Society for Endocrinology UK Guidance On The Initial Evaluation Of A Suspected Difference or Disorder Of Sex Development (DSD) (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.

Introduction & 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, IDSD, 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 re-published 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.

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 I). 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 as well as 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 optimised within the team. The role of a service manager or a clinic coordinator as well as 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.

**Networks & registers for clinical care, audit & research**

It is unrealistic to expect that every clinical centre can possess a comprehensive, multidisciplinary DSD team as outlined above. 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 (for example, 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 multi-disciplinary 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 organisation 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 ‘specialised service’ directly commissioned by NHS England but, currently, no formalised 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 utilisation 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).

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 and 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 organisations 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 utilises 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.
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). While 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 standardised 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.

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 normalise 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 dsdfamilies (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 as well as patients can also work in partnership with health care 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).

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 3,000 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 masculinisation 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 birth weight in XY DSD is very helpful given the well reported association between low birth weight, intra-uterine growth retardation and XY DSD (31, 47).

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 hrs, 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-hour 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 health care 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).

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

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 personalised 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 by chromosome analysis, either by karyotype, or more commonly, microarray, which identifies copy number variation (Fig.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).

Assessment of anatomy

Examination and assessment by a paediatric surgeon with experience of DSD is 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 Mülllerian 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 & 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.

Steroid measurement & 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-hour 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.
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 as well as 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 as well as hypogonadotrophic hypogonadism (73). In those cases, where there is a suspicion of hypogonadotrophic 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 standardised 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 hypogonadotrophic 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 (i.e. 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.

The human chorionic gonadotrophin (hCG) stimulation test

Stimulation with hCG allows the identification of functioning Leydig cells as well as biosynthetic defects in testosterone synthesis (Fig.3). However, it is an invasive test and its results require careful interpretation.
as outlined in Fig.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 1000-1500 units on 3 consecutive days (81) and this can be followed by further hCG
stimulation with 1500 units on two days a week for the following two weeks for a prolonged period of hCG
stimulation (82). The three week hCG stimulation test may be more appropriate in those cases where 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 hypogonadotrophic 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.

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 characterised 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 characterised 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 III.

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 virilisation 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).

### 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-Optiz/DHCR7); LH receptor defects (LHCGR); and partial and complete forms of gonadal dysgenesis (Table IV).

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, hyperresponsive 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 androgenisation. Genetic analysis and associated features may be useful in defining the molecular aetiology of gonadal dysgenesis (Table II). This group of conditions are also associated with a
risk of tumour development which may be related to the extent of androgenisation of the external genitalia in the XY child (95).

**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 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 IV).

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, while pregnenolone and progesterone and their metabolites accumulate, as expression of the combined block of CYP21A2 and CYP17A1 activities.

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 while 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.

**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 to 6 months, the defect should be easily identifiable simply on a urine sample which shows a decreased ratio for 5α:5β-reduced C<sub>21</sub> and C<sub>19</sub> steroids thus and can be reached 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).

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 androgenisation 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 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).

Conclusion

Whilst the overarching rationale for investigating a newborn or an adolescent with a suspected DSD is to minimise 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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<th>Role</th>
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| 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  
- Organise 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
- Organise 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
Oversee 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

Table I - The clinical members of the MDT and their potential roles in providing care to the patient and the parents.

<table>
<thead>
<tr>
<th>Inheritance</th>
<th>46 XX/46 XY/both</th>
<th>Gonadal dysgenesis</th>
<th>Testicular DSD</th>
<th>Ovotesticular DSD</th>
<th>Associated Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARX</td>
<td>XLD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>lissencephaly, epilepsy, learning difficulty</td>
</tr>
<tr>
<td>ATRX</td>
<td>XLD:del</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>learning difficulty, α-thalassemia</td>
</tr>
<tr>
<td>BMP15</td>
<td>XLD</td>
<td>XX</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBX2</td>
<td>AR</td>
<td>XY</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAX1</td>
<td>XL:dup</td>
<td>XY</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHH</td>
<td>AR/AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>minifascicular neuropathy</td>
</tr>
<tr>
<td>DHX37</td>
<td>AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMRT1</td>
<td>AD:del</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>learning difficulty</td>
</tr>
<tr>
<td>EMX2</td>
<td>AD:del</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>learning difficulty, renal agenesis</td>
</tr>
<tr>
<td>ESR2</td>
<td>AR/AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR2</td>
<td>AD</td>
<td>XX</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR2</td>
<td>AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>craniostenosis</td>
</tr>
<tr>
<td>FOXL2</td>
<td>AD</td>
<td>XX</td>
<td>+</td>
<td></td>
<td>blepharophimosis, epicanthus inversus and ptosis</td>
</tr>
<tr>
<td>GATA4</td>
<td>AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>congenital heart disease</td>
</tr>
<tr>
<td>HHAT</td>
<td>AR</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>short stature, generalised chondrodysplasia, muscle hypertrophy, myopia, intellectual deficiency</td>
</tr>
<tr>
<td>MAP3K1</td>
<td>AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>congenital heart defects, congenital diaphragmatic hernia, blepharophimosis-ptosis-epicanthus inversus syndrome</td>
</tr>
<tr>
<td>NR2F2</td>
<td>AD</td>
<td>+</td>
<td>+</td>
<td></td>
<td>rarely primary adrenal insufficiency</td>
</tr>
<tr>
<td>NR5A1</td>
<td>AD</td>
<td>XX</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NR5A1</td>
<td>AR/AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NUP107</td>
<td>AR</td>
<td>XX</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSPO1</td>
<td>AR</td>
<td>XX</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>SOX3</td>
<td>XL:dup</td>
<td>XX</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>SOX8</td>
<td>AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>skeletal anomalies, learning difficulty</td>
</tr>
<tr>
<td>SOX9</td>
<td>AD:dup</td>
<td>XX</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SOX9</td>
<td>AD</td>
<td>XY</td>
<td>+</td>
<td></td>
<td>campomelic dysplasia</td>
</tr>
<tr>
<td>SOX10</td>
<td>AD:dup</td>
<td>XX</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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</table>

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<table>
<thead>
<tr>
<th>Gene</th>
<th>Type</th>
<th>Sex</th>
<th>Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRY</td>
<td>T</td>
<td>XX</td>
<td>+</td>
</tr>
<tr>
<td>SRY</td>
<td>Del</td>
<td>XY</td>
<td>+</td>
</tr>
<tr>
<td>STARD8</td>
<td>XL</td>
<td>XY</td>
<td>+</td>
</tr>
<tr>
<td>TSPYL1</td>
<td>AR</td>
<td>XY</td>
<td>+</td>
</tr>
<tr>
<td>WNT4</td>
<td>AR/AD</td>
<td>XX</td>
<td>+</td>
</tr>
<tr>
<td>WNT4</td>
<td>AD:dup</td>
<td>XY</td>
<td>+</td>
</tr>
<tr>
<td>WT1</td>
<td>AD</td>
<td>XX</td>
<td>+</td>
</tr>
<tr>
<td>WT1</td>
<td>variable</td>
<td>XY</td>
<td>+</td>
</tr>
<tr>
<td>WWOX</td>
<td>AD;del</td>
<td>XY</td>
<td>+</td>
</tr>
<tr>
<td>ZFPM2</td>
<td>AD</td>
<td>XY</td>
<td>+</td>
</tr>
<tr>
<td>ZNRF3</td>
<td>AD</td>
<td>XY</td>
<td>+</td>
</tr>
</tbody>
</table>

Table II 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. AD: autosomal dominant; AR: autosomal recessive; del: deletion; dup; duplication; XLD: X-linked dominant.
<table>
<thead>
<tr>
<th>Inheritance &amp; Gene</th>
<th>Genitalia</th>
<th>Wolffian duct derivatives</th>
<th>Mullerian duct derivatives</th>
<th>Gonads</th>
<th>Typical signs and symptoms</th>
<th>Hormone profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-hydroxylase def</td>
<td>Autosomal Recessive, CYP21A2</td>
<td>Wide range of atypical genitalia</td>
<td>Absent</td>
<td>Normal</td>
<td>Ovary</td>
<td>Severe adrenal insufficiency in infancy ± salt loss; moderate to severe androgenisation at birth</td>
</tr>
<tr>
<td>11β-hydroxylase def</td>
<td>Autosomal Recessive, CYP11B1</td>
<td>Wide range of atypical genitalia</td>
<td>Absent</td>
<td>Normal</td>
<td>Ovary</td>
<td>Adrenal insufficiency in infancy; moderate to severe androgenisation at birth; arterial hypertension often developing at different ages</td>
</tr>
<tr>
<td>3β-hydroxysteroid dehydrogenase II def</td>
<td>Autosomal Recessive, HSD3B2</td>
<td>Commonly clitoromegaly or mild virilisation, can also be normal</td>
<td>Absent</td>
<td>Normal</td>
<td>Ovary</td>
<td>Severe adrenal insufficiency in infancy ± salt loss, androgenisation during childhood and puberty, premature pubarche</td>
</tr>
<tr>
<td>P450 oxidoreductase def</td>
<td>Autosomal Recessive, POR</td>
<td>Wide range of atypical genitalia including normal female</td>
<td>Absent</td>
<td>Normal</td>
<td>Ovary</td>
<td>Variable androgenisation at birth and puberty, glucocorticoid deficiency, features of skeletal malformations. Maternal androgenisation during pregnancy onset second trimester possible</td>
</tr>
<tr>
<td>P450 aromatase def</td>
<td>Autosomal Recessive, CYP19A1</td>
<td>Wide range of atypical genitalia</td>
<td>Absent</td>
<td>Normal</td>
<td>Ovary</td>
<td>Delayed bone age, development of ovarian cysts during infancy, childhood and puberty. Maternal androgenisation during pregnancy</td>
</tr>
<tr>
<td>Glucocorticoid insensitivity</td>
<td>Autosomal Dominant, GRα</td>
<td>Normal female</td>
<td>Absent</td>
<td>Normal</td>
<td>Ovary</td>
<td>Typically presents in young women with signs of androgen excess and</td>
</tr>
</tbody>
</table>
fatigue with or without mineralocorticoid excess, hypertension and bilateral adrenal hyperplasia.

dexamethasone

dexamethasone

Table III: Characteristics of 46, XX DSD associated with androgen excess

<table>
<thead>
<tr>
<th>Inheritance &amp; Gene</th>
<th>Genitalia</th>
<th>Wolffian duct derivatives</th>
<th>Mullerian duct derivatives</th>
<th>Gonads</th>
<th>Typical features</th>
<th>Hormone profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH deficiency or bioinactivity</td>
<td>Multiple causes of congenital hypogonadotropic hypogonadism (CHH)</td>
<td>Micropenis, undescended testes, rarely more atypical. Often normal male</td>
<td>Normal</td>
<td>Absent</td>
<td>Testes</td>
<td>May be associated with other features of hypopituitarism or syndromes associated with CHH. May present in early infancy or adolescence with pubertal delay.</td>
</tr>
<tr>
<td>Leydig cell hypoplasia</td>
<td>Autosomal Recessive, ( LH/HCG )</td>
<td>Wide range of atypical genitalia including normal female</td>
<td>Hypoplastic</td>
<td>Absent</td>
<td>Testes</td>
<td>Underandrogenisation with variable failure of sex hormone production at puberty</td>
</tr>
<tr>
<td>7-Dehydrocholesterol</td>
<td>Autosomal Recessive, ( LH/HCG )</td>
<td>Wide range of</td>
<td>Normal</td>
<td>Absent</td>
<td>Testes</td>
<td>Usually part of Smith Lemli Opitz</td>
</tr>
<tr>
<td>Condition</td>
<td>Genes</td>
<td>Gender</td>
<td>Testes</td>
<td>Syndrome</td>
<td>Associated features</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------------------------</td>
<td>-----------------</td>
<td>------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>reductase deficiency</strong></td>
<td>DHCR7</td>
<td>atypical genitalia</td>
<td></td>
<td>Syndrome</td>
<td>Maybe associated with adrenal insufficiency including mineralocorticoid deficiency</td>
<td></td>
</tr>
<tr>
<td><strong>Lipoid congenital adrenal hyperplasia</strong></td>
<td>Autosomal Recessive, STAR</td>
<td>Female, rarely atypical or male</td>
<td>Hypoplastic or normal</td>
<td>Testes</td>
<td>Severe adrenal insufficiency in infancy with salt loss, failure of pubertal development, rare cases associated with isolated glucocorticoid deficiency</td>
<td></td>
</tr>
<tr>
<td><strong>P450 side-chain cleavage deficiency</strong></td>
<td>Autosomal Recessive, CYP11A1</td>
<td>Female, rarely atypical or hypospadias</td>
<td>Hypoplastic or normal</td>
<td>Testes</td>
<td>Severe adrenal insufficiency in infancy with salt loss ranging to milder adrenal insufficiency with onset in childhood</td>
<td></td>
</tr>
<tr>
<td><strong>3β-hydroxysteroid dehydrogenase II deficiency</strong></td>
<td>Autosomal Recessive, HSD3B2</td>
<td>Wide range of atypical genitalia including apparently normal female genitalia</td>
<td>Normal</td>
<td>Testes</td>
<td>Severe adrenal insufficiency in infancy + salt loss, poor androgenisation at puberty with gynaecomastia</td>
<td></td>
</tr>
<tr>
<td><strong>Combined 17α-hydroxylase/17,20-lyase deficiency</strong></td>
<td>Autosomal Recessive, CYP17A1</td>
<td>Wide range of atypical genitalia</td>
<td>Absent or hypoplastic</td>
<td>Testes</td>
<td>Absent or poor androgenisation at puberty, gynaecomastia, hypertension</td>
<td></td>
</tr>
<tr>
<td><strong>Isolated 17,20-lyase deficiency</strong></td>
<td>Autosomal Recessive, CYP17A1, usually affecting key redox domains, alternatively caused by cytochrome b5 variants (CYB5)</td>
<td>Wide range of atypical genitalia</td>
<td>Absent or hypoplastic</td>
<td>Testes</td>
<td>Absent or poor androgenisation at puberty, gynaecomastia</td>
<td></td>
</tr>
<tr>
<td><strong>P450 oxidoreductase deficiency</strong></td>
<td>Autosomal Recessive, POR</td>
<td>Wide range of atypical genitalia</td>
<td>Absent or hypoplastic</td>
<td>Testes</td>
<td>Variable androgenisation at birth and puberty, glucocorticoid deficiency, features of skeletal malformations</td>
<td>Combined P450c17 &amp; P450c21 insuff, normal or low cortisol with poor response to ACTH stim, elevated 17-hydroxyprogesterone, T low</td>
</tr>
</tbody>
</table>

**Note:** Increased concentrations of Δ5 C21- & C19- steroids, 17 hydroxyprogrenenolone and DHEA suppressible by dexamethasone.
| 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 pregnenolone 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 | Androgenisation at puberty, gynaecomastia variable | Increased plasma estrone, decreased ratio of testosterone/ androstenedione and oestradiol after hCG stim, increased FSH & LH |
| 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 androgenise in puberty | Decreased ratio of 5α/5β C21. & C19. steroids in urine, increased T/DHT ratio before & 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 androgenise 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 & axillary hair, breast development & female body habitus at puberty, primary amenorrhea | Increased LH & T, increased oestradiol, FSH levels normal or slightly increased, resistance to androgenic & metabolic effects of T (may be normal in some cases) |
| Partial androgen insensitivity | X-linked recessive AR | Wide range of atypical genitalia including normal | Often normal | Absent | Testes | Decreased to normal axillary & pubic hair, facial & body hair, gynaecomastia common at puberty | Increased LH & T, increased oestradiol, FSH levels may be normal or slightly increased, partial resistance to androgenic & metabolic effects of T (may be normal in some cases) |
male with infertility, sometimes referred to as mild AIS (MAIS)
Legend To Figures

Fig.1  Approach to investigating adolescent girls with primary amenorrhea.

Fig.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.

Fig.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.
**Initial evaluation**
- Clinical phenotyping – history and examination
- Chromosome analysis (QF-PCR then Karyotype/Microarray)
- Consent for DNA storage and genomic testing

**Sex chromosome DSD or chromosome variation likely to explain DSD**

**46,XX DSD or 46,XY DSD**

**DSD Multidisciplinary Team Meeting**
- Clinical endocrinology
- Steroid & peptide biochemistry
- Clinical genetics
- Molecular genetics

**Genomic testing**
- Single gene analysis (only if monogenic condition clearly suspected)
- Microarray (if not done already, particularly if associated malformations)
- High Throughput Sequencing

**Liaise and discuss results with clinical geneticist in DSD team**

**Confirmatory result (Pathogenic/likely pathogenic gene variant)**

**VOUS or No causal genetic variants identified**

**Consider WES/WGS, especially if:**
- Associated malformations
- Endocrine abnormalities
- Positive family history
- Parental consanguinity

---

**Clinical Genetics**
- Genetic counselling
- Plan any further tests
- Cascade genetic testing in the family (if appropriate)
Low T & Low Precursors

Prolonged HCG stimulation*

Low T & Low Precursors

ACTH stimulated Cortisol Response**

Normal
Leydig cell hypoplasia
Gonadal dysgenesis

Low
Lipoid CAH
P450 SCC def
3β-HSD2 def
17β-HSD3 def
17,20-lyase def
Cytochrome B5 def
17α-OH/17,20-lyase def

Normal
17β-HSD3 def
17α-lyase def
17α-OH/17,20-lyase def

Low
P450 OR def
3β-HSD2 def

High/Normal T Low DHT

High/Normal T Normal DHT
Normal / High AMH
Poor/Normal functional response to T

Urinary 5β:5α

Normal
AKR def

High
5α-reductase2 def

Non-specific XY DSD

AIS