

CLINICAL PRACTICE GUIDELINE

Clinical practice guidelines for the management of differences of sex development in Japan

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Abstract. Differences of sex development (DSD) are congenital conditions in which chromosomal, gonadal, and anatomical sex characteristics are discordant with typical male or female development. These clinical practice guidelines provide evidence-based recommendations for the diagnosis and management of individuals with DSD across the lifespan. The guidelines were developed by a multidisciplinary committee of specialists representing pediatric endocrinology, adult endocrinology, urology, gynecology, psychiatry, and psychology. The committee employed a systematic review of the literature and used the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system to assess the strength of recommendations and the quality of evidence. Key areas addressed include the initial management of infants with atypical genitalia, diagnostic approaches, hormonal treatment, surgical interventions, gonadal tumor risk assessment, fertility preservation, and the transition from pediatric to adult care. The guidelines integrate international best practices with Japan's unique sociocultural, healthcare, and legal contexts for optimal DSD management and aim to improve clinical care for individuals with DSD while acknowledging the limited high-quality evidence in many aspects of DSD management.

Key words: Differences of sex development, Sex assignment, Hormone replacement therapy, Shared decision-making, Psychological support

List of Recommendations

1. Initial Management of Newborns with Atypical Genitalia

CQ1: Is it recommended to determine sex assignment shortly after birth?

Recommendation 1: We suggest that sex assignment is determined shortly after birth, following the completion of necessary medical examinations. (Strength of recommendation: Weak; Quality of evidence: expert opinion)

CQ2: Is rapid genetic testing recommended before sex assignment?

Recommendation 2: We suggest that rapid genetic testing before sex assignment may be useful for identifying the etiology in certain DSD conditions and should be considered in these cases. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

CQ3: What information should be initially provided to families when a child is born with atypical genitalia?

Recommendation 3: We recommend that healthcare providers explain the possibility of DSD and the necessity of further evaluation to determine appropriate sex assignment, while refraining from hastily communicating preliminary assigned sex. Information should be provided with sensitivity to the family's psychological state, and stigmatizing terminology or behavior should be avoided during consultations. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

CQ4: What findings in atypical external genitalia warrant deferring sex assignment?

Recommendation 4: We suggest deferring sex assignment when the external genitalia differ from typical male/female development in terms of gonadal position, penile/clitoral size, location of the urethral opening, degree of scrotal formation/labial fusion, and presence of vaginal opening. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

2. Diagnostic Approaches

CQ5: Are endocrinological stimulation tests, genetic testing, laparoscopy, and gonadal biopsy recommended for diagnosing DSD?

Recommendation 5a: We recommend endocrinological stimulation tests and genetic testing according to the suspected condition for diagnosing DSD. (Strength of recommendation: Strong; Quality of evidence: Low)

Recommendation 5b: We suggest that the utility of laparoscopy and gonadal biopsy is limited in diagnosing DSD and should be carefully considered according to the

suspected condition. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

CQ6: What are the causes and prevalence of DSD?

Recommendation 6: DSD has diverse etiologies, encompassing sex chromosome abnormalities, genetic variants, and environmental factors. The underlying etiologies remain unidentified in a significant proportion of cases. Although comprehensive epidemiological data on DSD prevalence in Japan has not been established, international data indicate that atypical external genitalia occur in 0.02–0.13% of births. Specific subtypes demonstrate varying incidence rates, with 46,XY DSD reported at 6.4 per 100,000 births and 46,XX DSD at 3.5–4.7 per 100,000 births. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

CQ7: What diagnostic approach is recommended for atypical external genitalia in newborns and infants?

Recommendation 7: We recommend visual inspection and palpation of the external genitalia, chromosomal analysis, endocrinological tests, imaging studies such as ultrasonography and MRI, and comprehensive evaluation by a multidisciplinary team of specialists. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

CQ8: What diagnostic approach is recommended for delayed puberty?

Recommendation 8: For males aged 15 years or older and females aged 13 years or older without signs of puberty, we recommend serum gonadotropin and sex hormone measurements, and as needed, GnRH stimulation test, hCG stimulation test, imaging studies of the uterus and ovaries or pituitary, and chromosomal analysis. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

CQ9: What diagnostic approach is recommended for delayed menarche or primary amenorrhea?

Recommendation 9: For females aged 15 years or older who have not experienced menarche, we strongly recommend a comprehensive evaluation, including assessment of secondary sexual characteristics (breast and pubic hair development), measurement of serum gonadotropin and estradiol levels, imaging of the uterus, ovaries, or pituitary gland, and chromosomal analysis. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

3. Hormone Replacement Therapy

CQ10: Is testosterone replacement therapy recommended for the induction of male secondary sexual characteristics?

Recommendation 10: For males with hypogonadism, we recommend testosterone replacement therapy for the induction of secondary sexual characteristics. (Strength of recommendation: Strong; Quality of evidence: Low)

CQ11: Is estrogen replacement therapy recommended for the induction of female secondary sexual characteristics?

Recommendation 11: For females with hypogonadism, we recommend estrogen replacement therapy for the induction of secondary sexual characteristics. (Strength of recommendation: Strong; Quality of evidence: High)

CQ12: Is testosterone replacement therapy recommended for adult males with hypogonadism?

Recommendation 12a: We recommend testosterone replacement therapy for adult males with hypogonadism to maintain sexual function and quality of life (QOL). (Strength of recommendation: Strong; Quality of evidence: Low)

Recommendation 12b: We suggest testosterone replacement therapy for adult males with hypogonadism may improve and maintain bone mineral density (BMD). (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

CQ13: Is estrogen replacement therapy recommended for adult females with hypogonadism?

Recommendation 13a: We recommend estrogen replacement therapy for adult females with Turner syndrome or complete androgen insensitivity syndrome (CAIS) following gonadectomy to improve and maintain BMD. (Strength of recommendation: Strong; Quality of evidence: High)

Recommendation 13b: We suggest estrogen replacement therapy for females with other forms of DSD and hypogonadism. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

CQ14: Which adult-onset complications should be monitored, and what management strategies are appropriate for addressing these sequelae?

Recommendation 14: Important adult complications include osteoporosis, metabolic syndrome, gonadal tumors, psychiatric disorders, and diminished QOL. We recommend establishing individualized care plans considering these complications to determine appropriate examinations and treatment strategies. (Strength of recommendation: Strong; Quality of evidence: Expert

opinion)

4. Surgical Management

CQ15: Is preoperative testosterone administration recommended before male genital reconstruction?

Recommendation 15: We suggest preoperative testosterone administration for individuals with micropallus undergoing hypospadias repair, as this may increase penile size and potentially facilitate earlier surgery. Both positive and negative effects on surgical outcomes have been reported; however, comprehensive evidence regarding patient characteristics, methods of administration, adverse effects, surgical complications, and reoperation rates is still lacking. (Strength of recommendation: Weak; Quality of evidence: Very low)

CQ16: When and which gonads should be removed in case at risk for gonadal tumors such as intra-abdominal gonads?

Recommendation 16: We recommend considering gonadal biopsy or gonadectomy as additional options alongside regular ultrasound monitoring in individuals with DSD who are at high risk for gonadal tumors. However, clear criteria regarding the indications and timing of prophylactic gonadectomy have not yet been established. (Strength of recommendation: Weak; Quality of evidence: Very low)

CQ17: When and what types of surgical procedures are recommended for females with clitoromegaly, labial fusion, urogenital sinus, vaginal agenesis, or vaginal agenesis or hypoplasia?

Recommendation 17a: We suggest an individualized approach, in consultation with an expert team, to determine the optimal timing of female external genital reconstruction or vaginoplasty, following the provision of comprehensive information to both parents and individuals. (Strength of recommendation: Weak; Quality of evidence: Very low)

Recommendation 17b: We suggest clitoroplasty with preservation of neurovascular bundles, labioplasty utilizing periclitoral skin, urogenital sinus mobilization with perineal skin flaps, and vaginal reconstruction using peritoneum or intestine as the main surgical procedures. (Strength of recommendation: Weak; Quality of evidence: Very low)

CQ18: Are female genitoplasty for clitoral hypertrophy, labial fusion, and urogenital sinus, and vaginoplasty for vaginal agenesis or hypoplasia recommended to improve sexual health in adulthood?

Recommendation 18: We suggest performing female genitoplasty and vaginoplasty to improve satisfaction

regarding sexual health in adulthood. We suggest providing continuous support and information through an expert team to address patient preferences regarding dissatisfaction with the appearance or function of external genitalia or vagina after reconstruction surgery. (Strength of recommendation: Weak; Quality of evidence: Very low)

CQ19: Is it recommended to delay surgical treatment until the patient reaches an age when they can make their own decisions?

Recommendation 19: There is insufficient evidence to recommend either early surgery based on parental decision-making or delayed surgery pending patient autonomy. We suggest a collaborative shared decision-making approach in consultation with an expert team, incorporating comprehensive risk-benefit analyses comparing early *versus* delayed surgical interventions. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

5. Psychological Support

CQ20: When and how is it recommended to disclose information directly to the individuals concerned?

Recommendation 20: We suggest disclosing information to individuals in a staged manner, accompanied by comprehensive psychological support, while acknowledging the substantial variability in clinical presentations and cognitive capacities among individuals. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

CQ21: Is psychological support recommended to improve QOL?

Recommendation 21: We suggest providing psychological support through a multidisciplinary expert team and considering peer support, as these may resolve various issues related to mental health and sexuality and improve QOL. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

CQ22: What types of psychological burdens or mental disorders may manifest in parents at the time of diagnostic disclosure of DSD in their neonates or during subsequent development stages?

Recommendation 22: Parents may experience significant stress related to “Illness Uncertainty” regarding their child’s future following disclosure, and may develop long-lasting symptoms of post-traumatic stress symptoms, anxiety symptoms, or depressive. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

CQ23: What is the prevalence of gender dysphoria and psychiatric disorders as comorbidities?

Recommendation 23: Gender incongruence may present as a comorbidity in 15% of cases (95% confidence interval: 13–17%). Certain pathological conditions and diseases are associated with psychiatric disorders, including depression and anxiety disorders. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

6. Gonadal Tumors

CQ24: Is gonadal biopsy recommended for the risk assessment of gonadal tumors?

Recommendation 24: While gonadal biopsy may be useful for assessing the risk of gonadal tumors in individuals with DSD who possess Y-chromosome material, clear evidence supporting routine use of gonadal biopsy is currently lacking. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

CQ25: What are the risk factors for gonadal tumors and biomarkers suggesting malignancy?

Recommendation 25: Risk factors for gonadal tumors include the presence of Y chromosome materials, intra-abdominal testes, post-pubertal age, gonadal dysgenesis, and the presence of undifferentiated germ cells. There are no effective laboratory biomarkers suggesting malignancy for early detection and diagnosis. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

CQ26: Is regular surveillance recommended for high-risk groups for gonadal tumors?

Recommendation 26: We suggest regular surveillance using ultrasound (US), magnetic resonance imaging (MRI), and tumor-specific biomarkers for high-risk groups under observation. (Strength of recommendation: Weak; Quality of evidence: Very low)

7. Reproductive Medicine

CQ27: Are assisted reproductive technologies and fertility preservation recommended for spermatogenesis defect?

Recommendation 27: We suggest artificial insemination and assisted reproductive technologies using ejaculated and testicular sperms for individuals desiring children. However, clear evidence regarding fertility preservation is currently lacking. (Strength of recommendation: Weak; Quality of evidence: Very low)

CQ28: Are assisted reproductive technologies and fertility preservation recommended for premature ovarian insufficiency?

Recommendation 28: We suggest assisted reproductive technologies and fertility preservation methods, including ovarian tissue cryopreservation, oocyte freezing, embryo freezing, for individuals with premature ovarian insufficiency who desire children currently or in the future, provided they have residual follicles. However, there is currently no definitive diagnostic method to confirm the presence or absence of residual follicles. (Strength of recommendation: Weak; Quality of evidence: Low)

CQ29: What is the potential for preserving spermatogenesis?

Recommendation 29: Evidence remains limited regarding the preservation potential of spermatogenesis. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

CQ30: What is the potential for preserving oogenesis?

Recommendation 30: In individuals with Turner syndrome, oogenic capacity is markedly reduced, leading to hypogonadism. However, some children with Turner syndrome retain sufficient ovarian follicles to allow for fertility preservation. In other conditions associated with hypogonadism, evidence regarding the extent to which oogenesis can be preserved remains limited. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

8. Transition to Adult Care

CQ31: Is transition from pediatric to adult care recommended to improve reproductive outcomes, life prognosis, and QOL?

Recommendation 31: We suggest implementing transition from pediatric to adult care with multidisciplinary medical care and psychological support by an expert team, as this may improve reproductive outcomes, life prognosis, and QOL. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Introduction

Differences of sex development (DSD) are defined as congenital conditions in which chromosomal, gonadal, internal, or external genital development is atypical [1, 2]. Individuals with DSD may present at various ages with diverse clinical manifestations, including atypical genitalia in newborns, delayed puberty in adolescents, or infertility in adults. They may seek care from multiple

specialties, including pediatrics, endocrinology, urology, gynecology, and psychiatry.

Despite significant advances in understanding the genetic and molecular mechanisms underlying many forms of DSD, clinical management remains challenging due to the rarity and complexity of these conditions. Historically, management approaches varied widely, and many interventions were based on limited evidence [3-6]. In recent years, there has been increased recognition of the need for evidence-based, patient-centered care that addresses both medical needs and psychological well-being.

These clinical practice guidelines aim to provide healthcare professionals with current evidence-based recommendations for the diagnosis and management of DSD. Japan-specific clinical practice guidelines for DSD management are essential due to distinct sociocultural perspectives on gender identity and family dynamics, unique healthcare system constraints, and specific legal requirements for gender designation that differ from other countries. These guidelines integrate international best practices with local sociocultural and administrative realities to ensure optimal patient care while meeting Japanese regulatory requirements. The guideline focuses on initial management of newborns, diagnostic approaches across the lifespan, hormone therapy, surgical management, tumor risk assessment, fertility preservation, and transition from pediatric to adult care. Where evidence is limited, the clinical guidelines provide expert consensus recommendations while acknowledging the need for further research.

Methods

Guideline Development Process

The guideline development committee was established in February 2023, comprising 23 members representing the Japanese Society for Pediatric Endocrinology, the Japan Endocrine Society, the Japanese Society for Pediatric Urology, the Japan Society of Reproductive Endocrinology, and the Japanese Society for Gender Incongruence. Committee members included specialists in pediatric endocrinology, adult endocrinology, pediatric urology, adult urology, gynecology, psychiatry, and psychology.

The committee identified 11 thematic areas for clinical guidance and formulated 31 clinical questions (CQs) related to DSD management. For each CQ, systematic literature reviews were conducted using PubMed and other databases, with searches limited to English-language publications from January 1990 to September 2023. The committee employed the GRADE methodology to assess the quality of evidence and the strength of

recommendations [7].

Recommendations were categorized as either “strong” (indicated by “recommend”) or “weak” (indicated by “suggest”). The quality of evidence was graded as very high (A), moderate (B), low (C), very low (D), or expert opinion. Six of the 31 CQs were identified as background questions and did not receive formal recommendations.

The draft guidelines underwent external review through public comments solicited from DSD support groups and relevant medical societies. The final guidelines were approved by the participating societies between January and February 2025.

1. Initial Management of Newborns with Atypical Genitalia

CQ1: Is it recommended to determine sex assignment shortly after birth?

Recommendation 1: We suggest that sex assignment be determined shortly after birth, following the completion of necessary medical examinations. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

DSD is often identified through atypical external genitalia during the neonatal period or early infancy. The inability to determine sex at birth places a significant psychological burden on caregivers, necessitating management as a condition of high psychosocial emergency. The selection and registration of binary sex designation are mandatory in many countries, including Japan, to register a newborn’s sex within a specified timeframe (until 13th day of life in Japan). Furthermore, social conventions that permit non-binary gender options in daily life have not yet been sufficiently established in Japan. Consequently, it is recommended to conduct necessary evaluations and determine the sex assignment as promptly as possible. Nevertheless, the ultimate determination of gender identity should be reserved for the individual, and families must be informed that the sex assigned at birth may change in the future. When providing medical information to families, it is important to note that research indicates both insufficient knowledge about DSD and excessive information provision can become barriers to family decision-making [8]. It is essential to create an environment where parents can understand the risks and benefits of available options and consult and share information with a specialist team.

CQ2: Is rapid genetic testing recommended before sex assignment?

Recommendation 2: We suggest that rapid genetic

testing before sex assignment may be useful for identifying the etiology in certain DSD conditions and should be considered in these cases. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

The determination of sex assignment in newborns with DSD represents a significant challenge with long-term psychosocial implications for both individuals and caregivers. In recent years, genetic testing has rapidly expanded its role in this decision-making process [9–11]. Although early integration of genetic testing with conventional assessments—including physical examination, endocrinological evaluation, and chromosomal analysis—may support more informed decision-making, several ethical concerns persist.

Sex assignment during the neonatal period or early infancy involves comprehensive evaluation of internal and external genitalia development, in addition to chromosomal and genetic assessments. A key objective in this process is to predict future gender identity, for which identifying the underlying etiology of the DSD can be of significant value. For instance, in CAIS, which presents with female external genitalia, only 2% of female individuals experience gender incongruence [12], thus female sex assignment is recommended. While elevated serum luteinizing hormone (LH) and testosterone are characteristic endocrinological features, diagnostic accuracy in the neonatal period remains limited [13]. In 5 α -reductase deficiency and 17 β -hydroxysteroid dehydrogenase deficiency, severe undermasculinization of the external genital resembling female phenotypes may occur. However, masculinization during puberty commonly occurs, with 13% of individuals with 5 α -reductase deficiency and 15% of individuals with 17 β -hydroxysteroid dehydrogenase deficiency initially assigned female later changing to male [14–16]. Although these conditions exhibit distinctive endocrinological profiles—such as an elevated serum testosterone to dihydrotestosterone (DHT) ratio following hCG stimulation in 5 α -reductase deficiency, and decreased serum testosterone to androstenedione and androstenediol to dehydroepiandrosterone (DHEA) ratios in 17 β -hydroxysteroid dehydrogenase deficiency [17–19]—diagnostic accuracy in neonates remains suboptimal [17, 20–22]. One may argue that CAIS and other forms of DSD are not usually suspected in individuals with complete female external genitalia in infancy, so that none of biochemical, radiological, and genetic studies will not be considered in such individuals. However, CAIS, 5 α -reductase deficiency, and 17 β -hydroxysteroid dehydrogenase deficiency are suspected when testis-like inguinal

masses are identified [23]. Therefore, rapid genetic testing to evaluate the possibility of AIS, 5 α -reductase deficiency, or 17 β -hydroxysteroid dehydrogenase deficiency in 46,XY DSD cases with complete or near-complete female external genitalia without identified uterus can significantly inform sex assignment.

Conversely, the utility of rapid genetic testing before sex assignment remains uncertain for other conditions. Individuals with partial AIS (PAIS) demonstrate diversity in both external genitalia and gender identity. Although pathogenic variants in *AR* are identified in 90–95% of CAIS, they are detected in only 56% of PAIS, which exhibits significant genetic heterogeneity [24]. Consequently, sex assignment depends not on genetic testing results but on comprehensive assessment of external and internal genitalia phenotypes. Similarly, in 46,XY DSD individuals with identified uterus, such as *SRY* abnormalities, disease-specific gender identity trends remain unclear, diminishing the influence of genetic testing on sex assignment. In 46,XX individuals with 21-hydroxylase deficiency, varying degrees of virilization of the external genitalia occur depending on residual enzyme activity. With relatively low rates of gender incongruity (approximately 4%) [12], female assignment is recommended [25]. While the *CYP21A2* genotype correlates with residual activity and clinical features, endocrinological tests provide high diagnostic accuracy, rendering genetic testing often unnecessary. For these DSD conditions, rapid genetic testing before sex assignment offers limited value in predicting long-term prognosis or complications.

Understanding the limitations of genetic testing is crucial when identification of disease etiology significantly impacts sex assignment. Currently, genetic testing for AIS, 5 α -reductase deficiency, and 17 β -hydroxysteroid dehydrogenase deficiency remains uncovered by medical insurance in Japan and is performed primarily at research level for reference. While rapid genetic testing would ideally yield prompt results to inform sex assignment, resource limitations often result in delayed diagnosis. Additionally, the sensitivity of genetic test is not 100%; failure to identify pathogenic variants does not exclude the underlying condition. Furthermore, the development of gender identity involves both genetic and environmental factors [26], making precise prediction of future gender identity through genetic test challenging. These limitations must be acknowledged when utilizing rapid genetic test as an aid in sex assignment.

Rapid genetic testing prior to sex assignment represents an important tool that may provide valuable information for predicting future gender identity in certain conditions. However, high-quality evidence supporting the utility of rapid genetic testing is currently lacking,

with recommendations based primarily on expert opinion and experience. The interpretation of genetic test results requires caution. Furthermore, decisions regarding sex assignment should be made through integrative approach that synthesizes genetic findings with comprehensive clinical assessment.

CQ3: What information should be initially provided to families when a child is born with atypical genitalia?

Recommendation 3: We recommend that healthcare providers explain the possibility of DSD and the necessity of further evaluation to determine appropriate sex assignment, while refraining from hastily communicating preliminary assigned sex. Information should be provided with sensitivity to the family's psychological state, and stigmatizing terminology or behavior should be avoided during consultations. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

Evidence

Approaches to communicating with families when a child is born with atypical genitalia are limited to expert opinions, as there are no randomized controlled trials or cohort studies with control on this topic.

When a child is born with atypical genitalia, families should be informed that the external genitalia are not fully developed, and the possibility of a DSD should be explained. The psychological impact on such parents of children diagnosed with DSD is significant (see CQ22). The proportion of post-traumatic stress symptoms in those parents is nearly as high as those experienced by parents of children diagnosed with pediatric cancer [27]. When explaining the basic concepts of DSD, terminology such as “differences” or “variations” is more acceptable than “disorders,” as these terms connote constitutional characteristics or individual variations rather than pathologic conditions [28]. Healthcare providers should be sensitive to the family's psychological stress and avoid terminology with negative connotations such as “abnormal,” “incomplete,” “indeterminate sex,” or “hermaphrodite.” In cases where the child's condition is exclusively related to sex differentiation, without accompanying renal or adrenal complications, healthcare providers should address to the family that the child is otherwise in good health.

Subsequently, families should be informed that “investigations are necessary to determine or confirm the sex, so please allow some time.” Healthcare providers should avoid hastily suggesting the possible sex at initial consultation. Explanations should ideally take place with both parents present, in a setting where privacy is

ensured. During this discussion, healthcare providers should outline the initial prognosis, including the timeframe for diagnosis. If a specialized DSD expert team is available, healthcare providers should explain that the team will be involved in the child's care. Rather than providing results incrementally, it is preferable to wait until all data necessary for sex assignment have been collected, discussed within the expert team, and a unified opinion regarding sex assignment has been reached before explaining and proposing options to the family. Regarding sex assignment, healthcare providers should listen attentively to the parents' and family's perspectives and engage in shared decision-making rather than unilaterally presenting determinations. In most cases, sex assignment can be determined by the 13th day of life to meet the legal deadline for birth registration in Japan. However, families should be informed that if the assignment cannot be completed within this timeframe, an extension for birth registration (including sex and name) is possible (though a date of registration is recorded). It is advisable to provide information about birth registration procedures in advance, possibly through a medical social worker. Note that, as the date of birth is considered as day 1, the 13th day of life corresponds to a 14-day deadline.

DSD should be managed at facilities with extensive experience in its treatment, and multidisciplinary care is recommended. Ideally, pediatric endocrinologists should lead family communications about DSD, with other specialists directly engaging with the family as needed [28]. Healthcare providers unfamiliar with DSD management should consider collaborating with core or semi-core institutions proposed by the Japanese Society for Pediatric Endocrinology (https://jspe.umin.jp/medical/files/map_ver.3_240725.pdf) or consider an early transfer to a specialized institution.

CQ4: What findings in atypical external genitalia warrant deferring sex assignment?

Recommendation 4: We suggest deferring sex assignment when the external genitalia differ from typical

male/female development in terms of gonadal position, penile/clitoral size, location of the urethral opening, degree of scrotal formation/labial fusion, and presence of vaginal opening. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

Sex assignment in newborns with DSD may significantly impact the long-term psychosocial adaptation of both individuals and their parents. In newborns with suspected DSD of certain severity, it is recommended to defer sex assignment until information regarding gonads, external genitalia, and internal genitalia has been collected and the etiology of DSD thoroughly investigated. Although delaying sex assignment beyond the 14-day birth registration deadline should be avoided when possible, investigations are not often completed within this timeframe (see CQ3). There are no high-quality evidence studies or comprehensive epidemiological data regarding which phenotypic severity thresholds warrant deferral.

The primary objective of sex assignment is to maximize consistency between the assigned sex and future gender identity [29]. The prevalence of gender incongruence varies depending on the underlying DSD etiology (Table 1) [12, 30]. Among 46,XY females, the likelihood of gender incongruence is relatively high in 5 α -reductase deficiency or 17 β -hydroxysteroid dehydrogenase deficiency, while those are low in CAIS [12, 14, 15]. Conversely, 46,XX females with 21-hydroxylase deficiency demonstrate a low prevalence of gender incongruence [12]. The prevalence in 46,XX individuals with cytochrome P450 oxidoreductase deficiency remains undetermined; however, female assignment is recommended, following clinical guidelines for 21-hydroxylase deficiency [25]. The correlation between the severity of undermasculinization of external genitalia and the likelihood of gender incongruence is inconsistent and varies according to the underlying condition. Therefore, in newborns with DSD presenting with atypical external genitalia, sex assignment can be determined after screening for these etiologies.

Table 1 The prevalence of incongruence between assigned sex and gender identity in disorders of sex development

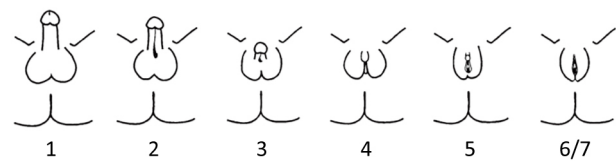
Condition	Sex Chromosomes	Males (%)	Females (%)
Cloacal exstrophy	46,XY	0	55
5 α -reductase deficiency	46,XY	0	13, 54
17 β -hydroxysteroid dehydrogenase deficiency	46,XY	ND	15, 53
Complete androgen insensitivity syndrome	46,XY	ND	2
21-hydroxylase deficiency	46,XX	15	4

ND: no data available

Table 2 Physical examination findings to be assessed in atypical external genitalia

Phenotype	Organ	Specific Assessment Items	Method
Male-like phenotype	Penis	Stretched penile length	Measuring tape
		Position of external urethral meatus	Visual inspection
	Testes	Testicular volume	Orchidometer
		Testicular position	Palpation, imaging
	Scrotum	Presence of scrotal hypoplasia	Visual inspection
Female-like phenotype	Clitoris	Clitoral width	Measuring tape
	Labia	Anogenital ratio	Measuring tape
	Vaginal introitus	Position of vaginal opening	Visual inspection

Atypical external genitalia refer to instances where gonadal position, penile/clitoral dimensions, external urethral meatus location, degree of scrotal formation/labial fusion, or vaginal orifice position differs from typical male/female presentations (Table 2). Standard values for stretched penile length and clitoral transverse diameter have been established for the Japanese population [31, 32]. Labial fusion is assessed by the anogenital ratio [33, 34]. However, no standardized criteria exist, even among expert opinions, regarding whether individual cases warrant deferral of sex assignment based on these evaluations. Deferral is generally unnecessary in cases with isolated mild hypospadias (glandular or coronal) or unilateral cryptorchidism without other atypical external genital findings. Conversely, deferral is recommended in cases with Grades 1–2 of Quigley classification [35] (Fig. 1) with male-typical genitalia and non-palpable gonads, Grades 3–5, or Grade 6/7 with female-typical genitalia and palpable gonads. Deferral is also recommended when external genitalia exhibit asymmetry, suggesting possible mixed gonadal dysgenesis, or when significant discrepancy exists between prenatal chromosomal karyotype and external genital findings. High-resolution fetal ultrasound, currently available in Japan, may raise suspicion of DSD during the fetal period. In such cases, it is essential to initiate prenatal evaluation and management to facilitate postnatal diagnosis and management, as well as to provide appropriate prenatal genetic counseling [36]. According to recently introduced assessment of the external genitalia score (EGS) [37] (Table 3), full-term infants scoring between 0.5–10.0 points should undergo comprehensive evaluation [38]. In very low birth weight or extremely low birth weight infants with DSD, genital assessment and sex assignment can be particularly challenging due to the lack of normative data and limitations in physical examination. These cases require heightened clinical caution and multidisciplinary evaluation to avoid premature conclusions. Accurate assessment of the external genitalia is crucial for appropriate deferral of sex assignment.

**Fig. 1** Quigley classification

Grade 6 with pubic hair development; Grade 7 without pubic hair development

This recommendation proposes deferring sex assignment in newborns with atypical external genitalia of certain severity and outlines assessment methodologies. Given the lack of standardized interpretive criteria, decisions regarding deferral should be individualized for each newborn. Collaboration with core or semi-core institutions for DSD management, as designated by the Japanese Society for Pediatric Endocrinology (https://jspe.umin.jp/medical/files/map_ver.3_240725.pdf), is advisable.

2. Diagnostic Approaches

CQ5: Are endocrinological stimulation tests, genetic testing, laparoscopy, and gonadal biopsy recommended for diagnosing DSD?

Recommendation 5a: We recommend endocrinological stimulation tests and genetic testing according to the suspected condition for diagnosing DSD. (Strength of recommendation: Strong; Quality of evidence: Low)

Recommendation 5b: We suggest that the utility of laparoscopy and gonadal biopsy is limited in diagnosing DSD and should be carefully considered according to the suspected condition. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

1) Endocrinological stimulation tests

Endocrine stimulation tests reported as useful in the diagnosis of DSDs include the hCG stimulation test and

Table 3 External genital score (EGS)

Score	Labial/Scrotal Fusion	Penile/Clitoral Stretched Length (cm)	External Urethral Meatus Position	Right Gonad Position	Left Gonad Position
3	Complete fusion	≥ 3.1	Tip of penis/clitoris		
2.5		2.6–3.0	Glans or near coronal sulcus		
2			Proximal to coronal sulcus on ventral aspect of penis/clitoris		
1.5	Posterior fusion	2.1–2.5	Base of penis/clitoris	Labial/scrotal	Labial/scrotal
1		1.0–2.0	Labial/scrotal	Between inguinal and labial/scrotal	Between inguinal and labial/scrotal
0.5				Inguinal	Inguinal
0	No fusion	<1.0	Perineal		

human menopausal gonadotropin (hMG) stimulation test. The utility of these tests is limited to specific conditions and purposes, and protocols (dosage and timing of blood sampling) and study ages vary across reports. These tests are typically performed during the neonatal period or prepubertal childhood, while their utility during mini-puberty or puberty when gonadotropins are physiologically elevated remains unclear.

The testosterone to DHT ratio after hCG stimulation test is useful for diagnosing 5 α -reductase deficiency [39, 40]. A testosterone to DHT ratio >10 (following urinary-derived hCG stimulation) or >30 (following recombinant hCG stimulation) is suggestive of 5 α -reductase deficiency. Similarly, the test can assist in diagnosing 17 β -hydroxysteroid dehydrogenase deficiency through assessment of testosterone to androstenedione and androstenediol to DHEA ratios [17, 22, 41]. However, the diagnostic accuracy of these tests is limited. The hCG stimulation test is also used to assess testosterone synthesis capacity and detect the presence of testicular tissue. Testosterone levels less than 1.1–1.5 ng/mL measured by immunoassay following urinary-derived hCG stimulation in prepubertal subjects are considered indicative of a poor response [42]. A Brazilian study involving 19 individuals with 46,XY DSD (five with PAIS [2–13 years], four with 5 α -reductase deficiency [1–10 years], and 10 unclassified [0–10 years]) reported that a recombinant hCG-stimulated testosterone level measured by LC-MS/MS >0.89 ng/mL at day 7 post-stimulation was the cutoff value for normal testosterone synthesis [40]. For evaluating the presence of testicular tissue, the measurement of anti-Müllerian hormone (AMH), although currently not covered by health insurance in Japan, has largely replaced the hCG stimulation test [43–45]. Basal serum AMH and inhibin B levels significantly correlate with post-hCG stimulated testosterone and DHT levels ($r = 0.64, 0.52, 0.62, \text{ and } 0.44$, respectively) [45]. Therefore, hCG stimulation test should be considered when

specific conditions such as 5 α -reductase deficiency or 17 β -hydroxysteroid dehydrogenase deficiency are suspected, or when evaluating testosterone synthesis capacity.

The hMG stimulation test is used to detect ovarian tissue. In a study of 11 infants with atypical external genitalia, a post-hMG stimulated estradiol (E2) level >80 pg/mL was reported as the cutoff value indicating the presence of ovarian tissue [46]. In another study of 7 individuals with ovotesticular DSD and 13 with DSD of unknown etiology, E2 and inhibin A levels increased significantly after hMG stimulation in ovotesticular DSD, with cutoff values suggesting the presence of ovarian tissue being 54 pg/mL for E2 and 20.0 pg/mL for inhibin A [47]. It should be noted that hMG preparations are used off-label, and the utility of the hMG stimulation test outside of ovotesticular DSD has not been established.

2) Genetic testing

Genetic testing plays a crucial role in the diagnosis of DSD. G-banding karyotype analysis or fluorescence *in situ* hybridization (FISH) for *SRY* are indicated in most individuals with DSD. A prospective study of 267 DSD individuals classified 46% as 46,XY DSD, 39% as 46,XX DSD, and 14% as sex chromosome DSD based on G-banding or *SRY*-FISH [48]. Other retrospective studies collecting over 200 cases classified 46–68% as 46,XY DSD, 22–39% as 46,XX DSD, and 8–29% as sex chromosome DSD [49–52]. Among 46,XX DSD, congenital adrenal hyperplasia represents the most common etiology, accounting for 32–60% [49–52]. 46,XY DSD encompass a diverse range of conditions. Next-generation sequencing has been performed in various cohorts, identifying causative disorders in 34–59% of cases [53–55]. Genetic testing is considered particularly useful in 46,XY DSD cases with moderate to severe undermasculinization of the external genitalia without an identifiable uterus, prior to sex assignment (see CQ2). While not covered by health insurance, genetic testing for 46,XY DSD has definite value for evaluating specific

disorders before sex assignment and for identifying the etiology in clinically challenging cases.

3) *Laparoscopy and imaging studies*

Given its invasive nature, less invasive alternatives should be considered before resorting to laparoscopy. It may be performed to search for non-palpable testes or Müllerian structures that cannot be identified by abdominal US or MRI; however, its utility in DSD diagnosis is limited.

US and MRI are less invasive than laparoscopy and frequently used in DSD to evaluate the morphology of gonads and determine the presence of Müllerian structures. Cystic structures in the gonads suggest the presence of ovarian components, while solid structures suggest testicular components. The presence or absence of Müllerian structures is useful in elucidating the pathophysiology of 46,XY DSD. Identification of a uterus suggests testicular dysgenesis, while absence of a uterus suggests disorders of androgen synthesis or action. However, it should be noted that US and MRI may sometimes be challenging for evaluating gonads and prepubertal uterus.

4) *Gonadal biopsy*

Gonadal biopsy is useful in diagnosing certain DSD conditions, such as ovotesticular DSD. It is considered when a diagnosis cannot be established through endocrinological tests, and imaging or genetic testing suggests the possibility of ovotesticular DSD or gonadal dysgenesis [56]. In Korea, gonadal biopsy or gonadectomy was performed in 23 individuals suspected of having ovotesticular DSD, with 57% confirmed as ovotesticular DSD, 22% as mixed gonadal dysgenesis, and 22% with other conditions [57].

CQ6: What are the causes and prevalence of DSD?

Recommendation 6: DSD has diverse etiologies, encompassing sex chromosome abnormalities, genetic variants, and environmental factors. The underlying etiologies remain unidentified in a significant proportion of cases. Although comprehensive epidemiological data on DSD prevalence in Japan has not been established, international data indicate that atypical external genitalia occur in 0.02–0.13% of births. Specific subtypes demonstrate varying incidence rates, with 46,XY DSD reported at 6.4 per 100,000 births and 46,XX DSD at 3.5–4.7 per 100,000 births. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

Evidence

DSD are classified based on sex chromosomes (Table 4) [1]. The etiology of DSD is diverse, encompassing sex chromosome abnormalities, genetic variants involved in

sex determination or differentiation processes, androgen production or action pathways, and environmental factors such as endocrine-disrupting chemicals. According to a survey of healthcare providers in the European Reference Network on Rare Endocrine Conditions (Endo-ERN), pathogenic variants are identified in 7–60% of DSD individuals (including 2,956 with congenital hypogonadotropic hypogonadism) among 6,720 who underwent next-generation sequencing genetic test. In studies analyzing more than 150 target genes, the diagnostic yield increases to 40–60% [58].

In 46,XX DSD, the most frequent causative gene is the *CYP21A2* responsible for 21-hydroxylase deficiency. In contrast, causative genes for 46,XY DSD are more diverse. A comprehensive study of causative genes in Japanese 46,XY DSD individuals with atypical external genitalia of Quigley grade 3 or higher identified single-gene disorders in 26% of individuals [55]. Large-scale studies from China report high proportions of variants in genes related to steroid hormone synthesis and action, such as *AR* and *SRD5A2*, as well as *NR5A1* involved in testicular development [59–61].

The prevalence or incidence of DSD in Japan has not been systematically studied. International reports indicate that the proportion of newborns presenting with atypical external genitalia at birth ranges from 0.02% to 0.13% [5, 62, 63]. A retrospective Danish registry data since 1968 reported the incidence of 46,XY DSD females as 6.4 per 100,000 (AIS: 4.1 per 100,000; gonadal dysgenesis: 1.5 per 100,000) [64], while the incidence of 46,XX DSD males was reported as 3.5–4.7 per 100,000 [65].

CQ7: What diagnostic approach is recommended for atypical external genitalia in newborns and infants?

Recommendation 7: We recommend visual inspection and palpation of the external genitalia, chromosomal analysis, endocrinological tests, imaging studies such as ultrasonography and MRI, and comprehensive evaluation by a multidisciplinary team of specialists. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

Evidence

DSD often present with atypical external genitalia. High-quality evidence regarding the diagnostic approach to atypical external genitalia is limited, and epidemiological data remain scarce.

International reports suggest an incidence of atypical external genitalia ranging from 0.02% to 0.13% (see CQ6). In Japan, where sex assignment is required on birth certificates, it is recommended to assign sex as early as possible (see CQ1). Therefore, evaluation

Table 4 Classification of differences of sex development

Sex Chromosome DSD	46,XY DSD	46,XX DSD
A: 45,X (Turner syndrome and variants)	A: Gonadal (testicular) dysgenesis 1. Complete gonadal dysgenesis (Swyer syndrome) 2. Partial gonadal dysgenesis 3. Testicular regression syndrome 4. Ovotesticular DSD	A: Gonadal (ovarian) dysgenesis 1. Ovotesticular DSD 2. Testicular DSD (SRY+, dupSOX9, <i>etc.</i>) 3. Gonadal dysgenesis
B: 47,XXY (Klinefelter syndrome and variants)	B: Androgen synthesis and action disorders 1. Androgen biosynthesis disorders 1. Congenital lipoid adrenal hyperplasia 2. 17 α -hydroxylase deficiency 3. 3 β -HSD deficiency 4. POR deficiency 5. Smith-Lemli-Opitz syndrome 6. 5 α -reductase deficiency 7. 17 β -HSD deficiency 2. Androgen action disorders 1. Androgen insensitivity syndrome (complete, partial) 3. LH receptor defects (Leydig cell hypoplasia/aplasia) 4. AMH and AMH receptor defects (persistent Müllerian duct syndrome)	B: Androgen excess 1. Fetal 1. 21-hydroxylase deficiency 2. 11 β -hydroxylase deficiency 3. 3 β -HSD deficiency 2. Fetoplacental 1. Aromatase deficiency 2. POR deficiency 3. Maternal (luteoma, exogenous)
C: 45,X/46,XY (mixed gonadal dysgenesis, ovotesticular DSD)	C: Other 1. Hypospadias, cloacal exstrophy 2. Other	C: Other 1. MRKH syndrome, MURCS, cloacal exstrophy, vaginal atresia 2. Other
D: 46,XX/46,XY (chimera, ovotesticular DSD)		

DSD: Differences of sex development; HSD: Hydroxysteroid dehydrogenase; POR: P450 oxidoreductase; LH: Luteinizing hormone; AMH: Anti-Müllerian hormone; MRKH: Mayer-Rokitansky-Küster-Hauser; MURCS: Müllerian duct aplasia, renal aplasia, and cervicothoracic somite dysplasia; SRY: Sex-determining region Y; SOX9: SRY-box transcription factor 9

necessary for sex assignment becomes the initial priority. Most experts recommend conducting a comprehensive assessment that includes family history (*e.g.*, maternal virilization during pregnancy), physical examination (particularly of the external genitalia), chromosome analysis, endocrinological tests, and imaging studies. The ideal approach involves management by a multidisciplinary team of specialists from endocrinology, surgery, urology, psychology, psychiatry, radiology, nursing, and clinical genetics [66]. Additionally, establishing a definitive diagnosis is crucial for individualized management,

genetic counseling, and predicting reproductive prognosis or tumor risk. DSD may be associated with primary adrenal insufficiency, which can potentially result in life-threatening adrenal crisis in the first days of life [67]. Therefore, prompt evaluation of primary adrenal insufficiency is essential in all individuals presenting with atypical genitalia. In recent years, genetic approaches (such as comprehensive gene analysis, whole-exome sequencing, and whole-genome sequencing) have significantly advanced, contributing substantially to diagnostic confirmation. However, high-quality evidence-based research

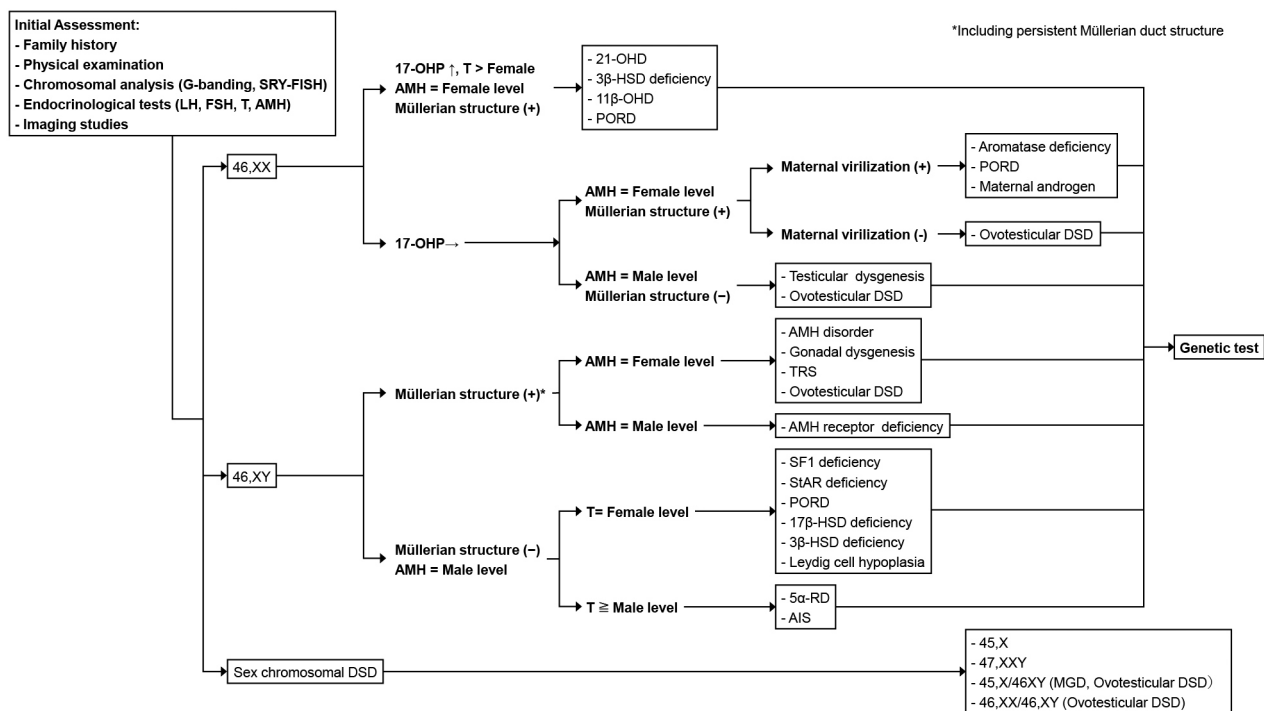


Fig. 2 Diagnostic approach for neonates and infants presenting with atypical external genitalia

LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, testosterone; AMH, anti-Müllerian hormone; DSD, differences of sex development; 17-OHP, 17 α -hydroxyprogesterone; 21-OHD, 21-hydroxylase deficiency; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 11 β -OHD, 11 β -hydroxylase deficiency; PORD, P450 oxidoreductase deficiency; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; 5 α -RD, 5 α -reductase deficiency

on diagnostic approaches to atypical external genitalia is lacking; therefore, current recommendations remain at the level of expert opinion.

According to various diagnostic approaches (Fig. 2), the proportions of 46,XX DSD, 46,XY DSD, and sex chromosome DSD among individuals with atypical external genitalia are reported as 18–30%, 51–81%, and 1–8%, respectively [62, 66, 68]. Among 46,XX DSD, congenital adrenal hyperplasia accounts for the majority at 65–74% [62, 66, 69]. In contrast, a definitive diagnosis is achieved in only 24% [62] to 59% [68] of 46,XY DSD.

1) Physical examination

Detailed and accurate evaluation of external genital phenotype is essential. Assessment should include the location of gonads, length and width of the phallus, position of the urethral meatus, status of the scrotum/labia, presence of vaginal opening, and pigmentation. Particularly important is whether gonads are palpable, contributing significantly to sex assignment [62]. While Prader or Quigley classifications can evaluate the degree of androgenic effect on the external genitalia, they cannot predict which sex should be assigned. For more objective description of the external genitalia, the External Masculinization Score (EMS) was proposed in 2000

[70], and the External Genitalia Score (EGS) in 2020 [37]. Reference values for Japanese individuals have been reported for both penile length and clitoral width [31, 32]. When evaluating labial fusion, the anogenital ratio is utilized [33, 34].

2) Chromosomal analysis

The etiology of DSD is classified according to sex chromosomes, making chromosome analysis indispensable for diagnosis. G-band analysis is typically requested. G-band analysis reveals chromosomal composition and can detect the presence of multiple cell populations (*e.g.*, mosaicism or chimerism) [71]. When examining karyotype, fluorescence *in situ* hybridization (FISH) analysis or polymerase chain reaction (PCR) for *SRY* may be submitted simultaneously [72]. This is because FISH or PCR yields results more rapidly than G-banding. FISH can detect low-frequency (<10%) mosaicism [73, 74].

3) Endocrinological tests (See CQ5)

Basal serum LH, follicle-stimulating hormone (FSH), and testosterone levels should be measured and evaluated according to age-specific reference ranges [62, 75, 76]. Serum E2 is elevated in both male and female neonates and remains low in females with normal ovarian function until puberty; therefore, its utility for DSD

diagnosis is limited. hCG stimulation test and serum AMH measurement may be performed depending on the clinical phenotype [44, 62, 77]. While AMH is not covered by health insurance, it serves as an excellent marker for testicular function [78, 79]. In 46,XX DSD (or cases with non-palpable gonads and detectable Müllerian structures), 21-hydroxylase deficiency must be differentiated by confirming whether serum 17 α -hydroxyprogesterone is elevated. Serum electrolytes should also be measured every 24 to 48 hours until 21-hydroxylase deficiency is excluded. Testosterone levels in early neonatal period are prone to false elevation with immunoassays, limiting their evaluation. Urinary steroid profiling is useful for diagnosing adrenal and gonadal disorders [80, 81] but is currently unavailable in Japan.

4) Imaging studies (See CQ5)

US of the pelvis, inguinal canal, or labial/scrotal fold is essential primarily for evaluating the presence of Müllerian structures and the location and component of gonads [82–86]. For cases with non-palpable gonads that cannot be identified by US, pelvic MRI should be performed [87, 88].

CQ8: What diagnostic approach is recommended for delayed puberty?

Recommendation 8: For males aged 15 years or older and females aged 13 years or older without signs of puberty, we recommend serum gonadotropin and sex hormone measurements, and as needed, GnRH stimulation test, hCG stimulation test, imaging studies of the uterus and ovaries or pituitary, and chromosomal analysis. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

Evidence

1) Male individuals

Pubertal onset is determined by the appearance of secondary sexual characteristics. In males, the puberty begins with testicular enlargement, followed by penile growth and pubic hair development. When pubertal onset is defined as the point at which one testis reaches a volume of 3 mL or greater, the mean age ($\pm$ SD) of pubertal onset in Japanese boys is 10.8 ± 1.3 years [89]. The +2 SD for age of pubertal onset is 13.4 years, and +2.5 SD is 14.1 years. Considering that constitutional delay of puberty is more common in boys [90], diagnostic evaluation should be considered if secondary sexual characteristics are not observed by 15 years of age. While constitutional delay of puberty is the most common cause, other differential diagnoses include hypergonadotropic or hypogonadotropic hypogonadism. The most common form of congenital hypergonadotropic hypogonadism is Klinefelter syndrome, which is

observed in 195–254 per 10,000 individuals [91]. Congenital hypogonadotropic hypogonadism such as Kallmann syndrome is reported in 1.2–10 per 100,000 individuals.

a. Family history

Constitutional delay of puberty or hypogonadotropic hypogonadism in siblings and family members should be inquired. It is important to note that Kallmann syndrome has been reported in siblings, and some affected individuals may present with milder phenotypes that require careful evaluation [92].

b. Medical history

Information regarding the use of assisted reproductive technologies during pregnancy should be obtained. Previous history of hypospadias or cryptorchidism should be confirmed. Kallmann syndrome is known to be associated with anosmia/hyposmia, renal malformations, hearing loss, cleft lip or palate, and mirror movements. In some cases, Kallmann syndrome may be accompanied by central adrenal insufficiency or other anterior pituitary hormone deficiencies [92].

c. Physical examination

Height and weight growth curves and external genital findings should be evaluated. In the absence of secondary sexual characteristics, the pubertal growth spurt will not occur, resulting in a gradual decline in height SDS relative to chronological age. For external genitalia examination, the following should be assessed: whether the external urethral meatus opens at the tip of the glans, absence of hypospadias, absence of bifid scrotum, presence of palpable testes in the scrotum, normal testicular consistency, testicular volume ≥ 3 mL, and presence of pubic hair and pigmentation. Additionally, the sense of smell, presence of gynecomastia, and history of intracranial organic disease should be evaluated [93, 94]. In cases of malnutrition due to wasting diseases or anorexia nervosa, treatment of the underlying condition should take priority.

d. Endocrinological tests

Serum LH, FSH, and testosterone should be measured. Inhibin B is a useful marker for evaluating Sertoli cell function in the testes but is not available for measurement in Japan. AMH may be an alternative to assess Sertoli cell function [94], although this is not fully covered by health insurance in Japan.

e. Chromosomal analysis

Chromosomal analysis is essential in adolescent males presenting with delayed puberty and suspected hypergonadotropic hypogonadism. G-band analysis should be performed to confirm the presence of structural abnormalities or mosaicism of sex chromosomes. Results may depend on the tissue examined, and the proportion of mosaicism may differ between leukocytes and

tissues such as buccal mucosa [95].

f. Endocrinological stimulation tests

If gonadotropins, especially FSH, are markedly elevated, hypergonadotropic hypogonadism is indicated. hCG stimulation test may be performed to evaluate Leydig cell function in the testes [96]. Conversely, if gonadotropins are age-appropriate or low, GnRH stimulation test should be performed to differentiate between constitutional delay of puberty and hypogonadotropic hypogonadism [97, 98]. Performing an hCG stimulation test following a GnRH stimulation test can be effective in differentiating constitutional delay of puberty from hypogonadotropic hypogonadism [99], although differentiation may be difficult in some cases.

g. Imaging studies

Bone age should be assessed [99]. In cases of delayed puberty, bone age will be significantly delayed compared to chronological age. If testes are palpable in the scrotum or inguinal region, US should be performed to evaluate internal echotexture [82]. If gonads are not palpable in the scrotum or inguinal region, abdominal MRI with diffusion-weighted imaging should be performed to confirm the presence and location of gonads and internal genitalia [100]. In cases with hypospadias and intra-abdominal testes, abdominal MRI should be performed to confirm the presence of Müllerian structures. If gonads are difficult to identify by imaging, laparoscopic investigation or biopsy may be considered. If anosmia is present or if the GnRH stimulation test shows a reduced gonadotropin response, brain MRI should be performed to confirm the presence of olfactory bulbs and olfactory sulci. However, a mild reduction in gonadotropin response to GnRH and mild hypoplasia of the olfactory bulbs on MRI cannot completely rule out constitutional delay of puberty [101].

2) Female individuals

Puberty in girls begins with breast development, followed by pubic hair development and menarche. When the onset of puberty is defined as breast development reaching Tanner stage II, the mean age ($\pm$ SD) of puberty onset in Japanese girls is 9.5 ± 1.1 years or 10.0 ± 1.4 years [89]. Since the +2 SD for age of puberty onset is 11.7 to 12.8 years, and +2.5 SD is 12.3 to 13.5 years, diagnostic evaluation should be considered if no secondary sexual characteristics are observed by age 13 years. Although constitutional delay of puberty is an important differential diagnosis for delayed puberty onset in girls, it is less common than in boys [90]. On the other hand, since girls may have hypogonadism due to Turner syndrome, the proportion of hypergonadotropic hypogonadism is higher in girls than in boys [90]. Functional hypogonadotropic hypogonadism should be considered when thinness, rapid weight loss, or excessive

exercise is observed.

a. Family history

The presence of amenorrhea or delayed puberty in siblings and family members should be confirmed. In cases of AIS, it is not uncommon for multiple sisters to be diagnosed with the same condition following the identification of AIS in one sibling.

b. Medical history

Perinatal information, such as birth length and weight, should be collected. Females with Turner syndrome have small length and weight at birth, with a mean birth length ($\pm$ SD) of 46.8 ± 2.7 cm and a mean birth weight ($\pm$ SD) of 2.68 ± 0.44 kg in Japanese individuals with Turner syndrome [102]. Recurrent otitis media and horseshoe kidney are relatively common complications in Turner syndrome. A history of inguinal hernia (especially bilateral) should raise the possibility for AIS.

c. Physical examination

Height and weight growth curves, breast development with nipple swelling, and pubic and axillary hair developments should be evaluated using Tanner staging. Palpable gonads in the external genitalia or inguinal region should be examined. Turner stigmata, such as webbed neck, cubitus valgus, or shield chest, should be evaluated. The virilization, such as clitoromegaly, hirsutism, or acne, should also be assessed.

d. Endocrinological tests

Serum LH, FSH, and E2 levels should be measured. Since gonadal dysfunction is the most common in females with DSD are hypergonadotropic, gonadotropins are often elevated after age 10 years. GnRH stimulation test is rarely necessary. Females with Turner syndrome who develop spontaneous and cyclic menstruation have serum FSH levels less than 10 mIU/mL at age 12 years [103]. AMH is a useful test for evaluating ovarian reserve, but health insurance coverage is limited to the evaluation of ovarian function and determination of treatment strategies for infertility.

e. Chromosomal analysis

Karyotype should be assessed by G-band analysis of peripheral blood in adolescent females presenting with delayed puberty and suspected hypergonadotropic hypogonadism. Even if G-banding shows a 46,XX karyotype and obvious Turner stigmata are present, additional testing should be considered for the possibility of low-frequency mosaic Turner syndrome, such as increasing the number of analyzed cells or performing interphase FISH analysis of buccal mucosa.

f. Imaging studies

Pelvic US or MRI should be performed to evaluate gonads and internal genitalia. It should be noted that the uterus may exist but may not be identifiable on imaging such as MRI before puberty [104].

CQ9: What diagnostic approach is recommended for delayed menarche or primary amenorrhea?

Recommendation 9: For females aged 15 years or older who have not experienced menarche, we strongly recommend a comprehensive evaluation, including assessment of secondary sexual characteristics (breast and pubic hair development), measurement of serum gonadotropin and estradiol levels, imaging of the uterus, ovaries, or pituitary gland, and chromosomal analysis. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

Evidence

According to the definition by the Japan Society of Obstetrics and Gynecology, delayed menarche is defined as the absence of menstruation in females aged 15 years and older, whereas primary amenorrhea is defined as the absence of menstruation in females aged 18 years or older, regardless of secondary sexual characteristics. The etiologies include dysfunction of the hypothalamic-pituitary-ovarian axis, gonadal abnormalities, endocrine disorders, and congenital anomalies of the uterus or vagina. The diagnostic approach requires physical examination, endocrinological test, imaging studies, and chromosomal analysis [105].

1) Physical examination

Secondary sexual characteristics of breast and pubic hair developments should be evaluated according to Tanner staging. The presence of breast development suggests that estrogen is acting or has at least acted [105]. In addition to breast development, the external genitalia should be examined to further assess estrogen effects. Estrogen effect is determined by thickening of the hymen and its light pink color. For the external genitalia, pigmentation of the labia, labial fusion, presence of a common urogenital sinus, or clitoral hypertrophy should also be evaluated. A dermatological assessment should include evaluation for hirsutism and acne [106].

Breast and external genital examinations should be performed only with explicit informed consent and in a manner that ensures privacy, sensitivity, and respect for developmental stage and autonomy, particularly in adolescent females. Adolescent female patients may express less reluctance to being examined by a female healthcare provider. When a detailed examination of the external genitalia is necessary—particularly when procedures such as labial separation are involved—referral to a gynecologist may be appropriate to ensure diagnostic accuracy and to prioritize patient comfort and psychological safety.

2) Endocrinological tests

In cases of delayed menarche or primary amenorrhea with breast development, pregnancy should be excluded.

Initial endocrine assessments for delayed menarche or primary amenorrhea should include assessment of thyroid function, prolactin, LH, FSH, and E2. If LH and FSH levels are low, hypothalamic-pituitary dysfunction should be considered. Potential causes include constitutional delay of puberty, chronic disease, congenital hypogonadotropic hypogonadism, functional hypogonadotropic hypogonadism, and pituitary tumors. In such cases, neuroimaging of the hypothalamus-pituitary region is recommended. If LH and FSH levels are high, ovarian dysfunction should be suspected. Potential causes include Turner syndrome and certain forms of congenital adrenal hyperplasia. In such cases, pelvic imaging to assess the uterus and ovaries, as well as chromosomal analysis, is warranted [105, 107].

Although not currently covered by health insurance in Japan, AMH measurement in delayed menarche or primary amenorrhea can help assess ovarian function in individuals with Turner syndrome before the onset of puberty [108]. Based on studies in healthy young women, AMH levels below detection or <-2 SD suggest decreased ovarian function [109]. In females with Turner syndrome before the onset of puberty, AMH <0.1 ng/mL indicates that menstruation is unlikely to develop [110], whereas the likelihood of spontaneous menstruation in females with detectable AMH level is 19 times higher compared to those with undetectable levels [111]. The international clinical practice guidelines for Turner syndrome recommend annual AMH measurement from 8–9 to 11–12 years of age to avoid missing the appropriate time for fertility preservation [112].

3) Chromosomal analysis

Chromosomal analysis should be considered in all cases of delayed menarche or primary amenorrhea [113]. In a retrospective observational study from Turkey, 37% of 94 individuals with primary amenorrhea had chromosomal abnormalities, 12 of whom were reported as a 46,XY karyotype [114], indicating that chromosomal analysis can be useful in diagnosing DSD.

4) Imaging studies

US is typically sufficient for evaluating the internal genitalia [115]. MRI may provide additional useful information in some cases. In all 14 individuals who required surgical treatment for Müllerian duct abnormalities (9 with Mayer-Rokitansky-Küster-Hauser syndrome, 3 with transverse vaginal septum, 1 with imperforate hymen, and 1 with cervical agenesis), MRI findings demonstrated complete concordance with the intraoperative surgical observations in all cases examined [116]. MRI enables non-invasive evaluation of Müllerian duct abnormalities and can preclude the need for diagnostic laparoscopy [117]. Hypothalamic-pituitary MRI should be considered when hypogonadotropic hypogonadism is

suspected, particularly in individuals with low serum gonadotropin levels [105].

3. Hormone Replacement Therapy

CQ10: Is testosterone replacement therapy recommended for the induction of male secondary sexual characteristics?

Recommendation 10: For males with hypogonadism, we recommend testosterone replacement therapy for the induction of secondary sexual characteristics. (Strength of recommendation: Strong; Quality of evidence: Low)

Evidence

Testosterone is essential for the development of secondary sexual characteristics, maintenance of appropriate body composition, including bone and muscle mass, and overall physical and social well-being [118]. It also plays a critical role in socioemotional and cognitive development [119]. Before initiating testosterone therapy, gender identity should be evaluated to ensure alignment with assigned sex [118].

1) Age at the start of treatment

For males with hypogonadism at risk, pubertal assessment should begin around age 9 and continue through follow-up [118]. This early monitoring allows time for patient and family education and shared decision-making regarding treatment options.

Some reports suggest initiating treatment by 12 years of age, which is close to the average age of puberty onset, as delayed puberty has been associated with increased risks of anxiety disorders, depression [120], and cardiometabolic diseases [121]. However, no studies have compared treatment initiation ages in high-risk children with hypogonadism, and the optimal timing remains uncertain. If the severity of hypogonadism is difficult to determine and spontaneous puberty remains possible, treatment may be delayed until age 14–15. Treatment initiation age should be individualized based on considerations including height prognosis and psychological maturity.

2) Likelihood of pubertal development

The pattern of pubertal development varies by condition. In Klinefelter syndrome, puberty often begins at an average age, but with small, firm testes, high gonadotropins, and relatively low testosterone levels [94, 122]. While testosterone levels may initially rise, they typically begin to decline by around 15 years of age. Approximately 80% of adult males with 47,XXY karyotype have testosterone levels below the reference range, necessitating ongoing monitoring [123, 124]. In males with 45,X/46,XY mosaicism, 80% of them experience spontaneous puberty, but those diagnosed in infancy

due to atypical genitalia have a lower likelihood of spontaneous puberty. More than 50% of those diagnosed early required testosterone therapy during puberty, compared to 15% of those diagnosed in adulthood [125]. In males with ovotesticular DSD, pubertal development depends on testicular component function and varies by case.

3) Testosterone preparations

The majority cases of hypogonadism in DSD present with hypergonadotropic profiles. This endocrinological pattern necessitates the implementation of testosterone as the primary therapeutic agent for the induction of secondary sexual characteristics. Multiple testosterone formulations exist, but no high-quality evidence demonstrates superiority of any specific formulation. In Japan, intramuscular injection of depot testosterone enanthate are widely used and covered by the health insurance.

4) Dosage determination

Testosterone should be initiated at a low dose and gradually increased. Typical regimens start with testosterone enanthate 25–50 mg every 4 weeks, increasing by 50–100 mg every 6–12 months, reaching adult doses of 150–250 mg every 2–4 weeks over 2–3 years [126, 127]. Starting with testosterone enanthate 100 mg/month results in a significantly lower ratio of change in bone age to change in chronological age (Δ bone age/ Δ chronological age) and twice the height gain compared to starting with 250 mg/month [128]. However, 100 mg/month accelerates progression to Tanner IV–V genital stages compared to spontaneous puberty [129]. Therefore, starting with a lower dose and slower titration favors height gain, while starting with a higher dose and faster titration favors more rapid secondary sexual development. The titration schedule should be individualized based on height, psychological maturity, and age.

CQ11: Is estrogen replacement therapy recommended for the induction of female secondary sexual characteristics?

Recommendation 11: For females with hypogonadism, we recommend estrogen replacement therapy for the induction of secondary sexual characteristics. (Strength of recommendation: Strong; Quality of evidence: High)

Evidence

The induction of secondary sexual characteristics in girls with hypogonadism aims to mimic physiological puberty in terms of physical and psychological development, including an adequate growth spurt, adult height attainment, uterine maturation, establishment of menstrual cycles, appropriate cognitive development, and adequate bone mass acquisition. Although the

effectiveness of estrogen replacement therapy for secondary sexual characteristic induction has been demonstrated [130–138], it should be noted that most studies have targeted Turner syndrome. Studies reporting height as an outcome have been limited to those focusing only on Turner syndrome. With recent advances in assisted reproductive technology, women with hypogonadism can now achieve pregnancy; therefore, it is important to include uterine maturation as a treatment goal and to provide opportunities for future pregnancy. Additionally, it is necessary to assess gender identity before initiating estrogen replacement therapy [118]. It is crucial to discuss with parents how to inform the patient at the earliest appropriate stage and to explain the condition through a continuous, age-appropriate approach [139]. Three clinically important challenges in inducing secondary sexual characteristics are the timing of treatment initiation, determination of the appropriate dosage, and selection of suitable formulations.

1) Age at the start of treatment

In females aged 13 years and older without signs of secondary sexual characteristics, evaluation of gonadal function is recommended (see CQ8). When diagnosed with hypogonadism, estrogen replacement therapy should be initiated. In cases where DSD are diagnosed during the neonatal or early childhood period, the risk of hypogonadism can sometimes be evaluated before 13 years of age. Spontaneous menarche can be expected in cases of Turner syndrome with FSH below 6.7 mIU/mL at ages 6–10 years [140], and regular menstrual cycles be predicted in those with FSH below 10 mIU/mL at ages 10–12 years [103]. Although not fully covered by health insurance in Japan, when AMH levels in the prepubertal period are below –2 SD of the age-specific reference value, spontaneous onset of secondary sexual characteristics is unlikely [141]. When seeking for more physiological induction of secondary sexual characteristics,

early initiation of estrogen replacement therapy at ages 10–12 years may be considered, using the mean age of secondary sexual characteristic onset in the general Japanese female population (mean $\pm$ SD) of 10.0 ± 1.4 years [89] as a reference. Early initiation of estrogen replacement therapy was previously thought to be disadvantageous for achieving height potential due to possible acceleration of bone maturation. However, a meta-analysis comparing adult heights between early initiation (10–12 years) and late initiation (12 years and older) in Turner syndrome showed no significant difference in adult height between the two groups (difference –1.0 cm, 95% CI: –4.0 to 1.9 cm) [118], suggesting that early initiation does not negatively impact height prognosis.

2) Estrogen preparations

E2 preparations are considered the most favorable option as they represent the physiological form of estrogen and allow for serum concentration monitoring. Regarding the choice between oral and transdermal administration, transdermal delivery is generally preferred, as it bypasses the first-pass hepatic metabolism associated with oral administration. Oral administration has been linked to an increased risk of thrombosis due to hepatic production of coagulation factors, adverse effects on lipid metabolism, and a potential association with breast cancer in postmenopausal women [142]. Comparative studies of oral conjugated estrogens, oral E2, and transdermal E2 preparations for inducing secondary sexual characteristic have demonstrated that transdermal E2 is significantly superior in promoting breast development, BMD acquisition, and uterine growth and maturity [133, 138].

When skin problems prevent the use of transdermal preparations, oral preparations must be selected. Many preparations used in other countries are not available in Japan, necessitating preparation selection adapted to current availability (Table 5).

Table 5 Estrogen preparations

Type	Route of Administration	Brand Name	Formulation	Clinical Features
CE	Oral	Premarin®	0.625 mg/tablet	• Extracted from pregnant mare urine • Contains 10 different estrogens
E2	Oral	Julianna®	0.5 mg/tablet	• Off-label use in adolescent population
E2	Transdermal	Estrana®	0.09, 0.18, 0.36, 0.72 mg/patch	• Avoids hepatic first-pass metabolism • Off-label use
EE2	Oral	Procexol®	0.5 mg/tablet	• Inhibits IGF-1 synthesis <i>via</i> GH pathway • More potent than E2

CE: conjugated estrogens; E2: estradiol; EE2: ethinyl estradiol

3) Dosage determination

The initial dose of estrogen preparations should be kept low (1/8–1/4 of the adult dose), with most reports gradually increasing the dose over 2–2.5 years at 6–12 month intervals until reaching serum E2 concentrations equivalent to the reference range for adult females [131–134, 137, 138, 143]. Many guidelines recommend that when initiating therapy before 12 years of age, an even lower dose (1/24 to 1/16 of the adult dose) should be used initially, with gradual increases [112, 118, 143] (Fig. 3). The goal of estrogen replacement therapy is to mimic physiological secondary sexual characteristics, adjusting supplementation levels while periodically evaluating breast Tanner stage, bone age, and uterine volume. While individualization of dosage and duration of incremental increases is considered desirable, studies comparing fixed protocols *versus* protocols individualized by body weight show no difference in the progression of secondary sexual characteristics [136].

Regarding the timing of progesterone introduction, although there are no prospective studies, it is generally recommended when breakthrough bleeding occurs, after 2–3 years of estrogen supplementation, or when US examination shows uterine maturation and endometrial thickening [112, 118, 143].

CQ12: Is testosterone replacement therapy recommended for adult males with hypogonadism?

Recommendation 12a: We recommend testosterone replacement therapy for adult males with hypogonadism to maintain sexual function and QOL. (Strength of recommendation: Strong; Quality of evidence: Low)

Recommendation 12b: We suggest testosterone replacement therapy for adult males with hypogonadism may improve and maintain BMD. (Strength

of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

1) Maintenance of sexual function and QOL

According to the Guidelines for Male Hypogonadism in Japan, testosterone replacement therapy, administered either *via* intramuscular injection or transdermal application, is recommended for males with hypogonadism accompanied by erectile dysfunction, decreased QOL (as assessed by patient-reported outcomes), increased visceral fat, reduced muscle mass, and diabetes (evidence level A). For late-onset hypogonadism in aging males, the diagnostic criteria including serum total testosterone levels (morning) <2.50 ng/mL and free testosterone levels <7.5 pg/mL are not mandatory requirements for determining the indication for testosterone replacement therapy. Rather, the decision to initiate testosterone replacement therapy should be primarily based on the symptoms and signs of late-onset hypogonadism. In Klinefelter syndrome, one of the most common conditions of DSD, there is substantial evidence supporting the effectiveness of testosterone replacement therapy in adulthood following fertility treatment. The therapeutic approach is similar to that used for general late-onset hypogonadism [144]. In Japan, intramuscular injection of testosterone enanthate (250 mg every 3–4 weeks) is common, and testosterone ointments are also available as over-the-counter medications.

There are extremely few reports regarding the indications and administration methods of testosterone replacement therapy for adult DSD cases other than Klinefelter syndrome. Unlike late-onset hypogonadism, these individuals are younger, necessitating consideration of external genital development and maintenance of masculinization, growth including bone metabolism, and psychological development. The content of QOL assessment differs from late-onset hypogonadism, which primarily aims to improve fatigue and libido; treatment goals vary by case, and even within the same case, changes occur according to the life cycle. Regarding QOL in adulthood, the Dutch Study Group reported on 14 individuals with DSD (mean age: 27 years) using the Male Sexual Health Questionnaire. Compared to controls, these individuals reported concerns about penile length, external genital morphology, and ejaculatory function, which were associated with reduced sexual activity [145]. According to the dsd-LIFE study in Europe, 78.1% of 197 individuals with DSD who received testosterone replacement therapy reported satisfaction with the treatment [146]. This suggests that maintenance of external genital development and improvement of sexual function through testosterone

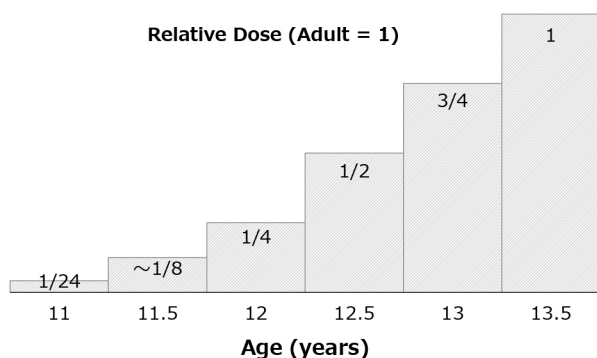


Fig. 3 An example of estrogen dose escalation

The numbers above the bars indicate the dosage as a fraction of the adult dose of estrogen preparations (adult dose = 1).

replacement therapy contribute to improved patient QOL.

The QOL of adult males with DSD is often discussed from the perspective of sexual function and is lower than that of control males but improves with testosterone replacement therapy [147]. On the other hand, it has been pointed out that because males with DSD are less likely to have partners, evaluation of sexual function may not represent an assessment of overall QOL [147]. Regarding testosterone replacement therapy, more comprehensive QOL-based assessment and consideration are necessary.

2) Improvement and maintenance of BMD

Testosterone plays an important role in bone growth and maintenance in males *via* its conversion to E2. In adult males with hypogonadism, reduced testosterone levels lead to decreased BMD. Randomized controlled trials have demonstrated that testosterone replacement therapy increases BMD, particularly in the lumbar spine and femur with a greater effect observed in trabecular bone compared to cortical bone [148]. However, there are currently no high-level evidence studies on whether testosterone replacement reduces fracture risk [149].

Despite limited evidence, testosterone replacement therapy in adult males with DSD and hypogonadism is considered effective and recommended for the maintenance of BMD [147, 150]. Osteoporosis or osteopenia has been reported in 42.5% of individuals with Klinefelter syndrome [151]. In individuals with hypogonadism due to Klinefelter syndrome, BMD in the lumbar spine and femur is significantly lower than in controls. Clinical studies have shown that testosterone replacement administered for three years [152] or for 48 weeks [153] significantly increases lumbar spine BMD. However, bone mass is not fully restored with testosterone replacement alone. This incomplete response is thought to be due to inadequate acquisition of peak bone mass [154] and coexisting vitamin D deficiency [155]. In an observational study including hypogonadotropic hypogonadism and Klinefelter syndrome, initiating testosterone replacement therapy at 18 years of age or younger was associated with a significantly greater increase in BMD compared to initiation at 19 years of age or older [156].

Although sufficient evidence has not been obtained, testosterone replacement may be effective in improving and maintaining BMD in males with DSD and hypogonadism. For those individuals, while discussion about fertility is necessary, we propose initiating testosterone replacement therapy as early as possible from the perspective of maintaining BMD.

CQ13: Is estrogen replacement therapy recommended for adult females with hypogonadism?

Recommendation 13a: We recommend estrogen replacement therapy for adult females with Turner syndrome or CAIS following gonadectomy to improve and maintain BMD. (Strength of recommendation: Strong; Quality of evidence: High)

Recommendation 13b: We suggest estrogen replacement therapy for females with other forms of DSD and hypogonadism. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

Adults with DSD often develop complications such as osteoporosis, metabolic syndrome, gonadal tumors, mental disorders, or decreased QOL. Empirical evidence supporting the efficacy of estrogen replacement therapy in ameliorating these complications remains limited [6].

In Turner syndrome, the risk of osteoporosis is increased due to estrogen deficiency secondary to hypogonadism [6]. Estrogen replacement therapy has been shown to be effective in maintaining and increasing BMD. Initiation is generally recommended between 12 and 15 years of age, with continuation advised throughout adulthood [157, 158]. However, estrogen monotherapy increases the risk of endometrial cancer; therefore, progesterone should be added once the estrogen dosage reaches adult replacement levels [159]. Turner syndrome increases the risk of insulin resistance and glucose intolerance; however, these conditions are not ameliorated by estrogen replacement therapy [160]. In another study, estrogen replacement therapy for Turner syndrome potentially improved endocrine abnormalities, hypertension, and vascular disorders, but did not improve mortality rates [161].

Female individuals with CAIS exhibit significantly lower serum estradiol level compared to adult female controls; the deficit of estrogenic effects, combined with the lack of androgenic effects, substantially increases their susceptibility to osteoporosis [162]. Therefore, regular evaluation of BMD is recommended regardless of a history of gonadectomy. After gonadectomy, BMD decreases but increases with estrogen replacement therapy [163]. Some reports suggest that testosterone replacement, instead of estrogen, may help maintain not only BMD but also sexual satisfaction; however, further investigation is needed [147, 164]. According to a short-term investigational study, both estrogen and testosterone replacement therapies for individuals with AIS potentially exacerbate lipid metabolism abnormalities. [165].

Only a few reports evaluated estrogen replacement therapy in other forms of DSD than Turner syndrome or CAIS. These reports suggest estrogen replacement

therapy for maintaining BMD in all cases with hypogonadism [6]. As with Turner syndrome, if the individual has a uterus, progesterone should be added once the estrogen dosage reaches adult replacement levels. Although 47,XXX often goes undiagnosed, premature ovarian insufficiency has been reported in some cases [166]. 48,XXXX is extremely rare, and while cases of premature ovarian insufficiency or secondary amenorrhea have been reported, the frequency of these complications remains unknown. Individuals with gonadal dysgenesis associated with Y-chromosomal materials (46,XY; 45,X/46,XY mosaicism; and related karyotypes) demonstrate increased susceptibility to gonadal tumor development. Prophylactic gonadectomy is often recommended for individuals with established risk factors for gonadal tumors, including young adulthood, undescended gonads, and specific genetic predispositions such as pathogenic variants in *SRY* or *WT1* [6, 147]. Estrogen replacement therapy becomes necessary after gonadectomy.

While estrogen replacement therapy for menopausal women shows several benefits, it may increase the risk of thromboembolic events, cardiovascular disorders, breast cancer, and endometrial cancer. The long-term outcomes of estrogen replacement therapy in individuals with DSD remain uncharacterized, necessitating further comprehensive investigation.

CQ14: Which adult-onset complications should be monitored, and what management strategies are appropriate for addressing these sequelae?

Recommendation 14: Important adult complications include osteoporosis, metabolic syndrome, gonadal tumors, psychiatric disorders, and diminished QOL. We recommend establishing individualized care plans considering these complications to determine appropriate examinations and treatment strategies. (Strength of recommendation: Strong; Quality of evidence: Expert opinion)

Evidence

Adult individuals with DSD may present with a wide range of complications, including osteoporosis, metabolic syndrome, gonadal tumor, psychiatric morbidities, and diminished health-related QOL (HRQOL), in addition to core sequelae such as hypogonadism, infertility, and psychosocial adaptation difficulties [6, 167]. Healthcare providers in adult specialty clinics, including endocrinology, obstetrics and gynecology, urology, and psychiatry, must possess comprehensive knowledge and clinical competence to identify, monitor, and manage these complex complications effectively through an integrated, multidisciplinary approach.

In individuals with DSD, decreased BMD due to sex hormone deficiency is a significant concern. The risk of osteoporosis is elevated in Turner syndrome as a consequence of hypogonadism. Estrogen replacement therapy has been shown to be effective in maintaining and improving BMD of the lumbar spine, and continuation of therapy into adulthood is recommended [158]. Since estrogen monotherapy increases the risk of endometrial cancer, progesterone should be added once estrogen reaches adult replacement dosage [159]. Similarly, decreased BMD is observed in Klinefelter syndrome, and testosterone replacement therapy has been shown to be effective in maintaining lumbar spine bone mass [168]. Reduced BMD is frequently documented in individuals with CAIS, with significant exacerbation observed following gonadectomy. Therefore, long-term estrogen replacement therapy is necessary to mitigate skeletal fragility [162]. Sex hormone replacement therapy in other forms of DSD is recommended for improving BMD.

In individuals with 21-hydroxylase deficiency, metabolic complications such as obesity, diabetes, hypertension, and dyslipidemia are observed, and cardiovascular disease is clearly increased [169]. These factors are not directly related to the pathophysiology or treatment of DSD but are believed to result from excessive glucocorticoid supplementation aimed at suppressing adrenal androgen excess. Individuals with Turner syndrome also exhibited increased risks of metabolic syndrome and cardiovascular disease. Although some of these complications improve with estrogen replacement therapy, mortality rates remain unaffected [161]. Metabolic syndrome is also seen in Klinefelter syndrome, requiring appropriate management [170].

Individuals with gonadal dysgenesis with Y chromosomes (46,XY, 45X/46,XY, etc.) demonstrate increased susceptibility to gonadal tumor development. Undescended gonads and specific genetic predispositions, such as pathogenic variants of *SRY* or *WT1*, increase the risk of gonadal tumor development. This risk is believed to rise during late adolescence to early adulthood. As there are no useful tumor biomarkers or imaging methods for early detection, gonadectomy may be considered [6, 167] (refer to CQ24, 25).

Psychological support is crucial for individuals with DSD, as these conditions are associated with an increased risk of anxiety disorders and depression [171]. Individuals with 21-hydroxylase deficiency demonstrate a higher risk of mental disorders and stress-related diseases, depending on age at diagnosis, treatment outcomes, and types of glucocorticoid preparations [172, 173]. In 46,XY DSD, impaired androgenic action is believed to increase the risk of depression, addiction, and eating disorders [147]. In Turner syndrome, a high

incidence of depression, reduced QOL, and low self-esteem has been reported [174]. In Klinefelter syndrome, increased risks of schizophrenia, bipolar disorder, autism spectrum disorder, and attention-deficit/hyperactivity disorder (ADHD) have been documented [175].

DSD encompasses a heterogeneous spectrum of complex conditions with multifaceted implications; current therapeutic interventions aimed at optimizing HRQOL remain inadequate according to contemporary outcome assessments. Since most studies target aggregates of various forms of DSD, QOL assessment for each specific condition has not been adequately conducted. Some reports suggest that reductions in QOL are more prominent in psychosocial domains than physical domains [176, 177].

As described above, various complications are observed in DSD during adulthood. Although clinical research remains limited due to the rarity of DSD, it is recommended to develop an individualized care plan that takes these complications into account, with corresponding diagnostic and therapeutic strategies.

4. Surgical Management

CQ15: Is preoperative testosterone administration recommended before male genital reconstruction?

Recommendation 15: We suggest preoperative testosterone administration for individuals with micropallus undergoing hypospadias repair, as this may increase penile size and potentially facilitate earlier surgery. Both positive and negative effects on surgical outcomes have been reported; however, comprehensive evidence regarding patient characteristics, methods of administration, adverse effects, surgical complications, and reoperation rates is still lacking. (Strength of recommendation: Weak; Quality of evidence: Very low)

Evidence

The most common and representative surgical procedure for male genital reconstruction in individuals with DSD is the surgical correction of hypospadias. Hypospadias is a condition where the urethral opening, normally located at the tip of the glans, is found on the ventral side of the penis due to a disruption in penile urethral development. This is one of the common signs observed in DSD. The position of the urethral opening varies widely among cases, ranging from the glans to the penile shaft, scrotum, or perineum. Hypospadias is typically associated with penile curvature and incomplete foreskin formation. In the context of DSD, it is frequently accompanied by reduced penile size (“micropallus”), which increases surgical complexity and often necessitates preoperative consideration of

testosterone administration. Testosterone administration is expected to enhance surgical conditions by promoting penile growth, particularly increasing glans width. Among the reviewed literature, although several prospective studies, systematic reviews, and meta-analyses with relatively high levels of evidence [178–187] are available, comprehensive evaluations of surgical outcomes, complications, reoperation rates, and adverse effects remain limited. Furthermore, indications for testosterone administration and treatment protocols vary considerably across studies.

A recent systematic review of 25 papers including 4,094 individuals found that short penile length is a crucial factor for the indication for preoperative testosterone administration, despite significant methodological heterogeneity among the included studies. The most commonly reported intramuscular testosterone injection regimen uses either 2 mg/kg or 25 mg monthly, with administration occurring 2–3 months before surgery. Intramuscular testosterone administration significantly promotes penile length development, with changes peaking after 2–3 months. The impact of preoperative testosterone administration on complications and side effects varies across studies. This variability may be attributed to differences in sensitivity to testosterone, severity of hypospadias, surgical techniques, and administration methods [178].

Li and colleagues evaluated the quality of existing literature reporting the effect of preoperative testosterone administration on postoperative complication rates in hypospadias repair using the fragility index, which assesses the robustness of statistical significance [179]. According to this report, the cited studies have methodological and statistical limitations that preclude drawing definitive conclusions regarding the impact of preoperative testosterone administration on hypospadias repair outcomes. Currently, there are no standardized criteria regarding the indication, dosage, or method of testosterone administration prior to hypospadias surgery. Therefore, as a recommendation for this clinical question, we “suggest” administering testosterone before performing hypospadias surgery in cases with micropallus, as it may increase penile size, empirically improve surgical difficulty, and potentially allow earlier surgical intervention. Both positive and negative effects on surgical outcomes have been reported; however, comprehensive evidence regarding patient characteristics, methods of administration, adverse effects, surgical complications, and reoperation rates is still lacking. Well-designed randomized controlled trials are needed to accurately assess the true effects of preoperative testosterone administration.

CQ16: When and which gonads should be removed in case at risk for gonadal tumors such as intra-abdominal gonads?

Recommendation 16: We recommend considering gonadal biopsy or gonadectomy as additional options alongside regular ultrasound monitoring in individuals with DSD who are at high risk for gonadal tumors. However, clear criteria regarding the indications and timing of prophylactic gonadectomy have not yet been established. (Strength of recommendation: Weak; Quality of evidence: Very low)

Evidence

1) Gonadectomy

The risk of gonadal tumors may vary depending on the specific DSD condition, although the natural history remains unclear. The incidence of germ cell neoplasia *in situ* (GCNIS) or germ cell tumors at the time of gonadectomy increases with age, with a notable acceleration in incidence between 15 and 20 years of age, regardless of gonadal tumor risk classification [188]. The 5-year recurrence-free survival and overall survival rates in individuals with GCNIS are equivalent to those without GCNIS/germ cell tumors; however, individuals with established germ cell malignancies exhibit significantly inferior survival outcomes [188].

Given the heterogeneity of conditions and varying risks of gonadal tumor development in DSD, tumor risk has recently been stratified according to specific conditions [189, 190]. However, although these risk classifications serve as references for determining treatment strategies, they cannot be considered sufficient evidence, and treatment approaches differ depending on the assigned gender, making the indications and timing for gonadectomy unclear [190]. Additionally, numerous factors must be taken into account when deciding whether to perform gonadectomy. These include patient age (with increased risk at older ages), race (higher risk among Caucasians), gonadal location (elevated risk for intra-abdominal gonads), genital phenotype, fertility potential, gonadal function (potential for development of secondary sexual characteristics), ability to perform self-examination, surgical risks, side effects of sex hormone replacement therapy, patient comprehension and cooperation, as well as the patient's gender identity [189, 191-193].

The Pediatric Urologic Oncology Working Group of the Societies for Pediatric Urology recently reported the incidence of GCNIS and germ cell tumors and long-term outcomes in 83 DSD individuals who underwent surgery. Fourteen individuals (16.9%) underwent gonadal biopsy, and 71 (85.5%) underwent gonadectomy (50/71 had bilateral gonadectomy). GCNIS or germ cell tumors

were identified in 8/83 individuals (9.6%) (7 with GCNIS, 1 with germ cell tumor). The median age at surgery was 2.95 years in individuals without GCNIS/germ cell tumors and 14 years in those with GCNIS/germ cell tumors. All 8 individuals with GCNIS/germ cell tumors were considered as high-risk or intermediate-risk. Among high-risk individuals, 8/54 (15%) had GCNIS/germ cell tumors. No individuals received post-operative adjuvant therapy or experienced recurrence, and all survived during a mean follow-up period of 6.4 years. Thus, most individuals with DSD who underwent gonadectomy showed no malignant pathological findings. All cases with GCNIS or germ cell tumors were classified as high or intermediate risk, and treatment was completed with surgery alone without recurrence [190].

2) Imaging studies

In individuals with DSD who underwent prophylactic gonadectomy, 40–50% of the gonads were confirmed upon surgical examination [85]. However, no imaging modality reliably detects precancerous lesions [85], and although follow-up using regular ultrasound and other imaging techniques is somewhat effective, inherent limitations remain

3) Gonadal biopsy (see CQ24)

Gonadal biopsy is one option for evaluating gonadal tumors since gonadal function can be preserved. However, gonadal biopsy does not necessarily reflect the histological appearance of the entire gonad and may miss the presence of GCNIS or germ cell tumors [194].

Based on the above considerations, this guideline proposes considering gonadal biopsy or gonadectomy as options in addition to regular US and other alternatives for DSD with high risk of gonadal tumor development, such as intra-abdominal gonads. However, clear criteria for the indications and timing of prophylactic gonadectomy have yet to be established, and further accumulation of evidence is needed.

CQ17: When and what types of surgical procedures are recommended for females with clitoromegaly, labial fusion, urogenital sinus, vaginal agenesis, or vaginal agenesis or hypoplasia?

Recommendation 17a: We suggest an individualized approach, in consultation with an expert team, to determine the optimal timing of female external genital reconstruction or vaginoplasty, following the provision of comprehensive information to both parents and individuals. (Weak recommendation, Very low evidence level)

Recommendation 17b: We suggest clitoroplasty with preservation of neurovascular bundles, labioplasty utilizing periclitoral skin, urogenital sinus mobilization with perineal skin flaps, and vaginal reconstruction

using peritoneum or intestine as the main surgical procedures. (Strength of recommendation: Weak; Quality of evidence: Very low)

Expert

The optimal timing of feminizing genitoplasty and vaginoplasty in individuals with DSD remains controversial. Traditionally, early surgery during infancy has been considered optimal from a psychosocial perspective [195]. However, recent perspectives suggest that irreversible surgical procedures should be delayed until the patient is sufficiently mature to make their own informed decision. [196].

In surveys of adolescent and young adult (AYA) with DSD who underwent early surgery, the majority shows understanding of their parents' decision to pursue early surgery and reported improved functional and cosmetic satisfaction, although some wish to have been involved in the surgical decision-making process [197]. The benefits of early surgery in infancy include faster healing due to tissue flexibility, reduced anxiety for both parents and individuals regarding genital appearance, and preferable effects on the development of gender identity [198]. The disadvantages of the early surgery include the potential gender incongruence and clitoral sensory impairment in later life. Some experts have noted a high frequency of additional surgery due to postoperative vaginal stenosis. However, no data are currently available comparing the long-term outcomes between individuals who underwent surgery in infancy and those who did not [198, 199]. A large-scale European survey of 459 individuals with DSD aged 16 years and older demonstrated diverse patient opinions regarding surgical timing; females with 21-hydroxylase deficiency tend to prefer early surgery, while females with 46,XY DSD tend to prefer delayed surgery [200]. Low risks and satisfactory results from early surgery were reported in females with 21-hydroxylase deficiency who underwent surgery in infancy regardless of age at surgery or severity of virilization [195]. An expert team should thoroughly explain the advantages and disadvantages of early surgery to parents, who are in a position to consider the best interests of individuals with DSD, when making decisions regarding early surgery [196, 201].

Conversely, some DSD patient advocacy groups argue that genital surgery should be deferred until individuals are able to actively participate in decision-making process [201]. Individuals and parents who collaborate with the advocacy groups often prefer later surgery [200]. The primary advantage of delaying surgery is that it allows individuals to participate in the decision-making process regarding their own bodies. Expert teams should provide comprehensive information about surgical options during

adolescence to both individuals and their parents, enabling informed decision-making. [198]. However, the age at which individuals are capable of making informed decisions varies, and the psychosocial impact of growing up without early surgery must be carefully weighed when determining the timing of surgical intervention on a case-by-case basis [198]. Notably, clitoroplasty performed during adolescence or later is associated with increased risks of bleeding and wound dehiscence [199].

Female genitoplasty for clitoral hypertrophy, labial fusion, and urogenital sinus in individuals with DSD typically includes clitoroplasty with preservation of the neurovascular bundles and reduction of the corporal bodies, labioplasty using periclitoral skin, cutback of the urogenital sinus, urogenital sinus mobilization with perineal flaps (either posterior or lateral), and formation of the anterior vaginal wall using the excess urogenital sinus tissue [195, 202]. Vaginoplasty techniques for vaginal agenesis or hypoplasia in DSD include reconstruction using substitute vagina with peritoneum or intestinal segments [203, 204]. Postoperative complications may include vaginal stenosis, delayed bleeding, wound dehiscence, urinary tract infection, urinary retention, urethrovaginal fistula, rectal injury, and labial atheroma [195, 199, 202, 203].

An important aspect of surgical outcomes is future individual satisfaction [195]. Among 500 individuals with DSD aged 16 years and older who underwent genital or breast surgery, approximately 20% express dissatisfaction with functional outcomes—such as vaginal stenosis or clitoral/glans sensation—and cosmetic appearance [205]. In AYA with DSD who developed vaginal stenosis following early vaginoplasty, additional vaginoplasty achieved adequate vaginal caliber and enabled sexual intercourse [203]. Urogenital sinus mobilization with perineal flaps is effective for treating postoperative vaginal stenosis. In cases of severe vaginal scarring or high vaginal stenosis, vaginal reconstruction using intestinal segments should be considered.

CQ18: Are female genitoplasty for clitoral hypertrophy, labial fusion, and urogenital sinus, and vaginoplasty for vaginal agenesis or hypoplasia recommended to improve sexual health in adulthood? Recommendation 18: We suggest performing female genitoplasty and vaginoplasty to improve satisfaction regarding sexual health in adulthood. We suggest providing continuous support and information through an expert team to address patient preferences regarding dissatisfaction with the appearance or function of external genitalia or vagina after reconstruction surgery. (Strength of recommendation: Weak; Quality of evidence: Very low)

Evidence

In 37 AYA with DSD who underwent female genitoplasty or vaginoplasty in infancy, the majority of individuals are functionally and cosmetically satisfied [197]. Among 500 individuals with DSD aged 16 years or older who had undergone genital or breast surgery, 22% reported dissatisfaction with appearance and 20% with function (*e.g.*, vaginal stenosis or reduced clitoral/glans sensation), while 13% were very dissatisfied with both aspects. However, many respondents reported that the surgical procedures had not disrupted their lives and had resulted in favorable outcomes [205].

Among 95 females with 21-hydroxylase deficiency, more than half of the 53 who were aware of clitoral hypertrophy reported anxiety and decreased self-esteem, along with tendencies toward negative feelings related to gender identity and body image [206]. In 459 individuals with DSD aged 16 years or older, females with 21-hydroxylase deficiency tend to prefer early surgery and clitoroplasty, although patient opinions regarding surgical timing are diverse [200]. According to a systematic review and meta-analysis of females with 21-hydroxylase deficiency who underwent feminizing genitoplasty or vaginoplasty, many were sexually active and expressed satisfaction with having undergone surgery; however, only about half reported satisfaction with their sexual lives [207]. Many individuals struggled with clitoral sensory impairment, dyspareunia, and infrequent sexual intercourse [207]. Surgical complications included vaginal stenosis in 27% of cases, while fistula formation, urinary incontinence, and urinary tract infections were infrequent [207].

de Neve-Enthoven *et al.* compared sexual self-concept and sexual function between 99 females with DSD (aged 17–60 years) and 589 control females (aged 18–68 years) [208]. Females with DSD demonstrated less interest in sexual activity, lower levels of sexual activity, reduced satisfaction with external genitalia, and lower sexual self-concept and sexual function compared to control subjects [208]. Conversely, sexually active females with DSD tended to have a more positive sexual self-concept, and cognitive-behavioral sex therapy was found to improve their sexual well-being [208]. As AYA with DSD grow older, they express a greater desire for information. Healthcare providers must acknowledge sexual diversity without bias and offer continuous support and information [209].

CQ19: Is it recommended to delay surgical treatment until the patient reaches an age when they can make their own decisions?

Recommendation 19: There is insufficient evidence to recommend either early surgery based on parental

decision-making or delayed surgery pending patient autonomy at the age of informed consent. We suggest a collaborative shared decision-making approach in consultation with an expert team, incorporating comprehensive risk-benefit analyses comparing early versus delayed surgical interventions. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

The optimal timing of surgical treatment such as female genitoplasty in DSD has been debated for many years [210, 211]. Traditionally, early surgery during infancy has been recommended with the goals of achieving genital appearance consistent with assigned gender and promoting psychosocial development [210, 211]. This approach is based on several advantages: favorable outcomes are more easily achieved in surgery during infancy due to tissue flexibility and high healing capacity; parental anxiety and psychological stress are reduced; children experience less psychological trauma with subsequent gender identity development less likely to be impaired; and risks of complications such as urinary tract infections are reduced [205, 211]. Recently, there has been growing advocacy for delaying irreversible surgeries until individuals can make autonomous decisions, based on the principles of respecting the autonomy of affected individuals and avoiding irreversible procedures in cases where gender incongruence may later emerge [197, 210–213]. However, the psychosocial impact of delayed surgery must be carefully considered, including concerns about effects on body image and self-esteem during adolescence, as well as the potential influence of parental anxiety and guilt on parent-child attachment formation [211, 214]. Therefore, current international guidelines recommend careful judgment according to individual cases, emphasizing the importance of balancing functional and psychosocial outcomes when deciding on surgical intervention [196, 215].

The shared decision-making process involving individuals, their parents, and the expert team is critical to determining the optimal timing of surgery [196, 211, 216, 217]. Under comprehensive support from the expert team, this process includes providing detailed information about diagnosis and prognosis to individuals, explaining the potential risks and benefits of surgery, considering factors influenced by delayed surgery, understanding the values and preferences of individuals and their parents, offering psychological support, and developing long-term follow-up plans [6, 211, 216]. Key principles include ensuring that individuals and their parents receive sufficient information and adequate time for

comprehension, offering comprehensive care that integrates psychological support, encouraging developmentally appropriate participation in decision-making, regularly reviewing and adjusting treatment plans as needed, and providing long-term follow-up that respects their values and preferences.

Currently, evidence remains insufficient to definitively determine whether early or delayed surgery is superior. Consequently, many experts now advocate for individualized approaches tailored to each case [210, 211, 215]. Careful consideration is especially necessary for irreversible surgeries. In contrast, early medical interventions are advisable for functional issues, such as surgery for hypospadias or gonadectomy when there is a high risk of malignancy. A large-scale European survey of 459 individuals with DSD demonstrated diverse patient opinions regarding the optimal timing of surgery [200]. Females with 21-hydroxylase deficiency and males with 46,XY DSD tend to prefer early surgery, whereas females with 46,XX DSD tend to prefer delayed surgery for vaginoplasty. Recently, the importance of genetic test in diagnosis and management of DSD has been highlighted [196]. New technologies such as whole exome sequencing or whole genome sequencing are expected to enable accurate diagnosis and individualized management [196]. Based on these research findings, establishing uniform criteria for the optimal timing of surgery is challenging and necessitates flexible responses tailored to individual cases. These guidelines do not provide definitive recommendations on the optimal timing of surgery; rather, they advocate for individualized clinical decision-making through a collaborative shared decision-making process.

Determining the optimal timing of surgical intervention in individuals with DSD requires meticulous, individualized assessment that encompasses a comprehensive evaluation of clinical circumstances specific to each individual, underlying diagnostic pathology, familial values and preferences, and available institutional healthcare resources. Both early surgery and delayed surgery have advantages and disadvantages, and evidence is currently insufficient to clearly recommend one approach over the other. Therefore, it is important to seek the best choice through comprehensive approaches by expert teams and shared decision-making processes with individuals and parents.

5. Psychological Support

CQ20: When and how is it recommended to disclose information directly to the individuals concerned?

Recommendation 20: We suggest disclosing information to individuals in a staged manner, accompanied by comprehensive psychological support, while

acknowledging the substantial variability in clinical presentations and cognitive capacities among individuals. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Expert

Studies addressing this CQ have been predominantly based on semi-structured interviews or questionnaire-based research. Due to the lack of before-and-after comparisons or control groups in the available studies, the evidence level for the recommendation addressing this clinical question is classified as expert opinion.

While there is consensus on the importance of disclosing information to individuals, clear evidence is lacking concerning who should disclose the information, as well as when and how it should be disclosed. A study involving 171 physicians from the Pediatric Endocrine Society examined responses to hypothetical cases based on a scenario of an 11-year-old female diagnosed with CAIS shortly after birth, in which the parents initially did not wish for the physician to explain the discordance between sex chromosomes and phenotype. The majority of respondents indicated that both chronological and developmental ages are important factors when deciding the timing of disclosing an individual's condition. While parental preferences also influence this decision, most respondents felt that disregarding parental preferences may sometimes be justified [218].

A semi-structured interview study involving 24 individuals with DSD, 19 parents, 37 healthcare providers, and 30 other stakeholders revealed no consensus regarding the timing of information disclosure. However, many respondents emphasized that information should be provided in accordance with the cognitive abilities of the individuals. The use of neutral and positive language, avoiding pathological terminology and refraining from using the word "normal," was considered desirable. Appropriate education was reported to facilitate health and lifestyle management, with adequate understanding contributing to positive behaviors. Divergent opinions emerged regarding whether comprehensive information should be provided solely by physicians or collaboratively by both parents and physicians [219].

Semi-structured interviews with nine individuals diagnosed with 46,XY or 46,XX gonadal dysgenesis, including four with CAIS (age at disclosure: 8–25 years; age at interview: 20–26 years), revealed that although all participants had been informed about their condition, few could recall the diagnostic terminology or specific details, and some reported difficulty processing the information. Life experiences prior to receiving a diagnosis influenced how individuals processed and responded to medical information at the time of disclosure. Although

physicians often attempt to normalize patient experiences as a form of support, this strategy is not always beneficial. Individuals commonly express concerns regarding potential social, practical, and existential challenges following a diagnosis [220].

A report analyzing data from 903 individuals with DSD aged 16 years or older (participants in the dsd-LIFE study: 284 with Turner syndrome, 233 with 46,XY DSD, 206 with congenital adrenal hyperplasia, and 180 with Klinefelter syndrome) found that greater openness was inversely associated with depressive symptoms and anxiety. These findings suggest that information disclosure should be guided by the individual's psychological state rather than by uniform criteria such as chronological age alone [221].

Based on these findings, the 2018 DSD consensus statement recommends initiating disclosure of the condition from an early stage using age-appropriate language, with the goals of fostering acceptance and minimizing fear and stigma [6]. This process is emphasized as being continuous and adaptive to the individual's developmental stage.

CQ21: Is psychological support recommended to improve QOL?

Recommendation 21: We suggest providing psychological support through a multidisciplinary expert team and considering peer support, as these may resolve various issues related to mental health and sexuality and improve QOL. (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

No empirical studies addressing this clinical question have employed before–after comparisons or case–control designs. Thus, the recommendation for this CQ is based on expert opinion.

The QOL of individuals with DSD is significantly lower compared to control groups [177, 222, 223]. A questionnaire survey involving 86 individuals with DSD aged 8 to 12 years revealed reduced QOL in areas such as self-esteem, physical health, and school functioning [224]. Mental health issues were identified as contributing factors to the diminished QOL. Some forms of psychiatric disorders are observed in 24% of those with 46,XY DSD (gonadal dysgenesis, AIS, 5 α -reductase deficiency) [225], 30.5% with 46,XX DSD [226], and 45% with 46,XY DSD. Sixty-eight percent of individuals with congenital adrenal hyperplasia, 5 α -reductase deficiency, and CAIS have one or more mental health problems during their lifetime [227]. Males with Klinefelter syndrome are more likely to have mental health problems compared to other males with DSD [171]. High

rates of suicidal ideation and frequent use of psychological counseling for mental health problems are observed in females with congenital adrenal hyperplasia, virilized 46,XX females, and 46,XY females [228]. Moreover, concerns surrounding sexuality are recognized as important determinants of QOL [229]. Limited sexual experiences such as romantic relationships and sexual activity [208, 230–232] and dissatisfaction with one's sexual life [233] are associated with reduced QOL in individuals with DSD.

Conversely, individuals with DSD receiving psychological support maintain their QOL [234–237]. Providers of psychological support are required to conduct regular assessments of mental health and gender–sexuality development in individuals with DSD [238, 239], as well as to possess an understanding of diverse expressions of sexuality, including gender identities beyond the male–female binary and same-sex relationships [230, 238–240]. Therefore, psychological support for individuals with DSD should ideally be provided by a multidisciplinary expert team [171, 208, 230–232, 241, 242].

Although the clinical community universally acknowledges the necessity of comprehensive psychosocial support for individuals with DSD, empirical data detailing specific individual needs and assessing the adequacy of existing support services remain critically limited. Although evidence demonstrating improvements in HRQOL through psychosocial interventions remains limited, individuals consistently emphasize the critical importance of structured peer support programs in their care experiences [243].

Based on these aforementioned findings, it is recommended that comprehensive psychological support services, delivered by multidisciplinary expert teams with specialized expertise in mental health and psychosexual development, be systematically integrated into routine clinical care pathways. Additionally, structured psychosocial support interventions, including evidence-informed peer support programs, should be implemented to optimize HRQOL outcomes in individuals with DSD.

CQ22: What types of psychological burdens or mental disorders may manifest in parents at the time of diagnostic disclosure of DSD in their neonates or during subsequent development stages?

Recommendation 22: Parents may experience significant stress related to “Illness Uncertainty” regarding their child's future following disclosure, and may develop long-lasting symptoms of post-traumatic stress symptoms, anxiety symptoms, or depressive. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

Evidence

The diagnosis of DSD generates personal, familial, and social concerns for parents, imposing a significant psychological burden [244]. Parents face various mental health challenges during their child's neonatal period and throughout subsequent development, experiencing elevated stress levels not only at the time of initial diagnosis but also long-term, including following sex assignment or surgical interventions [245]. When a child is diagnosed with DSD and sex of rearing cannot be immediately determined, parents experience psychological shock and intense stress due to their inability to predict their child's future psychosexual and psychosocial development [201]. This suggests that parents of children with DSD require timely provision of appropriate information and ongoing support from the earliest stages.

Among parents who have received disclosure regarding their child's DSD, anxiety and depressive symptoms are commonly observed initially [246, 247]. Although many parents adapt over time, some continue to experience persistent psychological distress, and others who initially appear to adapt may later develop progressively worsening depressive symptoms [248]. Parents experiencing high levels of stress tend to exhibit increased anxiety and depressive symptoms [249]. Additionally, heightened stigma directed toward both the parents and their children contributes to further elevation of parental anxiety and depressive symptoms [250]. Similar to parents of children with other chronic conditions, parents of children with DSD tend to perceive their children as more vulnerable [251]. Mothers of male children, in particular, report perceiving their children as more vulnerable [252] and experiencing greater stigma than fathers [253], along with higher levels of post-traumatic stress symptoms [212, 254], depressive symptoms [255], and anxiety symptoms [256].

Post-traumatic stress symptoms are associated with cognitive confusion about DSD [248]. DSD particularly tends to cause parents to experience "illness uncertainty." Parents of children with DSD worry more about their children's future compared to parents of children with other endocrine diseases [257]. Since this "uncertainty" is associated with anxiety and depressive symptoms [247], psychological interventions and support addressing DSD-related "uncertainty" may alleviate post-traumatic stress symptoms [201]. Additionally, insufficient knowledge about surgery contributes to "uncertainty," highlighting the need for written information to supplement limited consultation time [257]. Parents of male children tend to experience more uncertainty than parents of female children [245] and have stronger depressive symptoms [258].

The psychological and psychosocial support interven-

tions have been proposed therapeutic modalities to mitigate parental psychological distress and facilitate adaptive coping mechanisms [259, 260]. Support contents desired by parents includes mental health, advocacy for schools and communities, and fertility [259]. In addition to these needs, 40.4% of parents seek psychological counseling or psychotherapy. Particularly high needs are observed among parents of children with PAIS (65%), partial gonadal dysgenesis (54%), and androgen synthesis disorders (50%) [260]. Future development and validation of effective interventions to alleviate parental psychological burden are essential.

CQ23: What is the prevalence of gender incongruence and psychiatric disorders as comorbidities?

Recommendation 23: Gender incongruence may present as a comorbidity in 15% of cases (95% confidence interval: 13–17%). Certain pathological conditions and diseases are associated with psychiatric disorders, including depression and anxiety disorders. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

Evidence

Gender identity disorder, previously classified as a psychiatric disorder in ICD-10, was reclassified as gender incongruence in the revised ICD-11 (2022), removing it from the category of psychiatric disorders and repositioning it under "conditions related to sexual health." While gender identity disorder was characterized by identification with the opposite sex, gender incongruence is defined as a marked incongruence between experienced gender and assigned sex. This definition does not require identification with the opposite sex and includes individuals who identify as non-binary. All evidence relevant to this CQ is based on the diagnosis of gender identity disorder, not gender incongruence. Since the diagnostic term gender identity disorder is no longer in use, we will express this as gender incongruence in this chapter.

Sex assignment for newborns or infants with DSD represents a critical and challenging decision for healthcare providers, as some individuals may experience gender incongruence during or after puberty. The prevalence of gender incongruence in DSD varies according to specific underlying conditions. A 2021 systematic review examined 20 papers reported between 2005 and 2020, analyzing 21-hydroxylase deficiency, CAIS, PAIS, 5 α -reductase deficiency, 17 β -hydroxysteroid dehydrogenase deficiency, mixed gonadal dysgenesis, and complete gonadal dysgenesis [12]. Gender incongruence among individuals aged 12 years or older with these conditions

is estimated to occur in approximately 15% of cases (95% confidence interval: 13–17%) [12].

Females with sex chromosome DSD develop gender incongruence at a rate of 22%, whereas it is rarely observed in males. Among individuals with 21-hydroxylase deficiency, the rate of gender incongruence is 4% in females but significantly higher at 15% in males. All individuals with CAIS are phenotypically female, with a low rate of incongruence of 2%. In PAIS, the rate of gender incongruence is 12% in females and 25% in males [12].

No comprehensive studies exploring risk factors for psychiatric comorbidity in individuals with DSD have been published. A European cross-sectional cohort study involving 1,040 individuals with DSD, comprising 717 females, 311 males, and 12 individuals of other genders, reported that 5% had undergone sex change. Among the 39 participants (4%) exhibiting gender role change, including identification as male, female, neither male nor female, or differing gender expression, this subgroup demonstrated lower self-esteem and higher rates of comorbid anxiety disorders and depression compared to other participants [240].

The prevalence of psychiatric comorbidities differs by DSD condition. Eighty-five percent of females with CAIS or complete gonadal dysgenesis have at least one psychiatric disorder, most commonly depression and anxiety disorders [261]. Adults with DSD are significantly mentally distressed, with rates of suicidal tendencies and self-harm comparable to non-DSD females with histories of physical or sexual abuse [262]. Individuals with Mayer-Rokitansky-Küster-Hauser syndrome demonstrate significant negative correlations between sexual self-esteem and symptoms of depression, social introversion, and anxiety [263]. They commonly exhibit social anxiety, experience restricted sexual lives, and report decreased mental QOL [264].

Consequently, healthcare providers must recognize that certain conditions of DSD are associated with elevated prevalence rates of psychiatric comorbidities, including major depressive disorder and anxiety spectrum disorders. Furthermore, individuals with DSD may experience not only gender incongruence or desires for gender transition but may also identify along a broader gender spectrum beyond traditional binary classifications. This underscores the necessity for access to specialized psychological counseling and gender-affirming mental health services.

6. Gonadal Tumors

CQ24: Is gonadal biopsy recommended for the risk assessment of gonadal tumors?

Recommendation 24: While gonadal biopsy may be

useful for assessing the risk of gonadal tumors in individuals with DSD who possess Y-chromosome material, clear evidence supporting routine use of gonadal biopsy is currently lacking (Strength of recommendation: Weak; Quality of evidence: Expert opinion)

Evidence

Individuals having intra-abdominal gonads with Y chromosome materials have high risk of gonadal tumors. Prophylactic gonadectomy is generally performed in such individuals to reduce the risk of malignancy. Conversely, if the gonads are preserved for any reason, continuous monitoring for malignant transformation is essential, and biopsy may serve as a useful tool for tumor surveillance. The utility of gonadal biopsy must be validated through direct comparison with gonadectomy, as well as evaluated in conjunction with non-invasive modalities such as abdominal imaging, underlying pathological diagnosis, and patient age. A key limitation is that biopsy samples represent only a small portion of the gonad, potentially missing focal lesions. To date, no studies have examined the utility of biopsy for intra-abdominal gonads containing Y chromosome material based on these considerations, and there is currently no evidence demonstrating its effectiveness in assessing gonadal tumor risk in individuals with DSD.

A systematic review of 386 publications from 1951 to 2017 demonstrated that the risk of germ cell neoplasia *in situ* (GCNIS) or germ cell tumors at the time of surgical intervention increases with age, showing a marked rise between 15 and 20 years of age [188]. Histopathological examination of gonadal specimens from 118 females with DSD carrying Y-chromosomal material who underwent prophylactic gonadectomy revealed gonadal neoplasms in 17 cases (14.4%). Gonadal neoplasms were identified in 23.5% of females with 46,XY complete gonadal dysgenesis and in 14.3% of individuals with 45,X/46,XY mosaicism. Conversely, no malignant tumors were observed in AIS (0.0%), with one case showing benign Leydig cell tumor [265]. Multiple reports have described gonadal biopsy [190, 266–269], but all are retrospective studies and, as mentioned above, lack comparative studies with gonadectomy. Therefore, they do not directly contribute to answering this CQ.

CQ25: What are the risk factors for gonadal tumors and biomarkers suggesting malignancy?

Recommendation 25: Risk factors for gonadal tumors include the presence of Y chromosome materials, intra-abdominal testes, post-pubertal age, gonadal dysgenesis, and the presence of undifferentiated germ cells. There are no effective laboratory biomarkers

suggesting malignancy for early detection and diagnosis. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

Evidence

In DSD, the risk of gonadal tumor development increases depending on the underlying condition (Table 6) [1]. Major risk factors for tumor development include: 1) presence of Y chromosome materials, 2) presence of

undifferentiated germ cells, 3) gonadal location, and 4) age (Fig. 4) [270].

The presence of Y chromosome materials, particularly *TSPY* (testes-specific protein Y-linked region), constitutes a risk factor for gonadal tumor development. Even when Y chromosome cannot be identified by G-banding, the presence of *TSPY* still poses a risk. Pyle *et al.* conducted a systematic review of papers reported from January 2006 to January 2017, reporting detection rates

Table 6 Risk stratification for gonadal malignancies

Risk Category	Condition	Malignancy Risk (%)	Recommended Treatment	Number of Studies	Number of Patients
HIGH RISK GROUP	Gonadal dysgenesis (+Y chromosome), intra-abdominal	15–35	Gonadectomy	12	>350
	Partial androgen insensitivity syndrome, extra-scrotal	50	Gonadectomy	2	24
	Frasier syndrome	60	Gonadectomy	1	15
	Denys-Drash syndrome (+Y chromosome)	40	Gonadectomy	1	5
INTERMEDIATE RISK GROUP	Turner syndrome (+Y chromosome)	12	Gonadectomy	11	43
	17 β -hydroxysteroid dehydrogenase deficiency	28	Monitoring	2	7
	Gonadal dysgenesis (+Y chromosome), scrotal	Unknown	Biopsy and radiation?	0	0
	Partial androgen insensitivity syndrome, scrotal	Unknown	Biopsy and radiation?	0	0
LOW RISK GROUP	Complete androgen insensitivity syndrome	2	Biopsy and?	2	55
	Ovotesticular DSD	3	Testicular tissue removal?	3	426
	Turner syndrome (-Y chromosome)	1	None	11	557
NO RISK GROUP	5 α -reductase deficiency	0	Unclear	1	3
	Leydig cell hypoplasia	0	Unclear	2	

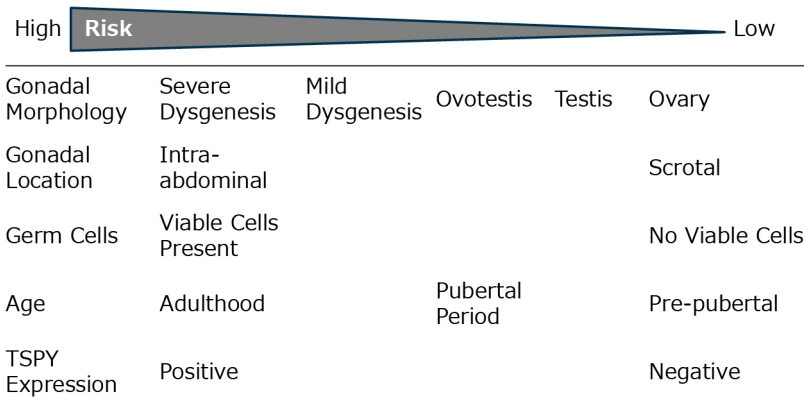


Fig. 4 Risk of gonadal tumors
(Modified from Pyle LC *et al. Am J Med Genet* 175C: 304–314, 2017)

of GCNIS and gonadoblastoma by pathological examination [270]. The presence of GCNIS or gonadoblastoma is considered a predisposing factor for future malignant gonadal tumor development. They reported potential risks for the malignancy of 40–60% in *TSPY*-positive WT1 abnormalities (Frasier syndrome, Denys-Drash syndrome), 12–40% in *TSPY*-positive gonadal dysgenesis, 15–20% in PAIS, and 0.8–15% in CAIS [270]. Denys-Drash syndrome is a genetic predisposition to Wilms tumor and renal dysfunction, requiring surveillance of both conditions [271]. Huang *et al.* conducted a retrospective study of 292 females with DSD with Y chromosome materials, reporting a detection rate of 15.4% for gonadoblastoma or malignant gonadal tumors [193]. The highest risk was observed in complete gonadal dysgenesis (23.3%), followed by CAIS (15.2%) [193]. Ouyang *et al.* reported the prevalence of gonadoblastoma or malignant gonadal tumors in 118 individuals with DSD as follows: 23.5% in those with 46,XY complete gonadal dysgenesis, 14.3% in those with 45,X/46,XY karyotype gonadal dysgenesis, and 2.9% in those with CAIS [265].

When markers of undifferentiated germ cells such as OCT3/4 or KITLG demonstrate persistent expression beyond one year of age, the incidence of gonadal neoplasm development is significantly increased [1]. Chemes *et al.* analyzed testicular tissues from 21 individuals with DSD possessing testes (aged newborn to 12 years, mean 4.7 years), including 13 with unilateral streak gonads and 9 with ovotestis. They reported that all individuals with testicular germ cell tumors and GCNIS tested positive for OCT3/4, an immature germ cell marker [272]. However, the differential diagnosis between maturational delay of germ cells and GCNIS based solely on OCT3/4 expression patterns remains diagnostically challenging, potentially resulting in the overdiagnosis of GCNIS [273]. Androgen receptor signaling correlates with the number of residual germ cells. Therefore, PAIS, in which a greater number of germ cells are potentially preserved, is considered to carry a higher tumor risk than CAIS, where germ cells have largely degenerated [273].

The anatomical location of the gonads is also a risk factor for tumor development, with intra-abdominal testes conferring increased risk. In male patients, orchiopexy has been shown to contribute to risk reduction [274].

Finally, age is also a risk factor, with the risk increasing in an age-dependent manner [274]. Chaudhry *et al.* performed pathological examination of resected testes in 133 individuals with CAIS (58 aged 16 years or older) [275]. Malignant tumors were identified in two individuals, both of whom underwent orchiectomy after the age

of 20 years. GCNIS was observed in six individuals, all but one of whom underwent orchiectomy after the age of 16 years [275].

Currently, no standardized surveillance protocols exist for the early detection and monitoring of gonadal neoplasm development. US and MRI of gonads have difficulty depicting GCNIS. Microcalcifications may be findings suggestive of germ cell tumors. Although not specifically examining DSD, Elzinga-Tinke *et al.* performed 176 testicular biopsies, observing microcalcifications by US in 76 individuals, with 20 of these diagnosed with GCNIS [276]. No reliable tumor biomarkers currently exist for the detection of early-stage gonadal lesions, and serum alpha-fetoprotein (AFP) and hCG levels typically remain within normal ranges in individuals with GCNIS.

CQ26: Is regular surveillance recommended for high-risk groups for gonadal tumors?

Recommendation 26: We suggest regular surveillance using US, MRI, and tumor-specific biomarkers for high-risk groups under observation. (Strength of recommendation: Weak; Quality of evidence: Very low)

Evidence

High-risk factors for gonadal tumors include 1) presence of Y chromosome materials, 2) presence of undifferentiated germ cells, 3) gonadal location, and 4) age (see CQ25). According to recommendations from the British Society for Paediatric Endocrinology and Diabetes, Ultrasonography (US) is the first-line imaging modality for evaluating the gonads, urogenital sinus, and Müllerian structures in individuals with DSD [277]. The examination should also encompass the adrenal glands, kidneys, pelvis, inguinal region, and scrotum. Important considerations include that structural identification does not guarantee future gonadal function and that intra-abdominal testes or streak gonads are difficult to detect by US.

When US visualization of gonadal structures proves inadequate, MRI is recommended as an alternative diagnostic modality [277]. High-resolution MRI is recommended to include T1- and T2-weighted images with and without fat suppression, obtained in three planes whenever possible. In adolescents, MRI is useful for detecting gonadal tumors, particularly those with cystic components.

Serum tumor biomarkers such as AFP or hCG are elevated in yolk sac tumors and choriocarcinoma, respectively, while serum LDH is mildly elevated in germ cell tumors and seminoma [193]. Huang *et al.* reported that among 292 individuals with DSD who underwent bilateral gonadectomy, serum tumor biomarkers were positive in 58.8% of cases [193]. Specifically, LDH

levels were elevated in 3 of 6 individuals with undifferentiated embryonal carcinoma and in 3 of 5 with testicular tumors. AFP levels were elevated in all cases of yolk sac tumors, while hCG elevation was observed only in choriocarcinoma. Elevated testosterone levels were found in individuals with gonadoblastoma and germ cell tumors. However, evidence on these biomarkers for the early detection of GCNIS and gonadoblastoma remains limited (see CQ25). Beyond conventional serum biomarkers, investigational research protocols include OCT3/4 immunohistochemical analysis as a marker for undifferentiated germ cell populations, molecular detection of Y-chromosomal material—particularly *TSPY*, which is associated with an elevated risk of gonadoblastoma—and comprehensive genotypic characterization of *WT1* [278].

7. Reproductive Medicine

CQ27: Are assisted reproductive technologies and fertility preservation recommended for spermatogenesis defect?

Recommendation 27: We suggest artificial insemination and assisted reproductive technologies using ejaculated and testicular sperms for individuals desiring children. However, clear evidence regarding fertility preservation is currently lacking. (Strength of recommendation: Weak; Quality of evidence: Very low)

Evidence

Assisted reproductive technologies such as *in vitro* fertilization and intracytoplasmic sperm injection (ICSI) have become widespread as standard medical practice in Japan. Combined with delayed marriage among women, an increasing number of couples are rapidly transitioning from non-assisted reproductive treatments such as timed intercourse and artificial insemination to assisted reproductive technology. Although the current status of reproductive medicine and fertility in individuals with DSD remains uncertain, sporadic case reports have documented successful paternity. Because the mechanisms and severity of spermatogenic dysfunction vary among conditions and individuals, it is challenging to universally recommend specific reproductive treatments. As the underlying pathology in DSD frequently involves gonadal dysfunction, most affected individuals exhibit non-obstructive azoospermia. This suggests that testicular sperm extraction (TESE), which involves random sampling of testicular tissue, or microdissection testicular sperm extraction (micro-TESE), may enable sperm retrieval even in individuals with DSD. In such cases, treatment for the female partner typically requires ICSI. For individuals with residual ejaculatory sperm, artificial insemination and *in vitro* fertilization may also be feasi-

ble and should be presented as additional treatment options alongside ICSI.

Successful pregnancies have been reported in case studies for specific conditions. Klinefelter syndrome is the most frequent DSD receiving reproductive treatment. Spontaneous pregnancies and instances in which gynecological infertility treatments are initiated without thorough evaluation of male factors in individuals with oligospermia are well-documented in clinical practice. Although the majority present with non-obstructive azoospermia, sperm retrieval rates by micro-TESE reach 40–66%, which is better than sperm retrieval rates in general non-obstructive azoospermia individuals without DSD [144, 279]. In 46,XX testicular DSD, germ cells are typically absent within the seminiferous tubules, consistent with Sertoli cell-only syndrome. To date, no cases of successful paternity have been reported, with the exception of a single case described by Słowikowska-Hilczner *et al.* [280, 281]. For ovotesticular DSD, successful paternity through TESE combined with ICSI has been reported in only a single case [282].

PAIS shows diverse phenotypes depending on residual androgen receptor function. The degree of spermatogenesis varies from non-obstructive azoospermia to complete spermatogenesis, and several cases of successful pregnancy and live birth have been reported [24, 283]. In males with 5 α -reductase deficiency, both natural conception and pregnancies resulting in live births *via* assisted reproductive technologies have been reported [284]. Pregnancy and birth cases have also been reported in congenital lipoid adrenal hyperplasia, 3 β -hydroxysteroid dehydrogenase deficiency, and LHCG receptor deficiency [285]. In cases of Leydig cell agenesis or hypoplasia, published case series have reported successful conception and live birth following TESE combined with ICSI, after optimizing intratesticular testosterone levels through hCG therapy [286]. In semen analysis of seven individuals with NR5A1 abnormalities, three had azoospermia while one demonstrated a sperm concentration of 12 million/mL, suggesting the possibility of achieving pregnancy without assisted reproductive technology [287]. In typical cases of mixed gonadal dysgenesis (MGD), such as 45,X/46,XY mosaicism, one gonad is streak-like while the contralateral gonad is an atrophic testis. Although testicular histopathology typically reveals hyalinization and Sertoli cell-only syndrome, published reports have documented successful offspring production *via* TESE combined with ICSI [288].

According to the dsd-LIFE study based on European DSD registries [281], among 1,039 individuals with DSD, 285 adult males (212 with Klinefelter syndrome, 41 with ovotestis or MGD, 18 with PAIS, 2 with androgen synthesis disorders, 6 with 46,XX testicular DSD,

and 6 others) were analyzed. Two individuals with Klinefelter syndrome achieved pregnancy without assisted reproductive technology, whereas 42 individuals with Klinefelter syndrome, one with partial gonadal dysgenesis, one with ovotestis, one with MGD, and one with 46,XX testicular DSD successfully underwent assisted reproductive technology following sperm retrieval. Identified barriers include insufficient provision of comprehensive information about assisted reproductive technologies and limited treatment implementation due to financial constraints. However, in Japan, insurance coverage for infertility treatment was introduced in April 2022, thereby improving accessibility to micro-TESE and assisted reproductive technologies for individuals with DSD, comparable to that for the general infertile male population. Future efforts should focus on building evidence through increased utilization of assisted reproductive technologies by individuals with DSD. Individuals with DSD often have not only spermatogenic dysfunction but also erectile dysfunction and ejaculatory disorders, with many cases not reaching marriage due to sexual dysfunction. Information about TESE and assisted reproductive technology should be provided to individuals with DSD not only in reproductive medicine settings but also at earlier stages [281].

In testicular tissue from individuals with DSD, the population of germ cells, including spermatogonia and spermatocytes, demonstrates a progressive, age-related decline, which may contribute to impaired spermatogenesis [289], thereby supporting the rationale for fertility preservation through early therapeutic intervention. However, given the heterogeneous patterns of spermatogenic dysfunction observed across individual cases, definitive evidence to support this approach remains lacking. In certain individuals with Klinefelter syndrome, theoretical models suggest that spermatogenesis is present during pubertal development but subsequently deteriorates with advancing age, prompting clinical consideration of the potential benefits of early micro-TESE [144]. However, in this condition, sperm retrieval rates do not decline significantly in individuals in their twenties [290], warranting caution when considering premarital micro-TESE for fertility preservation. Not limited to Klinefelter syndrome, sperm cryopreservation is possible when spermatozoa can be obtained *via* masturbation, based on patient preference and institutional judgment. However, this procedure is self-funded, with no public financial support similar to fertility preservation prior to anticancer drug administration. Testicular tissue cryopreservation may be performed for research purposes; however, successful spermatogenesis following transplantation has not yet been reported in humans.

CQ28: Are assisted reproductive technologies and fertility preservation recommended for premature ovarian insufficiency?

Recommendation 28: We suggest assisted reproductive technologies and fertility preservation methods, including ovarian tissue cryopreservation, oocyte freezing, embryo freezing, for individuals with premature ovarian insufficiency who desire children currently or in the future, provided they have residual follicles. However, there is currently no definitive diagnostic method to confirm the presence or absence of residual follicles. (Strength of recommendation: Weak; Quality of evidence: Low)

Evidence

Premature ovarian insufficiency is diagnosed in women under 40 years of age based on the presence of amenorrhea with high gonadotropin and low estrogen levels, presenting with infertility due to ovulatory dysfunction and various symptoms due to estrogen deficiency. Premature ovarian insufficiency occurs in 1–3% of all women, with known causes including chromosomal and genetic abnormalities, autoimmune diseases, and iatrogenic factors (ovarian surgery, anticancer drugs, and radiation therapy). Turner syndrome and mosaic Turner syndrome are frequently reported among chromosomal abnormalities. In premature ovarian insufficiency, these pathological factors reduce the number of residual ovarian follicles to levels comparable to menopause.

The established treatment for premature ovarian insufficiency in individuals desiring children involves assisted reproductive technology using donor oocytes; however, this approach presents ethical and immunological challenges due to the entirely non-autologous nature of the pregnancy. In individuals with residual follicles, numerous cases of pregnancy and live birth following infertility treatment using assisted reproductive technologies with autologous oocytes have been reported. Although pregnancy using autologous oocytes can be expected, no definitive diagnostic method exists to determine the presence or absence of residual follicles.

A recent retrospective cohort study of 466 individuals with premature ovarian insufficiency reported no pregnancies among those treated with hormone replacement therapy alone (0/37 individuals). In contrast, 11.7% (50/429 individuals) achieved pregnancy and live birth through assisted reproductive technology following ovarian stimulation with high-dose FSH or hMG preparations. [291]. In premature ovarian insufficiency caused by chromosomal abnormalities, a before-and-after comparison study demonstrated that 6.1% (3/49 individuals) achieved pregnancy and live birth through assisted reproductive technology [292]. However, in individuals with

premature ovarian insufficiency, regular activation of dormant primordial follicles does not occur. Instead, only a small number of follicles sporadically activate and develop, resulting in absent or minimal antral follicle response to FSH during ovarian stimulation cycles. Therefore, retrievable oocytes are minimal. Consequently, follicle activation therapy, including laparoscopic ovarian tissue removal followed by *in vitro* pharmacological or physical stimulation to promote activation of dormant primordial follicle activation and early follicle development [293–296], as well as methods involving ovarian injection of platelet-rich plasma [297–301] or mesenchymal stem cells [302, 303], are being explored; however, sufficient evidence to support their efficacy has not yet been established.

Published reports of fertility preservation interventions for premature ovarian insufficiency in individuals with DSD are predominantly limited to females with Turner syndrome and mosaic Turner syndrome, with multiple case series documenting successful oocyte cryopreservation and ovarian tissue cryopreservation procedures. Assisted reproductive technology using frozen oocytes is widely implemented; however, thawing and transplanting cryopreserved ovarian tissue for oocyte retrieval and subsequent achievement of offspring through *in vitro* fertilization remains at the research stage. Regarding oocyte cryopreservation, published case series have reported controlled ovarian stimulation and oocyte retrieval in three individuals with Turner syndrome aged 13–14 years, yielding 10–12 mature oocytes per individual for cryopreservation [304]. Similar procedures in three individuals aged 18–30 years resulted in the cryopreservation of 9–20 mature oocytes per patient [305]. Additionally, case reports of individuals with mosaic Turner syndrome who had undergone secondary sexual development (aged 14–25 years) have documented successful oocyte retrieval following ovarian stimulation, enabling cryopreservation of a substantial number of mature oocytes (12–29) in all cases [306–308]. A retrospective study evaluating the safety and efficacy of ovarian stimulation and oocyte cryopreservation in individuals with mosaic Turner syndrome demonstrated that oocyte retrieval rates (mean 9 ± 3.16) were comparable to those of healthy women. Importantly, no cases of ovarian hyperstimulation syndrome—a serious complication of ovarian stimulation and oocyte retrieval—were observed. These findings suggest that oocyte cryopreservation following ovarian stimulation is a safe and effective fertility preservation option for females with mosaic Turner syndrome [309]. Regarding ovarian tissue cryopreservation, although based on a single case report, laparoscopic cryopreservation of ovarian tissue from a Turner syndrome patient shortly after secondary sexual

development, followed by transplantation of the thawed tissue, resulted in the first reported pregnancy worldwide [310]. This suggests that ovarian tissue cryopreservation may become a potential option for fertility preservation in individuals with Turner syndrome.

CQ29: What is the potential for preserving spermatogenesis?

Recommendation 29: Evidence remains limited regarding the preservation potential of spermatogenesis. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

Evidence

Given the heterogeneous pathophysiological mechanisms of DSD, a unified explanation for spermatogenic dysfunction remains elusive. Approximately 80% of cases of male infertility involve spermatogenic dysfunction, the majority of which are idiopathic or of unknown etiology. Among patients undergoing micro-TESE for non-obstructive azoospermia, the most common histopathological finding is Sertoli cell-only syndrome. Thus, it remains challenging to determine whether the absence of germ cells is attributable to specific genetic abnormalities or represents a characteristic feature of DSD. Sertoli cell-only syndrome or defects in sperm maturation in individuals with DSD may arise through mechanisms similar to those observed in the general male infertility population.

Historically, the evaluation of spermatogenesis has relied on the presence of sperm in semen analysis. However, in individuals with DSD, sperm may be present only in minimal amounts within the testes, making detection difficult without performing micro-TESE. Even in cases previously considered to lack spermatogenesis, micro-TESE may demonstrate the presence of sperm production, suggesting that the potential for spermatogenesis in individuals with DSD may be greater than previously recognized.

Most individuals with Klinefelter syndrome present with non-obstructive azoospermia; however, sperm retrieval rates by micro-TESE range from 40% to 66%, which is higher than the rates observed in individuals with non-obstructive azoospermia without DSD [144, 279]. Although progressive testicular atrophy has been documented to occur following pubertal onset—prompting consideration of semen analysis and TESE in adolescent individuals—comparative studies have demonstrated no statistically significant differences in sperm retrieval rates between peripubertal and adult cohorts undergoing TESE procedures [144, 290]. However, marked decline in spermatogenic function is presumed to begin around age 30 [290]. In 46,XX testicular

DSD, germ cells are absent within the seminiferous tubules, essentially representing Sertoli cell-only syndrome characterized by the absence of spermatogenesis [280]. This reflects the absence of gene clusters important for spermatogenesis in the Y chromosome, particularly the AZF region on the long arm.

In PAIS, 5 α -reductase deficiency, 3 β -hydroxysteroid dehydrogenase deficiency, LHCG receptor deficiency, and NR5A1 deficiency, published case reports encompass a spectrum of reproductive outcomes ranging from successful sperm retrieval *via* micro-TESE to documented cases of spontaneous pregnancy [24, 283–285, 287]. In cases of Leydig cell agenesis or hypoplasia, published case reports have documented successful offspring outcomes following TESE combined with ICSI, after optimization of intratesticular testosterone concentrations *via* hCG therapy [286]. In pathological conditions characterized by steroidogenesis abnormalities, improvement of the intratesticular endocrine environment may induce spermatogenesis. However, due to the lack of published data on the prevalence of preserved spermatogenesis in these specific conditions, the extent to which spermatogenic function is maintained across the broader spectrum of DSD remains inadequately characterized.

In 45,X/46,XY mosaicism, various degrees of spermatogenic dysfunction that progress over time have been reported. These variations appear to correlate with differences in the mosaic tissue distribution [125, 311].

In individuals with DSD in whom genetic abnormalities have been confirmed, the specific step at which abnormalities cause spermatogenic dysfunction can be explained to some extent, as described above. However, in most cases, predicting spermatogenic dysfunction based solely on the genetic diagnosis remains challenging. In individuals with DSD, multiple factors influence reproductive outcomes, including alterations in the intratesticular endocrine milieu secondary to testosterone deficiency, testicular dysfunction associated with comorbid metabolic conditions, progressive changes in gonadal architecture, and vas deferens obstruction resulting from congenital anatomical anomalies or prior testicular surgical interventions. Consequently, determining the etiology of spermatogenic dysfunction requires individualized assessment of these multifactorial contributors in each case. Individuals with DSD have high rates of comorbidities, including endocrine diseases, which may also contribute to elements of spermatogenic dysfunction [312].

CQ30: What is the potential for preserving oogenesis?

Recommendation 30: In individuals with Turner syn-

drome, oogenic capacity is markedly reduced, leading to hypogonadism. However, some children with Turner syndrome retain sufficient ovarian follicles to allow for fertility preservation. In other conditions associated with hypogonadism, evidence regarding the extent to which oogenesis can be preserved remains limited. (Strength of recommendation: not applicable; Quality of evidence: not applicable)

Evidence

DSD is a clinical entity encompassing a variety of pathological conditions. Oogenetic capacity should be evaluated according to each pathological condition. Turner syndrome shows low oogenetic capacity, resulting in hypogonadism. Multiple studies have evaluated fertility preservation in individuals with Turner syndrome.

A comparative study involving 15 individuals with Turner syndrome (aged 5–22 years) and a control group without Turner syndrome assessed ovarian tissue cryopreservation, histological examination, and endocrinological test values. Follicles were detected in 60% (9/15) of individuals with Turner syndrome; however, many follicles were abnormal, and the *in vitro* maturation rate of immature cumulus–oocyte cell complexes obtained during ovarian cryopreservation was low at 16% [313]. Ovarian tissue cryopreservation was performed in five individuals with mosaic Turner syndrome and one individual with non-mosaic Turner syndrome, with histological examination confirming residual follicles in all cases [314]. In an individual with mosaic Turner syndrome who underwent ovarian tissue cryopreservation at 3 years of age, the preserved tissue demonstrated follicular density comparable to that observed in age-matched controls without Turner syndrome [315].

Regarding fertility in other DSD females, there are no comprehensive reports, and it is considered to vary greatly depending on the pathological condition. While fertility may be reasonably preserved in some females with congenital adrenal hyperplasia, oocyte formation cannot be expected in females with DSD where gonads have differentiated into testes. Additionally, oogenesis is not expected in many pathological conditions with gonadal dysgenesis.

8. Transition to Adult Care

CQ31: Is transition from pediatric to adult care recommended to improve reproductive outcomes, life prognosis, and QOL?

Recommendation 31: We suggest implementing transition to adult care with multidisciplinary medical care and psychological support by an expert team, as this may improve reproductive outcomes, life prognosis, and QOL. (Strength of recommendation: Weak;

Quality of evidence: Expert opinion)

Evidence

Transition from pediatric to adult care in individuals with DSD is a critical process for supporting the progression from childhood to adulthood and ensuring optimal lifelong medical management [167, 316–319]. This is not merely transfer between medical institutions or departments, but a continuous and complex process spanning several years, aimed at improving patient independence and self-management capabilities [316, 318]. The psychosocial well-being and QOL of individuals with DSD tend to be lower compared to the general population, and many clinical needs during the transition period involve psychological support [167, 319]. Therefore, in transition to adult care for DSD, continuous psychological support forms the foundation of the entire transition process [10, 147, 316, 319].

The main elements of transition to adult care for DSD based on psychological support include ensuring continuity of medical care, improving self-management capabilities, addressing challenges including reproductive medicine and complications during adulthood, and improving QOL [147, 167, 316–319]. To achieve these goals, comprehensive approaches are recommended including: 1) multidisciplinary medical care by an expert team, 2) planned transition programs, 3) patient education and empowerment, 4) access to reproductive medicine, 5) long-term follow-up, 6) family support, and 7) establishment of adult medical systems post-transition [147, 167, 316–320]. Optimal multidisciplinary care for individuals with DSD involves collaboration among endocrinologists, urologists, gynecologists, psychiatrists, psychologists, genetic counselors, and specialized nurses to provide comprehensive management that addresses complex medical and psychosocial needs [10, 147, 317, 319]. The success of planned transition programs requires close collaboration between pediatric and adult departments [147, 316–318]. In Western countries, expert consensus recommends initiating the transition to adult care around 12–13 years of age, presenting a comprehensive transition plan by 14–15 years, and gradually completing the process between 18 and 21 years of age [316, 318]. The deployment of transition coordinators or joint clinics between pediatric and adult departments, as reported in other diseases like diabetes mellitus, may also be effective [321]. Patient education and empowerment involves supporting understanding of complex medical information specific to DSD (chromosomes, gonads, internal and external genital status, *etc.*) and participation in treatment decision-making. The graduated provision of disease-specific knowledge and treatment information, coupled with the systematic enhancement of

individual self-management competencies, is essential for optimal care outcomes. The timing of information disclosure to individuals is also a critical issue. Since individuals with DSD often do not fully understand their diagnosis or treatment history, appropriate information provision during transition becomes necessary [167, 316–319]. Access to reproductive medicine is also an important element. Given that infertility is a significant concern for many individuals with DSD, early provision of information and timely intervention regarding reproductive options—such as gamete cryopreservation and adoption—are essential [167, 281, 317, 318]. In individuals with 21-hydroxylase deficiency, reproductive capacity may improve with appropriate glucocorticoid replacement therapy. However, those with Turner syndrome or Klinefelter syndrome require more complex interventions [167]. Additionally, appropriate support and intervention for sexual function and satisfaction are required [167, 319]. Long-term follow-up includes the management of complications that affect life expectancy and QOL, such as hypogonadism, gonadal tumors, osteoporosis, cardiovascular disease, and metabolic abnormalities [167, 319]. These risks are particularly elevated in individuals with Turner syndrome and Klinefelter syndrome [167, 322]. Family support is also an important element, with psychological support for parents and siblings contributing to successful transition care for individuals [319, 323]. Establishment of adult medical systems is also a challenge. Training adult physicians familiar with DSD and establishing specialized clinics are necessary to create medical systems that serve as receiving structures after transitioning [316, 317, 319]. Although empirical evidence demonstrating the effectiveness of comprehensive transition care programs incorporating these elements remains limited, studies examining individuals with Mayer-Rokitansky-Küster-Hauser syndrome have documented significant reductions in psychosocial distress and improvements in HRQOL outcomes. [324, 325]. However, DSD constitute a rare and heterogeneous group of conditions, making it challenging to establish standardized transition programs. Treatment interruption during transition must be avoided at all costs, requiring flexible responses according to individual needs and circumstances.

Optimization of transition from pediatric to adult care for individuals with DSD likely contributes to improving reproductive outcomes, life prognosis, and QOL. However, at present, recommendations based on high-quality evidence are limited, and clinical practice is largely guided by expert opinion and clinical experience. Evaluating the effectiveness of transition care program requires consideration of both patient-related outcomes, including biological and psychosocial indicators, and

program-related outcomes, such as user satisfaction. Future accumulation of research on transition care for individuals with DSD is expected to establish effective transition care.

Conclusion

These clinical practice guidelines provide evidence-based recommendations for the diagnosis and management of DSD across the lifespan. The guidelines emphasize the importance of multidisciplinary care, shared decision-making, and individualized approaches to management. Given the rarity and heterogeneity of DSD conditions, many recommendations are based on low-quality evidence or expert opinion, highlighting the need for further research.

Key recommendations include balanced approaches to sex assignment in newborns, comprehensive diagnostic workup, individualized decisions regarding surgical interventions, appropriate hormone replacement therapy, gonadal tumor surveillance, consideration of fertility preservation options, and structured transition to adult care. Throughout the guidelines, the psychological and social dimensions of DSD care are emphasized as essential components of comprehensive management.

Healthcare providers caring for individuals with DSD should recognize the complex and evolving nature of these conditions and work collaboratively with individuals and families to optimize physical health, psychological well-being, and QOL. These guidelines aim to standardize and improve DSD care in Japan while acknowledging that management approaches will continue to evolve as new evidence emerges.

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