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REQUESTED REVIEW

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Society for Endocrinology UK Guidance on the initial evaluation of a suspected difference or disorder of sex development (Revised 2021)

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Abstract

It is paramount that any child or adolescent with a suspected difference or disorder of sex development (DSD) is assessed by an experienced clinician with adequate knowledge about the range of conditions associated with DSD and is discussed with the regional DSD service. In most cases, the paediatric endocrinologist within this service acts as the first point of contact but involvement of the regional multidisciplinary service will also ensure prompt access to specialist psychology and nursing care. The underlying pathophysiology of DSD and the process of delineating this should be discussed with the parents and affected young person with all diagnostic tests undertaken in a timely fashion. Finally, for rare conditions such as these, it is imperative that clinical experience is shared through national and international clinical and research collaborations.

KEYWORDS
DSD, genitalia, gonads, puberty

1 | INTRODUCTION AND DEVELOPMENT OF GUIDANCE

Differences or disorders of sex development (DSD) are a wide range of conditions with diverse features and pathophysiology[1] that most often present in the newborn or the adolescent. Newborns with DSD usually present with atypical genitalia whereas adolescents present with atypical sexual development and maturation during the pubertal years. These clinical situations can often be challenging to evaluate as they can present in a wide range of paediatric and adult settings. Developing a logical and pragmatic management plan for investigations whilst establishing a dialogue and building rapport with the child or adolescent and the parents are central to the initial approach and ongoing management.

The consensus reached in 2005 on the general principles of managing patients with DSD represented a historic milestone for international collaboration in this area.[2] Over the last two decades, and as a direct result of the above initiative, multidisciplinary and international collaborative projects such as EuroDSD, I-DSD, DSDlife and DSDnet have promoted greater engagement with patients and parents and continued to generate new knowledge and guidance.[3] Since guidance on the initial evaluation of a complex condition is often influenced by local provision of health care, it was felt that reaching a consensus at a national level was the most effective means of improving care in the UK. A UK DSD taskforce was initially formed in 2009 under the auspices of the UK Society for Endocrinology with the remit of formulating guidance on the initial evaluation process and the diagnostic approach.[4] After completion, the document was subject to open external review by the involved professional societies, patient group representatives and wider open consultation. The guidance was subsequently revised and republished in 2015.[5] The current, second, revision of the guidance was initiated in 2020 and has also taken a similar path as described above. The main focus of this guidance has remained on the initial approach to the care of the infant or adolescent who presents with a suspected DSD.

2 | THE MULTIDISCIPLINARY TEAM

Optimal care for infants and adolescents with DSD requires an experienced multidisciplinary team (MDT) that is accessible through regional centres or clinical networks that link between one or more specialist centres. It is clear that many centres that deliver care in DSD do not have access to the whole range of expertise that may be required.[6,7] As a minimum standard, the clinical team for children should include specialists in paediatric endocrinology, paediatric urology, paediatric clinical psychology, paediatric radiology, paediatric nursing and, in the case of newborn infants, neonatology and, in the case of the older child, an adolescent gynaecologist. All patients should also have a named keyworker which could be the specialist nurse in the team, but the family and young person should also be able to directly contact the lead clinician. In addition, the core MDT should have links to a wider MDT which includes specialists from clinical genetics, clinical biochemistry, adult endocrinology, adult urology, assisted conception, plastic surgery, gynaecology, adult clinical psychology, psychiatry, social services, sex therapy and if possible a clinical ethics forum (Table 1). The parents and the young person should be informed of the range of support that is available. The MDT has a responsibility to learn and develop their practice and that of other services in their network and region including non-specialists who are often the first point of contact in a new presentation. The network should have a forum to meet regularly, in the context of a clinic or an educational meeting where the team can review and discuss its own performance. Members of the MDT should also be aware of how their own values and beliefs are played out in the clinical setting. Engagement of the MDT in quality improvement exercises and in building collaborative working partnerships, attendance at joint clinics and education events are crucial if knowledge and information sharing as well as care is to be optimized within the team. The role of a service manager or a clinic coordinator and a database administrator has not been sufficiently emphasized in the past. Whilst it is possible that some of these roles can be assumed by one of the existing members of the MDT, there is still a need for dedicated time for overseeing and/or performing these tasks that allow structured management within a complex service. For certain rare conditions associated with DSD, the need for an out of region referral or discussion may be required. Virtual electronic platforms such as the clinical patient management system (CPMS) that is available to centres affiliated to the European Reference Network (ERN) for rare endocrine conditions (Endo-ERN) can provide a forum where a complex case can be remotely and securely discussed with several experts at short notice.[8] The use of secure NHS-approved web-based platforms may also obviate the need for immediate physical transfer of a patient or a family for specialist MDT input.

3 | NETWORKS AND REGISTERS FOR CLINICAL CARE, AUDIT AND RESEARCH

It is unrealistic to expect that every clinical centre can possess a comprehensive, multidisciplinary DSD team as outlined above.

TABLE 1 The clinical members of the MDT and their potential roles in providing care to the patient and the parents

	Role
Neonatologist or General Paediatrician	- Initial explanation - Management of the unwell child - Initiation of first-line investigations - Seek advice from paediatric subspecialist (endocrine or surgical) with an interest in DSD
Paediatric Endocrinologist	- Detailed explanation over multiple visits - Management of the unwell child - Interpreting first-line investigations and planning second-line investigations - Organize timely and appropriate involvement of other members of MDT - Act as the link between the parents and MDT - Initiate and monitor long-term medical therapy such as steroid or sex steroid therapy
Paediatric Radiologist	- Interpret and often perform ultrasound scans in the newborn - Judge the reliability of ultrasound scans in the newborn esp when the results may influence sex assignment
Paediatric Urologist	- Assessment of external anatomy - Explanation of the anatomy and results of imaging - Explanation of pros and cons of surgery - Develop a plan for complex imaging (other than pelvic ultrasound) and further assessment of the anatomy - Perform procedures such as laparoscopy, biopsy, reconstructive surgery and gonadectomy - Organize timely and appropriate involvement of other members of MDT
Paediatric Specialist Nurse	- Provide general support to the patient and parents in addition to that provided by other members of the MDT - Arrange specialist investigations - Liaise with the rest of the DSD team, including the clinical psychologist
Clinical Psychologist	- Provide specialist support to parents soon after birth - Provide support to the growing up child and the parents - Develop an individualized plan for each family - Guide the MDT on timing and tempo of explanation of the condition to the older child and adolescent
Clinical Endocrine Biochemist*	- Facilitate timely analysis of samples - Provide specialist support and interpretation of results - Guide subsequent biochemical tests - Facilitate storage of samples for analysis at a later stage
Clinical Geneticist	- Facilitate timely analysis of chromosome analysis - Closer involvement in the child with dysmorphic features - Oversee the process of genetic analysis - Facilitate storage of samples for analysis at a later stage - Genetic counselling
Gynaecologist	- Availability at an early stage to discuss future outcome and map long-term care pathway in the affected girl - Discuss issues related to sexual function, reproductive function and surgery - Assess the understanding & review the diagnosis - Assess the need for psychology support in the adolescent girl - Initiate and monitor long-term sex steroid therapy - Perform examination, investigative and therapeutic procedures in the adolescent girl - Oversee vaginal dilator training with specialist nurse
Adult Endocrinologist	- Investigate and manage the adolescent presenting for the first time after the age of 16 yrs - Liaise with other members of the MDT - Act as the link between the patient and MDT - Initiate and monitor long-term medical therapy such as steroid or sex steroid therapy - Act as the transition link for adolescents under paediatric care
Service Coordinator	- Oversees the coordination of a complex clinical service - Responsible for data management - Oversees activities related to audit and benchmarking of services - Oversees public and professional engagement of the service

Furthermore, in many cases, care at a local hospital with early telephone or secure video-link consultation with the regional centre may be more appropriate for reasons of both convenience and necessity (eg adrenal crisis in CAH). For the less complex case of hypospadias, immediate multidisciplinary input may not be necessary and initial discussion and explanation of the condition with the parents does not require urgent transfer of the baby at an emotionally sensitive period. Similarly, some investigations can also be performed at local centres that are affiliated to a regional centre. However, all centres managing children and young people with DSD should have a specialist multidisciplinary DSD team that can be accessed by its regional network and as described earlier. It is also important that all personnel who may be involved in the care of an affected person have access to the regional DSD team and have the opportunity to develop themselves professionally. Recent international surveys show that engagement in research and quality care improvement as well as participation in registries and continuous professional development activities is variable amongst centres that deliver DSD care.[6] Some regions in the UK, such as Scotland, have attempted to overcome these hurdles with the development of a managed clinical network.[91] A service model such as this is aimed at ensuring the provision of a high-quality and equitable service for all affected children and adolescents in a region. A formal organization such as this has the potential to develop a structured referral pathway within the region as well as beyond and can provide the infrastructure for better long-term care of the patient as close to home as possible. In England, DSD care is considered a 'specialized service' directly commissioned by NHS England but, currently, no formalized national network exists.

In addition to 'getting it right the first time', networks can have several other benefits in the field of DSD. A network can facilitate the creation of widely agreed protocols for the care of the affected newborn, set and monitor national standards of care, inform the rational utilization of other services such as clinical genetics and clinical biochemistry and provide a forum for education and professional development. More recently, European networks such as Endo-ERN and a urology network (eUROGEN) of reference centres for rare conditions provide a forum to promote best practice for these rare conditions. Some networks such as the Scottish DSD network and Endo-ERN have ongoing surveillance capability through projects such as the Scottish Audit of Atypical Genitalia (SAAG)[9] and EuRRECa[10] that allow continuous monitoring of clinical activity. Professional scientific societies also play an important role and many such as the BSPED and ESPE have a dedicated special interest group for DSD. Under the aegis of the BSPED, the BSPED DSD working group has developed auditable standards of care.[11]

Research and audit are vital for the management of DSD, and clinical networks have a strong potential to drive these activities with the development of care standards including patient experience data and peer-observation of clinical care provision. Following the 2005 Consensus Workshop that stressed the need for the regular collection and sharing of data across geographical boundaries, the current I-DSD registry was initially launched in 2008.[12] Over a decade later, this registry and its associated network, I-DSD play an increasingly important role in supporting research, training and benchmarking of care and service.[13] Patient registries can also facilitate the development of local circles of patients and parents with similar conditions who can support each other. The case for participating in standardized data collection and exchange for DSD has now been made at several levels and should be standard practice in centres that care for people with DSD.[2,3,11,14,15]

4 | COMMUNICATION

Ideally, discussions with the family are led by one professional. In most situations, particularly in the case of the newborn, the paediatric endocrinologist assumes the role of clinical lead and oversees the timely involvement of other members of the team. Other team members should be discouraged from providing results as soon as they are received. For infants, this team should develop a plan for clinical management with respect to diagnosis, sex assignment, management choices band psychosocial care. The lead clinician should process this information and take responsibility for sharing the information with the team and the parents so that informed decisions can be reached in a timely manner.[16] The process of informing parents, children and young people of the various investigations and results should be discussed and documented such that the whole MDT team is aware of the status of new or ongoing conversations with the family. Ongoing communication with the family's general practitioner is important and consent for sharing information should be discussed with parents and young person. The patient and family should also be provided details of resources that can provide peer support that is independent of the clinical service. A record of early discussions, either as audio recordings or a letter, which is shared between the parents and other immediate members of the MDT and the general practitioner are helpful for all. The decision-making history that is captured in these records can be especially important for parents who can then revisit the processes that led to any critical decisions in the neonatal period. Some services may have access to translation services to ensure the letter is accessible to the patient or parents. Use of drawings and written material during discussion as well as information sheets are also useful aids for families.

Discussions with parents and young people need to occur on multiple occasions in a quiet and peaceful setting, with enough time for the family and MDT to develop a shared understanding of investigations, results, diagnosis, management and the value of ongoing psychological care for both themselves and/or their child. The pace at which information is shared should be set by the family, and issues of confidentiality should be discussed and respected.[17] Parents' and young people's initial recollections of conversations with professionals may have long-lasting effects on them and their relationship with their child and health professionals and, therefore, MDT training is required in the use of appropriate language that can contribute to psychologically appropriate care and avoid unnecessary harm.[18] The use of phrases such as 'diverse' or 'variations' in sex development may help to introduce the concept of the range of variation that may occur in typical sex development. Some peer information and support organizations prefer terms such as 'intersex' or 'variations in sex characteristics'. The young person and their family may adopt terms that best suit their psychosocial position and these choices need to be respected. The team should be cognizant of the needs of the parents who do not use English as their first language. It is also possible that for rare dialects and languages, the interpreter may originate from the same community.

From the outset, parents and young people need to be aware that the management of the condition will require a stepwise approach that first targets short-term goals without compromising more distant goals that together achieve optimal long-term well-being.[19] Families' contributions to decisions on care will be shaped by their own expectations, experiences and their understanding of sex and gender roles within the religious and cultural context of their own social networks. The complexity of the psychological and physical impact of intervention genital difference will require a thorough discussion with several members of the MDT so that the parents are fully informed and can understand the care plan to which they are asked to consent.[20,21] In cases where a high value is placed on religious opinions, centres may choose to involve an experienced religious leader such as a hospital chaplain, imam or rabbi to provide help and understanding when addressing the patient's or parents' concerns. The parents and young people may need support and guidance as to how to share essential understanding within their close community and those in trusted caring relationships with the child in a way that both utilizes existing support whilst preserving the child's need for privacy, dignity and self-autonomy.[22,23] In addition, adolescents will need direct support in navigating complex issues such as the potential for any intervention before embarking on sexual activity, decisions regarding gonadectomy, medication management and the potential for fertility preservation prior to any irreversible procedure.

5 | PSYCHOLOGICAL CARE

When a child or young person is identified for investigation regarding a possible DSD, immediate psychological care for patient and family should be provided by the whole team and led by the clinical psychologist. The initial aim is to orientate the family to the psychological tasks and practical demands ahead. There is good evidence in a wide range of chronic childhood conditions that the early involvement of the psychologist can be helpful and information giving combined with psychological techniques focussed on parents' thinking can help with parental adaptation.[24] Whilst there is a need to study these interventions in more detail within the field of DSD, early provision of psychosocial support is increasingly becoming standard practice in the newborn period [25] and it should also be considered good practice in the setting of DSD. Decision-making processes and tools have been suggested as useful methods of engaging parents in a way that provides information and is supportive.[22] Such psychosocial support will allow those impacted to examine and understand their early emotional reactions as well as explore present and future worries, adjust to the period of uncertainty during the initial diagnosis process and prepare for ongoing engagement with healthcare, whilst facilitating inclusion in informed decision-making about themselves or their child.[26] The clinical psychologist is also well placed to assess the level of care the family needs, assess and facilitate the bonding of the parents with the newborn, and, in the case of the young person, perform an assessment of gender identity, when appropriate. The psychological care that is provided as part of the initial approach should always be considered as a routine part of the care that is available and offered to the child or the parent. Although parents of all infants with atypical genitalia may need psychological care,[27] as a minimum, the parents of every newborn with suspected DSD where there has been a delay in sex assignment should be provided immediate clinical psychology input. An approach which is more appropriate for an adolescent needs to be adopted in the case of a new diagnosis in this age group.[28] All adolescents with a newly diagnosed DSD or existing DSD requiring medical or surgical attention should also receive clinical psychology input in addition to any support provided to their parents or wider family. A standardized assessment of the need for future clinical psychology input should also become routine at the point of transfer from paediatric to adult services. In the MDT, the clinical psychologist has important additional roles that include the training of team members in communication and provision of input into tools that are used to collect patient/parent-reported experience of the care received.

6 | THE ROLE OF PEER SUPPORT AND ADVOCACY GROUPS

Based on lived experiences across the life span, peer groups can provide ongoing support to parents and the affected individual, including opportunities to gather and explore practical information, promote autonomy, and build knowledge and self-confidence regarding the diagnosis of DSD.[29] For parents, gathering, using and questioning information will shape their understanding as they often act as the advocate for their child or young person and therefore need to be fully informed about DSD practice, short- and long-term outcomes of treatments and health risks and psychological challenges for their child. By being in touch with others with a similar condition and engaging with a peer group, people can gain a sense of empowerment and the whole experience may also normalize a condition which may have previously been perceived as a source of stigma. Peer groups can provide a range of such information via websites and newsletters as well as via telephone and online forums and group meetings for both families and professionals.[30] Contact details of national peer groups and web resources such as CAH Support Group (livingwithcah.com) and dsdfamiles (dsdfamilies.org) should be supplied as routine as part of any written information. Whilst such groups and resources are not subject to a standard process of national accreditation in the UK, the co-involvement of local clinical experts as advisors means that many groups function within the framework of clinical practice in the UK. It is possible that families or individuals may prefer to talk to others known to the MDT or regional services. The creation of a local pool of support volunteers, contributing to education and support events is a valuable adjunct to a regional service. A patient's interest in peer contact may change over time and therefore this should be reviewed intermittently by the MDT. Peer support groups and patients can also work in partnership with healthcare providers at several levels in improving the quality of care and research.[30] For instance, through international projects such as DSDnet, I-DSD, I-CAH and, more recently Endo-ERN, patients and peer groups have been able to provide guidance on health care and research.[14]

7 | WHICH NEWBORN SHOULD BE INVESTIGATED?

It is generally accepted that investigations are necessary in those cases where the appearance of the genitalia is such that sex assignment is not possible at birth or the appearance is not consistent with any prenatal genetic tests. However, the interpretation of the genital appearance and the ability to assign sex in some cases may depend on the expertise of the observer. Whilst the label of 'ambiguous genitalia' has often been assigned to newborns in whom the most appropriate sex of rearing is not immediately clear to those present at the child's birth, in most cases, the genitalia are not 'ambiguous' but simply 'atypical' and we recommend that the term 'atypical' should be used instead of 'ambiguous'. The birth prevalence of atypical genitalia may be as high as 1 in 300 births [31] but the birth prevalence of infants who require specialist input in the neonatal period is about 1 in 3000 and the birth prevalence of infants in whom sex assignment is delayed beyond birth may be as low as 1 in 11,000 births.[8]

When evaluating infants, the clinical features of the external genitalia that require examination include the presence of gonads in the labioscrotal folds, the fusion of the labioscrotal folds, the size of the phallus and the site of the urinary meatus on the phallus, although the real site of the urinary meatus may, sometimes, only become clear on surgical exploration.[32] These external features can be scored to provide an aggregate score, the external masculinization score (EMS).[33] More recently, the external genitalia score (EGS), has been developed as a gender-neutral alternative to the EMS.[34] The EGS describes the site of urethral meatus and genital tubercle length in greater detail but continues to have a high level of correlation to the original EMS tool.[34] In boys with atypical genitalia, a chromosomal anomaly may be present in approximately 3% of those with isolated cryptorchidism, 7% of those with hypospadias and 13% of those with a combination of cryptorchidism and hypospadias.[35] In boys with XY DSD, comprehensive investigations will reveal an endocrine or genetic abnormality in at least a quarter of cases.[36–39] Routine systematic examination of 423 consecutive, apparently healthy, term newborn boys revealed that 412 (98%) had the maximum EMS of 12, 10 had an EMS of 11 and only 1 out of 423 had an EMS of less than 11.[33] Thus, infants who require further clinical evaluation and need to be considered for investigation for a suspected DSD should include those with isolated perineal hypospadias, isolated micropenis, isolated clitoromegaly, any form of familial hypospadias, isolated bilateral undescended testes and those who have an EMS of less than 11 or an EGS of less than 10.5.[33,34] This will avoid unnecessary detailed investigations of boys with isolated glandular or mid-shaft hypospadias and boys with unilateral inguinal testis. In newborn girls, the length of the clitoris does not seem to be dependent on gestation and a newborn with a length greater than 8mm requires further evaluation.[40] Micropenis is defined as a stretched penile length of less than 2.5SD from the mean and based on contemporary studies from a wide range of countries, a stretched penile length of less than 2cm would represent a reasonable cut off for micropenis in the newborn.[34,41–45] In approximately 25% of affected cases, XY DSD is part of a complex multi-system condition [37,46] and the coexistence of a systemic metabolic disorder, other associated conditions or dysmorphic features would lower the threshold for investigation as would a family history of consanguinity, stillbirths, multiple miscarriages, fertility problems, genital abnormalities, hernias, delayed puberty, genital surgery, unexplained deaths and the need for steroid replacement. Knowledge of birthweight in XY DSD is very helpful given the well-reported association between low birthweight, intra-uterine growth retardation and XY DSD.[31,47]

8 | WHAT INVESTIGATIONS SHOULD BE PERFORMED?

In all infants with atypical genitalia and/or bilateral impalpable gonads, a first tier of investigations should be undertaken to define the sex chromosomes and delineate, by pelvic ultrasound, the internal genitalia and exclude congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency—the commonest cause of a life-threatening condition that is associated with atypical genitalia in the newborn. This first tier should, therefore, also include plasma glucose, serum 17OH-progesterone (17OHP) and serial measurement of electrolytes. Serum 17OHP is usually unreliable before the age of 36 h, and in the salt losing form of CAH, serum electrolytes usually do not become abnormal before day 4 of life. Furthermore, 17OHP concentrations may be falsely elevated in premature or sick neonates and can also be elevated in rarer forms of CAH. The results of PCR or FISH analysis using Y and X-specific markers and the 17OHP results should be available within a maximum of two working days in all specialist centres. In situations where the level of suspicion of CAH is very high and the infant needs immediate steroid hormone replacement therapy, further serum and urine samples should be collected and stored before starting therapy. These should be of a sufficient volume to assess 17OHP, testosterone, androstenedione, renin activity and aldosterone, in that order of priority. Baseline or stimulated serum cortisol concentrations can be difficult to interpret in the newborn especially in the premature infant or following prenatal or postnatal exposure to glucocorticoids. At least one spot or 24-h urine sample (at least 5ml) for a urine steroid profile (USP) should be collected before starting therapy. The results of these initial investigations, and especially the karyotype, shall often dictate the second tier of investigations. It is imperative that a regional protocol that is easily available within the region by all healthcare staff has been developed for these first-tier investigations and this protocol should include details of sample collection and transport as well as contact details of key staff.[48]

In an infant with atypical genitalia, impalpable gonads and a karyotype of 46, XX, and the presence of a uterus, the diagnosis of CAH due to 21-hydroxylase deficiency is very likely. A significantly elevated serum 17OHP as well as a wider range of androgens and a urine steroid profile can confirm this diagnosis and can also identify other rare forms of CAH such as 11β–hydroxylase deficiency. In other infants who are not 46, XX and have had CAH excluded, it is necessary to determine the presence of testes as well as the adequacy of androgen production and at the initial stage this will include the measurement of gonadotrophins, testosterone, serum anti-Müllerian hormone (AMH) and/or serum inhibin B as well as further detailed imaging and laparoscopy. A urine sample should also be collected to assess proteinuria. However, the onset of proteinuria in the glomerulopathy associated with *WT1* variants is very variable and if there is a high suspicion then there may be a need for repeated assessments.[49]

9 | WHICH ADOLESCENT SHOULD BE INVESTIGATED AND HOW EXTENSIVELY?

The initial assessment in an affected adolescent should not only start the process of diagnosis but should also be used to develop a rapport with the patient and where appropriate, their parents. The delivery of medical, nursing and psychological care needs to be undertaken within a hospital setting that is sensitive for the needs of the young person. The explanation of the diagnosis to the patient and the family is critical and needs to be performed sensitively and carefully over a period of time for reflection and this can be facilitated via expert psychological input. Adolescents usually present to paediatric or adult healthcare teams with a suspected DSD in three ways—a girl with primary amenorrhea (with or without breast development), a girl who virilises at puberty or a boy with pubertal delay (Figure 1). The need for any physical examination or imaging should be discussed with the adolescent and conducted by the most appropriate health professionals within the MDT and with the help of the psychologist or specialist nurse, if necessary. Any examination in clinic should be deferred to subsequent consultations once a rapport has developed with the adolescent. In some cases, this may be best performed under an anaesthetic by a surgeon and/or a gynaecologist. In adolescents with an existing DSD, the period of transition to adult services is an opportunity to review the diagnosis and consider further investigations. The joint DSD clinic serves as the forum where this can be reviewed. This clinic can also function as the forum where new cases in older adults can be discussed within the wider MDT.

In girls with primary amenorrhoea, investigations should be considered at the age of 13 years if there is no breast development and by 15 years if other aspects of puberty, particularly breast development, have progressed normally. History should include a family history and an assessment of coexisting chronic disease, exercise and weight changes. Physical examination should include measurement of blood pressure, height and weight and assessment of secondary sexual characteristics including clitoral enlargement. Vaginal examination to assess vaginal length is only indicated when considering vaginal dilation as it is not of diagnostic value alone and should be performed by a gynaecologist. The procedure is only required when the individual wishes or when sexual activity is contemplated. An initial investigation screen should combine a transabdominal pelvic ultrasound to identify a uterus with measurements of serum electrolytes, LH, FSH, prolactin, TSH, FT4, SHBG, androstenedione, oestradiol, testosterone, AMH or Inhibin B. Ultrasound scans do not yield information on vaginal anatomy and this is better obtained from magnetic resonance imaging (MRI), which is most useful in cases where there is menstrual obstruction. Raised gonadotrophins or an absent uterus in the presence of normal breast development necessitate chromosome analysis (by karyotype or microarray).

The appearance of clitoromegaly and hirsutism at puberty in the presence of primary amenorrhoea is a classical presentation of 17β-hydroxysteroid dehydrogenase type 3 deficiency and 5α-reductase type 2 deficiency and can also be seen in SF-1 deficiency.[50] It is less typical of partial androgen insensitivity syndrome (PAIS) which is usually associated with atypical genitalia at birth. In all these conditions, Müllerian structures will not be detectable. Also, in partial gonadal dysgenesis and ovotesticular DSD, the mild clitoromegaly that may have been present at birth but overlooked, may become a more prominent feature at adolescence.[50] The differential diagnosis would also include 46, XX CAH and androgen-secreting tumours of the ovary or adrenal gland; in all these cases, Müllerian structures are present. Investigations include serum measurements of LH, FSH, DHEAS, SHBG, androstenedione, testosterone, dihydrotestosterone and 17OHP. A USP can confirm a diagnosis of 5α-reductase type 2 deficiency, disorders of androgen excess or adrenocortical tumour. A pelvic ultrasound will assess the presence of a uterus and determine the need for a chromosome analysis.

Although the commonest cause of delayed puberty is constitutional delay, all boys with delayed puberty who are over the age of 14 years should be assessed carefully.[51] Boys who are overweight or who have penoscrotal webbing need careful examination so that a normal penis is not mistaken for micropenis. Rarely, PAIS, a disorder of testosterone biosynthesis or mild forms of testicular dysgenesis can present in this age group, especially if there is a history of hypospadias repair, orchidopexy or gynaecomastia. First-line investigations include a bone age and serum measurements of LH, FSH and testosterone. For those with raised gonadotrophins, chromosome analysis should be performed to exclude

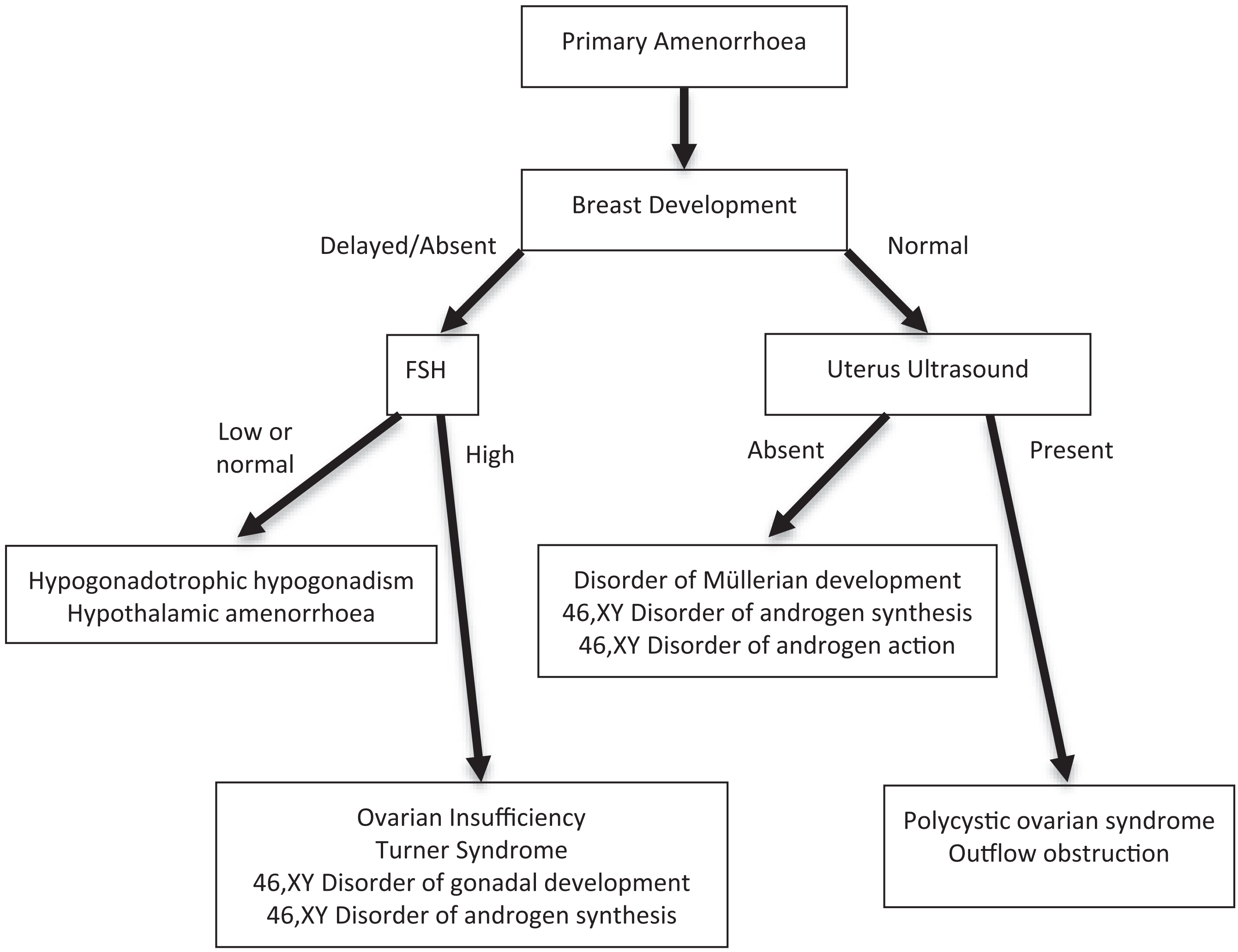

FIGURE 1 Approach to investigating adolescent girls with primary amenorrhoea

conditions such as Klinefelter's syndrome (47, XXY and mosaic variants) or 45, X/46, XY mosaicism.

10 | THE ROLE OF THE CLINICAL GENETICIST

Establishing a specific molecular diagnosis is helpful in the clinical management of cases and in offering accurate genetic counselling for the family. In those cases, where a clear steroidogenic defect has been identified biochemically, targeted single-gene analysis will confirm the diagnosis in most cases. Whilst the yield from diagnostic genetic testing maybe less than 50% in those who are 46, XY DSD and have no clear abnormality of steroidogenesis, with ongoing advances in genomic medicine,[52] the ability to better diagnose, predict and treat disease is anticipated to transform many aspects of care. For a significant number of individuals with a DSD, its utility may reside in ending diagnostic uncertainty and delivering personalized care.

Diagnostic genetic laboratories are moving from single-gene sequencing to high-throughput sequencing (HTS) assays (parallel testing), designed to sequence multiple DSD-related genes on a targeted panel in one analysis or through whole genome or exome sequencing (WGES) with predetermined filters that target DSD-related genes.[6,53] A targeted panel is advantageous as it yields high-quality coverage of the genes of interest, whilst minimizing the risk of incidental findings. Recent surveys of practice show the increasing use of HTS at an earlier stage of the diagnostic pathway in XY DSD[6] and with greater familiarity with this approach, as well as faster turnaround times, it is clear that practice is changing, such that, the choice of second-line endocrine investigations may be influenced by the results of the genetic analysis.[54]

The clinical geneticist at the specialist DSD centre can evaluate complex genetic syndromes and advise which genetic testing technique is appropriate and cost-effective for each clinical situation, following urgent confirmation of chromosomal sex. Initial testing is usually performed by quantitative fluorescence polymerase chain reaction (QFPCR) due to the rapid turnaround time and followed

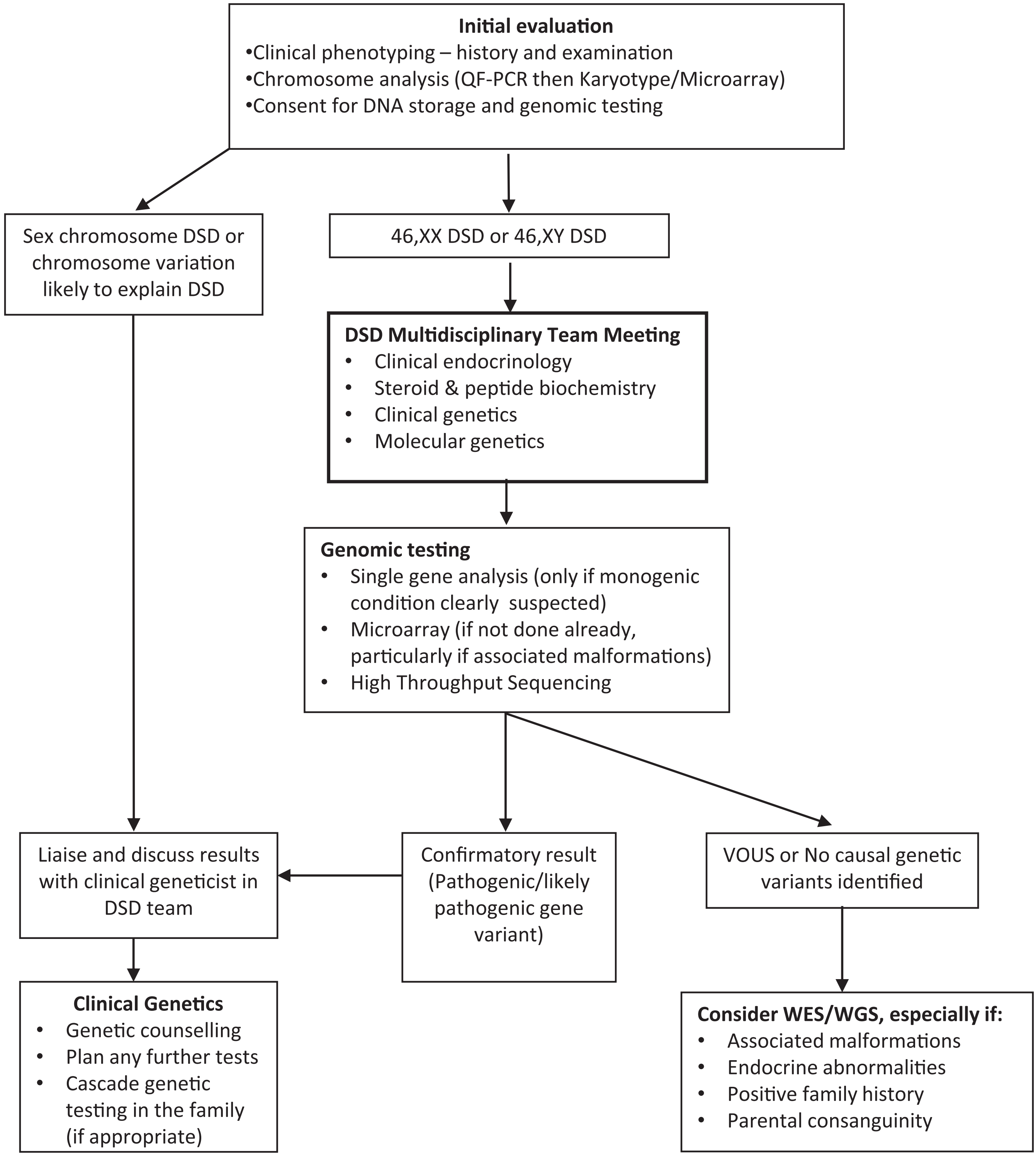

FIGURE 2 An integrated pathway for genomic and phenotypic evaluation of DSD. VOUS—variant of unclear significance. A multidisciplinary diagnostic team that has detailed knowledge of the DSD field as well as the diagnostic tools plays a central role in the diagnostic process

by chromosome analysis, either by karyotype, or more commonly, microarray, which identifies copy number variation (Figure 2). With the advent of targeted panels and WGES, more extensive biochemical and radiological investigations might be reserved until answers from analysis are obtained, with the potential to avoid further costly and invasive investigations. However, there are existing challenges in bringing HTS and WGES into mainstream practice.[53,54] Pre-test counselling needs to be broader, to cover all potential test outcomes, including the identification of gene variants of uncertain significance (VOUS) whilst explaining the limitations of this approach such as the variable coverage of the genes of interest in WGES. In addition, in line with international recommendations, patients and parents should be informed about the possibility of unsolicited findings of medically relevant disease variants. In NHS England, diagnostic genetic testing for DSD is included in the National Genomic Test Directory (https://www.england.nhs.uk/publication/national-genomic-test-directories/) and in both England and Scotland it is provided through a network of NHS genomic laboratory centres. Diagnostic interpretation of the genetic findings requires a very careful and methodical approach and, to deliver a high-quality service, centres that provide a diagnostic genetic service for DSD should have detailed phenotypic information in addition to the genetic findings and which can be discussed at a regular meeting of a diagnostic board and which, as a minimum, consists of the clinical geneticist, the molecular geneticist, the clinical biochemist and the paediatric endocrinologist with a special interest in DSD at the diagnostic centre.[54] This diagnostic board should have the capacity to review their own activities and remain up to date with continuing advances in this field.

Close involvement of the clinical genetics service can ensure that the MDT covers all aspects of genetic counselling including provision of information to the family, the mode of inheritance of the disorder and the choices or options available for dealing with this risk. Established links with the clinical genetics service are also useful when considering prenatal testing or interventions such as steroid hormone therapy in CAH or interruption of pregnancy. As the scope for non-invasive prenatal diagnosis (NIPD) using free floating foetal DNA continues to increase, the close involvement of the clinical geneticist at a very early stage in at-risk pregnancies will become even more important.[55] Abnormalities of sex chromosomes are identified in approximately 1% of all pregnancies that undergo prenatal karyotype and although 40% of these pregnancies may be associated with a termination, early referral for genetic counselling seems to be associated with a lower likelihood of termination.[56]

11 | ASSESSMENT OF ANATOMY

Examination and assessment by a paediatric surgeon with experience of DSD are critically important in the affected newborn. Combining expert physical examination with radiological assessment and endoscopic visualization, when necessary, can provide information on the location and state of the gonads, the urogenital sinus and Müllerian structures. During this initial assessment, the anatomy and drainage of the renal tract should also be assessed.

Ultrasonography is the first-line imaging modality and should include the adrenals, kidneys, pelvis, inguinal regions and labioscrotal folds where appropriate.[57] In the neonate, the uterus, ovaries and adrenals should be identifiable, but the likelihood of success is dependent on the state of the child and the expertise of the operator. It should also be borne in mind that the presence of a uterine structure does not guarantee later function and intra-abdominal testes and streak gonads are difficult to identify ultrasonography. In the prepubertal adolescent, it may be difficult to confirm the presence of a uterus by ultrasonography and repeat imaging after a 6-month course of oestrogen may be required. Although MRI seems to be used more often these days, in children, its use should be considered ancillary to ultrasound [58] and it should be reserved for cases where ultrasonography has failed to delineate the relationship of the Müllerian structures and where there are abnormalities of the urinary tract. High-resolution MRI should include the pelvis and perineum with and without fat saturation and T1 in three planes where possible. MRI can identify extra-abdominal ectopic testes and the presence of the spermatic cords, but may not be superior to ultrasound examination for identifying the presence and character of intra-abdominal testes or streak gonads.[59] In adolescents, MRI can delineate structural anomalies such as haematocolpos or hydronephrosis, identify secretory tumours and identify the location of the intra-abdominal gonads. However, the value of this modality, as well as that of ultrasound scanning in identifying early neoplasia in retained testes remains questionable.[60,61] Apart from the above, MRI imaging of the upper abdomen in adolescents is not required unless there is an adrenal mass in which case contrast enhancement shall also be required.

Nowadays, the 'genitogram' is not routinely performed for diagnostic purposes. It has been superseded by endoscopic examination of the genital tract (genitoscopy), which provides a more detailed and thorough assessment. However, a genitogram, performed in preparation for surgery allows the placing of stents in various internal structures to allow a more focused radiological examination. These investigations should provide information on the length of the urogenital sinus, the associated Müllerian structures and the relationship of the urethra and its sphincter. In 46, XX DSD, genitoscopy can assess drainage of both the bladder and Müllerian structures and provide a detailed assessment of the urogenital anatomy. In 46, XY DSD, endoscopic examination can be used to identify any Müllerian remnants that arise from the posterior urethra.

Genitoscopy can be combined with laparoscopy, but this is not necessary in all cases of DSD. Laparoscopy is a very effective method of inspecting the internal sex organs and facilitates manipulation or biopsy of intra-abdominal gonads.[62,63] However, as laparoscopy can only visualize intraperitoneal structures, Müllerian remnants deep within the pelvis or closely attached to the bladder may not be seen. In 46, XY DSD, laparoscopy is clearly indicated in all infants with impalpable testes where the gonads need to be identified and brought down to the scrotum if possible. Laparoscopy can also be used in adolescents who present with a DSD. However, MRI may be a more suitable first-line investigation for defining the anatomy.

12 | STEROID MEASUREMENT AND ITS INTERPRETATION

Steroid hormone analysis is a vital component of the biochemical evaluation, but the method of analysis can have a significant impact on the result. Liquid chromatography linked with tandem mass spectrometry (LC-MS/MS) allows multiple analyte analysis from a single sample whilst maintaining analytical specificity[64] and, in cases of DSD, plasma or serum steroids should be measured by LC-MS/MS which is available increasingly widely in the UK.[65] It is expected that over the next few years, further advances in the range of steroids that can be routinely measured in diagnostic laboratories in the UK will lead to greater diagnostic accuracy. For instance, LC-MS/MS-based analysis of multiple steroids including 17OHP, 21-deoxycortisol and 11-deoxycortisol may provide greater diagnostic precision at an earlier stage in a newborn with CAH.[66] However, there is a need for a sustained effort at ensuring that these diagnostic services contribute to external quality assessment. Close communication between the clinical and biochemistry personnel within the DSD team is vital to enable correct interpretation of laboratory results and awareness that results should be available in a timely manner. In addition to serum steroid analysis, USP analysis by gas chromatography mass spectrometry (GC-MS) can provide additional and more comprehensive qualitative and quantitative data on excretion of steroid metabolites. As gonadotrophins, androgens and precursors, fluctuate markedly over the first few months of life and may lead to a diagnostically blind window there is a place to consider an early neonatal collection as well as further samples at a later stage. A urine sample can be frozen and stored for many years and may help with a review of the diagnosis at a later stage. USP is not appropriate for suspected cases of 5α-reductase type 2 deficiency until after 3 months of age as diagnostic pairs of 5β to 5α reduced metabolites are not detectable until then.[67] As urine metabolites may also fluctuate during the day,[68] in cases where there is a high level of suspicion, the clinician should consider a 24-h urine collection. The number of steroid metabolites that can be measured on a USP has also increased dramatically over the past few years and whilst ratios of individual metabolites may provide greater discriminatory power[69] their utility in routine clinical practice needs further review.[70] Normally, infants, particularly boys, have significant changes in steroid and other endocrine hormone concentrations during the first 100 days of birth.[64] In boys, serum testosterone and DHT may initially be high at birth but decline to less than 1 nmol/L or undetectable, respectively. Concentrations then rise from around day 30 after birth to peak at day 70 before declining to normal prepubertal concentrations.[71] These normal variations may influence the interpretation of sex steroid and gonadotrophin measurements as well as the results of the hCG stimulation test. Furthermore, the actual value for the hormone concentration will vary depending on the assay methodology.

13 | PEPTIDE HORMONES

Analysis of peptide hormones is important in investigation of suspected DSD, and these hormones include the gonadotrophins, LH and FSH, anti-Müllerian hormone (AMH) and inhibin B. The absolute levels of gonadotrophins and the ratio of LH:FSH show sexually dimorphic patterns during the first year of life.[72] In addition, they are helpful in the assessment of primary hypogonadism and hypogonadotropic hypogonadism.[73] In those cases, where there is a suspicion of hypogonadotropic hypogonadism, an LHRH stimulation test may need to be considered as well as investigations that exclude other pituitary hormone deficiencies. AMH is strongly expressed in Sertoli cells from the time of testicular differentiation to puberty and to a much lesser degree in granulosa cells from birth to menopause and is widely used nowadays to assess ovarian reserve.[74] In the past, circulating AMH concentrations had to be interpreted with caution due to differences in the way immunoassays were standardized but nowadays commonly used assays show very low inter-assay variance.[75] In boys, AMH is detectable at birth at a much higher circulating concentration in boys than in girls and these concentrations rise over infancy before gradually declining at puberty. Therefore, up to date, age, sex and method-related reference ranges are necessary for interpretation.[73] In male neonates, levels that are close to the lower end of the normal range should be repeated later in infancy as they should rise further in boys with normal testes. The measurement of AMH is a powerful tool to assess Sertoli cell activity in children with suspected DSD and may also have a diagnostic utility in conditions associated with androgen deficiency or insensitivity where AMH may be raised and in hypogonadotropic hypogonadism where AMH may be low.[76] The discriminant value of AMH in cases of bilateral anorchia is so high that an undetectable AMH in such a case may avoid the need for invasive surgical exploration.[77] Inhibin B is a dimeric disulphide-linked glycoprotein consisting of two subunits (ie α and β) and like AMH, it is part of the TGFβ protein family. The main role of inhibin is the down-regulation of FSH synthesis, and like AMH, it can act as a marker of functioning testicular and ovarian tissue.[78] However, unlike AMH, the peptide hormone assays for inhibin B are not currently included in any external quality control exercise in the UK.[73] The utility of measuring circulating inhibin B maybe greatest from late childhood when unlike AMH which falls to low levels during adolescence, inhibin B levels rise higher.[76,78] Recently, INSL3 has been reported as a marker of Leydig cell activity [79] and its utility in the evaluation of conditions associated with DSD needs further exploration.

14 | THE HUMAN CHORIONIC GONADOTROPHIN STIMULATION TEST

Stimulation with human chorionic gonadotrophin (hCG) allows the identification of functioning Leydig cells as well as biosynthetic defects in testosterone synthesis (Figure 3). However, it is an invasive test and its results require careful interpretation as outlined in Figure 3 and it should only be performed at a later stage in the diagnostic pathway under the direction of the regional DSD centre.[54] Generally, in routine cases of XY DSD, serum AMH has a high predictive value for a post-hCG testosterone.[80] Most protocols for hCG stimulation in the UK use intramuscular hCG 1000-1500units on three consecutive days [81] and this can be followed by further hCG stimulation with 1500 units on two days a week for the following 2 weeks for a prolonged period of hCG stimulation.[82] The 3 week hCG stimulation test may be more appropriate in those cases where

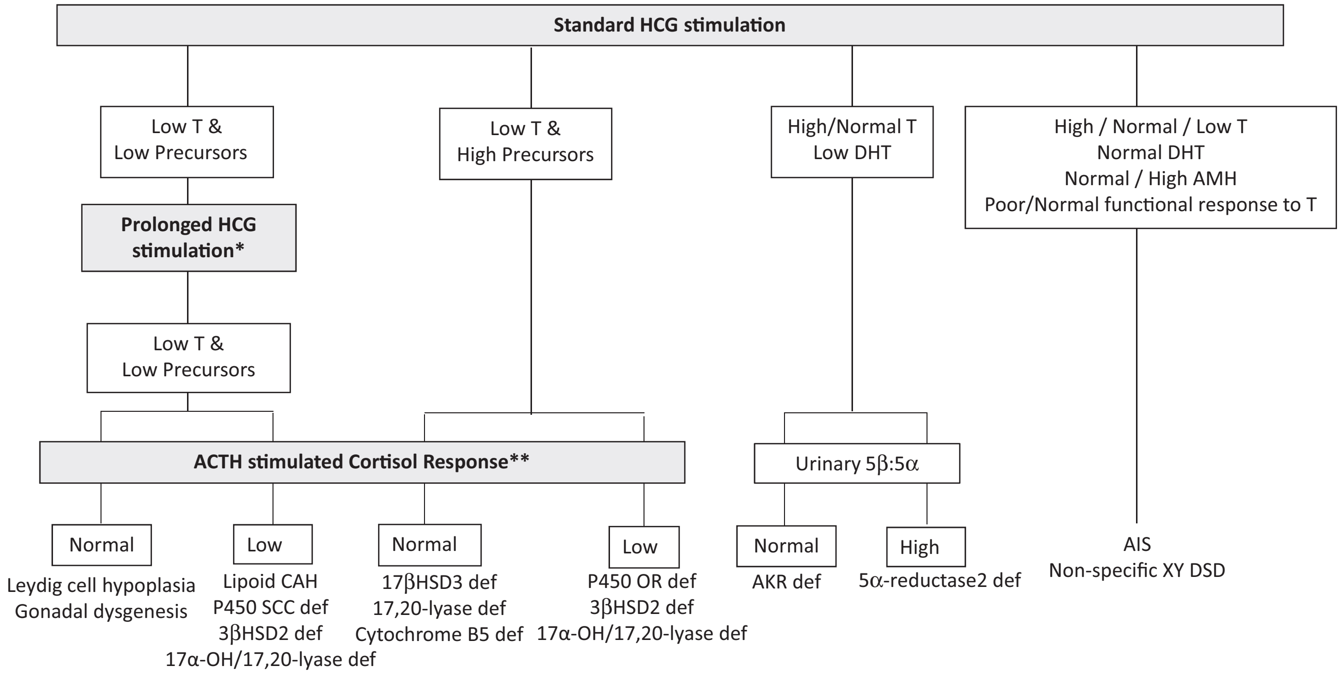

FIGURE 3 Interpretation of the results of the hCG stimulation test when investigating XY DSD and pointers for consideration of prolonged hCG stimulation and ACTH stimulation. *A prolonged hCG stimulation test should be considered in those cases where there is a poor testosterone response to a standard hCG stimulation test or where a poor response is anticipated. **A synacthen stimulation test should be considered in those cases who show a poor testosterone response to hCG stimulation or if there is a clinical or biochemical suspicion of adrenal insufficiency. 17α-OH—17α hydroxylase, 17bHSD3—17β-hydroxysteroid dehydrogenase type 3, 3βHSD2 def—3β-hydroxysteroid dehydrogenase II, AIS—androgen insensitivity syndrome, AKR, aldoketoreductase; CAH, congenital adrenal hyperplasia; LCH, Leydig Cell Hypoplasia; P450 OR, P450 oxidoreductase; SCC, side-chain cleavage; def, deficiency

there is a high suspicion that a functioning gonad may not be present such as in bilateral cryptorchidism or where there is a high suspicion of hypogonadotropic hypogonadism where the Leydig cells may require more prolonged stimulation. In young infants and adolescents, 3 days of hCG stimulation may be sufficient and in the very young infant with an intrinsically active gonadal axis, an hCG stimulation test may not be necessary if serial blood samples show raised serum testosterone concentrations. A testosterone response to hCG may be labelled as normal if absolute testosterone concentrations reach a level that is above the upper limit of the normal prepubertal range or rise by more than twice the baseline value.[83] Other androgens that should also be measured include androstenedione and dihydrotestosterone and with the use of LC-MS/MS, the sample volumes have become lower. For these two metabolites, the post-hCG, day 4 sample is more important than the pre-hCG sample on day 1. If a prolonged hCG stimulation test is performed, the day 22 sample that is collected at the end for testosterone measurement should be stored and can be used to measure DHT or androstenedione if a sufficient sample was not available on day 4. There is no evidence that a urine steroid profile or a serum AMH checked after hCG stimulation has any added diagnostic value. There is less experience as well as a lower demand for a corresponding test to assess ovarian tissue or reserve in DSD. Whilst reports of ovarian hormones following stimulation with FSH need further exploration, none of the current tests of ovarian reserve are reliable predictors of reduced ovarian function.[84] In the presence of a poor testosterone response following hCG stimulation, assessment of adrenal function by a standard short synacthen stimulation test should be considered. There is currently insufficient evidence to recommend that everybody with XY DSD should have a ACTH stimulation test but clinicians should be aware of the clear association between some forms of DSD and primary adrenal insufficiency and should consider thorough assessment of adrenal function in those diagnoses where an association has already been described[85] and in those with any clinical suspicion of adrenal insufficiency, especially those with low steroid precursors on USP.

15 | XX DSD

46,XX DSD can be classified into disorders of ovarian development, conditions with androgen excess and other syndromes, which are often associated with other developmental abnormalities.

21-hydroxylase deficiency CAH with androgen excess is the commonest cause of 46, XX DSD with atypical genitalia in the neonatal period or early infancy and is characterized by androgen excess and a variable alteration in glucocorticoid and mineralocorticoid function and a specific profile of steroid hormones.[86,87] This profile can identify the enzyme defects including deficiency of 21-hydroxylase (90%–95% of cases), 11β-hydroxylase (4%–8% of cases), 3β-hydroxysteroid dehydrogenase type 2 (rare) and P450 oxidoreductase (unknown prevalence). P450 oxidoreductase deficiency (PORD) biochemically manifests as apparent combined CYP17A1 and CYP21A2 deficiency, sometimes also resembling CYP19A1 (aromatase) deficiency. Unlike other forms of CAH, PORD is characterized by increased androgen concentrations only during the prenatal and early neonatal period, but rapidly develop sex hormone deficiency. Further details of these enzyme defects as well as others that can cause 46, XX DSD are outlined in Table 2.

46, XX DSD also includes disorders of gonadal development including 46, XX ovotesticular DSD and 46, XX testicular DSD. 46, XX ovotesticular DSD commonly presents at birth with atypical genitalia and progressive virilization during puberty. In contrast, individuals with 46, XX testicular DSD usually have a male phenotype and absent Müllerian structures and are often diagnosed after karyotype analysis during work-up for infertility.[88] In 46, XX testicular DSD, about 80%–90% of patients will have Y chromosomal material including a translocated *SRY* gene, which is only rarely detected in 46, XX ovotesticular DSD. In other cases of 46, XX testicular DSD, duplications involving regulatory genes, *SOX9* and *SOX3* have been described. Gene variants in *NR5A1* and *NR2F2* have also been reported in 46, XX testicular and ovotesticular DSD with the *NR2F2* variants being associated with cardiac defects as well as other features.[89] Rarely, *RSPO1* and *WNT4*, and more recently *WT1* variants have also been described.[89,90] In those with a suspicion of 46, XX ovotesticular DSD, functional testing will require detection of testicular and ovarian tissue by a combination of biochemical testing, imaging and surgical exploration.

Disorders of Müllerian development are another group of 46, XX DSD, and in these cases, ovarian function is usually normal but often associated with cloacal anomalies and other characteristic malformations. Novel and recurrent copy number variations have been reported to be associated with a third of Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome and other Müllerian abnormalities.[91,92] Although most cases of Müllerian development disorders are not associated with androgen excess, the presence of the latter, particularly in the adolescent, should alert the clinician to a possible abnormality of the *WNT4* gene. Variants in a wide range of genes have now been described to be associated with uterine abnormalities and often these conditions are associated with multiple other anomalies.[93]

16 | XY DSD WITH LOW TESTOSTERONE AND LOW PRECURSORS

The differential diagnosis of 46, XY DSD associated with low testosterone and low precursors includes high defects in steroid synthesis (steroidogenic acute regulatory (StAR) protein, P450 side-chain cleavage (P450scc) enzyme/*CYP11A1*, sometimes Smith-Lemli-Opitz/*DHCR7*); LH receptor defects (*LHCGR*); and partial and complete forms of gonadal dysgenesis (Table 3).

Of note, complete or partial combined 17α-hydroxylase/17,20-lyase deficiency (*CYP17A1*) may also present with 'low testosterone and low precursors' if DHEAS and androstenedione are the only intermediates measured. The actual diagnosis can be reached by assessment of adrenal function by measuring ACTH, ACTH-stimulated cortisol, plasma renin activity (PRA), 11-deoxycorticosterone (DOC), corticosterone, aldosterone, measurement of Δ5 (pregnenolone, 17OHPreg) and Δ4 (progesterone, 17OHP) precursors or urine steroid analysis. Isolated 17,20-lyase deficiency, cytochrome b5 deficiency and PORD might also be diagnosed by this approach. Proximal blocks (StAR, P450scc) in the pathway affect steroidogenesis in the adrenal gland as well as the developing gonad.

LH receptor defects ('Leydig cell hypoplasia') typically result in elevated basal LH, hyper-responsive LH to GnRH stimulation, low precursors and testosterone, and impaired androgen response to hCG stimulation. No Müllerian structures will be present and adrenal function is normal. A spectrum of phenotypes has been reported including atypical genitalia and micropenis. In some cases, basal LH may not be elevated at times when the HPG axis is quiescent (6 months to late childhood).

In complete gonadal dysgenesis ('Swyer syndrome'), affected people will usually have a female phenotype with intra-abdominal dysgenetic streak gonads and a risk of tumour development. In some situations, ovotestes or even undifferentiated gonadal tissue may be found.[94] Müllerian structures are usually present due to impaired AMH secretion in early foetal life. Androgens and their precursors will be low, LH elevated, depending on age and a poor or absent testosterone response to hCG stimulation is seen. AMH concentrations will be low or undetectable, and adrenal function is usually normal unless the underlying defect is in steroidogenic factor-1 (*NR5A1*) or related adrenal or gonadal factors.

Partial gonadal (testicular) dysgenesis can present with a spectrum of phenotypes ranging from clitoromegaly, to atypical genitalia or severe hypospadias. Müllerian structures may or may not be present and testes of variable size and architecture are present along the path of descent. The biochemical profile is similar to complete gonadal dysgenesis, but generally less severe. If mild degrees of clitoromegaly in infancy are overlooked, a 46, XY child with partial gonadal dysgenesis may first present at puberty with progressive androgenization. Genetic analysis and associated features may be useful in defining the molecular aetiology of gonadal dysgenesis (Table 4). This group of conditions are also associated with a risk of tumour development which may be related to the extent of androgenization of the external genitalia in the XY child.[95]

17 | XY DSD WITH LOW TESTOSTERONE AND HIGH STEROID PRECURSORS

46, XY DSD with low testosterone and increased precursors can be caused by several variants of CAH, namely by 17α-hydroxylase (CYP17A1) deficiency, PORD and 3β-hydroxysteroid dehydrogenase

TABLE 2 Characteristics of 46, XX DSD associated with androgen excess

	Inheritance & Gene	Genitalia	Wolffian duct derivatives	Mullerian duct derivatives	Gonads	Typical signs and symptoms	Hormone profile
21-hydroxylase def	Autosomal Recessive, *CYP21A2*	Wide range of atypical genitalia	Absent	Normal	Ovary	Severe adrenal insufficiency in infancy ± salt loss; moderate-to-severe androgenization at birth	Decreased cortisol and/ or mineralocorticoids. Increased 17-hydroxyprogesterone, 21-deoxycortisol, androstenedione, testosterone, and/ or plasma renin (activity)
11β-hydroxylase def	Autosomal Recessive, *CYP11B1*	Wide range of atypical genitalia	Absent	Normal	Ovary	Adrenal insufficiency in infancy; moderate-to-severe androgenization at birth; arterial hypertension often developing at different ages	Decreased cortisol, corticosterone, aldosterone, and or plasma renin (activity) Increased 11-deoxycortisol, 11-deoxycorticosterone, androstenedione, testosterone
3β-hydroxysteroid dehydrogenase II def	Autosomal Recessive, *HSD3B2*	Commonly clitoromegaly or mild virilization, can also be normal	Absent	Normal	Ovary	Severe adrenal insufficiency in infancy ± salt loss, androgenization during childhood and puberty, premature pubarche	Increased concentrations of Δ^5 C_{21-} and C_{19-} steroids, 17 hydroxypregnenolone and DHEA suppressible by dexamethasone
P450 oxidoreductase def	Autosomal Recessive, *POR*	Wide range of atypical genitalia including normal female	Absent	Normal	Ovary	Variable androgenization at birth and puberty, glucocorticoid deficiency, features of skeletal malformations. Maternal androgenization during pregnancy onset second trimester possible	Combined P450c17 and P450c21 insuff, normal or low cortisol with poor response to ACTH stim, elevated 17-hydroxyprogesterone, testosterone, progesterone and corticosterone; low oestradiol
P450 aromatase def	Autosomal Recessive, *CYP19A1*	Wide range of atypical genitalia	Absent	Normal	Ovary	Delayed bone age, development of ovarian cysts during infancy, childhood and puberty. Maternal androgenization during pregnancy	High androgens in cord blood, androgens may stay elevated or normalize soon after birth
Glucocorticoid insensitivity	Autosomal Dominant, *GRα*	Normal female	Absent	Normal	Ovary	Typically presents in young women with signs of androgen excess and fatigue with or without mineralocorticoid excess, hypertension and bilateral adrenal hyperplasia.	High/normal ACTH and high serum and urinary cortisol and lack of suppression to dexamethasone

type 2 (HSD3B2) deficiency, caused by inactivating gene variants in the corresponding genes *CYP17A1*, *POR* and *HSD3B2*, respectively. In addition, 46, XY DSD with low testosterone and increased precursors can typically be found in individuals affected by 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3) deficiency, caused by *HSD17B3* variants (Table 3).

Deficiency of CYP17A1 leads to CAH in about 1% of cases of 46, XY DSD. Characteristically, affected individuals present with external female genitalia and low DHEA, androstenedione and testosterone. There is an increase in mineralocorticoid synthesis and although there may be cortisol deficiency this is rarely manifested, as corticosterone can also bind and activate the glucocorticoid receptor. In PORD, sex steroids are characteristically low, sometimes low normal, whilst pregnenolone and progesterone and their metabolites accumulate, as expression of the combined block of CYP21A2 and CYP17A1 activities. Though there is often a relative preponderance of mineralocorticoid over glucocorticoid metabolites in affected cases, hypertension only manifests in adolescence or later. Although baseline glucocorticoid secretion is usually sufficient, in the majority of cases, the stress response to ACTH is significantly impaired, requiring at least stress dose hydrocortisone cover or permanent glucocorticoid replacement. 3β-HSD2 (also termed Δ4–Δ5 isomerase) deficiency invariably leads to glucocorticoid deficiency and as well as a variable degree of mineralocorticoid deficiency and its characteristic features are outlined in Table 4. 17β-HSD3 deficiency is responsible for the conversion of androstenedione to testosterone in the gonad and has no effect on adrenal steroidogenesis. Plasma steroids characteristically show increased androstenedione levels whilst testosterone levels are concurrently low, particularly after hCG stimulation. However, a low testosterone to androstenedione ratio may also occur in cases of gonadal dysgenesis and the reliability of a low ratio in identifying 17β-HSD3 deficiency is unclear. In urine, the typical finding is an increase in the androgen (and androstenedione) metabolites, androsterone and etiocholanolone, but this may only become apparent after puberty.

18 | XY DSD WITH NORMAL TESTOSTERONE, NORMAL PRECURSORS AND LOW DHT

The type 2 isoenzyme of 5α-reductase type 2 (SRD5A2) is highly expressed in androgen-sensitive tissues and converts testosterone to the more potent androgen, dihydrotestosterone (DHT) required for the development of external male genitalia. At birth, the external appearance of the genitalia of an infant with SRD5A2 deficiency can range from a completely female phenotype to a range of hypospadias severity or, rarely, isolated micropenis. A positive family history is often present in this autosomal recessive condition. In serum, the testosterone: DHT ratio following hCG stimulation often exceeds 30:1 but there are several reports of cases with a lower ratio.[96] In infants over 3–6 months, the defect should be easily identifiable simply on a urine sample which shows a decreased ratio for 5α:5β-reduced C_{21} and C_{19} steroids. This biochemical abnormality will also be present in a child who had early gonadectomy. Early diagnosis of this condition is important for sex assignment, and definitive diagnosis in a highly suspicious case may require access to a diagnostic genetics service with a quick turnaround time. With the wide availability of rapid genetic testing, the diagnosis of SRD5A2 deficiency is an example of a condition where molecular genetics is superseding detailed biochemistry as the preferred diagnostic tool.[6] In the infant raised as a boy, application of topical DHT may be a method of assessing the potential of the genitalia to virilise over the longer term. With the discovery of the alternative 'back door' pathway to DHT synthesis, there is a possibility that defects in the aldoketo reductase pathway may also lead to XY DSD [97] but the clinical significance of this defect in the presence of normal 5α-reductase remains debatable.[98]

19 | XY DSD WITH NORMAL TESTOSTERONE, NORMAL PRECURSORS AND NORMAL DHT

A defect in androgen signalling is most likely due to dysfunction of the androgen receptor protein (AR) and gene variants resulting in a complete lack of function of the *AR* cause Complete Androgen Insensitivity Syndrome (CAIS).[99] This presents in the newborn infant as a discordance between a female phenotype and a prenatal karyotype of 46, XY, a postnatal check because of a positive family history, or as inguinal or labial swellings in a girl. CAIS usually presents in adolescence as primary amenorrhoea with normal breast development and absent uterus. The presence of pubic hair is often reported in CAIS and should not be used to exclude the diagnosis. AR gene variants that result in some residual AR function and varying degrees of androgenization cause partial androgen insensitivity syndrome (PAIS). Although children with AIS typically have normal testosterone and DHT response to hCG stimulation and a normal urinary steroid profile, some demonstrate a poor response to hCG stimulation.[81] The serum AMH concentration is normal or may even be elevated. LH levels are increased in the face of normal or elevated serum testosterone, reflecting a state of androgen resistance. A family history of X-linked inheritance is informative although one-third of cases are the result of spontaneous new gene variants.

A functional assessment of androgen sensitivity may include assessing the clinical effect of a short course of testosterone or dihydrotestosterone applied on the phallus or by the effect of systemic testosterone following hCG stimulation. However, there is no consensus on the choice of androgen, dosage, method of administration, timing and duration of treatment as well as the definition of an optimal response in the growth of the phallus. Androgen sensitivity can be also assessed by measuring change in androgen responsive circulating proteins such as SHBG following androgen exposure but this is rarely performed in clinical practice as the response can be very variable. There may be other methods of assessing tissue responsiveness to androgens including the measurement of androgen responsive proteins in genital skin fibroblasts[100] or the assessment

TABLE 3 Characteristics of 46, XY disorders of sex development associated with deficiency of androgens or androgen action

	Inheritance & Gene	Genitalia	Wolffian duct derivatives	Mullerian duct derivatives	Gonads	Typical features	Hormone profile
LH deficiency or bioinactivity	Multiple causes of congenital hypogonadotropic hypogonadism (CHH)	Micropenis, undescended testes, rarely more atypical. Often normal male	Normal	Absent	Testes	May be associated with other features of hypopituitarism or syndromes associated with CHH. May present in early infancy or adolescence with pubertal delay.	Poor response to LHRH stimulation test, low AMH, variable testosterone response to hCG stimulation test.
Leydig cell hypoplasia	Autosomal Recessive, *LH/HCGR*	Wide range of atypical genitalia including normal female	Hypoplastic	Absent	Testes	Underandrogenization with variable failure of sex hormone production at puberty	Low T and DHT, elevated LH and FSH, exaggerated LH response to LHRH, poor T and DHT response to hCG stimulation
7-Dehydrocholesterol reductase deficiency	Autosomal Recessive, *DHCR7*	Wide range of atypical genitalia	Normal	Absent	Testes	Usually part of Smith-Lemli-Opitz syndrome	Variable elevation of 7-dehydrocholesterol. Maybe associated with adrenal insufficiency including mineralocorticoid deficiency
Lipoid congenital adrenal hyperplasia	Autosomal Recessive, *STAR*	Female, rarely atypical or male	Hypoplastic or normal	Absent	Testes	Severe adrenal insufficiency in infancy with salt loss, failure of pubertal development, rare cases associated with isolated glucocorticoid deficiency	Usually deficient of glucocorticoids, mineralocorticoids and sex steroids
P450 side-chain cleavage deficiency	Autosomal Recessive, *CYP11A1*	Female, rarely atypical or hypospadias	Hypoplastic or normal	Absent	Testes	Severe adrenal insufficiency in infancy with salt loss ranging to milder adrenal insufficiency with onset in childhood	Usually deficient of glucocorticoids, mineralocorticoids and sex steroids
3β-hydroxysteroid dehydrogenase II deficiency	Autosomal Recessive, *HSD3B2*	Wide range of atypical genitalia including apparently normal female genitalia	Normal	Absent	Testes	Severe adrenal insufficiency in infancy ± salt loss, poor androgenization at puberty with gynaecomastia	Increased concentrations of Δ^5 C_{21-} and C_{19-} steroids, 17 hydroxypregnenolone and DHEA suppressible by dexamethasone
Combined 17α-hydroxylase/17,20-lyase deficiency	Autosomal Recessive, *CYP17A1*	Wide range of atypical genitalia	Absent or hypoplastic	Absent	Testes	Absent or poor androgenization at puberty, gynaecomastia, hypertension	Decreased T, increased LH & FSH, low cortisol, increased plasma deoxycorticosterone, corticosterone and progesterone, decreased plasma renin activity, low renin hypertension with hypokalaemic alkalosis

(Continues)

TABLE 3 (Continued)

	Inheritance & Gene	Genitalia	Wolffian duct derivatives	Mullerian duct derivatives	Gonads	Typical features	Hormone profile
Isolated 17,20-lyase deficiency	Autosomal Recessive, CYP17A1, usually affecting key redox domains, alternatively caused by cytochrome b5 variants (CYB5)	Wide range of atypical genitalia	Absent or hypoplastic	Absent	Testes	Absent or poor androgenization at puberty, gynaecomastia	Decreased T, DHEA, androstenedione and oestradiol, abnormal increase in plasma 17-hydroxyprogesterone and 17-hydroxypregnenolone, increased LH and FSH, increased ratio of C_{21}-deoxysteroids to C_{19} steroids after hCG stim. Normal glucocorticoid and mineralocorticoid levels
P450 oxidoreductase deficiency	Autosomal Recessive, *POR*	Wide range of atypical genitalia	Absent or hypoplastic	Absent	Testes	Variable androgenization at birth and puberty, glucocorticoid deficiency, features of skeletal malformations (Antley-Bixler syndrome) Maternal androgenization during pregnancy onset second trimester possible	Combined P450c17 and P450c21 insuff, normal or low cortisol with poor response to ACTH stim, elevated 17-hydroxyprogesterone, T low
Cytochrome b5 deficiency	Autosomal Recessive	Wide range of atypical genitalia including normal female	Hypoplastic or normal	Absent	Testes	Associated with clinical and biochemical features of methemoglobinemia. Low methemoglobin reductase activity and low red cell cytochrome b5.	Low androgen metabolite excretion with increased excretion of pregnenetriol and normal mineralocorticoid and glucocorticoid metabolite excretion. Ratio of corticosterone over cortisol metabolites normal and elevated ratio of 17-alpha-hydroxyprogesterone over androgen metabolites
17β-hydroxysteroid dehydrogenase type 3 deficiency	Autosomal Recessive *HSD17B3*	Wide range of atypical genitalia including normal female	Present	Absent	Testes	Androgenization at puberty, gynaecomastia variable	Increased plasma oestrone, decreased ratio of testosterone/ androstenedione and oestradiol after hCG stim, increased FSH and LH

(Continues)

TABLE 3 (Continued)

	Inheritance & Gene	Genitalia	Wolffian duct derivatives	Mullerian duct derivatives	Gonads	Typical features	Hormone profile
5α-reductase-2 deficiency	Autosomal Recessive *SRD5A2*	Wide range of atypical genitalia including normal female	Normal or hypoplastic	Absent	Testes	Decreased facial and body hair, no temporal hair recession, prostate not palpable. Will androgenize in puberty	Decreased ratio of 5α/5β C_{21-} and C_{19-} steroids in urine, increased T/DHT ratio before and after hCG stim, modest increase in LH, decreased conversion of T to DHT in vitro
Aldo-keto reductase deficiency	Autosomal Recessive *AKRC2*, *AKRC4*	Wide range of atypical genitalia including normal female	Normal or hypoplastic	Absent	Testes	Similar features to 5α-reductase-2 deficiency but not expected to androgenize in puberty.	DHT deficiency and apparent 17,20-lyase deficiency but normal *CYP17A1* and *SRD5A2*
Complete androgen insensitivity	X-linked recessive *AR*	Female with blind vaginal pouch	Often normal or hypoplastic	Absent	Testes	Scant or absent pubic and axillary hair, breast development and female body habitus at puberty, primary amenorrhea	Increased LH and T, increased oestradiol, FSH levels normal or slightly increased, resistance to androgenic and metabolic effects of T (may be normal in some cases)
Partial androgen insensitivity	X-linked recessive *AR*	Wide range of atypical genitalia including normal male with infertility, sometimes referred to as mild AIS (MAIS)	Often normal	Absent	Testes	Decreased to normal axillary and pubic hair, facial and body hair, gynaecomastia common at puberty	Increased LH and T, increased oestradiol, FSH levels may be normal or slightly increased, partial resistance to androgenic and metabolic effects of T

TABLE 4 A selection of genes associated with disorders of gonadal development in XX and XY DSD. It is beyond the scope of this table to include all the congenital conditions and syndromes that are associated with atypical genitalia

	Inheritance	46 XX/46 XY/both	Gonadal dysgenesis	Testicular DSD	Ovotesticular DSD	Associated Features
ARX	XLD	XY	+			lissencephaly, epilepsy, learning difficulty
ATRX	XLD:del	XY				learning difficulty, α-thalassemia
BMP15	XLD	XX	+			
CBX2	AR	XY	+			
DAX1	XL:dup	XY	+			
DHH	AR/AD	XY	+			minifascicular neuropathy
DHX37	AD	XY	+			
DMRT1	AD:del	XY	+			learning difficulty
EMX2	AD:del	XY	+			learning difficulty, renal agenesis
ESR2	AR/AD	XY	+			
FGF9	AD	XX		+		
FGFR2	AD	XY	+			craniosynostosis
FOXL2	AD	XX	+			blepharophimosis, epicanthus inversus and ptosis
GATA4	AD	XY	+			congenital heart disease
HHAT	AR	XY	+			short stature, generalized chondrodysplasia, muscle hypertrophy, myopia, intellectual deficiency
MAP3K1	AD	XY	+			
NR2F2	AD			+	+	congenital heart defects, congenital diaphragmatic hernia, blepharon-phimosis-ptosis-epicanthus inversus syndrome
NR5A1	AD	XX	+	+	+	
NR5A1	AR/AD	XY	+			rarely primary adrenal insufficiency
NUP107	AR	XX	+			
RSPO1	AR	XX		+	+	palmoplantar hyperkeratosis, squamous cell carcinoma of skin
SOX3	XL:dup	XX		+	+	
SOX8	AD	XY	+			skeletal anomalies, learning difficulty
SOX9	AD:dup	XX		+	+	
SOX9	AD	XY	+			campomelic dysplasia
SOX10	AD:dup	XX		+	+	Waardenburg and Hirschsprung syndromes, peripheral neuropathy
SRY	T	XX		+	+	
SRY	Del	XY	+			
STARD8	XL	XY	+			
TSPYL1	AR	XY	+			abnormal brain stem development, sudden infant death
WNT4	AR/AD	XX		+	+	sex reversal dysgenesis of kidneys, adrenals and lung, lethal when biallelic
WNT4	AD:dup	XY	+		+	may also only have ovarian tissue
WT1	AD	XX		+		
WT1	variable	XY	+			Deletion—WAGR syndrome, inactivation—Denys Drash syndrome, splicing—Frasier syndrome
WWOX	AD;del	XY	+			epilepsy
ZFPM2	AD	XY	+		+	congenital heart disease
ZNRF3	AD	XY	+			

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; del, deletion; dup, duplication; XLD, X-linked dominant.

of the androgen responsive transcriptome[101] but their clinical utility requires further exploration. *AR* analysis may reveal a causative gene variant in over 90% of cases with a CAIS phenotype but given that only 20% of cases with a PAIS phenotype have a variant in the coding region of *AR*,[102] there is a need to improve the diagnosis of this condition especially as it has been reported that the gene variants in *AR* may exist beyond the coding sequence.[100] A number of newborns with XY DSD are loosely labelled as 'PAIS' when no conclusive biochemical or genetic abnormalities are identified in gonadal function, androgen synthesis or androgen action. However, the term PAIS should only be used in the context of a molecular confirmation of a likely causative *AR* variant as there is great prognostic value in having a genetically confirmed diagnosis of PAIS.[103] The majority of infants with XY DSD encountered in a DSD clinic and who are systematically investigated do not have an endocrine disorder of androgen synthesis and do not have a variant in *AR*.[37] Whilst in some of them, there may be other phenotypic clues, such as in the case of persistent Mullerian duct syndrome,[104] in others, the aetiology may only become clear with further clinical follow-up. The use of HTS in such cases has also started to identify variants in a wide range of genes that may have a role to play in genital tubercle development or testis migration.[105]

20 | CONCLUSION

Whilst the overarching rationale for investigating a newborn or an adolescent with a suspected DSD is to minimize the level of uncertainty, more specifically and commonly, it will include the need to work with the family to provide information and discuss whether the child is brought up as a boy or a girl, anticipate early medical problems, explain the aetiology to the young person or the parents of an affected newborn, support them psychologically in assimilating this knowledge and, finally, to develop a management plan that leads to optimal long-term outcome. A rational, stepwise and empathic approach that relies on the skills and knowledge of the experts within the MDT is essential for achieving these goals. The ultimate ambition of preserving a physically and psychologically well adult must be held in mind from the earliest care of the newborn, child or adolescent with a suspected DSD.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

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