 6 mo by the Greulich and Pyle atlas) for her chronological age of 11 yr and 0 mo. She had no growth problems other than being overweight due to excess hydrocortisone at 11 yr of age (**Fig. 1a**). At the last visit (age 12 yr), she received hydrocortisone (23 mg/m^2/d) and fludrocortisone (0.05 mg/d); the doses were administered based on the assessment of overnight fasting pregnanetriol levels (21) and plasma renin activity before the drugs in the morning.

Case 2

The patient was a female born at 39 wk of gestation to healthy, nonconsanguineous parents, and she had a birth weight of 3,278 g. At birth, she showed no signs of 21-OHD, such as virilization of the external genitalia, pigmentation, or SW. Neonatal screening at 6 d of age using DBS demonstrated a 17-OHP level of 8.2 ng/mL, 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, 4.7 mEq/L, 51.6 pg/mL, 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 5 mo of age revealed elevated 17-OHP levels (212 ng/mL) and decreased serum cortisol levels (11.8 μg/dL), both of which were obtained 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/m^2/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

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Table 1. Characteristics of the cases

Case	1	2	3	4
Genotype	P30L, del	P30L, IVS2-13C>G	P30L, R356W	P30L, R356W
Sex	Female	Female	Male	Male
Gestation/Birth weight	39wk-2d/2,925 g	39wk-1d/3,278 g	38wk-5d/2,404 g	Term/2,745 g
Chief finding	Virilization	Abnormality on neonatal screening	Abnormality on neonatal screening	Sibling of Case 3
[At first visit]				
Age	4 d	30 d	26 d	4 d
Virilization	+	-	-	-
Failure-to-thrive	+	-	-	-
Na (mEq/L)	140	140	135	142
K (mEq/L)	6.3	4.7	5.6	4.4
17-OHP (ng/mL)	10.4	12.3	13.4	2.8
[At treatment initiation]				
Age	8 d	7 mo	2 yr 9 mo	6 mo
Na (mEq/L)	136	141	137	138
K (mEq/L)	6.1	4.4	4.5	5.6
17-OHP (ng/mL)	68.6	214	140	140
First morning P3/Cr (mg/gCr)	N.E.	9	N.E.	7.8
PRA (ng/mL/h)	>18.0	N.E.	6.3	16.8
[At last visit]				
Age	12 yr 8 mo	1 yr 11 mo	7 yr 2 mo	1 yr 9 mo
First morning P3/Cr (mg/gCr)	13.6	3.28	7.7	N.E.
HDC dosage (mg/m^2/d)	23	5.3	12	15
FC dosage (mg/d)	0.05	-	-	0.1

P3, pregnanetriol; PRA, plasma renin activity; HDC, hydrocortisone; FC, fludrocortisone; N.E., not examined; del, deletion. The reference range for First morning P3/Cr was 2.2–3.3 mg/gCr, as reported by Izawa *et al*. (21).

of the indices of 21-OHD status in our hospital protocol, was 4.9 mg/m^2/d (optimal range: 1.2–2.1 mg/m^2/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), and 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

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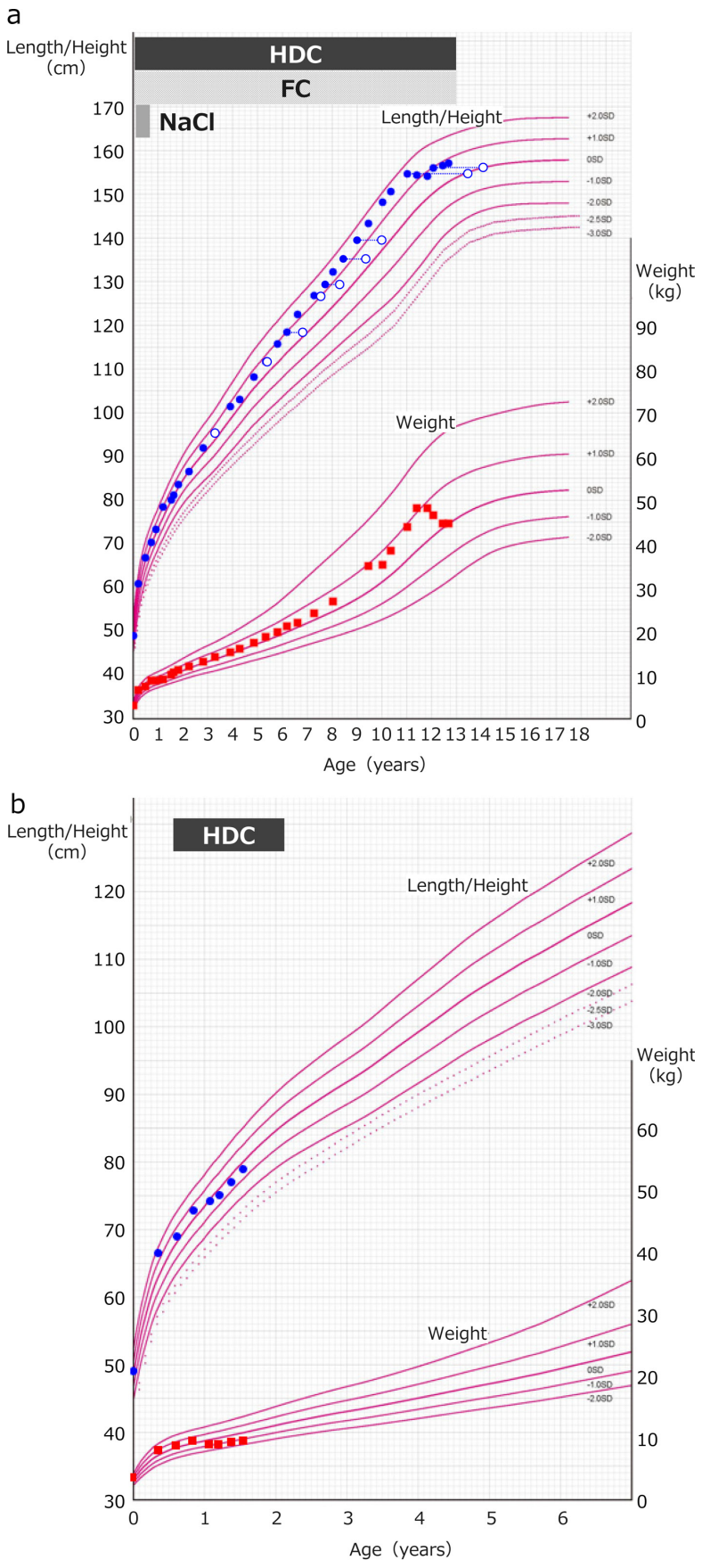

Fig. 1. Clinical course of each patient. (a) Case 1, (b) Case 2, (c) Case 3, and (d) Case 4. Growth curves are based on a cross-sectional growth chart for Japanese children of both sexes. Open circles indicate bone age by the Greulich and Pyle atlas. In Case 1, bone age at 3 yr and 3 mo and 5 yr and 4 mo did not differ from the chorological age.

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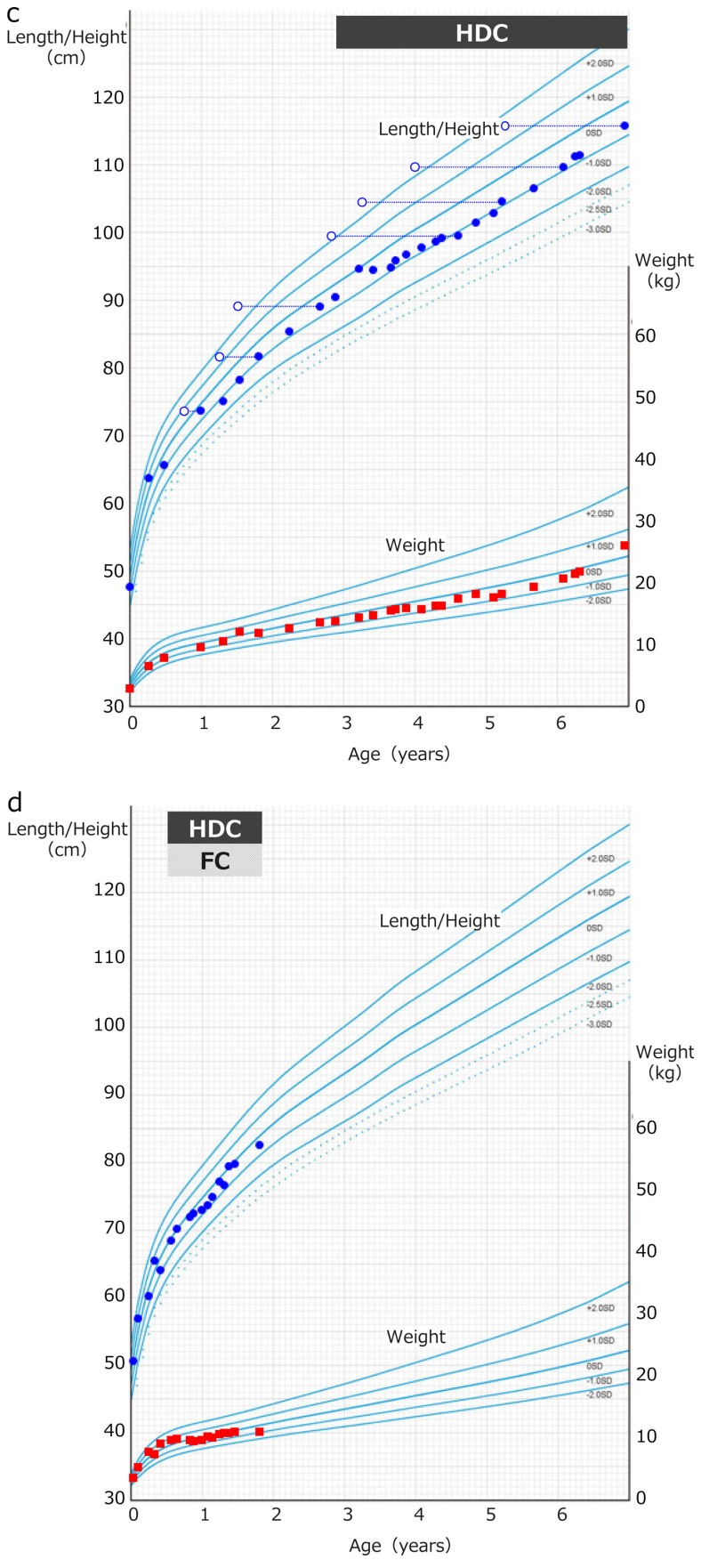

Fig. 1. continued.

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Table 2. Rapid ACTH stimulation test findings

Case	2	3	
Age at examination	5 mo	26 d	2 yr and 9 mo
17-OHP (ng/mL)			
Basal	214	13.4	68
Peak	212	119	408
Cortisol (μg/dL)			
Basal	9.4	4.2	13.72
Peak	11.8	25.5	14.66

patients had an affected siblings(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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References

1. Speiser PW, White PC. Congenital adrenal hyperplasia. N Engl J Med 2003;349: 776–88. [Medline] [CrossRef]
2. Merke DP, Bornstein SR. Congenital adrenal hyperplasia. Lancet 2005;365: 2125–36. [Medline] [CrossRef]
3. Chiou SH, Hu MC, Chung BC. A missense mutation at Ile172----Asn or Arg356----Trp causes steroid 21-hydroxylase deficiency. J Biol Chem 1990;265: 3549–52. [Medline] [CrossRef]
4. Tusie-Luna MT, Traktman P, White PC. Determination of functional effects of mutations in the steroid 21-hydroxylase gene (CYP21) using recombinant vaccinia virus. J Biol Chem 1990;265: 20916–22. [Medline] [CrossRef]
5. Higashi Y, Fujii-Kuriyama Y. Functional analysis of mutant P450(C21) genes in COS cell expression system. Methods Enzymol 1991;206: 166–73. [Medline] [CrossRef]
6. Tusie-Luna MT, Speiser PW, Dumic M, New MI, White PC. A mutation (Pro-30 to Leu) in CYP21 represents a potential nonclassic steroid 21-hydroxylase deficiency allele. Mol Endocrinol 1991;5: 685–92. [Medline] [CrossRef]
7. Speiser PW, Dupont J, Zhu D, Serrat J, Buegeleisen M, Tusie-Luna MT, *et al*. Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Invest 1992;90: 584–95. [Medline] [CrossRef]
8. Hsu LC, Hsu NC, Guzova JA, Guzov VM, Chang SF, Chung BC. The common I172N mutation causes conformational

change of cytochrome P450c21 revealed by systematic mutation, kinetic, and structural studies. J Biol Chem 1996;271: 3306–10. [Medline] [CrossRef]
9. Krone N, Braun A, Roscher AA, Knorr D, Schwarz HP. Predicting phenotype in steroid 21-hydroxylase deficiency? Comprehensive genotyping in 155 unrelated, well defined patients from southern Germany. J Clin Endocrinol Metab 2000;85: 1059–65. [Medline] [CrossRef]
10. Weintrob N, Brautbar C, Pertzelan A, Josefsberg Z, Dickerman Z, Kauschansky A, *et al*. Genotype-phenotype associations in non-classical steroid 21-hydroxylase deficiency. Eur J Endocrinol 2000;143: 397–403. [Medline] [CrossRef]
11. Soardi FC, Barbaro M, Lau IF, Lemos-Marini SH, Baptista MT, Guerra-Junior G, *et al*. Inhibition of CYP21A2 enzyme activity caused by novel missense mutations identified in Brazilian and Scandinavian patients. J Clin Endocrinol Metab 2008;93: 2416–20. [Medline] [CrossRef]
12. Tardy V, Menassa R, Sulmont V, Lienhardt-Roussie A, Lecointre C, Brauner R, *et al*. Phenotype-genotype correlations of 13 rare CYP21A2 mutations detected in 46 patients affected with 21-hydroxylase deficiency and in one carrier. J Clin Endocrinol Metab 2010;95: 1288–300. [Medline] [CrossRef]
13. Kashimada K, Ishii T, Nagasaki K, Ono M, Tajima T, Yokota I, *et al*. Clinical, biochemical, and genetic features of non-classical 21-hydroxylase deficiency in Japanese children. Endocr J 2015;62: 277–82. [Medline] [CrossRef]
14. Wilson RC, Mercado AB, Cheng KC, New MI. Steroid 21-hydroxylase deficiency: genotype may not predict phenotype. J Clin Endocrinol Metab 1995;80: 2322–9. [Medline]
15. Krone N, Rose IT, Willis DS, Hodson J, Wild SH, Doherty EJ, *et al*. United Kingdom Congenital adrenal Hyperplasia Adult Study Executive (CaHASE). Genotype-phenotype correlation in 153 adult patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency: analysis of the United Kingdom Congenital adrenal Hyperplasia Adult Study Executive (CaHASE) cohort. J Clin Endocrinol Metab 2013;98: E346–54. [Medline] [CrossRef]
16. New MI, Abraham M, Gonzalez B, Dumic M, Razzaghy-Azar M, Chitayat D, *et al*. Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Proc Natl Acad Sci USA 2013;110: 2611–6. [Medline] [CrossRef]
17. Riedl S, Röhl FW, Bonfig W, Brämswig J, Richter-Unruh A, Fricke-Otto S, *et al*. AQUAPE CAH Study Group. Genotype/phenotype correlations in 538 congenital adrenal hyperplasia patients from Germany and Austria: discordances in milder genotypes and in screened versus prescreening patients. Endocr Connect 2019;8: 86–94. [Medline] [CrossRef]
18. Kohn B, Levine LS, Pollack MS, Pang S, Lorenzen F, Levy D, *et al*. Late-onset steroid 21-hydroxylase deficiency: a variant of classical congenital adrenal hyperplasia. J Clin Endocrinol Metab 1982;55: 817–27. [Medline] [CrossRef]
19. Tsuji A, Konishi K, Hasegawa S, Anazawa A, Onishi T, Ono M, *et al*. Newborn screening for congenital adrenal hyperplasia in Tokyo, Japan from 1989 to 2013: a retrospective population-based study. BMC Pediatr 2015;15: 209. [Medline] [CrossRef]
20. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, *et al*. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103: 4043–88. [Medline] [CrossRef]
21. Izawa M, Aso K, Higuchi A, Ariyasu D, Hasegawa Y. The range of 2.2-3.3 mg/gCr of pregnanetriol in the first morning urine sample as an index of optimal control in CYP21 deficiency. Clin Pediatr Endocrinol 2008;17: 75–80. [Medline] [CrossRef]
22. Izawa M, Aso K, Higuchi A, Ariyasu D, Hasegawa Y. Pregnanetriol in the range of 1.2-2.1 mg/m(2)/day as an index of optimal control in CYP21A2 deficiency. Clin Pediatr Endocrinol 2007;16: 45–52. [Medline] [CrossRef]
23. Baumgartner-Parzer SM, Schulze E, Waldhäusl W, Pauschenwein S, Rondot S, Nowotny P, *et al*. Mutational spectrum of the steroid 21-hydroxylase gene in Austria: identification of a novel missense mutation. J Clin Endocrinol Metab 2001;86: 4771–5. [Medline] [CrossRef]
24. Dolzan V, Stopar-Obreza M, Zerjav-Tansek M, Breskvar K, Krzisnik C, Battelino T. Mutational spectrum of congenital adrenal hyperplasia in Slovenian patients: a novel Ala15Thr mutation and Pro30Leu within a larger gene conversion associated with a severe form of the disease. Eur J Endocrinol 2003;149: 137–44. [Medline] [CrossRef]
25. Araujo RS, Billerbeck AE, Madureira G, Mendonca BB, Bachega TA. Substitutions in the CYP21A2 promoter explain the simple-virilizing form of 21-hydroxylase deficiency in patients harbouring a P30L mutation. Clin Endocrinol (Oxf) 2005;62: 132–6. [Medline] [CrossRef]
26. Moura-Massari VO, Cunha FS, Gomes LG, Bugano Diniz Gomes D, Marcondes JA, Madureira G, *et al*. The presence of clitoromegaly in the nonclassical form of 21-hydroxylase deficiency could be partially modulated by the CAG polymorphic tract of the androgen receptor gene. PLoS One 2016;11: e0148548. [Medline] [CrossRef]
27. Rocha RO, Billerbeck AE, Pinto EM, Melo KF, Lin CJ, Longui CA, *et al*. The degree of external genitalia virilization in girls with 21-hydroxylase deficiency appears to be influenced by the CAG repeats in the androgen receptor gene. Clin Endocrinol (Oxf) 2008;68: 226–32. [Medline]
28. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, *et al*. Diagnosis and treatment of primary adrenal insufficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2016;101: 364–89. [Medline] [CrossRef]

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