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Special Report

Clinical guidelines for the diagnosis and treatment of 21-hydroxylase deficiency (2021 revision)

Tomohiro Ishii[1, 2], Kenichi Kashimada[1, 3], Naoko Amano[2, 4, 5], Kei Takasawa[3, 4], Akari Nakamura-Utsunomiya[4, 6], Shuichi Yatsuga[4, 7], Tokuo Mukai[1, 8], Shinobu Ida[1, 9], Mitsuhisa Isobe[10, 11], Masaru Fukushi[10, 12], Hiroyuki Satoh[13, 14], Kaoru Yoshino[13, 15], Michio Otsuki[16, 17], Takuyuki Katabami[16, 18], and Toshihiro Tajima[4, 19]

[1]*Differences of Sex Development (DSD) and Adrenal Disorders Committee, Japanese Society for Pediatric Endocrinology*
[2]*Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan*
[3]*Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University (TMDU), Tokyo, Japan*
[4]*Committee on Mass Screening, Japanese Society for Pediatric Endocrinology*
[5]*Department of Pediatrics, Saitama City Hospital, Saitama, Japan*
[6]*Department of Pediatrics, Hiroshima Prefectural Hospital, Hiroshima, Japan*
[7]*Department of Pediatrics, Fukuoka University School of Medicine, Fukuoka, Japan*
[8]*Department of Pediatrics, Asahikawa Red Cross Hospital, Asahikawa, Japan*
[9]*Department of Gastroenterology and Endocrinology, Osaka Women's and Children's Hospital, Osaka, Japan*
[10]*Japanese Society for Neonatal Screening*
[11]*Saitama City Institute of Health Science and Research, Saitama, Japan*
[12]*Sapporo Immuno Diagnostic Laboratory (IDL), Sapporo, Japan*
[13]*Japanese Society for Pediatric Urology*
[14]*Division of Urology, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan*
[15]*Department of Urology, Aichi Children's Health and Medical Center, Obu, Japan*
[16]*Japan Endocrine Society*
[17]*Department of Endocrinology, Tokyo Women's Medical University, Tokyo, Japan*
[18]*Division of Metabolism and Endocrinology, Department of Internal Medicine, St. Marianna University School, Yokohama Seibu Hospital, Yokohama, Japan*
[19]*Department of Pediatrics, Jichi Medical University Tochigi Children's Medical Center, Tochigi, Japan*

Highlights

- The working committee updated clinical guidelines for diagnosis and treatment of 21-hydroxylase deficiency based on recent evidence and knowledge related to this disorder.
- The clinical guidelines provide 45 recommendations with their strength and quality of evidence graded using the GRADE framework.
- The recommendations can be applied to children and adults with 21-hydroxylase deficiency considering the balance of benefits and risks for each patient.

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Corresponding author: Tomohiro Ishii, M.D., Ph.D., Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
E-mail: tishii@1992.jukuin.keio.ac.jp

Abstract. Congenital adrenal hyperplasia is a category of disorders characterized by impaired adrenocortical steroidogenesis. The most frequent disorder of congenital adrenal hyperplasia is 21-hydroxylase deficiency, which is caused by pathogenic variants of *CAY21A2* and is prevalent between 1 in 18,000 and 20,000 in Japan. The clinical guidelines for 21-hydroxylase deficiency in Japan have been revised twice since a diagnostic handbook in Japan was published in 1989. On behalf of the Japanese Society for Pediatric Endocrinology, the Japanese Society for Mass Screening, the Japanese Society for Urology, and the Japan Endocrine Society, the working committee updated the guidelines for the diagnosis and treatment of 21-hydroxylase deficiency published in 2014, based on recent evidence and knowledge related to this disorder. The recommendations in the updated guidelines can be applied in clinical practice considering the risks and benefits to each patient.

Key words: 21-hydroxylase deficiency, congenital adrenal hyperplasia, neonatal mass screening, guideline

Introduction

Neonatal mass screening for 21-hydroxylase deficiency (21-OHD) has been implemented in Japan since January 1989 and has detected 21-OHD in approximately 1 in 18,000–19,000 live births (1–3). 21-OHD requires continuous lifelong treatment. It is recommended that definite diagnosis and treatment for individuals identified as positive by mass screening be conducted by specialists or experts, such as pediatric endocrinologists, to avoid unnecessary treatment. In 1989, the first clinical practice guidelines for 21-OHD in Japan were published as a diagnostic handbook focusing mainly on severe cases (4, 5). The guidelines for the treatment of neonatal mass screening were revised in 1999 to enable the treatment of mild or asymptomatic cases (6, 7). In addition, with the subsequent development of international guidelines (8, 9), these guidelines were further revised to include diagnostic or therapeutic management in 2014 (10). In this edition, the content has been updated based on new knowledge and the revision of the international guideline (11). Furthermore, topics that contribute to the improvement of the patient's quality of life (QOL), such as genital reconstructive surgery or gender identity, were added, and the new guidelines were developed by consensus of the working committee members composed of the Japanese Society for Neonatal Screening, the Japanese Society of Pediatric Urology, the Japan Endocrine Society, and the Japanese Society for Pediatric Endocrinology (Disorders of Sex Development and Adrenal Disorders Committee, Committee on Mass Screening).

This guideline presents standard medical care to physicians involved in the management of 21-OHD, aiming to equalize medical care and further improve patient QOL while supporting diagnosis and treatment. The guidelines do not constrain physicians' practice policies, and actual practice should be judged accordingly depending on the patient's condition.

These guidelines include recommendations for each topic. "Strength of recommendation" and "quality of evidence" have been described throughout the text. The quality of evidence indicated the level of validity of the study on which it was based. The strength of recommendation is based on evidence from the literature in principle; however, expert opinions have been provided where evidence is insufficient.

Strength of Recommendation

1. Strong recommendation. "Will produce benefits for most patients"
2. Weak recommendation. "Should be considered because they often benefit patients"

Quality of Evidence

●○○ Low: Case series without controls
●●○ Middle: Cohort study without controls
●●● High: Cohort study with a control, non-randomized controlled trial
Consensus: Widely recognized opinion despite no direct research

Outline of the Targeted Disorder: 21-OHD

A group of diseases with cortisol deficiency and adrenal enlargement due to adrenocorticotropic hormone (ACTH) overproduction is collectively referred to as congenital adrenal hyperplasia (CAH). CAH is an autosomal recessive disorder with an incidence of 1 in 14,000–20,000 in most ethnic groups (1, 2, 11). 21-OHD is the most frequent disorder among CAH, with an incidence of approximately 1 in 18,000–19,000 in Japan (1, 3). 21-OHD is caused by biallelic loss-of-function pathogenic variants of the *CYP21A2* gene, which encodes steroid 21-hydroxylase (P450c21) (11, 12). This enzyme converts 17-hydroxyprogesterone (17-OHP) to 11-deoxycortisol (11-DOF) and progesterone to deoxycorticosterone (DOC). Furthermore, because 11-DOF and DOC are ultimately converted to cortisol and aldosterone, respectively, both hormones are deficient in 21-OHD (**Fig. 1**). The lack of cortisol leads to the accumulation of cortisol precursors upon stimulation by ACTH, and these accumulated precursors are directed toward the adrenal androgen production pathway (**Fig. 1**). Therefore, one of the important symptoms of this condition in female neonates is virilization of the external genitalia. If the disease is not diagnosed during the early neonatal period in males or females, poor feeding and

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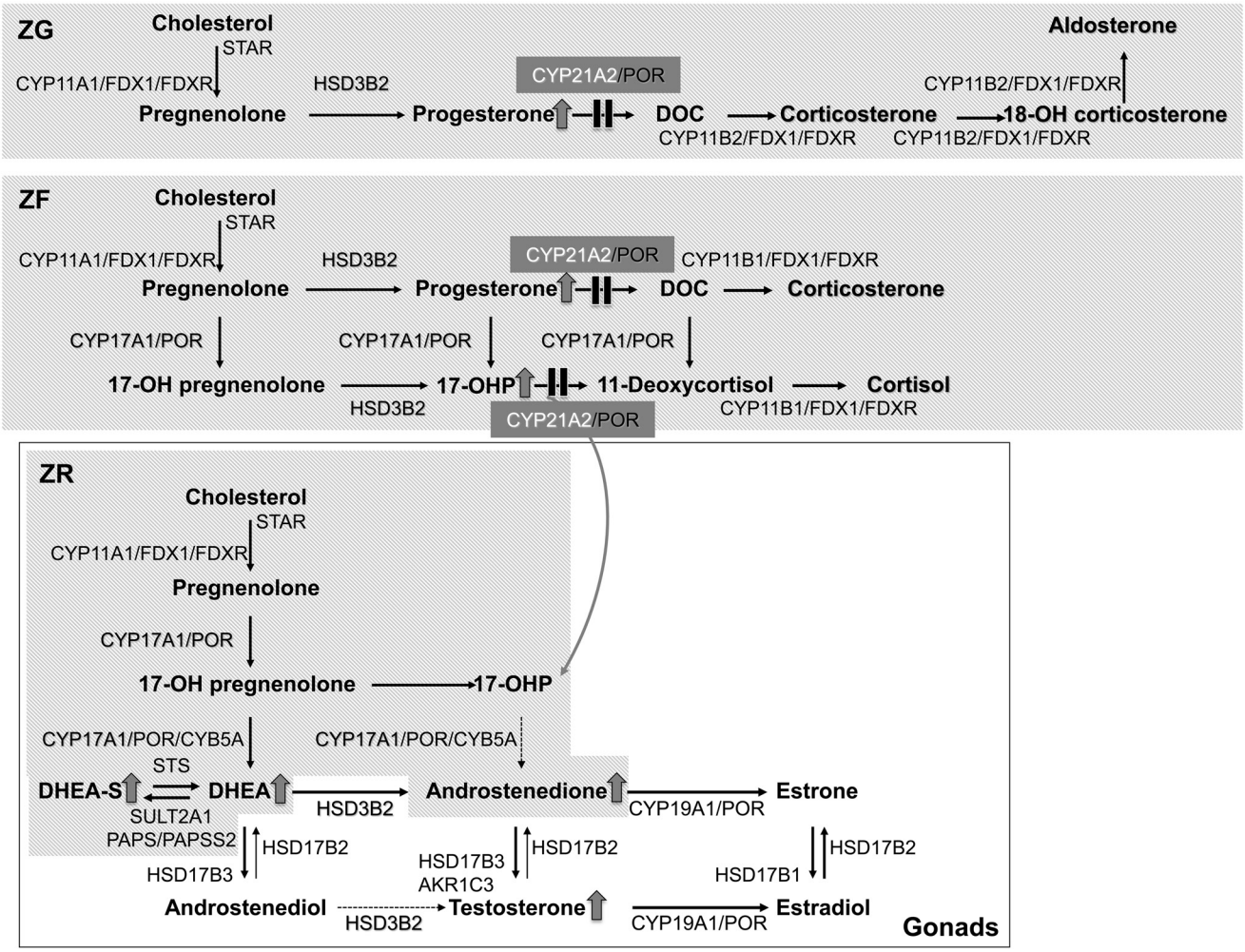

Fig. 1. Steroidogenesis in 21-hydroxylase deficiency. DOC: deoxycorticosterone, 17-OHP: 17-hydroxyprogesterone, DHEA: dehydroepiandrosterone, DHEA-S: dehydroepiandrosterone sulfate, ZG: zona glomerulosa, ZF: zona fasciculata, ZR: zona reticularis.

poor weight gain may occur, and an adrenal crisis may develop after the first week of life (13, 14). 21-OHD is a disorder affecting not only growth and sexual maturation but also the quality and prognosis of life.

21-OHD is classified into classic and non-classic forms according to the severity of manifestations. The classic form is subclassified into salt-wasting and simple-virilizing forms. In the salt-wasting form, symptoms of deficiencies in both cortisol and aldosterone are observed alongside symptoms of adrenal androgen excess. In the simple-virilizing form, only cortisol deficiency and adrenal androgen excess symptoms were observed. Finally, in the non-classic form, relatively mild cortisol deficiency and adrenal androgen excess symptoms were observed. Patients with the salt-wasting or simple-virilizing form present with symptoms during the neonatal period. On the other hand, those with the non-classic form often present symptoms after infancy. Among these three forms, the salt-wasting form is the most prevalent, accounting for 75% of cases (1, 11, 12). The non-classic form of adrenal androgen excess is diverse and may be asymptomatic until adulthood (15–17). There is overlap in clinical manifestations or age at diagnosis among the three forms, but there is an association between the disease form and *CYP21A2* genotype (11, 18, 19).

1. Neonatal Mass Screening

Recommendations

1. We recommend that a screening program for 21-OHD be conducted as part of the neonatal mass screening. 1 (Consensus)
2. We recommend that neonatal mass screening for 21-OHD be performed via immunoassays of 17-OHP [e.g., enzyme-linked immuno sorbent assay (ELISA)], with the use of the direct procedure for the first-tier screen and the extraction procedure for the second-tier screen. To reduce the false-positive rate, cut-off values should be modified according to gestational age. 1 (●●○)
3. To further reduce the false-positive rate and improve the positive predictive value of 21-OHD neonatal mass

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screening, we suggest that the second-tier screen be performed by measuring steroid profile with liquid chromatography-tandem mass spectrometry (LC-MS/MS), which has better specificity than immunoassay with extraction procedure. 2 (●●○)

4. To enable neonates who test positive for neonatal mass screening to receive prompt and appropriate medical care, we recommend that prefectures and ordinance-designated cities establish an outline for conducting neonatal mass screening for inborn errors of metabolism and develop a specific medical care protocol. 1 (Consensus)

Evidence

1-1. Outcomes of neonatal mass screening

Neonatal mass screening for 21-OHD began in 1989 in Japan. The incidence of 21-OHD before the initiation of screening was estimated to be approximately 1 out of 43,674 persons, but an epidemiologic study conducted after neonatal mass screening showed that the incidence was 1 out of 18,827 (1, 3, 20). In this study, the salt-wasting form was more abundant than the simple-virilizing form in both sexes, and the male-female ratio was 1:1 in both salt-wasting and simple-virilizing forms. Other reports indicate that the incidence of salt-wasting forms diagnosed by neonatal mass screening is similar in both sexes, although the disease is more frequently detected in females by clinical diagnosis before neonatal mass screening (21, 22). Thus, it seems that before the start of neonatal mass screening, males of the simple-virilizing form were overlooked and males of the salt-wasting form might have been incorrectly diagnosed as cases of sudden unexpected death.

The cost-benefit analysis is important for neonatal mass screening as a maternal and newborn health project in prefectures and ordinance-designated cities. The expenses required for screening or management of patients are calculated as a cost, while the facility fees, nursing expenditure, and special education expenditure, which are avoided through early detection, are calculated as a benefit (23). This evaluation resulted in the highest net benefit for screening for congenital hypothyroidism (¥3.1 billion) compared with that for 21-OHD (¥200 million).

Although the effectiveness of neonatal mass screening for the diagnosis of 21-OHD is obvious, the major concern is the high false-positive rate (low positive predictive value) (24–31). The aforementioned cost-benefit analysis did not consider the cost of re-testing a child with a false-positive result or nursing a child if admitted to the hospital. In addition, it is anticipated that parents will be anxious that their children may be affected by chronic illnesses (32). To circumvent this issue, two-step screening, including a second-tier screen with a higher positive predictive value, should be considered for those with a positive test on the first-tier screen via ELISA (11, 33–36).

1-2. Current status in neonatal mass screening

In Japan, the cut-off value for 17-OHP levels has not been standardized nationwide. This is attributed to the diverse attitudes toward mass screening for this disorder held by consultant physicians or prefectures and ordinance-designated cities that are responsible for implementing each mass screening (27–29, 37). In addition, how individuals are recalled and evaluated with positive tests varies widely between regions. Accordingly, specific follow-up procedures for positive screenings should be set out in the protocol in each local region so that neonates who test positive for mass screening can be adequately managed anywhere. In particular, if an extremely high 17-OHP level or clinical symptoms of adrenal insufficiency are observed, immediate consultation with a pediatric endocrinologist is necessary. For this reason, "a cut-off value for an immediate work-up" in case of extremely elevated 17-OHP in the first blood sampling and "a cut-off value for a post-retest work-up" in case of consistently elevated 17-OHP in the second blood sampling should be established.

As the current situation in Japan as of June 2019, an assay for 17-OHP level using ELISA with antibodies against the position 7 of the 17-OHP molecule was performed by 33 out of 36 laboratories, and using time-resolved fluorescent immunoassay kits was performed by 3 laboratories in the country. The first-tier screen was measured using a direct procedure in all laboratories. However, in the second-tier screen, only three laboratories adopted the direct procedure, and one of them used kits from two manufacturing companies. Of the remaining 33 laboratories, 28 used the extraction procedure and 5 used LC-MS/MS (confirmed in September 2021, https://www.jsms.gr.jp/contents03-05.html). It is necessary to understand how to determine 17-OHP levels and screen patients for 21-OHD in each region.

17-OHP levels are often falsely high in preterm and low-birth-weight neonates. One reason is that a large amount of undetermined steroids from the fetal cortex show a cross-reaction with the 17-OHP assay. Second, 17-OHP is high in preterm and low-birth-weight neonates because of the various stresses that they are subjected to (27–29, 31). For this reason, a cut-off value for gestational age has been established in Europe and the United States to reduce the recall rate and improve the positive predictive value (38–41). In Japan, a cut-off value for gestational age was established in Tokyo and Chiba to reduce the false-positive rate in preterm and low-birth-weight neonates (42–44). In Tokyo, the positive predictive value for 1989–2013 was 33.3% for neonates born after 37 weeks, but it was 2% for those born at less than 37 weeks and 25.8% overall (42, 43). However, similar efforts have not yet been made in other regions.

The 17-OHP cut-off level for each gestational age is used only in the Tokyo metropolitan and Chiba prefectures, and not nationwide. Thus, the current standard protocol of neonatal mass screening for low-birth-weight neonates is to perform blood resampling either 30 days after birth, when the weight reaches 2500

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g, or at discharge (45). For low-birth-weight neonates (in Japan, this is applied only to low-birth-weight neonates) with high 17-OHP at 4 to 6 days of life, it is often difficult to determine whether the neonate is affected at that time; therefore, it is important to follow up on the possibility that the neonate may have 21-OHD. Since birth weight ≤ 1500 g is often managed in the neonatal intensive care unit for a long period, blood sampling and examination may be required at the discretion of the attending physician while using the standard protocol for the second blood sampling as a principle. In addition, if a detailed examination is deemed necessary, it is important to promptly refer patients to specialists for further investigation.

In recent years, LC-MS/MS has enabled the accurate determination of steroid hormone concentrations. The use of LC-MS/MS as the second-tier test for mass screening has been reported to reduce the recall rate and increase the positive predictive value (33–35, 46). Neonatal mass screening with LC-MS/MS has also been introduced in Japan. Yamagishi *et al.* (47) measured five steroids (cortisol, 21-deoxycortisol (21-DOF), 11-DOF, androstenedione (AD), and 17-OHP) in filter paper blood by LC-MS/MS and set 17-OHP ≥ 50 ng/mL or gestational age ≥ 37 weeks and 17-OHP ≥ 20 ng/mL or 21-DOF ≥ 2 ng/mL as a cut-off value for an immediate work-up. 17-OHP ≥ 2.5 ng/mL and 11-DOF/17-OHP ≤ 0.2 and (17-OHP + AD)/cortisol ≥ 0.1 were set as cut-off values for resampling. As a result, the blood resampling rate was reduced to one-tenth of the previous rate (0.061%). By setting 17-OHP ≥ 2.5 ng/mL and 11-DOF/17-OHP ≤ 0.2 in particular, the false-positive rate can be kept low even if the cut-off for 17-OHP is lowered, thus allowing us to detect 21-OHD without overlooking the affected cases (47). Isobe *et al.* compared the rate of blood resampling using an immunoassay with an extraction procedure versus LC-MS/MS for a second-tier screen. Using LC-MS/MS as an indicator of 11-DOF/17-OHP reduced the rate of blood resampling to 0.15%, while it was 1.45% for the immunoassay with extraction (48).

It is technically feasible to extract DNA from filter paper blood and perform *CYP21A2* genetic analysis as a confirmatory test (49, 50) based on previous studies in Japan (51, 52). However, there are many difficulties in genetic testing as routine practice. No large studies have evaluated the usefulness of genetic testing in second-tier screening.

2. Diagnosis

Recommendations

1. If the 17-OHP level is above the cut-off value for an immediate work-up in neonatal mass screening, we recommend a detailed endocrinological examination of the neonate, regardless of the presence or absence of clinical symptoms, such as atypical external genitalia, hyperpigmentation of the skin, or symptoms or signs of adrenal insufficiency. 1 (Consensus)
2. If the 17-OHP level is above the cut-off value in the neonatal mass screening and remains above the cut-off value for the repeated test, we recommend endocrinological examination of the neonate in detail, regardless of the presence or absence of clinical symptoms such as atypical external genitalia, hyperpigmentation of the skin, or symptoms or signs of adrenal insufficiency. 1 (Consensus)
3. To diagnose 21-OHD, physicians should pay careful attention to symptoms of adrenal insufficiency, such as poor feeding, weight loss, and vomiting. When symptoms of adrenal insufficiency or biochemical abnormalities occur, including hyponatremia, hyperkalemia, or metabolic acidosis, glucocorticoid treatment should be initiated prior to confirmation of diagnosis of 21-OHD. 1 (Consensus)

Evidence

2-1. Clinical presentation

Nearly all cases of classic 21-OHD in children have been identified through neonatal mass screening in Japan (43, 53). When 17-OHP in neonatal mass screening is above the cut-off value for an immediate work-up, careful monitoring of the clinical manifestations and immediate examinations are required.

According to the pathophysiology, the clinical manifestations of 21-OHD can be categorized into three groups: 1) symptoms caused by adrenal insufficiency or salt-wasting, 2) virilization of the external genitalia due to excessive androgens in 46,XX cases, and 3) skin hyperpigmentation due to excessive ACTH. Adrenal insufficiency can be diagnosed based on general condition, including poor feeding. Body weight gain between 7 and 14 days of age is another informative finding for identifying salt-wasting (13). The severity of virilization of the external genitalia was assessed using the Prader classification (54). In most 46,XX cases of 21-OHD, serum 17-OHP levels were measured just after birth because of virilized external genitalia (13, 53). Approximately half of 46,XX cases have been reported to be detected earlier than screening (21, 22, 55). In other words, 17-OHP levels in all neonates with atypical external genitalia should be measured immediately after birth. Although some 46,XX cases exhibit complete male-type external genitalia, such cases can be differentiated from 46,XY cases by the absence of testes in the labioscrotal folds. The 46,XX cases have ovaries as the gonads, which are located in the abdominal cavity. The stretched penile length is not an appropriate index for identifying virilization of the external genitalia in 46,XY cases (21, 22). Skin hyperpigmentation can be recognized in the external genitalia, axilla, lip, and mouth and is the only manifestation of 46,XY cases. Skin hyperpigmentation rapidly disappeared after initiation of glucocorticoid therapy.

2-2. Biochemical, endocrinological, and imaging studies

There are four purposes for the detailed

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examination of neonates with elevated 17-OHP levels: first, the differentiation between false positives (normal) and affected individuals, and, in affected individuals, categorizing the severity of the disease, that is, non-classic (mild) and classic (severe); second, confirming 21-OHD by excluding other types of CAH in which 17-OHP levels can be elevated; third, the swift and appropriate detection of adrenal insufficiency or salt-wasting, which is mainly observed in the most severe forms; and fourth, the appropriate gender assignment of 46,XX cases with virilized external genitalia. Clinicians are required to evaluate clinical problems individually and weigh the priority of the problems to be examined.

1) Diagnostic tests

The most valuable diagnostic test is the measurement of serum 17-OHP levels (11, 56). The major concerns regarding 17-OHP levels are potentially elevated in other types of CAH compared to 21-OHD (11β-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase deficiency, and cytochrome P450 oxidoreductase deficiency) and falsely elevated because of cross-reactivity (57, 58). To properly diagnose 21-OHD, careful interpretation of the 17-OHP levels is required. Repeated 17-OHP and ACTH stimulation tests, if equivocal, will assist in the interpretation.

There are two major methodologies for 17-OHP measurement: immunoassays and mass spectrometry. The major concern in immunoassays is the cross-reactivity of other steroid compounds. Preterm neonates in whom the fetal cortex remains are likely to exhibit falsely elevated 17-OHP levels, leading to positive results in neonatal mass screening. In contrast, 17-OHP measurement using LC-MS/MS is highly specific and reliable (59, 60). Currently (April, 2022), medical insurance in Japan does not cover LC-MS/MS, and ELISA is widely used. 17-OHP levels should be interpreted according to the characteristics of each measurement method.

Other endocrinological tests that help diagnose 21-OHD include measurement of 21-DOF, urinary steroid profile, and ACTH stimulation test. 21-DOF is formed by 11-hydroxylase from 17-OHP without undergoing 21-hydroxylation. 21-DOF is a valuable indicator for the diagnosis of 21-OHD (61, 62). However, the measurement of 21-DOF is not covered by medical insurance in Japan. Currently, LC-MS/MS for the screening of 21-OHD is introduced in certain regions in Japan, and the LC-MS/MS panel includes measurements for 21-DOF, 11-DOF, AD, cortisol, and 17-OHP. The urinary steroid profile is the simultaneous measurement of multiple urinary steroid metabolites using gas chromatography-mass spectrometry-selected ion monitoring. Although not covered by medical insurance, the urinary steroid profile can differentiate 21-OHD from other disorders of CAH or false positives in preterm and term infants (63–65). Because sample collection is noninvasive, even for preterm infants, the urinary steroid profile is a useful test for neonates suspected of having 21-OHD. Serum-steroid ratios such as 17-OHP/11-DOF are potentially excellent indices for diagnosing 21-OHD. As described above, 11-DOF is included in the LC-MS/MS panel for neonatal mass screening. In regions where LC-MS/MS screening for 21-OHD is introduced, the serum-steroid ratio will assist in the diagnosis of 21-OHD (47). The ACTH stimulation test is classically established to distinguish 21-OHD from other CAH disorders, enabling a definite diagnosis. The ACTH stimulation test also indicates the severity of 21-OHD (classic, non-classic, false-positive) (11, 56). Generally, ACTH is administered intravenously at a pharmacological dose of 250 μg/m^2 (66), but in neonates and preterm infants, after 24–48 h from birth or later, intramuscular injection would be another option (11).

To distinguish 21-OHD from other types of CAH, multiple steroid measurements including cortisol, DOC, 11-DOF, 17-OH pregnenolone, 17-OHP, dehydroepiandrosterone (DHEA), and AD are necessary (66). However, many of these are not covered by medical insurance in Japan.

2) Examinations to evaluate the state of illness

a) Initial assessment of disorders of sex development (DSD).

The presence of atypical external genitalia indicates DSD. Although most cases of atypical genitalia with ambiguity of sex are 21-OHD, immediate examinations to obtain comprehensive clinical data for identifying the pathophysiology of DSD are essential. In addition to chromosomal analysis (G banding), physical examination assessing the virilization of the external genitalia and ultrasonography localizing the gonads and the size and shape of the internal genitalia should be performed. In particular, in 46,XX cases with 21-OHD or 11β-hydroxylase deficiency, the gonads differentiated normally, the ovaries remained in the abdominal cavity, and the testes descended into the scrotums. It should also be noted that complete male-type external genitalia can be observed in 46,XX cases, although the frequency is extremely low. Ultrasonography is a noninvasive test that helps determine the localization of the gonads and the anatomical evaluation of the internal genitalia.

b) Assessment of salt-wasting and dehydration

Careful examination of the general condition and state of illness, including the symptoms of adrenal insufficiency, is indispensable in infants with high 17-OHP levels. Severe salt-wasting, that is, Na < 130 mEq/L or K > 6.0 mEq/L, generally occurs during the second week of life (13). Specifically, these include measurements of plasma ACTH, serum electrolytes, plasma glucose, plasma aldosterone, plasma renin, and blood gas analyses (67). Aldosterone levels may be elevated in patients with poorly controlled or untreated diseases (67). These laboratory findings will be used as supporting data to diagnose 21-OHD and differentiate the disease form, for example, a salt-wasting or simple-virilizing form. If a case is strongly suspected of 21-OHD based on the clinical symptoms and usual laboratory findings and exhibits signs of adrenal insufficiency, glucocorticoid treatment should be initiated even before

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definitive diagnosis of 21-OHD (66). Serum and urine samples collected before treatment are helpful for further detailed endocrinological examinations that enable the diagnosis of 21-OHD.

3) Diagnosis of non-classic form

Non-classic 21-OHD is a milder form of 21-OHD that usually does not require intervention during the neonatal period, infancy, and childhood. Although the non-classic form is not the primary target of neonatal mass screening, a certain number of non-classic cases can be detected by screening. The prevalence of the non-classic form in Japan is estimated to be less than that in other countries, and the prevalence of the non-classic form discovered by neonatal mass screening is considered approximately 1 in 0.5 million births (68).

The non-classic form was originally defined by 46,XX cases without virilization of the external genitalia at birth and with an endocrinological abnormality of 21-OHD. In males, distinguishing the non-classic form from the simple-virilizing form is challenging, and it can be diagnosed by family history or the peak 17-OHP level after the ACTH stimulation test (56). The most prevalent genotype of the non-classic form in Japanese patients is p.Pro31Leu, and genetic testing assists in diagnosis (68, 69). Non-classic 21-OHD should be differentiated from polycystic ovary syndrome in adulthood. In a prospective study from Spain, 2.2% of adult females with hyperandrogenism had non-classic 21-OHD (70). In this study, a serum 17-OHP cut-off of 1.7 ng/mL provided 100% sensitivity for diagnosing non-classic 21-OHD.

4) Genetic testing

Genetic testing is not mandatory, as 21-OHD diagnosis is possible solely based on endocrinological data (11). Genetic testing would be useful to confirm the diagnosis of 21-OHD without a detailed endocrinological examination, distinguish between non-classic and classic forms in men, and provide information on genetic counseling (56). Although some cases have been reported to exhibit discrepancies between the genotype and phenotype, the extent of 21-OHD caused by pathological variants of *CYP21A2* is fairly correlated with clinical severity; thus, the genotype would enable the prediction of disease severity (19, 71).

CYP21A2 is tandemly surrounded by its pseudogene, *CYP21AP1*, and gene conversion and unequal crossover between the two genes frequently occur. Therefore, Sanger and next-generation sequencing methods cannot be applied, and methods of analysis that combine MLPA and nested PCR are widely used (72, 73). Because specific procedures are required, genetic analysis of *CYP21A2* is available in a limited number of laboratories. Because the interpretation of the results is extremely complex, a set of trio samples from the proband and their parents is desirable. Moreover, the percentage of pathological *de novo* variants is higher in this disease than in other autosomal recessive disorders (11).

5) Differential diagnosis

Other than 21-OHD, the serum 17-OHP level can be elevated in preterm birth, very early postnatal period, other types of CAH, adrenal tumors, and syndromes with overgrowth (27–29, 74, 75). In preterm neonates, the residual fetal cortex, in which 21-hydroxylase is expressed at a low level, leads to increased 17-OHP synthesis compared to that in full-term neonates. In addition, it is believed that some unidentified steroid metabolites from the fetal adrenal cortex cross-react with 17-OHP immunoassays (27–29, 76). This accounts for the majority of false positives in neonatal mass screening for 21-OHD, yielding an extremely low positive predictive value at <37 weeks gestational age (43). Serum 17-OHP levels are elevated transiently in other conditions, such as the early neonatal period during the first 48 h of life and physical stresses due to crucial illness and infectious diseases. False-positive cases due to cross-reactions of immunoassays can be reduced using LC-MS/MS (11, 47).

In three disorders of CAH other than 21-OHD, 11β-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase deficiency, and cytochrome P450 oxidoreductase deficiency, serum 17-OHP levels can be elevated. Although these disorders share common clinical features, such as glucocorticoid deficiency, their clinical features differ, and different therapeutic approaches are required. All three types of CAH, 46,XX cases with 11-OHD, and 46,XY and 46,XX cases with 3β-hydroxysteroid dehydrogenase deficiency or cytochrome P450 oxidoreductase deficiency, cause atypical external genitalia at birth. Although most cases with high 17-OHP levels are 21-OHD, a detailed investigation to confirm the diagnosis of 21-OHD is indispensable.

Generally, the Δ5/Δ4 steroid ratio (e.g., 17-hydroxypregnenolone/ 17-OHP) (77) in 3β-hydroxysteroid dehydrogenase deficiency, urinary steroid profile (64, 78) in cytochrome P450 oxidoreductase deficiency, serum levels of DOC and 11-DOF, and urinary steroid profile in 11β-hydroxylase deficiency are helpful in differential diagnosis. Multiplex steroid analysis using LC-MS/MS is also applicable for differential diagnoses.

Although its prevalence is extremely rare, syndromes with overgrowth or adrenal gland tumors can be detected by neonatal mass screening because of high 17-OHP levels (74, 75). A retrospective study of 0.3 million neonates who underwent screenings for 15 years reported that two of the nine full-term infants born with large-for-gestational-age who exhibited high 17-OHP levels were diagnosed with syndromes with overgrowth (one Beckwith-Wiedemann syndrome and one Perlman syndrome).

a) 3β-hydroxysteroid dehydrogenase deficiency (OMIM:201810)

This condition is caused by a deficiency in type II 3β-hydroxysteroid dehydrogenase (3β-HSD), which is expressed in the adrenal cortex and gonads. However, 3β-HSD deficiency is rare. A study from the UK estimated an incidence of 1 in 1 million, comprising approximately 1% of all CAH cases (79). In this disorder, the production of the Δ4-steroid 17-OHP is impaired and is predicted to be low in the serum; however, paradoxically high

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levels of 17-OHP have been documented, and in some 3β-HSD deficiency cases, the elevated 17-OHP level was comparable to that of the classic form of 21-OHD (57). The paradoxical elevation of circulating 17-OHP levels is probably due to peripheral conversion of high levels of accumulated Δ5-steroids by isoenzyme type I 3β-HSD in extra-adrenal tissues, including the liver and skin (57).

b) 11β-hydroxylase deficiency (OMIM:202010)

This disease is characterized by the activity of 11β-hydroxylase (P450c11) in the zona fasciculata, and the reticular layer of the adrenal cortex is impaired by a pathological variant of *CYP11B1*. The incidence of this disease is 1 in 1 million, accounting for approximately 1% of all CAH cases (80). P450c11 converts 11-DOF to cortisol and DOC to corticosterone. Unlike 21-OHD, two-thirds of individuals develop low-renin hypertension, possibly because DOC, which is excessively high in this disorder, has mineralocorticoidal effects. However, hypertension is not evident during the neonatal period and infancy, but is obvious during childhood.

c) Cytochrome P450 oxidoreductase deficiency (OMIM:201750)

This condition is caused by impaired cytochrome P450 oxidoreductase (POR) activity due to pathogenic variants of the *POR* gene. Since POR acts as a coenzyme for various microsomal enzymes, the activity of enzymes such as 17α-hydroxylase (P450c17), P450c21, and aromatase (P450arom) is affected by POR deficiency (58). Although the exact prevalence is unknown, it has been speculated to account for 2%–3% of CAH cases (81). It may be accompanied by skeletal malformations, such as craniosynostosis, central facial hypoplasia, radio-humeral fusion, arthrogryposis, and elongated fingers, also known as Antley-Bixler syndrome (58).

3. Initial Treatment for Neonates and Maintenance Therapy for Pediatric Patients with Classic Form (Table 1)

Recommendations

Glucocorticoids:

1. We suggest that high-dose glucocorticoid administration as an initial treatment for classic 21-OHD during the neonatal period can rapidly suppress excessive adrenal androgen production. 2 (●○○)
2. We recommend the use of hydrocortisone (HC) for maintenance therapy in pediatric patients with classic 21-OHD. 1 (●●○)
3. We recommend against the use of long-acting glucocorticoids for maintenance therapy in pediatric patients with classic 21-OHD. 1(●●○)
4. We recommend that the dosage of glucocorticoids during maintenance therapy be carefully individualized to avoid undertreatment or overtreatment. 1 (Consensus)

Mineralocorticoids:

5. We recommend the administration of fludrocortisone (FC) and sodium chloride to neonates, infants, and children with the salt-wasting form of 21-OHD. 1 (●●●)

Evidence

3-1. Treatment principles

The principles of treatment for 21-OHD are to supplement insufficient glucocorticoids and mineralocorticoids, suppress excessive adrenal androgen production, and ensure appropriate growth and maturation comparable to that of healthy children. Lifelong treatment is required to maintain a stable condition. Insufficient treatment causes adrenal crisis because of decreased tolerance to physical stress and reduced adult height due to bone age advancement. Overtreatment with glucocorticoids causes iatrogenic Cushing's, including growth impairment, obesity, and hypertension. Even if we could supplement HC physiologically, this would not be able to suppress ACTH completely, consequently leading to increased adrenal androgen levels due to the blockage of 21-hydroxylation. This means that an ideal physiological HC dosage, if possible, cannot perfect disease control. Therefore, pediatric endocrinologists should manage infants and children with 21-OHD.

3-2. Glucocorticoids: initial treatment in neonates and infants

In classic 21-OHD, it is important to maintain a balance between hyperandrogenism and hypercortisolism (11). The previous Japanese guidelines for treatment recommend initial treatment with high-dose HC (100–200 mg/m^2/d) to suppress excessive adrenal androgen production in the neonatal period (7). In contrast, the European and American guidelines set the dosage of initial treatment to a maximum of 25 mg/m^2/d, typically as low as 10–15 mg/m^2/d, and recommend rapidly reducing HC dosages when adrenal androgen levels reach the target range (8, 11). Because attempts to completely normalize 17-OHP levels typically result in overtreatment with features of Cushing's syndrome, frequent medical assessments, such as blood tests, are recommended (11). 17-OHP levels remain high until 3 months after birth in females and 6 months after birth in males treated with the initial HC dosage in Europe and the US, suggesting insufficient suppression of adrenal androgens (82). At 3 years of age, however, the height SDS of patients who were treated with initial low-dose HC therapy approached the genetic height potential without bone age advancement (82). Therefore, there is no clear evidence that initial low-dose HC therapy confers a disadvantage, such as bone age advancement, due to insufficient suppression of adrenal androgens.

In children with classic 21-OHD, height SDS decreases from birth to 1–2 years old, and height SDS at 2–3 years of age is significantly correlated with adult height SDS (83–86). The decrease in height SDS during the first two years was also significantly correlated with the dosage of glucocorticoids (83–86).

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Table 1. Suggested dosages of glucocorticoid and mineralocorticoid for initial treatment and maintenance therapy

	Stage	Hydrocortisone (HC) (mg/m^2/day, 3 times day)	Fludrocortisone (FC) * (mg/day, twice a day)	Sodium chloride (NaCl)* (g/kg/day, 3–8 times a day)
Initial treatment	Neonate	25–100 **	0.025–0.2	0.1–0.2
Maintenance therapy	Neonate Infant	10–20	0.025–0.2	0.1–0.2
	Toddler Preschooler School child Adolescent	10–15	0.025–0.2 ****	
	Adult	10–15 ***	0.025–0.2 ****	

* FC and NaCl are almost always required for classic salt-wasting 21-OHD. The dosages of FC and NaCl are chosen based on serum sodium and potassium levels, plasma renin activity or active renin concentration, body weight gain, and blood pressure. ** The dosage is managed based on the severity of clinical symptoms or signs. If adrenal crisis is suspected, bolus intravenous injection of HC (25–50 mg/m^2) should be performed immediately. The maintenance dosage of HC (10–20 mg/m^2/d) can be acceptable for initial treatment unless patients show salt-wasting or failure to thrive. *** Adult patients may be given prednisolone or dexamethasone (see section 5. Treatment and Monitoring for Adult Patients with Classic Form). **** The required dosage may decrease with age, and FC may be discontinued.

Adult height can be compromised with HC dosages >20 mg/m^2/d during infancy (11). Therefore, after the initial dose, endocrinological and electrolyte balances should be carefully evaluated and the appropriate HC dosage should be adjusted for each individual (87).

In contrast, in studies using initial treatment with low-dose HC (9–15 mg/m^2/d) [8] and high-dose HC in accordance with the Japanese treatment guidelines (88, 89), the height SDS at 1 year of age corresponded to -1 SD. There was also no significant difference in height SDS at 1, 2, and 3 years of age between groups initially treated with > 150 and 100 mg/m^2/d of HC (90). Therefore, the relationship between decreased height SDS early after birth and the dosage of glucocorticoids in the initial treatment is uncertain, and there is no clear evidence that initial treatment with high-dose HC worsens the prognosis for height. Height SDS at the age of 1 year or over could be affected by factors other than the HC dosage during the neonatal period. All studies regarding initial HC dosages were retrospective; thus, prospective studies are required in the future.

There is no clear evidence for the optimal dosage of glucocorticoids in the initial treatment. Following the 2014 version of the guidelines (10), the recommended initial dosage of HC in the neonatal period is 25–100 mg/m^2/d (**Table 1**). In cases of probable or suspected adrenal crisis, 100 mg/m^2/d of HC should be initiated immediately after parenteral bolus administration of HC. If adrenal crisis is not suspected, lower initial dosages, as described in European and American guidelines, are acceptable. In the absence of signs of adrenal insufficiency, clinical manifestations and biochemical data should be evaluated carefully to consider the need for treatment, as this may be a non-classic 21-OHD. Once adrenal androgen production is suppressed or body weight gain is expected after treatment begins, the dosage of HC is promptly reduced every 5–7 days and shifted to maintenance therapy by 3–4 weeks of age. The dosages and modes of administration should be adjusted individually based on the patient's condition and clinical experience.

3-3. Glucocorticoid: maintenance therapy in pediatric patients

HC is used for glucocorticoid maintenance therapy in pediatric patients. Owing to its short half-life, HC has been reported to be associated with a lower risk of adverse effects caused by more potent long-acting glucocorticoids, including growth impairment, obesity, and reduced bone mineral density (91). Prednisolone and dexamethasone are reported to exhibit growth suppression effects in 15 fold (92) and 70–80 fold (93), respectively, compared to HC. Thus, long-acting glucocorticoids should not be used as maintenance therapy in pediatric patients.

HC is usually administered three times per day. No clear benefit has been identified with increasing morning or evening doses (94). Physiological cortisol production is estimated to be 5–6 mg/m^2/d in HC-equivalents (95–97). Adult height is reduced when the dosage exceeds 20 mg/m^2/d during infancy and 15–17 mg/m^2/d during adolescence (83–85, 98, 99). Height has been reported to increase at doses < 20 mg/m^2/d at the onset of puberty (100). Negative correlations were reported between adult height and glucocorticoid dosage during early puberty (84–86, 101). In contrast, in a meta-analysis of adult height in 21-OHD, no significant correlation was found between adult height SDS adjusted for parental height and the total dosage of glucocorticoid (102). Although the correlation between glucocorticoid dosage in maintenance therapy and height prognosis is uncertain, it is reasonable to treat children in the prepubertal stage with as low a dose as possible to avoid bone age advancement.

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The appropriate dosage for maintenance therapy differs among individuals, for unknown reasons. In addition, there are few long-term studies regarding the safety and efficacy of glucocorticoid treatment (HC, prednisolone, and dexamethasone), and no conclusion has been reached (103). The appropriate dosage for maintenance therapy fluctuates with growth rate. In the pubertal stage, higher doses (up to 17 mg/m^2/d) and more frequent dosing of HC may be required owing to enhanced cortisol clearance (104). Although some patients who reach adult height are controlled well for a short to medium period with the administration of intermediate- and long-acting glucocorticoids such as prednisolone and dexamethasone, it is necessary to be cautious that the use of these synthetic steroids is associated with adverse effects, such as bone density reduction and obesity (91, 105, 106).

As described above, undertreatment leads to adrenal insufficiency and reduced adult height due to premature induction of puberty, while overtreatment leads to obesity and Cushing's syndrome and inhibits growth. Therefore, it is crucial to control the dosage balance during the treatment of patients, and practical administration should be individually dependent on the patient's condition and age.

3-4. Mineralocorticoid

Patients with classic salt-wasting 21-OHD are not treated sufficiently with HC alone and require FC (7, 10, 11, 107, 108). Owing to insufficient sodium intake from breast milk and bottle formula for treatment, sodium chloride replacement is necessary in neonates and infants with the salt-wasting form (7, 8, 10, 11). Aldosterone deficiency is clinically evident in the salt-wasting form and may occur sub-clinically in the simple-virilizing form (107). Maintenance of an adequate sodium balance can decrease vasopressin and ACTH levels, reduce the dosage of HCs, and improve adult height (109). In a meta-analysis, adult height SDS adjusted for parental height was significantly higher in the group with FC treatment than in the group without FC (110).

The recommended dosage of FC followed the guidelines of the 2014 revision (10) (**Table 1**). In European and American guidelines, the administration of FC is recommended in all patients with classic 21-OHD (7, 8, 11); however, there is no clear evidence for the benefit of FC in all patients. Even if FC is not administered earlier, patients with poor body weight gain, high plasma renin activity or concentration, and electrolyte imbalance (hyponatremia and hyperkalemia) should be diagnosed with salt-wasting 21-OHD and treated with FC. If glucocorticoid treatment starts at a high dose (100 mg/m^2/d HC), mineralocorticoid deficiency sometimes appears when the dosage of glucocorticoids is reduced for replacement with maintenance therapy (7).

During the neonatal and early infantile periods, relative mineralocorticoid resistance occurs because of immature tubular reabsorption of sodium; thus, administration of sodium chloride in addition to FC is required for salt-wasting forms (10, 11, 111, 112). Sodium chloride replacement is generally not required beyond infancy, because sensitivity to FC increases with age. Although it is reported that a high-dose FC does not require sodium chloride supplementation (113), hypertension due to excessive FC has been observed after infancy (114, 115). It is important that the practical administration of FC and sodium chloride be individually dependent on the patient's condition and age.

4. Monitoring for Pediatric Patients with Classic Form

Recommendations

1. We recommend comprehensive monitoring of treatment in pediatric patients with 21-OHD using growth velocity, pubertal stage, and bone advancement, in addition to endocrinological data. 1 (Consensus)
2. We recommend regular assessment of height, weight, and blood pressure in pediatric patients of all ages and bone age after 2 years of age. 1 (Consensus)
3. We suggest consistently timed endocrinological data such as just before taking glucocorticoids early in the morning for monitoring therapy. 2 (●○○)
4. We recommend adjusting the dosage of glucocorticoids to avoid adrenal insufficiency and adverse events due to iatrogenic Cushing's syndrome. 1 (Consensus)
5. We recommend adjusting the dosage of mineralocorticoids to avoid salt-wasting episodes and adverse events due to excess mineralocorticoids, such as hypertension. 1 (●●○)
6. We suggest regularly screening testicular adrenal rest tumor (TART) by ultrasonography in males after 10 years of age. 2 (●●○)

Evidence

Proper monitoring of treatment and adjustment of medications for patients with 21-OHD is difficult (7, 8, 10, 11, 116). These guidelines also recommend comprehensive monitoring of treatment in pediatric patients with 21-OHD using growth velocity, bone age advancement, and endocrinological data based on the 2014 version of the guidelines (10).

Overtreatment with glucocorticoids causes a reduction in growth velocity and obesity, whereas undertreatment with glucocorticoids causes adrenal insufficiency, accelerated growth velocity, and bone age advancement. Insufficient dosage of mineralocorticoids causes salt-wasting episodes and failure to thrive. To avoid these adverse events, we should regularly assess growth parameters such as height, weight, bone age, and sexual maturation. We should also regularly evaluate the degree of obesity and height-age adjusted BMI. In children with 21-OHD, obesity can become more apparent, and adiposity rebound is observed earlier than in healthy children, regardless of HC or FC dosing

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(117–119). Bone age should be assessed in patients after 2 years of age because even in untreated patients with classic 21-OHD, the bone age does not advance until 1–1.5 years of age (120). Annual evaluation is usually appropriate (7, 10, 11); however, bone age should be examined twice per year when abnormal changes in growth velocity or pubertal maturation are observed. Age-appropriate growth and maturation indicate long-term optimal therapy.

Endocrinological data should be assessed using a highly accurate assay and evaluated based on the appropriate reference values. The levels of adrenocortical steroid hormones may differ according to the method used. Therefore, immunoassays with organic solvent extraction or LC-MS/MS should be used. One of the suitable biochemical markers for monitoring glucocorticoid treatment is serum 17-OHP level (7, 10, 11, 121, 122). Serum AD and testosterone levels can be measured to monitor therapy in prepubertal males and females and in pubertal females (7, 10–12, 123). However, AD measurement is not covered by medical insurance in Japan, and standard reference ranges for sex, age, and testosterone have not been established. When LC-MS/MS becomes widely available, intermediate metabolites such as serum 21-DOF and 11-oxygenated androgens are promising candidates as indicators of treatment status (11, 124).

Plasma ACTH has a robust circadian rhythm and is not useful for therapy monitoring. Serum 17-OHP also exhibits a circadian rhythm and daily variance. It is preferable to consistently measure serum 17-OHP before taking glucocorticoids early in the morning (10–12, 121). The target range of serum 17-OHP levels just before taking the morning dose was 4–12 ng/mL in both childhood and adulthood (12, 122) and < 5.9 ng/mL during puberty (82). However, these target ranges were not derived from studies that have investigated auxological data. Normalization of 17-OHP suggests overtreatment with glucocorticoids. A urinary steroid profile is a useful indicator of treatment status (11, 125). In particular, the measurement of pregnanetriol (PT), a urine metabolite of 17-OHP, has been proposed for therapy monitoring (7, 10, 126–128). In Japanese children with 21-OHD excluding neonatal and pubertal patients, the optimal range of urine PT was 1.2–2.1 mg/m^2/d and 2.2–3.3 mg/gCr in the first morning sample based on auxological data (127, 128). Measuring the first morning urine PT value would be more practical and useful for monitoring 21-OHD biochemically because the first morning PT correlated with 17-OHP before taking the morning dose of glucocorticoid (129). In addition to PT, total androgen and AD metabolites are also useful as indicators of treatment status (130). However, in Japan, blood sampling before taking medication early in the morning is difficult. In poorly controlled patients, serum 17-OHP levels may be measured before glucocorticoids and urine PT are administered. Most endocrinological data are short-term indicators and could be different at the time of sampling and under different sampling conditions. Therefore, the dosages of medications should be adjusted by comprehensively considering the total endocrinological data measured repeatedly.

Indicators for mineralocorticoid treatment include blood pressure and serum electrolyte and plasma renin levels. Because excessive FC increases systolic blood pressure, even in infancy (130–133), regular measurement of blood pressure is recommended. Plasma renin levels were monitored considering age-specific reference ranges. Plasma renin is not always a reliable indicator in neonates and infants because it shows a physiologically high renin activity or concentration. Low plasma renin levels suggest excessive FC or NaCl (114). Plasma renin levels should not be suppressed but should be around the upper limit or mildly high (112, 134). Hypertension and lower limb edema were observed in 10 of 134 infants having the medication of FC, in 7 of whom, the dosage of FC was 0.025–0.05 mg/d (135). Some cases required FC administration during the neonatal and early infantile periods because of the immaturity of the reabsorption of sodium in renal tubules, and subsequently reduced the FC dosage (11). Thus, sensitivity to FC could differ among individuals and change with age. Therefore, it is necessary to assess the FC dosage repeatedly, even after early childhood.

The prevalence of TART has been reported to increase after 10 years of age and to be 20%–30% under 18 years of age (136–138). Regular screening TART by testicular ultrasonograms should be considered every 1–2 years in children at and older than the age of 10 years (11).

5. Treatment and Monitoring for Adult Patients with Classic Form

Recommendations

1. Patients with classic 21-OHD receive maintenance therapy depending on their individual glucocorticoid deficiency status. We suggest that HC or prednisolone as a glucocorticoid replacement therapy be preferred over dexamethasone from the viewpoint of obesity and insulin resistance. 2 (●●○)
2. We recommend regular monitoring, such as physical and endocrinological examinations, when glucocorticoid is prescribed alone or in combination with mineralocorticoids. 1(●●○)
3. Glucocorticoid overtreatment (iatrogenic Cushing's syndrome) should be avoided because overtreatment (especially with dexamethasone) causes decreased bone mineral density, obesity, increased insulin resistance, and compromised QOL. 1 (●●○)
4. We suggest that serum 17-OHP level before taking glucocorticoid in the early morning is a useful marker of treatment monitoring. 2 (●○○)
5. We recommend glucocorticoid preparation with low placental transfer for maintenance therapy in adult females who wish to conceive. 1(●○○)
6. We recommend a planned, systematic transition

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medicine from pediatric to adult health care service. 1 (Consensus)

Evidence

The therapeutic goals for adult patients with classic 21-OHD are to prevent adrenal insufficiency and control adrenal hyperandrogenism. Since there are no evidence-based data on replacement regimens for patients with 21-OHD, it is necessary to determine the dosages of glucocorticoid and mineralocorticoid supplementation for each patient. In childhood, HC is selected because of the risk of impaired growth, whereas in adulthood, prednisolone and dexamethasone are options for controlling adrenal hyperandrogenism (**Table 2**) (11, 139).

In the United Kingdom Congenital Adrenal Hyperplasia Adult Study Executive (CaHASE), which targeted adults with 21-OHD in the UK, dexamethasone reduced adrenal androgens and ACTH more than HC and prednisolone, but increased insulin resistance (140). Moreover, deterioration in QOL has been associated with obesity, insulin resistance, and the use of prednisolone or dexamethasone. In a meta-analysis of 19 studies, dexamethasone significantly suppressed adrenal androgen production to a greater extent than HC and prednisolone; patients taking dexamethasone also had a significantly higher BMI and significantly lower bone mineral density (87). Subjects treated with dexamethasone had significantly higher HC equivalent doses than those treated with prednisolone or HC. In contrast, there were no differences in adrenal androgen production, BMI, or bone mineral density between the HC and prednisolone groups. In the previous study investigating metabolic changes in 60 adults with good control on HC that were later changed to dexamethasone (dexamethasone treatment period 11.5 ± 4.9 years), waist/height ratio, which is an index of metabolic syndrome, and HOMA-IR, which is an index of insulin resistance, were significantly elevated, but the frequency of metabolic syndrome and hypertension did not differ before and after the change (141). To date, no randomized controlled trials on the long-term course of classic-form patients treated with HC, prednisolone, or dexamethasone have been reported (11). However, as dexamethasone has been reported to have adverse metabolic and QOL effects, HC or prednisolone is recommended as a first-line glucocorticoid replacement. If dexamethasone is used, it should be initiated at a lower dosage.

Symptoms or signs of glucocorticoid overtreatment (clinical features of Cushing's syndrome), such as skin thinning, moon facies, central obesity, osteoporosis, insulin resistance, impaired glucose tolerance, and adrenal hyperandrogenism, including hirsutism, amenorrhea, and infertility, have been evaluated to optimize treatment (142). In particular, it should be noted that complete normalization of 17-OHP indicates glucocorticoid overtreatment (11). Hypertension is an important phenotype of iatrogenic Cushing's syndrome (143). Therefore, regular monitoring of the blood pressure is essential. Excess adrenal androgen causes irregular menstruation in adult females and impairs spermatogenesis via gonadotropin suppression in adult males, both of which can result in infertility. Menstrual history and semen analysis should also be monitored regularly.

Recently, new delivery techniques for glucocorticoids have been investigated and developed: two-phase release oral tablets of HC and continuous subcutaneous infusion-pump therapy of HC for more physiologic replacement, in addition to selective corticotropin-releasing hormone type 1 receptor antagonists and melanocortin type 2 receptor antagonists to control adrenal androgen overload (144).

If adult females with classic 21-OHD wish to conceive, adrenal-derived progesterone should be suppressed (target <0.6 ng/mL). Dexamethasone is contraindicated during pregnancy because it is not metabolized in the placenta and passes through the fetus. Thus, HC, prednisolone, or both in fractions 2–3 were administered during pregnancy. In contrast, for adult males with classic 21-OHD who develop TART, intensification of glucocorticoid therapy (such as dexamethasone twice a day) is required to suppress ACTH and reduce TART (144).

The optimal FC dosage for classic 21-OHD during adulthood has not yet been reported. The requirement for mineralocorticoid replacement decreases with increasing age. In nonhypertensive adults with classic 21-OHD, continued FC treatment is desirable. However, the need for mineralocorticoid replacement should be re-evaluated during transitional care (11).

21-OHD is a target disease for transition medicine

Table 2. Maintenance therapy for adult patients with 21-hydroxylase deficiency (11)

	Suggested dosage (mg/day)	Number of doses per day
Glucocorticoid		
Hydrocortisone	15–25	2–3
Prednisolone	4–6	1–2
Dexamethasone	0.25–0.5	1
Mineralocorticoid		
Fludrocortisone	0.05–0.2	1–2

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from pediatric to adult healthcare. It is necessary to provide appropriate medical care according to age-related changes in the pathological condition, complications in individual patients, and physical and psychological maturation. In addition, consideration should be given to appropriately shifting the decision maker in medical management from the caregiver to the patient him/herself. Therefore, it is recommended to consider planned transition medicine for adult healthcare (145). After the transition to adult health care, there are many life events, such as going on to college, getting a job, getting a partner, and raising a child. In particular, when wishing to conceive, it is recommended to provide appropriate genetic counseling on recurrence risk for 21-OHD in the next generation, which can be a source of anxiety for patients, and the neonatal mass screening system in Japan.

6. Stress Dosing during Maintenance Therapy

Recommendations

1. We recommend increasing the dosage of glucocorticoids for patients with 21-OHD in cases of substantial physical stress, such as febrile illness (>38.5 °C), gastroenteritis, surgery under general anesthesia, and major trauma. 1 (●●○)
2. We recommend patients with 21-OHD to always wear or carry a medical identification card indicating adrenal insufficiency. 1 (●○○)
3. We suggest that an increased dosage of glucocorticoids is not always necessary for patients with 21-OHD in cases of mental and emotional stress, minor illness, or light exercise. 2 (●○○)
4. We suggest prescribing a glucocorticoid self-injection kit for emergency use and providing patients with 21-OHD or their parents (or guardians) with instructions for self-injection. 2 (●○○)

Evidence

Patients with 21-OHD cannot produce adequate cortisol in response to physical stress and are at a risk of adrenal crisis. Adrenal crisis is more frequent in patients younger than 10 years, especially in those younger than 1 year. Gastroenteritis is a common precipitating factors (146, 147). Therefore, it is necessary to temporarily increase the dosage of glucocorticoids in situations of substantial physical stress, such as febrile illness, gastroenteritis, surgery under general anesthesia, or major trauma. Insufficient glucocorticoid dosage during physical stress poses a high risk of mortality due to adrenal crisis, and adequate glucocorticoid escalation to match the severity of stress is required (148). When a stress dose of HC is administered, FC administration is unnecessary because abundant HC can exert mineralocorticoid activity. Patients should resume maintenance therapy promptly when the physical stress has subsided. Owing to the risk of hypoglycemia and electrolyte imbalances, younger children should avoid prolonged starvation and be given intravenous glucose and sodium as needed (147, 149, 150). Patients should constantly wear or carry a medical identification card indicating adrenal insufficiency to receive prompt and appropriate treatment for adrenal crises. Physicians must consider prescribing a glucocorticoid (hydrocortisone sodium succinate) kit, which has been covered by the medical insurance in Japan since 2020, and provide guidance on the emergency use of this kit to patients and their parents. It is important to instruct patients and their parents on the aforementioned sick day rules and HC self-injection regularly and repeatedly (151).

It has been reported that increasing the dosage of glucocorticoids for light exercise and psychological stress (e.g., anxiety and entrance examinations) is not always necessary (152, 153). However, some reports recommended that the dosage of glucocorticoids should be increased for strenuous exercise, such as a marathon or triathlon (154). We should assess the degree of physical stress and the necessity of increasing the dosage of glucocorticoids in each case.

There is an ongoing debate regarding the types of physical stress for which the glucocorticoid dosage should be increased, the actual dose, and the route of administration. The updated guidelines follow the 2014 version of the guidelines and exemplify the types of physical stress and recommended HC dosages (**Table 3**). Because the proposed situation and dosage were set empirically, the dosage should be adjusted individually for each patient. Large-scale clinical studies of stress dosing during physical activity are warranted.

7. Treatment for Pediatric Patients with Non-classic Form

Recommendations

1. We suggest that maintenance therapy in non-classic 21-OHD be initiated when the symptoms of adrenal hyperandrogenism, such as increase in growth velocity, advancement of bone age, virilization of female external genitalia, and irregular menstruation, are present. 2 (●○○)
2. We recommend against maintenance therapy for asymptomatic patients with non-classic 21-OHD. 1 (●○○)
3. We recommend that a stress dose of glucocorticoid be administered for non-classic 21-OHD during glucocorticoid maintenance therapy or in which cortisol levels fall below the borderline (15–18 mg/dL) after the ACTH stimulation test in the following situations: febrile illness (38.5 °C), gastroenteritis with possible dehydration, surgery under general anesthesia, and major trauma. 1 (●○○)

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Table 3. Stress dosing

Degree of physical stress	Situations	Suggested dosage of hydrocortisone (HC)
Mild	Vaccination, upper respiratory infection up to low-grade fever	Maintenance dose
Moderate *	Infection with high fever (> 38.5°C) Gastroenteritis, minor surgery, trauma, burn, dental treatment	3- to 4-fold maintenance dose or 50–100 mg/m^2/day **
Severe *	Sepsis, major surgery	100 mg/m^2/day **

* In cases of suspected adrenal crisis, surgery under general anesthesia, or difficulty in taking the stress dose of HC orally, a parenteral bolus administration of HC 50 mg/m^2 (infant: 25 mg, child: 50 mg; adult: 100 mg) is recommended. If an intravenous line is difficult to place, hydrocortisone sodium succinate can be intramuscularly injected (hydrocortisone sodium phosphate can be administered only by intravenous injection in Japan). ** For severe physical stress, after first bolus injection, continuous infusion is preferable to intermittent injections at 6-h intervals (184, 219).

Evidence

In non-classic 21-OHD patients with endocrinological abnormalities but without any symptoms or signs of glucocorticoid or mineralocorticoid deficiency, it is necessary to periodically evaluate the physical examination, height, weight, and bone age to determine the indication for glucocorticoid or mineralocorticoid replacement (7, 11, 68). No large studies have shown the benefits of treatment for the subclinical non-classic form; therefore, we do not recommend treatment of the non-classic form similar to the classic form. For children, when height growth and bone age are accelerated and when adult females have symptoms of adrenal hyperandrogenism, such as virilization, maintenance therapy should be initiated (155). The age at onset of non-classic 21-OHD when diagnosed in childhood in Japan was between 2 and 8 years, and it manifested as advancement of bone age or virilization of the external genitalia in females (68). No premature pubarche, hirsutism, acne, or menstrual irregularities during childhood have been reported for the non-classic form in Japan. There have been a few cases of adrenal crisis under physical stress after the initiation of glucocorticoid replacement therapy, suggesting the importance of stress dosing during maintenance therapy (68, 156). In both the classic and non-classic forms, it is necessary to determine an appropriate dosage for each case so that the progression of height growth and bone age is equivalent to that in the reference age and sex.

8. Treatment for Adult Patients with Non-classic Form

Recommendations

1. We suggest that non-classic 21-OHD patients who have been treated with glucocorticoids since childhood should be considered for treatment discontinuation when adult height is reached or when symptoms disappear. 2 (●○○)
2. We suggest glucocorticoid treatment for adult females with non-classic 21-OHD presenting with hirsutism*1 or infertility due to hyperandrogenism*2. 2 (●○○)
3. We recommend glucocorticoid preparation with low placental transfer in adult females with non-classic 21-OHD who wish to conceive*3. 1 (●○○)
4. We recommend against the use of glucocorticoid therapy in non-pregnant and asymptomatic adult females with non-classic 21-OHD who do not wish to conceive. 1 (●●○)
5. We suggest against the use of glucocorticoid therapy in males with non-classic 21-OHD. 2 (●○○)
6. We recommend that a stress dose of glucocorticoid be administered to patients with non-classic 21-OHD during glucocorticoid maintenance therapy or whose serum cortisol levels after the ACTH stimulation test fall below the borderline range (15–18 mg/dL) in the following situations: febrile illness (>38.5°C), gastroenteritis with possible dehydration, surgery under general anesthesia, major trauma, or childbirth. 1 (●○○)

*1: In cases at high risk of hirsutism with a family history, we should examine 17-OHP to screen for non-classic 21-OHD.
*2: Treatment is recommended for premenopausal adult females who consider hirsutism cosmetically important. However, treatment has not been proposed for mild cases or for cases without endocrine abnormalities.
*3: The type and dosage of glucocorticoids administered during pregnancy are similar to those in the classic form. However, given the possibility of glucocorticoid-related complications, the minimum amount needed should be considered.

Evidence

Although there is little evidence for the treatment of non-classic 21-OHD in adulthood, continuation, initiation, or cessation of glucocorticoid therapy should be considered individually. In patients who have been treated with glucocorticoids since childhood,

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discontinuation of the treatment at puberty can be considered when adult height is reached or when symptoms or signs of adrenal insufficiency are no longer apparent (11). Glucocorticoid therapy is generally not needed in adult men with the non-classic form, with no TART or compromised fertility.

Glucocorticoid therapy is necessary for adult females with non-classic 21-OHD with hirsutism, infertility, or abortion due to androgen excess. Although the spontaneous pregnancy rate for females with non-classic 21-OHD does not decrease, infertility (mainly due to anovulation) is observed in 10%–30% of those female of reproductive age, and glucocorticoid therapy improves the fertility rate (155, 157). Eyal *et al.* (158) retrospectively analyzed 75 traceable patients with non-classic 21-OHD who wished to conceive. Pregnancy and normal birth rates of the patients were similar to those of the general population. Glucocorticoid therapy did not improve the rate of miscarriages. However, the period from initiation of glucocorticoid administration to conception was significantly shorter than that from the conception trial to initiation of glucocorticoid administration. Therefore, glucocorticoid treatment should be considered for adult females with non-classic 21-OHD with hirsutism who have no spontaneous pregnancy and overt or subclinical ovulation disorders.

Although there is no good quality of evidence, we suggest glucocorticoid treatment for adult females with non-classic 21-OHD experiencing infertility or hyperandrogenic symptoms. Adult females with non-classic 21-OHD who present with infertility and a history of miscarriage should use glucocorticoids that do not traverse the placenta. Replacement therapy during pregnancy should be performed according to the classic form. In the event of major surgical procedures, trauma, or delivery, a stress dose of glucocorticoids should be administered to patients with ACTH-stimulated serum cortisol levels below the borderline range (15–18 mg/dL). For adult females with non-classic 21-OHD who might have psychological and psychiatric problems (elevated anxiety score or subjective feeling of poor health), adequate psychological diagnoses and support are necessary (159).

In principle, glucocorticoids should not be administered to non-pregnant and asymptomatic adult females (160). Glucocorticoid administration for symptomatic non-classic 21-OHD improves symptoms, but the minimum dosage should be used because of concerns regarding glucocorticoid-related complications (e.g., adrenal crisis, cardiovascular events, metabolic disorders, and osteoporosis) (133, 161). However, because some drug-related complications are similar to comorbidities of the non-classic form, they should be interpreted with caution.

9. Prenatal Diagnosis and Treatment

Recommendations

1. Prenatal diagnosis and treatment are not yet well-established. 1 (●○○)
2. We recommend that prenatal diagnosis and treatment be performed at institutions with experts after genetic counseling and approval from the institutional ethics committee. 1 (●○○)
3. We suggest that prenatal diagnosis and treatment should not be performed in institutions with no long-term follow-up system for safety or ethical approval of clinical studies. 2 (●○○)

Evidence

Maternal administration of dexamethasone has been shown to suppress the production of excessive adrenal androgens in fetuses with 21-OHD through the placenta (162–164). The purpose of prenatal treatment is to reduce virilization of the external genitalia in affected females, avoid surgery, and reduce the social and psychological burden on patients and their families (162–165). However, the optimal dosage and duration of dexamethasone administration have not yet been established (162, 165). In addition, prenatal treatment does not completely cure the disease, requires regular follow-up and lifelong treatment, and does not eliminate the risk of adrenal crises.

In a meta-analysis on prenatal diagnosis and treatment of 21-OHD, 323 pregnancies in four studies were examined for therapeutic efficacy and fetal and maternal side effects (165). As a result, it was effective for the control of virilization of female external genitalia with no side effects on the fetuses and mothers, except for a significant increase in edema and skin striation in mothers. However, the limited literature and evidence on this topic require further investigation.

Virilization of the external genitalia in female fetuses with 21-OHD occurs 6 weeks after conception. Prenatal treatment must be initiated as early as possible when pregnancy is noticed and at the latest by 8 weeks when the external genitalia are fully formed (166). However, genetic diagnosis using normal villous puncture cannot be performed until approximately 10 weeks. Dexamethasone must be administered to the mother in all pregnancies at risk, although only the affected females need treatment. Therefore, only one-eighth of all pregnancies require treatment. Dexamethasone will be administered in seven of eight pregnancies that do not require treatment. In an attempt to determine fetal sex earlier and shorten dexamethasone treatment in males, there have been reports applying methods for determining the fetal *SRY* gene from maternal blood at 6 weeks of gestation (167–169) and *CYP21A2* genetic analysis of fetal cell-free DNAs in maternal blood by 8 weeks (170, 171). There are inconsistent results regarding development, psychological prognosis,

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and impact on advanced brain function in prenatally treated infants (172–176). Moreover, there are many unclear points regarding the long-term effects of obesity, dyslipidemia, hypertension, cardiovascular disease, and other risks (177). Safety information from a large number of cases and long-term follow-up is lacking, including not only prenatally treated affected females but also fetuses exposed to unnecessary dexamethasone. In addition, there are reports of weight gain, edema, and Cushing's signs in mothers receiving dexamethasone, and management of maternal complications is required (102). Under these circumstances, prenatal diagnosis and treatment of 21-OHD are regarded as treatments with many ethical problems that need to be considered (8, 109).

In recent publications, such as the guidelines of the German Society for Pediatric Endocrinology and Diabetes and the American Endocrine Society, prevention of unnecessary maternal and fetal dexamethasone exposure and avoidance of possible disadvantages are prioritized over the mental burden incurred by parents and patients due to virilization of the external genitalia. Therefore, studies with long-term follow-up should be performed only in countries that allow this type of treatment, in well-established facilities that can handle these cases, and after obtaining approval from the institutional ethics committee and informed consent from parents (11, 178, 179).

10. Surgical Treatment

Recommendations

1. We recommend that the advantages and disadvantages of surgery be assessed in each individual case through shared decision making with a multidisciplinary team. 1 (Consensus)
2. We recommend that glucocorticoid is administered in increasing dosages during the intraoperative and postoperative periods. 1 (●●○)
3. We recommend that clitoroplasty be performed in females with cosmetic problems and clitoromegaly. A neurovascular bundle-preserving procedure is recommended as standard surgery. 1 (●○○)
4. We suggest that vaginoplasty be performed for females who have a urogenital sinus with no visible vaginal opening and are supposed to have future problems regarding the difficulty in menstrual blood discharge or sexual intercourse. 2 (●○○)

Evidence

The objectives of female genital surgery are to (1) ensure a gender-matched genital appearance, (2) preserve sexual and reproductive function in adulthood, and (3) minimize complications of sexual or voiding function related to surgery. There are no randomized controlled trials on the timing of surgery (early versus late) or operative modality (one-stage versus two-stage). In fact, the literature is limited to case series or pre- and postoperative comparisons, with long-term results unknown in terms of the degree of preoperative virilization, details of surgical methods, and endocrinological control.

10-1. Timing of surgery

In Japan, it is common to perform surgery starting from 6 months of age (when replacement therapy is stable) to before the development of gender labeling (1 year and 6 months to 2 years old). Clitoroplasty and vaginoplasty are simultaneously performed in the majority of cases; however, vaginoplasty may be delayed until puberty in selected cases. In Europe and the United States, whether surgery is performed early, before the age of 2 years or later, when the patient is able to choose is debated (180), since patient participation has been considered for the decision-making process of treatment since the consensus statement of DSD was published in 2006. Early surgery has been criticized because of the lack of evidence regarding the effects of early surgery on gender identity, possibility of loss of clitoral sensation, and high frequency of vaginal reconstruction. In contrast, there is no evidence that late surgery, in which children spend their childhood with virilized external genitalia, has a better outcome (181). The ultimate goal of genitoplasty is patient satisfaction with the maintenance of urogenital function and body image. Among patients who underwent genitoplasty, both parents and individuals were satisfied with their appearance and encouraged earlier surgery before the age of 2 years (182, 183).

10-2. Perioperative glucocorticoid replacement

In 21-OHD, cortisol is not adequately secreted in response to physical stress, and adrenal crises may develop. Invasive surgery is considered a type of physical stress that increases the risk for the development of adrenal crisis. Indeed, in the perioperative period of surgery with incision or general anesthesia, the peak serum cortisol concentration in healthy adults increases to an average of 18.9 μg/dL (184). Therefore, in patients with 21-OHD, an increased dosage of glucocorticoids may be needed transiently during the perioperative period, which is also recommended by the British-Irish Society of Anesthesiologists (185) and Endocrine Society guidelines (11). An adequate glucocorticoid dosage that can completely prevent adrenal crisis has not been determined because of multiple predisposing or precipitating factors, including reduced clearance of cortisol due to general anesthesia (186), differences in the type of procedure and anesthetic, and individual differences in stress response. Generally, at the time of major surgery, HC 50 mg/m^2 (100 mg in adults) is administered as a bolus at the induction of anesthesia, followed by continuous 50–100 mg/m^2/d (200 mg/d in adults), and the dosage is adjusted according to the degree of surgical invasion (183,185). Therapy was maintained at 50–100 mg/m^2/d (200 mg/d in adults)

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after surgery. Once the patient can take oral HC, the glucocorticoid dosage is tapered and returned to maintenance therapy. Genital surgery in females with 21-OHD is often treated as a major surgery. See "6. Stress dosing during maintenance therapy" for details.

10-3. Clitoroplasty

1) Surgical procedure

Clitoral amputation (clitorectomy) performed before 1970 was based on the misconception that clitoris is not required for sexual function. As the function, anatomy, and development of the clitoris have been elucidated, the standard procedure for modern clitoroplasty is to preserve the neurovascular bundle and remove the enlarged corpus cavernosum (187). There are variations in the approaches and procedures. The guidelines of the Endocrine Society recommend that clitoroplasty be postponed for patients with mild clitoral hypertrophy smaller than 2 cm, whereas clitoroplasty should be considered for patients with moderate to high clitoral hypertrophy as long as it is performed by an experienced surgical team (11), because adult females who underwent amputation or old clitoroplasty in childhood were reported in the 2000s to have altered their perception of the clitoris and orgasms compared to controls.

2) Postoperative complications

Modern clitoroplasty has few short-term postoperative complications (181). Infection or hemorrhage, labial fusion, scarring, and clitoral atrophy have been reported (188). Although long-term evaluation of sexual function is lacking, many patients who undergo the procedure are sexually active and are satisfied with the surgical results (133, 188). There are variable reports comparing clitoral perception with controls, ranging from less and mild postoperative clitoral dysesthesia to more often and more severe (133, 189). However, clitoral perception is difficult to evaluate. It is unclear how compromising clitoral perception affects sexual satisfaction.

10-4. Vaginoplasty

1) Surgical procedure

Flap vaginoplasty is widely performed, when the vagina joins the urethra in low confluence (190–192). When the vagina joins the urethra at a high confluence and the urogenital sinus is long, total urogenital mobilization (TUM), partial urogenital mobilization (PUM), pull-through vaginoplasty, and anterior sagittal transrectal approach (ASTRA) techniques are used (190–192).

2) Postoperative complications

Bleeding, flap necrosis, wound infection, and lower extremity nerve palsy have been reported, with low rates of 1.2%–12.2% (190–192). Long-term complications have been reported, including urinary tract infection, lower urinary tract dysfunction, vaginal stenosis, and decreased sexual function. Urinary tract infection was reported to be 0%–8.5% (190, 191, 193), but the incidence of urinary tract infection did not differ from that in 21-OHD patients without surgery (193), suggesting that adult females with 21-OHD are more prone to urinary tract infection. Postoperative daytime urinary incontinence is observed in approximately 30% of patients with lower urinary tract dysfunction (194). The risk of daytime urinary incontinence differs according to the type of surgery. Vaginal stenosis is present in 6%–57% with a reoperation rate of 3%–36% (190, 191, 195–204). The stricture rate according to the procedure is unclear. The remnant urogenital sinus in 85% of cases and vaginal orifice stenosis in 15% of cases suggest inadequate surgery in early life (205). Reoperation of the remnant urogenital sinus and vaginal orifice stenosis were successful (205). However, the long-term results according to the type of surgery are unclear.

3) Postoperative sexual function and delivery

Regarding postoperative sexual function, sexual arousal is good; 80% of patients experience regular sexual intercourse, and sexual desire, sexual stimulation, and orgasm achievement are not reduced (206, 207). In a report of 299 pregnancies in patients with 21-OHD, the adjusted odds ratio of cesarean section was 2.10 (95% confidence interval, 1.44 to 3.07), suggesting a good pregnancy course and delivery even after vaginoplasty (208). As this study did not collect information regarding classic and non-classic forms, a specific understanding of the prognosis for each form will require further investigation.

11. Gender Identity

Recommendations

1. In 46,XX cases with classic 21-OHD, we recommend assigning sex as female at birth. 1 (Consensus)
2. In 46,XX cases of classic 21-OHD, we recommend that continuous psychosocial support and education regarding gender identity be provided by a multidisciplinary team. 1 (Consensus)

Evidence

The majority of 46,XX patients with 21-OHD assigned as female at birth were reported to have female gender identity, and no correlations between gender identity and virilization scores of the external genitalia were documented (209–212). Approximately 5% of 46,XX patients assigned as adult females had gender dysphoria, and the prevalence was significantly higher than that of the general population (209, 212). In contrast, more than 10% of 46,XX patients assigned as male at birth had gender dysphoria (209, 213). The proportion of 46,XX adult females with 21-OHD whose sexual orientation is not heterosexual is approximately 20%, which is higher than that of the general population (214, 215). Male gender role behavior is often observed (211, 216), but there is no significant correlation between the degree of male gender roles and male gender identity (210). In addition, fertility in females can be expected because

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the gonads of 46,XX cases of 21-OHD are ovaries and the internal genitalia include the uterus. Given these facts, we recommend that it is valid to advise female assignment for 46,XX patients with 21-OHD, although there are still some concerns to be discussed regarding 46,XX cases of 21-OHD with significant virilization of the external genitalia (217).

The findings on gender issues in 21-OHD are based on clinical studies investigating the effects of androgen excess during fetal life. The effect of postnatal androgen exposure on gender identity has not been examined (218). Accordingly, regarding the effects of postnatal hyperandrogenism on gender identity, there is no optimal dosage of glucocorticoids or biomarkers for appropriate treatment in adult females with 21-OHD. Furthermore, it should be noted that gender identity could be affected not only by the classic form but also by the non-classic form (215). Customized and careful support is necessary for 46,XX male patients with 21-OHD.

Physicians should be aware that 21-OHD with 46,XX is DSD. It is essential to establish a health support system provided by a multidisciplinary team for these patients, even in adulthood (11). In addition, there are various methods for evaluating gender identity that could be improved in the future (212). Psychological problems are more common in 21-OHD than in other chronic childhood illnesses (216). Taken together, comprehensive and continuous support care for gender issues throughout life, including transitional care, is required.

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Appendix

1. Diagnostic criteria of 21-OHD

Definite: I + II (if II-1 is observed) + III + IV or I + III + IV
Probable: I + II (if II-1 is observed) + IV

I. Clinical symptoms and signs (Note #1)
1. Adrenal insufficiency: poor sucking, poor weight gain, nausea/vomiting, dehydration, impaired consciousness, or shock.
2. Virilization: clitoromegaly, labial fusion, common urogenital sinus, or hirsutism in females and increased penile length or azoospermia in males.
3. Skin hyperpigmentation: diffuse skin hyperpigmentation throughout the body or intense hyperpigmentation in the oral mucosa, lips, areola, umbilicus, and vulva.
4. Short stature: excess adrenal androgens in both sexes promote premature growth spurt, but premature epiphyseal closure eventually results in short stature.

II. Laboratory findings
1. Increased serum 17-OHP level.
(Reference laboratory findings)
1. High urine PT level (Note #2).
2. High urinary pregnanetriolone level and 11-hydroxyandrosterone/pregnanediol ratio (Note #3).
3. High plasma ACTH level.
4. High plasma renin activity or level.
5. Hyponatremia and hyperkalemia.

III. Genetic testing
Pathogenic variants of *CYP21A2* encoding P450c21 (Note #4).

IV. Exclusion criteria
- 3β-hydroxysteroid dehydrogenase deficiency
- POR deficiency
- 11β-hydroxylase deficiency

(Note #1) Not all symptoms of 1–4 are always observed.
(Note #2) Low specificity during the neonatal period.
(Note #3) In Japan, urinary pregnanetriolone can be measured by urinary steroid profile of gas chromatography-mass spectrometry-selective ion monitoring. However, gas chromatography alone can result in high false values.
(Note #4) Genetic testing can be useful in cases that are difficult to diagnose by clinical information only.

2. Source of funds for preparation

Funding for the preparation of the updated guidelines was provided by the Japanese Society for Pediatric Endocrinology and a Scientific Grant-in-Aid for the Ministry of Health, Labour and Welfare's Research Project on Refractory Disease Policy (Research on Abnormal Adrenal Hormone Production).

3. Methods of literature review

The titles and abstracts of articles were extracted and eligibility was assessed in the full text according to the following methods:

1) Search time: 2013/1/1 to 2020/3/31 (papers on operative treatment and gender identity were retrospectively collected, some of which have publication dates prior to 2013)
2) Search source: PubMed (https://pubmed.ncbi.nlm.nih.gov) and Ichushi Web (https://login.jamas.or.jp)
3) Search words: 21-hydroxylase deficiency and congenital adrenal hyperplasia
4) Search languages: English and Japanese
5) Additional search conditions: Human

4. Development process

As this is a minor update of the 2014 version of the guidelines, further clinical questions were not established. Based on the GRADE system, the strength of recommendation and the quality of evidence were established after discussions at meetings with the working committee members.

1. Progress of revision work
1) Suggestion for revision of guidelines at the Joint Meeting of Disorders of Sex Development and Adrenal Disorders Committee and Committee on Mass Screening in the Japanese Society for Pediatric Endocrinology (September 26, 2019)

2) Approval for establishment of the Guideline Revision Working Group by the Council of the Japanese Society for Pediatric Endocrinology (January 18, 2020)
3) Confirmation of Conflict of Interest of the working committee members by the COI Committee of the Japanese Society for Pediatric Endocrinology (April 10, 2020)
4) Determination and dissemination of methods for systematic reviews (May 15, 2020)
5) The first web meeting of the Guideline Revision Working Group (November 2, 2020)
6) The second web meeting of the Guideline Revision Working Group (March 22, 2021)
7) Draft guidelines completed (June 30, 2021)

2. External evaluation
1) Hearing of public opinions from members of the Japanese Society for Pediatric Endocrinology (July 1–31, 2021)
2) Hearing of public opinions from members of the Japanese Society for Neonatal Screening (July 25 to August 30, 2021)
3) Hearing of public opinions from members of the Japanese Society of Pediatric Urology (August 1–15, 2021)
4) Hearing of public opinions from members of the Japan Endocrine Society (July 28 to August 11, 2021)
5) Hearing of public opinions from the patient association of Osaka CAH (July 1–31, 2021)
6) Hearing of public opinions from the patient association of Congenital Adrenal Hyperplasia (July 1–31, 2021)
7) Evaluation and recommendation of the Guidelines Committee of the Japanese Society for Pediatric Endocrinology (August 31, 2021).
8) Approval by the Council of the Japanese Society for Pediatric Endocrinology (September 22, 2021).
9) Approval by the Council of the Japanese Society of Pediatric Urology (October 1, 2021)
10) Approval by the Board of the Japanese Society of Neonatal Screening (October 5, 2021)
11) Approval by the Board of the Japan Endocrine Society (October 5, 2021)

5. Timing of revision

The timeline for revision of the updated guidelines is approximately 5 years after release. In particular, for areas with low-quality evidence, we will examine the literature and studies that may cause breakthroughs in the field as they are published, and we will revise the guidelines as appropriate. The working committee members for the revision will be organized according to the consensus of affiliated academic societies. If a new fact that has a significant impact on the content of the guidelines is discovered and judged to be urgent, it may be revised or added as a "recommendation."

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