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Original Article

Guidelines for diagnosis and treatment of 21-hydroxylase deficiency (2014 revision)

Mass Screening Committee, Japanese Society for Pediatric Endocrinology, and
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Abstract. Purpose of developing the guidelines: The first guidelines for diagnosis and treatment of 21-hydroxylase deficiency (21-OHD) were published as a diagnostic handbook in Japan in 1989, with a focus on patients with severe disease. The "Guidelines for Treatment of Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency) Found in Neonatal Mass Screening (1999 revision)" published in 1999 were revised to include 21-OHD patients with very mild or no clinical symptoms. Accumulation of cases and experience has subsequently improved diagnosis and treatment of the disease. Based on these findings, the Mass Screening Committee of the Japanese Society for Pediatric Endocrinology further revised the guidelines for diagnosis and treatment. Target disease/conditions: 21-hydroxylase deficiency. Users of the guidelines: Physician specialists in pediatric endocrinology, pediatric specialists, referring pediatric practitioners, general physicians; and patients.

Key words: 21-hydroxylase deficiency, mass screening, guideline

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Introduction

Mass screening for 21-hydroxylase deficiency (21-OHD) started in Japan in January 1989, and one per 18,000 to 19,000 infants are found to have 21-OHD (1–3). Many patients with 21-OHD have skin pigmentation, virilization of the external genitalia (females), poor suckling and poor weight gain, but others have 21-OHD with only very mild clinical symptoms. 21-OHD requires continuous treatment and it is recommended that specialized medical facilities make a differential diagnosis and treat subjects found to be positive in mass screening to avoid unnecessary treatment. The first guidelines for diagnosis and treatment of 21-OHD were published as a diagnostic handbook in Japan in 1989, and were developed for severe patients (4, 5). The "Guidelines for Treatment of Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency) Found in Neonatal Mass Screening (1999 revision)" published in 1999 were revised to include 21-OHD patients with very mild or no clinical symptoms (6, 7). However, problems in endocrine tests, new specialized endocrine tests, and new diseases to be differentiated have arisen. Furthermore, long-term courses of 21-OHD patients have been reported, and problems in the adult stage have emerged. The Lawson Wilkins Pediatric Endocrine Society (the present Pediatric Endocrine Society) and the European Society for Paediatric Endocrinology proposed a consensus statement in 2002 (8), and the Endocrine Society (USA) also published new clinical practice guidelines in 2010 (9). Therefore, we revised the previous diagnosis and treatment guidelines and developed the current guidelines (hereinafter referred to as the Guidelines) based on newly emerging information.

A "grade" and "evidence level" are given for each recommendation in the guidelines. The grade indicates the recommendation level based on published studies, and the evidence level reflects the level of the study used as a rationale. Expert opinions are used in the Guidelines if there no appropriate findings in published articles. Surgery for external genitalia; and complications and prognosis are not described in the Guidelines. However, other guidelines are under development in cooperation with the Sex Differentiation Committee of the Japanese Society for Pediatric Endocrinology.

Grade level

1. Major recommendation: Most patients receive benefits.
2. Minor recommendation: Many patients receive benefits. Requires consideration and selection based on the patient's conditions.

Evidence level

●○○ Low: Evaluation of case reports without controls

●●○ Medium: Cohort study without controls

●●● Cohort study with controls, nonrandomized comparative study

Consensus: Widely recognized ideas, even if a study has not been performed

Pathophysiology of 21-OHD

Congenital adrenal hyperplasia (CAH) refers to a range of autosomal recessive diseases resulting from deficiency of cortisol secretion. The incidence is 1/10,000–20,000 globally and in Japan (1, 2, 9). 21-OHD is the most frequent disease among CAH cases, and develops due to mutation or deficit of the *CTYP21A2* gene, which encodes the steroid 21-hydroxylase (p450c21) (9–11). This enzyme converts 17-hydroxyprogesterone (17-OHP) into 11-deoxycortisol, and progesterone into 11-deoxycorticosterone, which are then converted into cortisol and aldosterone, respectively. Therefore, production of cortisol and aldosterone is disturbed in patients with severe 21-OHD.

Disturbance of cortisol production causes accumulation of precursors of cortisol by stimulation of ACTH, and these precursors lead to a pathway for adrenal androgen (Fig. 1). Virilization of the external genitalia is one of the most important symptoms in female neonates

with salt-wasting (SW) or simple virilizing (SV) disease. Rapid accelerated growth is stimulated, and sexual precocity develops in both male and female patients who are not treated after the neonatal stage; and serious SW may be fatal. Approximately 75% of 21-OHD cases have the serious SW type (1, 9, 10). In addition to the classical SW and SV types, a nonclassical (NC) type is also found (12–15). NC patients develop excessive adrenal androgen after birth; however, the severity differs, and there are asymptomatic patients. Steroid synthesis pathways are shown in Fig. 1. Backdoor pathways have been implicated in virilization of the external genitalia of girls with 21-OHD (refer to the Fig. 1 legend) (16–18).

21-OHD results from genetic abnormalities of *CYP21A2,* and the disease severity is likely to correlate with the *CYP21A2* genotype (9, 19, 20). The 21-OHD genotype requires careful determination because genetic results can be complicated due to duplication, deletion and recombination of *CYP21A2* in the 6q21.3 region. More than 100 *CYP21A2* gene mutations have been found, and mutation of intron 2 (mutation from C/A to G between the splice acceptor site and –13 bp upstream) causing a deletion and splicing variant is found in one allele in about 50% of SW patients. An Ile172Asn mutation in exon 4 giving an enzyme with 1–2% activity is common in SV patients. A Val281Leu mutation in exon 7 giving an enzyme with 20–50% activity is found in an allele in about 70% of Caucasian NC patients (12, 13), and a Pro30Leu mutation is common in Japanese NC patients (14). Many patients have heterozygosity of at least two mutations and variable remaining enzyme activity; thus, the phenotype can be diverse, and the *CYP21A2* genotype (extent of loss of enzyme activity) does not always correspond to the phenotype (13, 21).

1. Neonatal Mass Screening

Recommendation

1. We recommend that 21-OHD mass screening be conducted as part of a series of neonatal mass screenings. 1 (Consensus)
2. We recommend that an immunological measurement (e.g., ELISA) be used for 21-OHD mass screening, with direct determination in the first tier test and use of an extraction procedure in the second tier test. To decrease the false-positive rate and increase the positive predictive value, steroid profiles should be measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the second tier test. 1 (●○○)
3. We recommend that to provide rapid and appropriate treatment for neonates found to be positive in mass screening, prefectural and major city governments that conduct mass screening establish procedures for screening inborn errors of metabolism and develop a practical treatment protocol. 1 (Consensus)

Explanation

1-1. Outcomes of neonatal mass screening

Neonatal mass screening of 21-OHD started in 1989 in Japan. The incidence of 21-OHD before mass screening was conducted was estimated to be 1/43,674, based on the patient survey by Suwa *et al.* (22). However, in mass screening from 1981 to the end of 1987 in about 500,000 subjects in Sapporo, Tokyo (partial), Kanagawa and Shizuoka (western region), 16 SW, 7 SV and 2 unknown type cases (25 in total) were found, indicating a rate of 1/20,570 (23). Suwa *et al.* reported the findings of a survey in 51 testing institutions nationwide (1, 3). Of 4,085,448 mass screening cases over 10 yr from April 1982 to March 1992, 271 were found to have 21-OHD, i.e., an incidence of 1/18,827. In this survey, there were more SW cases than SV cases regardless of sex, and the male-female ratio was 1:1 for both types. Other studies show that the incidence of clinical diagnosis before mass screening is higher in girls, but that SW 21-OHD has a similar incidence in males and females (24, 25). Therefore, males with SV21-OHD seem to have been missed prior to mass screening, while patients with severe SW

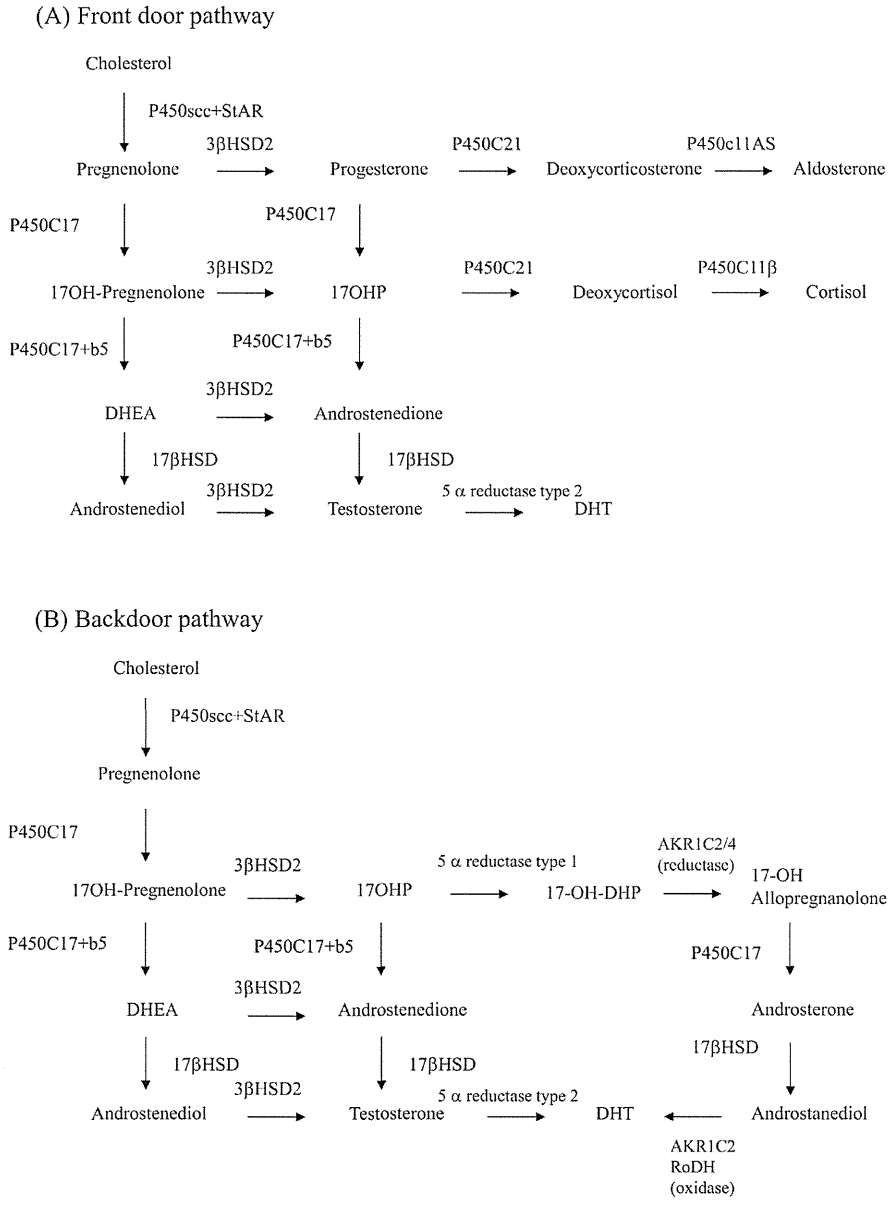

Fig. 1. (A) Front door pathway. Steroid production in the normal fetus. 3β-HSD activity is low in the fetal adrenal gland, and the most produced adrenal steroid is DHEA (and DHEA-S). However, small amounts of steroids go into the aldosterone and cortisol pathways. 21-hydroxylase in the adrenal gland is required for both pathways. The adrenal gland can produce small amounts of testosterone through the activity of 17β-HSD. (B) Backdoor pathway. If 21-hydroxylase (P450c21) is deficient, three pathways produce androgen. The first is a pathway from cholesterol to DHEA. Large amounts of DHEA are converted to DHEA-S and inactivated, while some DHEA is converted to testosterone and dihydrotestosterone (DHT). In the second pathway of the normal adrenal gland, a tiny amount of 17-OHP is converted to androstenedione, small amounts of 17-OHP produced in CAH are converted to androstenedione, and testosterone is produced. The third pathway is a backdoor pathway in which 17-OHP undergoes by 5α- and 3β-reduction and is converted to 17-OH allopregnanolone. This steroid is converted to androstanediol, oxidized by 3α-HSD oxidoreductase and converted to DHT. This pathway was first found in Marsupialia and mammals and is also present in humans based on urine steroid analysis using mass spectrometry. This pathway is thought to be involved in virilization of the external genitalia in girls with 21-OHD.

21-OHD were incorrectly diagnosed as cases of idiopathic sudden death.

In a collaborative study in 15 medical institutions from 1990 to 1995, 70 patients who were identified after the start of mass screening were followed up (26). For 49 patients identified in mass screening and 21 who visited a hospital due to symptoms, the mean ages at the first visit were 17.6 and 7.4 d, respectively. Many patients (about 30%) who visited the hospital before receiving mass screening results were girls with a chief complaint of external genitalia anomaly. Clitoromegaly was found in all girls, but half of these patients did not visit a hospital until the results of mass screening were obtained.

The results of mass screening show a national incidence of 21-OHD of 1/19,000–20,000, which is similar to those in other countries (27–30). For neonatal mass screening performed by maternal and child health services of prefectural and major city governments, the cost-benefit ratio is also important. In Japan, the cost includes expenses for mass screening tests and treatment and management of patients who are found to have 21-OHD, but the benefit is the reduced expenses of facilities, child rearing and special education facilitated by early diagnosis (31). The net benefit of screening for congenital hypothyroidism is the highest among diseases (3.1 billion Yen), and the net benefit of 21-OHD screening is 0.2 billion Yen.

The major problem of 21-OHD mass screening is the high false-positive rate [low positive predictive value (PPV)] (27, 32–40). In the prescribed cost-benefit analysis, the costs of testing false-positive subjects for diagnosis and nursing costs of in-patients are not considered. Parents also have psychological anxiety that their child has a lifelong, life-threatening chronic disease (41). Improvement of this problem may require two-step mass screening in which the second tier test has a high PPV in ELISA-positive subjects in the first tier test (9, 42–45).

1-2. Current status of mass screening

In December 2010, 17-hydroxyprogesterone (17-OHP) was determined in 45 test facilities in Japan using ELISA kits produced by two Japanese manufacturers. In the first tier test, all facilities used a direct method. In the second tier test, only two used the direct method, and one of the two used kits produced by the two manufacturers. Extraction was used in 42 testing facilities, and 3 set a cutoff value in combination with the direct method. The remaining test facility used high performance liquid chromatography (HPLC) (46).

The cutoff value for 17-OHP is not standardized in Japan because local governments that implement mass screening, consultant physicians and liaison councils have different purposes for mass screening (34–37, 46, 47). The contact procedures for positive subjects and detailed examinations depend on the region, and particularly on the pediatric endocrinologists in each region. Therefore, local governments should establish procedures for mass screening that permit neonates who are positive in mass screening to be appropriately treated in any region. If a neonate has an extremely high 17-OHP level or clinical symptoms of adrenal insufficiency, the neonate should be seen by a pediatric endocrinologist as soon as possible.

Premature and low birth weight infants are likely to give false positive 17-OHP findings. This is because abundant steroids are secreted from the adrenal gland of neonates and cross-react with antibodies to 17-OHP; and because 17-OHP secretion is enhanced by stress in premature and low birth weight infants (34–47). Therefore, in Europe and the United States, cutoff values by birth weight or gestational week have been established, and this has decreased the rate of recall and improved the PPV (48–51). Cutoff values for low birth weight infants based on modified gestational weeks have also reduced the rates of recall and detailed examinations in Japan (36). Cutoff values by birth weight and gestational week determined in Tokyo and

Niigata also reduced false-positive rates (30, 34). However, test facilities for mass screening do not always use this approach (46).

Standard procedures for low birth weight infants include blood sampling at 30 d after birth, at discharge; or when the body weight reaches 2,500 g; based on guidelines on secondary blood sampling in low birth weight infants (52). It is difficult to determine if 21-OHD is present in low birth weight infants with a high 17-OHP level in blood sampling 4 to 6 d after birth. Therefore, it is important for the physician in charge of the results of the first test to be informed about the possibility of CAH and to request careful follow-up of clinical symptoms (46, 52). Many infants with a birth weight ≤ 1,500 g are cared for in a neonatal intensive care unit (NICU) for a long period, and continuous blood sampling and tests may be requested by the physician in charge, although the guidelines for the second blood sampling are used as a general rule. It is also important to perform a detailed examination immediately after this is determined to be necessary (46, 52).

Steroid hormones can be accurately determined by LC-MS/MS, and many studies worldwide have shown decreased rates of recall and an increased PPV using LC-MS/MS as a second tier test after mass screening (42–44). However, a study in the US showed that false-positive rates increased when using LC-MS/MS as a second tier test (53), suggesting the difficulty of setting suitable cutoff values. Fujikura et al. reported the results of secondary tests after mass screening in Japan; and suggested that setting of appropriate cutoff values is important because LS-MS/MS may also be used in Japan (45). *CYP21A2* mutation can also be examined by DNA extraction from filter paper blood; this method is technically feasible (54, 55) and has been validated in Japan (56, 57). However, there are many difficulties with routine genetic testing, and no large-scale studies have evaluated the utility of this procedure as a secondary test after mass screening.

2. Diagnosis of 21-OHD

Recommendation

1. We recommend that if the 17-OHP level is high in neonatal mass screening (beyond the reference value in detailed tests at a mass screening facility), a detailed examination should be conducted regardless of external genitalia anomaly, pigmentation and adrenal insufficiency symptoms. 1 (Consensus)
2. We recommend that if the 17-OHP level is high in neonatal mass screening and does not return to normal at recall (beyond the upper limit in a mass screening facility), a detailed examination should be conducted regardless of external genitalia anomaly, pigmentation and adrenal insufficiency symptoms. 1 (Consensus)
3. We recommend that 21-OHD be diagnosed with utmost care based on adrenal insufficiency symptoms including poor suckling, body weight loss and vomiting and that treatment start immediately after these symptoms and hyponatremia, hypokalemia and metabolic acidosis are found, even if endocrinological test results are not available. 1 (Consensus)

Explanation

A summary of disease diagnosis (Table 1) and a flow chart (Fig. 2) are provided.

2-1. Clinical symptoms

If the 17-OHP level is extremely high, patients should be examined for hyperpigmentation, virilization of female external genitalia and signs of adrenal insufficiency including poor suckling and dehydration. Testes should not be palpable in virilizing girls with 21-OHD. Patients should also be examined for the presence or absence of a common urogenital sinus including the vaginal and urethral opening. Those with virilization of the external genitalia including clitoromegaly and labial fusion should undergo a detailed examination prior to mass screening, since such patients are suspected to have 21-OHD. Therefore, filter paper blood should be

Table 1 Handbook for diagnosis of 21-hydroxylase deficiency in the neonatal stage

A. Clinical manifestations
1. Adrenal insufficiency symptoms
   Failure to thrive, poor body weight gain, frequent vomiting, dehydration, impaired consciousness and shock
2. Virilization
   Virilization of the external genitalia in girls[1] includes clitoromegaly, labial fusion and common urogenital sinus. Virilization of the external genitalia in boys, including increased stretched penile length.
3. Skin hyperpigmentation
   Diffuse systemic pigmentation or potent pigmentation in the oral mucosa, labium, areola, hilum and external genitalia

B. Test findings
1. High serum 17-OHP
   1a. Random blood sampling
   1b. After ACTH stimulation
2. High urine PTL and 11-OHAn/PD5 ratio[2]
3. High 21-DOF[3]

C. Reference findings
1. High plasma ACTH

D. Test findings for classification
1. Hyponatremia, hyperkalemia
2. Metabolic acidosis
3. Abnormally high plasma renin activity or concentration[4]

E. Exclusions[5]
1. 3β-hydroxysteroid dehydrogenase deficiency
2. 11β-hydroxylase deficiency
3. P450 oxidoreductase deficiency
4. Steroid hormone-producing tumor
5. Effect of extrinsic drugs

Diagnostic criteria

Neonatal 21-hydroxylase deficiency is diagnosed by a combination of clinical manifestations and endocrinological findings. The disease can be diagnosed in the following situations after items in E above are excluded.

1. The presence of symptoms of adrenal insufficiency or SW[6],
   i) Girl with virilization
      (1) B-2 is found.
   ii) Boy or girl without virilization
      (1) B-1 (a or b) and skin hyperpigmentation are found.
      (2) B-1 (a or b) and B-2 are found.
      (3) B-1 (a or b) is repeatedly found[7].
2. The absence of adrenal insufficiency symptoms and SW
   i) Girl with virilization
      (1) B-1 (a or b) and skin hyperpigmentation are found.
      (2) B-1 (a or b) and B-2 are found.
      (3) B-1a and B-1b are found.
   ii) Boy or girl without virilization[8]
      (1) B-1a and 1b and skin hyperpigmentation are found.
      (2) B-1a and 1b and B-2 are found.

[1] If an external genitalia anomaly is found, karyotype analysis is necessary to rule out other diseases.

[2] For classical 21-hydroxylase deficiency, P450 oxidoreductase deficiency and transient hyper-17-hydroxyprogesteronemia, differentiation procedures at 100% sensitivity and specificity regardless of gestational week and days old have been reported using urinary steroid profiles in the neonatal and infant stages (16). First indicator: pregnanetriolone (PTL). Second indicator: 11β-hydroxyandrosterone (11-OHAn)/pregnanediol (PD5) ratio. Urinary steroid profiles are not covered by insurance.

[3] 21-DOF is measured by a commercial laboratory (ASKA Pharmaceutical Medical Co., Ltd.). This procedure is not covered by insurance.

[4] There are data on the reference plasma renin activity. Reference data: 0–6 days after birth 8.83 ± 8.67 ng/ml/h, 7–27 days after birth 7.40 ± 3.74 ng/ml/h (Inada H, Imamura T, Nakajima R. Pediatric Endocrine Test Manual. Medical Review Co., Ltd., revised version 2003, Osaka)

[5] Diseases with increased 17-OHP levels in screening include 3β-hydroxysteroid dehydrogenase deficiency, 11ß-hydroxylase deficiency, P450 oxidoreductase deficiency, and steroid hormone-producing tumor. Definitive diagnosis of these diseases in the neonatal stage is difficult, and comprehensive differential diagnosis should be conducted using clinical symptoms and endocrine tests. P450 oxidoreductase deficiency is distinguishable by urinary steroid profile analysis using gas chromatography-mass spectrometry, which determines urine steroid metabolites by selective ion monitoring.

[6] A patient with adrenal insufficiency should be treated immediately. Treatment should not wait for the results of blood and urinary steroid tests after understanding the pathophysiology based on the findings in D in the table above. If a patient does not meet endocrinological criteria, an ACTH stimulation test should be performed after symptoms stabilize. If an abnormal increase in 17-OHP is found in this test, the patient is diagnosed with 21-hydroxylase deficiency.

[7] Measurement is repeated on other days.

[8] Mild SV boys are difficult to differentiate from NC type patients by endocrinological tests alone, and follow-up may be required. If a *CYP21A2* gene deletion or mutation in genetic analysis is identified as definite homozygosity or complex heterozygosity, the findings are useful for diagnosis.

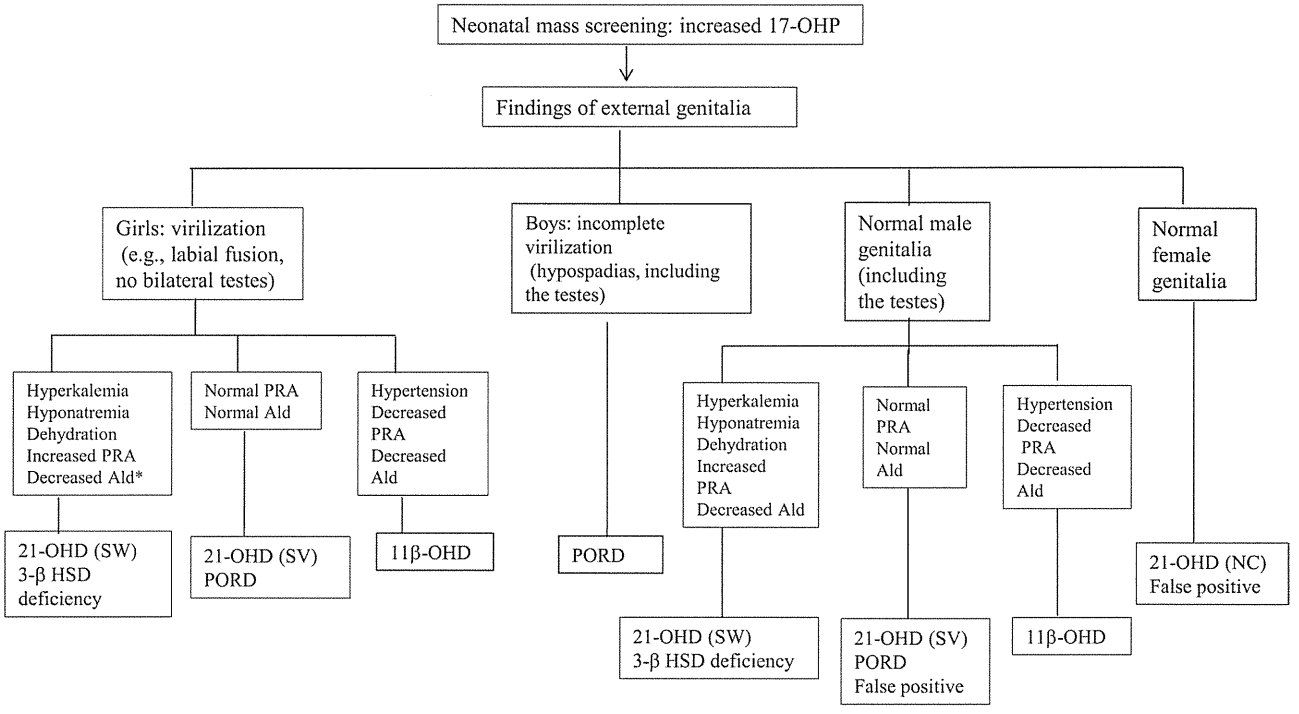

Fig. 2. Flow chart of diagnosis of individuals with increased 17-OHP levels detected by neonatal mass screening. *Ald, aldosterone. (Amano N, Hasegawa T. Diagnostic algorithm for adrenocortical insufficiency. Jpn J Pediatr Med 2012;44:588-92).

drawn for 17-OHP testing at a mass screening facility. About half of girls with 21-OHD undergo a detailed examination prior to mass screening due to an external genitalia anomaly (24, 25, 30, 58). Boys with normal external genitalia and a high 17-OHP level should be similarly examined for adrenal insufficiency and pigmentation to diagnose 21-OHD. It is difficult to evaluate increased penile length, a virilizing symptom, in boys with SW and SV 21-OHD (24, 25, 27, 30).

2-2. Biochemical and endocrine tests and imaging

In addition to the above examinations, biochemical and endocrine tests can be conducted. If virilization of the external genitalia in girls or under masculinization of the external genitalia in boys is suspected, karyotype analysis is required, in addition to differential diagnosis of other diseases. Blood 17-OHP is the most useful test item for diagnosis, and determination of serum or filter paper blood 17-OHP using approved kits is covered by insurance. Therefore, it is preferable to use kits at a test institution or mass screening facility. Repeated determination of the 17-OHP level is required. 21-Deoxycortisol (21-DOF) is an 11-hydroxyl derivative of 17-OHP without a 21-hydroxyl group and is useful for diagnosis (see note 3 in Table 1, not covered by insurance). Urine pregnanetriol (PT) is a direct urine metabolite of 17-OHP, and increased PT may also be helpful for diagnosis of 21-OHD. However, overlap with normal infants, transient hyper-17-hydroxyprogesteronemia, and increased 17-OHP in premature infants have been shown, which makes PT less useful for diagnosis (59).

Diagnosis can be confirmed using gas chromatography-mass spectrometry, in which urine steroid metabolites are determined by selective ion monitoring (urine steroid profile) (note 2 in Table 1). Homma *et al.* determined the urine pregnanetriolone (PTL)/Cr ratio in 59

patients with 21-OHD, 83 patients with transient hyper-17-hydroxyprogesteronemia; and 62 normal subjects, and found that 21-OHD was distinguishable in full-term and preterm infants (59, 60). An increased ratio of urine metabolites of androstenedione and pregnenolone, 11β-hydroxyandrosterone/pregnanediol, was also useful for diagnosis. This test is not covered by insurance; but is useful for diagnosis of 21-OHD and is described in the "Handbook of Diagnosis of 21-Hydroxylase Deficiency of the Study Group for Adrenal Hormone Production Abnormality" prepared by the Ministry of Health, Labour and Welfare Project on Intractable Disease (61).

Tests for diagnosis and understanding of the pathological condition include plasma ACTH, serum electrolytes, plasma glucose, plasma aldosterone, plasma renin activity or concentration, and blood gas (6, 9). Findings in these tests can identify SW and SV cases and improve diagnostic reliability. It has been suggested that all 21-OHD patients are unresponsive to mineralocorticoids (62) and mineralocorticoid deficiency in the neonatal stage (63). The flow chart in Fig. 2 shows the differentiation of SW and SV types, but the type is sometimes indistinguishable. If 21-OHD is suspected due to clinical symptoms and abnormal test results, and a manifestation of adrenal insufficiency is found, treatment must be the priority.

Imaging by ultrasonography can rapidly detect enlargement of adrenal glands, but experience is required to determine adrenal enlargement accurately. However, endocrine tests and karyotype analysis are time-consuming, and imaging findings can be used to suggest the presence of 21-OHD in 46,XX girls if the uterus is visualized in addition to the adrenal gland.

2-3. Genetic diagnosis

Genetic diagnosis is currently performed in commercial laboratories and not covered by insurance. A genetic abnormality can be detected in about 90% of 21-OHD patients (9), and analysis of parents improves the accuracy of diagnosis of 21-OHD. However, 21-OHD can be diagnosed based on the above clinical symptoms and test findings, and evaluation of the *CYP21A2* gene is not always necessary. However, genetic analysis can assist diagnosis of patients without typical clinical symptoms, including girls without marked virilizing symptoms, boys with mild SV21-OHD, and NC patients. Genetic diagnosis also provides important information for genetic counseling. However, the higher rates of *de novo* mutation in comparison with other autosomal recessive diseases require attention (64–66). The structures of the *CYP21A2* gene and its pseudogene *CYP21A1P* are also complicated, and deletion or point mutations may not be detected. Southern blotting and restriction fragment length polymorphism (RFLP) analysis are sometimes needed (67, 68). Also, as described above, the genotype does not always correspond to the phenotype.

2-4. Differential diagnosis

Other CAH conditions with high 17-OHP levels in mass screening include P450 oxidoreductase (POR) deficiency, 3β-hydroxysteroid dehydrogenase (3β HSD) deficiency, and 11β-hydroxylase deficiency (11β OHD). Virilization of the external genitalia develops in 46,XX girls with POR deficiency, while incomplete virilization in 46,XY boys with POR deficiency is frequently associated with early craniosynostosis, characteristic facies, humeral-radial synostosis, and joint contracture (69, 70). Electrolytes are normal in POR deficiency, and neonatal adrenal insufficiency is rarely found. POR deficiency and 21-OHD can be differentiated by analyzing urinary steroid profiles (60). 3β HSD deficiency causes virilization of the external genitalia in 46,XX girls and incomplete virilization in 46,XY boys, resulting in adrenal insufficiency, and may increase the 17-OHP level (71, 72). Serum steroid metabolites should be determined, and 3β HSD deficiency is diagnosable from the ratios of pregnenolone/

progesterone, 17-OH pregnenolone/17-OHP, and dehydroepiandrosterone (DHEA)/androstenedione (11, 61). 11β OHD is characterized by hypertension, but some neonates do not have hypertension (11, 73, 74). In contrast to 21-OHD, 11β OHD is endocrinologically diagnosed by a decreased plasma renin concentration or activity, decreased level of plasma aldosterone and increased baseline and ACTH-stimulated levels of deoxycorticosterone and 11-deoxycortisol (61). Diagnosis of 3β HSD deficiency and 11β OHD by urinary steroid profile analysis, in contrast to 21-OHD and POR deficiency, should be made based on several test results until 3 to 4 mo after birth. Genetic diagnosis is also useful for differentiation of POR deficiency, 3β HSD deficiency and 11β OHD. These genetic tests are conducted by research institutions.

Cases of adrenal tumor due to high 17-OHP levels have also been reported (75, 76). As described above, false-positive results in mass screening are common in preterm and low birth weight infants, and the urinary steroid profile is useful for differentiation regardless of gestational week and days old (59, 60).

2-5. Diagnosis of NC and very mild SV types found in mass screening

The major purpose of mass screening is early detection of severe SW and SV 21-OHD. Very mild SV and NC types with unremarkable increased levels of androgen are also found in mass screening (69, 77–81). Ishii *et al.* estimated that the prevalence of the NC type was 1/2,000,000 (81). A total of 15 patients were identified in a national survey, including 11 found in mass screening, 3 diagnosed due to virilizing symptoms or accelerated growth, and 1 identified due to an affected sibling (80).

These diagnoses require confirmation based on a high 17-OHP level using various methods. If the baseline 17-OHP level is not high in blood sampling, the test should be repeated (6). For very mild SV and NC type patients, increased 17-OHP levels should be confirmed in an ACTH stimulation test. The NC type sometimes shows a normal 17-OHP level in random tests, and the baseline 17-OHP level should be determined before 8 a.m. (9, 82, 83). The ACTH stimulation test is then performed for differentiation from other CAHs. Data for 17-OHP before and after an ACTH stimulation test have been reported in overseas studies (82, 83). In a test that is not performed for diagnosis of 21-OHD alone, Cortrosyn®.

(ACTH 1-24) at 0.25 mg/m^2 is intravenously administered for 90 min at intervals of 30 min. In addition to 17-OHP, cortisol, deoxycorticosterone, 11-deoxycortisol, 17-OH pregnenolone, DHEA and androstenedione are determined after stimulation with Cortrosyn® to differentiate 21-OHD from other CAH conditions (some test items are not covered by insurance).

Boys with classical (and particularly very mild SV) 21-OHD are difficult to differentiate from NC cases using endocrinological and genetic tests alone, and follow-up may be required. Patients with virilizing symptoms (e.g. , accelerated growth, accelerated bone maturation) require treatment. Those who are followed up without treatment should be monitored for adrenal insufficiency symptoms and SW findings (78, 80).

3. Initial Treatment in the Neonatal Stage and Maintenance Therapy in the Childhood Stage (Table 2)

Recommendation

Glucocorticoids:

1. We suggest that initial treatment of classical 21-OHD in the neonatal stage requires administration of glucocorticoids at a dose higher than that of maintenance therapy to rapidly reduce enhanced adrenal androgen production. 2 (●○○)
2. We recommend use of hydrocortisone (HC) for maintenance therapy in growing children with classical 21-OHD. 1 (●○○)
3. We recommend against use of long-acting

Table 2 Glucocorticoid and mineralocorticoid dosages for initial treatment and maintenance therapy

		HC (mg/m^2/day, 3 times a d)	FC* (mg/day, 2–3 times a d)	Sodium chloride* (g/kg/day, 3–8 times a d)
Initial treatment	Neonatal stage	25–100**	0.025–0.2	0.1–0.2
Maintenance therapy	Neonatal stage Infant stage	10–20	0.025–0.2	0.1–0.2
	Preschool child stage Schoolchild stage Pubertal stage	10–15	0.025–0.2	
	Adult stage	10–15***	0.025–0.2****	

* FC and sodium chloride are almost always required for classical SW 21-OHD. The doses of FC and sodium chloride are chosen based on serum sodium and potassium levels, plasma renin activity or concentration; and body weight gain. Reference values for plasma renin activity for one month in the neonatal stage are described in Note 4 of Table 1. ** The dose is managed based on the severity of clinical symptoms. If adrenal crisis is suspected, a bolus intravenous injection of HC (50 mg/m^2) should be performed immediately. *** Patients in the adult stage may be given prednisolone or dexamethasone (see section 7 on treatment of adult classical CAH). **** The required dose may decrease with age, and FC may be discontinued.

glucocorticoids for maintenance therapy in growing children with classical 21-OHD. 1 (●○○)

4. We recommend that the dose of glucocorticoids during maintenance therapy be carefully chosen to prevent overdose and underdose. 1 (Consensus)

Mineralocorticoids:

5. Administration of fludrocortisone (FC) and sodium chloride is recommended for SW neonates and infants. 1 (●●●)

Explanation

3-1. Treatment principles

The principles of treatment of 21-OHD are to supplement insufficient glucocorticoid and mineralocorticoid levels, inhibit enhanced adrenal androgen production, and maintain growth and maturation similar to those of healthy children. Treatment continues for life. Insufficient treatment causes adrenal crisis (acute and severe adrenal insufficiency) due to decreased tolerance to physical stress and short stature due to bone age advancement. Excessive treatment causes iatrogenic Cushing's syndrome, including short stature, obesity and hypertension; 21-OHD is managed by pediatric endocrinologists when possible.

3-2. Glucocorticoids: initial treatment in the neonatal stage

In the neonatal stage of classical 21-OHD, adrenal androgen production is markedly enhanced. The previous Japanese treatment guidelines recommended initial treatment with high-dose HC (100–200 mg/m^2/d) to inhibit adrenal androgen production (7). In contrast, the European and American guidelines include initial treatment with up HC 25 mg/m^2/d, typically with a low-dose of HC of 10–15 mg/m^2/d (8–10). The 17-OHP level remains high until 3 mo after birth in females and 6 mo after birth in males treated with HC at the initial dose in Europe and the US (84). This suggests that this dose does not sufficiently inhibit adrenal androgen production. However, the target height SDS was reached

at 3 yr old without bone age advancement, and adrenal insufficiency occurred in only one subject with gastroenteritis (84). Therefore, low-dose HC does not rapidly reduce adrenal androgen production, but there is no clear evidence that initial low-dose HC is disadvantageous.

In children with classical 21-OHD, height SDS decreases from birth to 1–2 yr old, and height SDS at 2–3 yr old significantly correlates with adult height (85–89). The decrease in height SDS during this period is also significantly correlated with the dose of glucocorticoids (85–89). However, in studies using initial treatment with low-dose HC (9–15 mg/m^2/d) (84) and a high dose in accordance with the Japanese treatment guidelines (90, 91), the height at 1 yr old corresponded to –1 SD. There was also no significant difference in height SDS at 1, 2 and 3 yr old between groups initially treated with > 150 and 100 mg/m^2/d HC (92). Therefore, the relationship of decreased height SDS early after birth with the dose of glucocorticoids in initial treatment is uncertain, and there is no clear evidence that initial treatment at a high dose worsens the prognosis for height.

There is also no clear evidence for the optimal dose of glucocorticoids in initial treatment. Therefore, the initial dose in neonatal treatment is defined as HC 25–100 mg/m^2/d in the Guidelines based on a survey (hereinafter referred to as the JSPE survey) of councilors of the Japanese Society for Pediatric Endocrinology (Table 2). A patient with adrenal crisis or a similar condition should be treated with 100 mg/m^2/d HC after bolus administration. This treatment is used by many pediatric endocrinologists. A patient with no adrenal crisis may start treatment at a lower dose. Since a patient with no manifestation of adrenal insufficiency may have NC 21-OHD, treatment does not start immediately, but symptoms and biochemical data should be carefully evaluated. After adrenal androgen production is reduced by the treatment, the dose is immediately decreased at intervals of 5–7 d, and maintenance therapy begins 3–4 wk after birth. These doses and modes of administration are considered to be targets, and practical administration is dependent on the individual patient and clinical experience.

3-3. Glucocorticoids: maintenance therapy in growing children

HC is also used as a glucocorticoid for maintenance therapy in growing children with 21-OHD. HC causes fewer adverse reactions than more potent long-acting glucocorticoids, particularly risks for growth retardation, because of its short half-life. Prednisolone and dexamethasone produce growth suppression that is 15-fold (93) and 70- to 80-fold (94) higher than that with HC. Thus, long-acting glucocorticoids should not be used for maintenance therapy in growing children. HC is often administered three times a day, and there is no clear advantage of increasing the dose in the morning or evening (95).

Physiological cortisol production is estimated to be 5–6 mg/m^2/d as HC (96–98). Adult height decreased in patients given >20 mg/m^2/d in the infant stage and > 15–17 mg/m^2/d in the pubertal stage (85–89, 99). Adult height of patients with 21-OHD is also negatively correlated with the dose of glucocorticoids in the early pubertal stage (87–89). However, in a meta-analysis of patients with 21-OHD, adult height SDS adjusted for parents' height had no significant correlation with the total dose of glucocorticoids (100). Thus, there is no clear correlation between the dose of glucocorticoids in maintenance therapy and the height prognosis; however, it is reasonable to treat children before the pubertal stage with as low a dose as possible.

Based on the JSPE survey, the Guidelines recommend a dose of HC for maintenance therapy similar to that in the European and American guidelines (Table 2). The appropriate dose in maintenance therapy differs among individuals for unknown reasons. In the pubertal stage, patient control is sometimes insufficient because cortisol clearance increases, even if replacement

therapy is performed and compliance is good (101). The JSPE survey showed that some patients require a dose exceeding the recommended dose for HC maintenance therapy; and that some patients are controlled well for a short to medium period with long-acting glucocorticoids, including dexamethasone.

As described above, underdosing causes adrenal insufficiency and excessive adrenal androgen and inhibits growth, while an overdose causes Cushing's syndrome and inhibits growth. Therefore, it is important to control the dose balance in treatment of patients. The dose and mode of administration are targets, and practical administration is dependent on the individual patient and the patient's age.

3-4. Mineralocorticoids

Classical SW 21-OHD is not sufficiently treated with HC alone and requires FC (7, 9, 63, 102). Sodium intake from breast milk and bottle formula is insufficient for treatment in the infant stage, and sodium chloride replacement is necessary (7–9). Aldosterone resistance may occur in the neonatal stage (62). Aldosterone deficiency is found in SW cases; and may also occur in SV cases (63). Sodium balance is appropriately maintained by decreasing vasopressin and the ACTH level, reducing the HC dose, and improving adult height (103). The results of a meta-analysis of patients with CAH showed that adult height SDS adjusted for parents' height was significantly higher in patients treated with FC than in those without FC (100).

The Guidelines recommend a dose of FC in maintenance therapy based on the previous Japanese guidelines, American guidelines; and the results of the JSPE survey (Table 2) (7, 9, 104). The European and American guidelines recommend FC administration to all patients (8, 9), but there is no clear evidence for a benefit of FC in all patients. A patient with poor body weight gain, high plasma renin activity or concentration; and electrolyte imbalance (hyponatremia and hyperkalemia) should be diagnosed with SW 21-OHD and treated with FC, even if FC was not administered earlier. The FC dose is determined based on the plasma renin activity or concentration, electrolyte levels; and body weight gain. Adverse reactions including increased blood pressure and edema should be monitored. If glucocorticoid treatment starts at a high dose (100 mg/m^2/d HC), mineralocorticoid deficiency sometimes appears when reducing the dose of glucocorticoids for replacement with maintenance therapy (7). The proposed dose and mode of administration are targets, and practical administration is dependent on the individual patient and the patient's age.

4. Stress Dosing During Maintenance Therapy (Table 3)

Recommendation

1. We recommend that the dose of glucocorticoids be increased for febrile illness (>38.5°C), gastroenteritis with dehydration, surgery under general anesthesia, and major trauma. 1 (●●○)
2. We suggest that a patient have a medical identification tag indicating adrenal insufficiency. 2 (●○○)
3. We suggest not routinely increasing the dose of glucocorticoids in patients with mental and emotional stress or slight disease or before mild exercise. 2 (●○○)

Explanation

Cortisol does not respond sufficiently to physical stress in patients with 21-OHD, resulting in adrenal crisis. Adrenal crisis frequently occurs in children < 10 yr old and particularly in those < 1 yr old; and is likely to be associated with gastroenteritis (106). Therefore, if a patient has febrile illness, gastroenteritis with dehydration, surgery or trauma, it is necessary to increase the dose of glucocorticoids transiently. A sufficient dosage of HC has the effect of a mineralocorticoid; therefore, FC administration is unnecessary.

Table 3 Stress dosing

Physical stress	Conditions	HC dose
Mild	Vaccination Upper respiratory infection up to low-grade fever	Maintenance dose
Moderate*	Infection associated with high fever (> 38.5°C) Vomiting, diarrhea, poor feeding, sluggishness Minor surgery, trauma, dental treatment, burn	3- to 4-fold maintenance dose or 50-100 mg/m^2/d**
Severe*	Sepsis, major surgery	100 mg/m^2/d**

* If adrenal crisis is suspected, prior to surgery under general anesthesia or stress is difficult to control orally, a parenteral bolus administration of HC 50 mg/m^2 (infant, 25 mg; child, 50 mg; adult, 100 mg) is first performed (9). If an intravenous line is difficult to place, succinate ester of HC can be intramuscularly injected (only phosphate ester of HC is allowed to be intravenously administered in Japan). ** For intravenous injection, continuous administration is preferable to bolus injection at 6-h intervals (105).

Maintenance therapy is resumed immediately after conditions are stabilized. A child at an early age has a risk for hypoglycemia and electrolyte imbalance; therefore, long-term fasting conditions should be avoided, and intravenous administration of glucose and sodium should be performed as required. For rapid and appropriate treatment of adrenal crisis, the patient should have a medical identification tag indicating adrenal insufficiency.

Mild exercise and mental stress (e.g., anxiety and tests) do not routinely require an increased dose of glucocorticoids (107), but based on the JSPE survey, the dose should be increased in patients before strenuous exercise (e.g., a marathon) associated with wasting. It is important to understand the level of physical distress for each patient to determine the appropriate dose increase. The relationships of the type of physical stress with the dose and mode of administration are under discussion. The Guidelines recommend stress-based HC doses based on the JSPE survey and European and American guidelines (Table 3). The proposed doses and modes of administration should be used for each patient based on clinical experience. A large-scale comparative study is required to determine the appropriate doses and modes of administration.

5. Treatment Monitoring for Growing Children

Recommendation

1. We recommend that monitoring of treatment in children be determined with consideration of changes in growth rate and bone age, in addition to test findings. 1 (Consensus)
2. We suggest that routine evaluation of height, body weight and blood pressure be performed at all ages in growing children and that bone age be determined after one year old. 2 (●○○)
3. We suggest that treatment be evaluated by endocrine test at a constant time (before administration of glucocorticoids early in the morning). 2 (●○○)
4. We recommend that monitoring of adverse events (Cushing's syndrome) due to excessive glucocorticoids and avoidance of complete inhibition of intrinsic adrenal steroid secretion are required. 1 (●○○)

Explanation

It is not easy to monitor treatment of 21-OHD (7–10). The JSPE survey suggested that there is no endocrinological indicator for complete monitoring and determination of the optimal dose of glucocorticoids. Thus, the appropriateness of treatment has to be judged based on clinical symptoms and test

findings. Therefore, the Guidelines recommend comprehensive monitoring of treatment in children based on changes in growth rate and bone age, in addition to test findings.

Excessive glucocorticoid levels cause a reduced growth rate and obesity, while insufficient levels enhance the growth rate and bone age. Insufficient mineralocorticoids reduce the growth rate and cause poor body weight gain. Therefore, height and body weight should be routinely evaluated. Untreated patients with classical 21-OHD do not show increased bone age until 1–1.5 yr old; therefore, bone age is evaluated after one year old. Evaluation once a year is usually sufficient (7, 9, 108), but bone age should be determined twice a year when the growth rate is rapidly changing or the patient is in the pubertal stage. Growth and maturation that proceed with age are important as long-term indicators.

Endocrinological tests should be conducted using precise methods and evaluated based on appropriate references. Adrenal steroid hormone levels depend on the measurement method, and it is desirable to determine adrenal steroid hormones by extracted immunological assays and LC-MS/MS. The best indicator for treatment with glucocorticoids is serum 17-OHP (7, 9, 109, 110). Determination of serum 17-OHP using approved kits is covered by insurance, and it is preferable to use a kit at a test institution or mass screening facility. Serum androstenedione and testosterone (prepubertal males and females, pubertal females) can be used for treatment monitoring (7, 9, 10, 111), but measurement of the serum androstenedione level is not covered by insurance in Japan, and standard references by sex and age have not been established for androstenedione.

Plasma ACTH has a robust circadian rhythm and is difficult to use for monitoring. Serum 17-OHP also has a circadian rhythm and daily variance, and thus it is preferable to determine serum 17-OHP before administration of glucocorticoids early in the morning (9. 10, 109, 110). However, in the JSPE survey, most institutions determined casual 17-OHP in outpatient consultations. The target for serum 17-OHP is 400–1200 ng/dL before administration early in the morning in both the child and adult stages (10, 110) and < 590 ng/dL in the pubertal stage (84). Normalized 17-OHP suggests an overdose of glucocorticoids. Monitoring using pregnanetriol (PT), a urinary metabolite of 17-OHP, by urine collection has also been proposed (7, 112–114). In a study of 21-OHD in Japanese children, excluding the neonatal and pubertal stages, a PT range of 1.2–2.1 $mg/m^2/d$ was shown to indicate good control (113). If 21-OHD cannot be determined before administration of glucocorticoids early in the morning, determination of the 17-OHP level and monitoring of PT by urine collection may be useful, while keeping the circadian rhythm and daily variance of 17-OHP in mind.

If treatment to control of 21-OHD is difficult, determination of the 17-OHP level before administration of glucocorticoids and monitoring of PT by urine collection may be considered.

Endocrinological tests include short-term indicators that may be changed by sampling conditions; therefore, tests should be performed several times and comprehensively evaluated. Indicators for monitoring mineralocorticoid treatment include blood pressure, serum electrolytes and plasma renin. Excessive mineralocorticoids increase systolic blood pressure (115). Plasma renin should be close to the average level for each age, but it is also frequently high in healthy children in the neonatal and infant stages; therefore, it should be used only as a reference. Decreased plasma renin suggests an overdose of FC or sodium chloride. Ten of 134 infants who were given FC were found to have hypertension and leg edema (104). The FC dose was 0.025–0.05 mg/d in 7 of the 10 patients. These patients were neonates who were given FC due to immature sodium reabsorption in the renal tubule, and required subsequent reduction of the FC dose. These findings reflect individual