

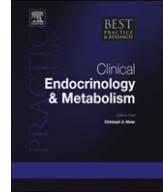


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### Investigation and initial management of ambiguous genitalia

S. Faisal Ahmed, Professor, MD, FRCPCH\*,  
Martina Rodie, BSc, MBChB, MRCPCH

*Department of Child Health, Royal Hospital For Sick Children, University of Glasgow, Yorkhill, Glasgow G3 8SJ, United Kingdom*

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Infants rarely present with truly ambiguous genitalia and such children should be evaluated by experts who work within a multidisciplinary team that is dedicated for evaluation and management of children and adults with suspected and confirmed disorders of sex development. The paediatric endocrinologist who is a vital and often the central member of this clinical team not only needs to lead the clinical evaluation of the infant systematically but also needs to be sensitive to the needs of the infant, the parents and the rest of the team. A thorough knowledge of the underlying pathophysiology and the strengths and weaknesses of the investigative tools that are available for reaching a diagnosis is crucial.

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The birth of a new baby is one of the greatest wonders of nature and one of the most exciting events known to mankind. The first question that is usually posed by the new parent is “is it a boy or a girl?”; without this information the parents cannot even formulate the second question which is usually “is he/she alright?”. It is no wonder that the birth of a child with an abnormality of genital development, where the sex of rearing is uncertain at birth, presents difficult clinical and ethical issues. However, the extent of genital ambiguity may depend on the expertise of the observer; whilst the prevalence of genital anomalies at birth may be as high as 1 in 300 births<sup>1</sup>, the birth prevalence of complex anomalies that may lead to true genital ambiguity may be as low as 1 in 5000 births.<sup>2</sup> Rather than treating every affected child as a medical emergency, it is paramount that such a child is firstly assessed by an expert with adequate knowledge about the range of variation in the physical appearance of genitalia, the underlying pathophysiology of disorders of sex development and the strengths and weaknesses of the tests that can be performed in early infancy. Such an expert should be able to ensure that the parents’

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\* Corresponding author. Tel.: +44 141 201 0571; Fax: +44 141 201 0837.

E-mail address: [s.f.ahmed@clinmed.gla.ac.uk](mailto:s.f.ahmed@clinmed.gla.ac.uk) (S.F. Ahmed).

needs for information are comprehensively addressed whilst appropriate investigations are performed in a timely fashion. Further, the expert also needs to have immediate access to the multidisciplinary team that is essential for the management of such a child.<sup>3</sup>

Terminology

The use of terminology which is clear and easy to use and understand by all health professionals, patients and their families is fundamental to the understanding, investigation and management of affected newborns and children. In addition, terminology should respect the patient. The term ‘intersex’ has had variable connotations even within professionals; some employed it as a term that covered all affected newborns whilst, at the other end of the spectrum, some believed that the term should only apply to those where there is complete mismatch between chromosomal and anatomic sex. The consensus reached in Chicago in 2005 on management of these patients stressed the importance of the aspect of terminology and recommended the substitution of the term ‘intersex’ with ‘disorder of sex development (DSD)’ which is defined as any congenital condition in which development of chromosomal, gonadal or anatomic sex is atypical.<sup>4</sup> It also recommended the abandonment of terms such as ‘pseudohermaphroditism’ and ‘true hermaphroditism’. Whilst the new nomenclature (Table 1) is easier to use and understand and helps the professional to plan investigations, it will nevertheless evolve over time as our understanding of long-term outcome as well as molecular aetiology improves in the future. Given that genital anomalies may occur as commonly as 1 in 300 births and may not always be associated with a functional abnormality, some have advocated the use of ‘differences’ in preference to the term ‘disorder’.<sup>5,6</sup> The strength of the acronym ‘DSD’ is that it can be used to cover both differences and disorders of sex development. However, the likelihood of this difference existing as a disorder will depend on the functional implications of the condition which may be heavily influenced by the social and cultural framework within which the child exists.

General principles of management

Optimal clinical management of infants with DSD should comprise the following principles:

**Table 1**  
The classification of DSD. The karyotype is the primary root, the disorders are in the shaded row are the secondary root and the actual diagnoses which will often be based on molecular, biochemical or histological examination are listed under each secondary root.

Primary Root	Disorder of gonadal development	Disorder of androgen synthesis	Disorder of androgen action	Disorder of androgen excess	Leydig cell defect
	Complete gonadal dysgenesis Partial gonadal dysgenesis Gonadal regression Ovotesticular DSD Testicular DSD	StAR P450 scc 3β-HSD CYP17 17βHSD 5α reductase P450 OR	PAIS CAIS Other	21αhydroxylase 11βhydroxylase Aromatase P450 OR Maternal androgens	Leydig cell hypoplasia LH deficiency
Secondary Root					
Primary Root	Persistent Mullerian Duct Syndrome	Defects of Mullerian development	Non-specific disorder of undermasculinisation	Actual Diagnosis	
	AMH low AMH normal AMH not known	MURCS MRKH Uterine Didelphys Other	Isolated hypospadias Isolated bilat cryptorchidism Isolated micropenis Anomalies EMS >8 Anomalies EMS 5-8 Anomalies EMS <5	Cloacal Anomaly Bladder Exstrophy Smith Lemli Opitz Synd Other	

1. All newborn infants should receive a male or female sex assignment.
2. When there is any doubt about sex assignment, a hasty decision must be avoided prior to expert evaluation
3. Whilst all specialist neonatal units should be expected to be able to stabilise the critically unwell infant with a DSD, comprehensive evaluation and the development of a plan for long-term management must be completed at a specialist centre with an experienced multidisciplinary team.
4. The specialist centre should be able to quickly complete first-line investigations that are sufficient for deciding sex assignment and excluding immediate medical concerns; the centre should then be able to develop a plan for second-line investigations that will guide long-term management of the child.
5. Management should be patient-centred and holistic and, as far as possible, evidence based. Decisions which are not evidence based should be explained to the family.
6. Patient and family concerns should be respected and addressed in strict confidence.
7. Open communication with patients and families is essential and participation in decision making is encouraged.
8. The multidisciplinary specialist team should have the ability to arrange or, preferably, provide long-term care from infancy to adulthood for the affected individual.

## Communication

The initial contact with the parents of a child with a DSD is important as first impressions from these encounters often persist. A key point to emphasise is that the child with a DSD has the potential to become a well-adjusted, functional member of society. The use of the phrase 'differences in sex development' may be particularly beneficial in introducing the concept of the range of variation in sex development that can be encountered to those with little prior knowledge of the field. The analogy between a common condition such as variations in stature and associated functional disability may be easy to explain and understand, both for the parent as well as the health professional. Most differences in stature do not have any consequence but marked tall or short stature can affect function. In addition, in many cases, although the abnormality in stature itself may not be profound and may not have a functional consequence, it may be a pointer towards other more important co-existing health issues and, thus, requires thorough clinical evaluation. Whilst it is likely that many conditions that may be associated with DSD are much more complex and ethically challenging, discussions that use the above approach as the first step may reduce the stigma that is often experienced by families and emphasise that DSD is not shameful.<sup>7</sup> In those cases where there are no doubts about sex assignment, it should not be assumed that the parents' need for information and psychological help are any less; the parents' perception of risk may be quite different from the clinical perception of the severity of illness.<sup>8</sup> In those cases where there is true genital ambiguity, it should be explained to the parents that the best course of action may not initially be clear, but the health-care team will work with the family to reach the best possible set of decisions in the circumstances. The health-care team should discuss with the parents the information to be shared in the early stages with family members and friends. Parents need to be informed about sex development; they should be provided written information and directed to Internet-based information (Table 2). Ample time and opportunity should be provided for continued discussion with review of information previously provided.

## The multidisciplinary team

Optimal care for children with DSD requires an experienced multidisciplinary team that is generally found in regional centres. The team may exist as a clinical network with links to other children's centres; for instance, see [www.sgan.nhsscotland.com](http://www.sgan.nhsscotland.com). Ideally, the team includes paediatric sub-specialists in endocrinology, surgery and/or urology, psychology/psychiatry, gynaecology, genetics, neonatology, nursing and, if possible, social work and medical ethics. Core composition will vary according to DSD type, local resources, developmental context and location. Ongoing communication with the family's primary care physician is important. The team has a responsibility to

**Table 2**  
Online information on DSD for Patients, Parents & Professionals.

General Information about Sex Development
• Syndromes of Abnormal Sex Differentiation – <a href="http://www.hopkinschildrens.org">www.hopkinschildrens.org</a>
• UK Intersex Association – <a href="http://www.ukia.co.uk">www.ukia.co.uk</a>
• Intersex Society of North America – <a href="http://www.isna.org">www.isna.org</a>
• Child Physiology – <a href="http://www.sickkids.ca">www.sickkids.ca</a>
Congenital Adrenal Hyperplasia
• Congenital Adrenal Hyperplasia Education & Support Network – <a href="http://www.congenitaladrenalhyperplasia.org">www.congenitaladrenalhyperplasia.org</a>
• Climb Congenital Adrenal Hyperplasia UK Support Group – <a href="http://www.livingwithcah.com">www.livingwithcah.com</a>
• Your Child with Congenital Adrenal Hyperplasia – <a href="http://www.rch.org.au/cah_book/index.cfm?doc_id=1375">www.rch.org.au/cah_book/index.cfm?doc_id=1375</a>
• Adrenal Hyperplasia Network – <a href="http://www.ahn.org.uk">www.ahn.org.uk</a>
Androgen Insensitivity Syndrome
• Androgen Insensitivity Syndrome Support Group – <a href="http://www.aissg.org">www.aissg.org</a>
• eMedicine – <a href="http://emedicine.medscape.com/article/924996-overview">http://emedicine.medscape.com/article/924996-overview</a>
• Complete Androgen Insensitivity Syndrome – <a href="http://www.rch.org.au/publications/CAIS.html">www.rch.org.au/publications/CAIS.html</a>
XY/XO Gonadal Dysgenesis
• xyTurners – <a href="http://www.xyxo.org">www.xyxo.org</a>
Hypospadias
• Hypospadias Support Group – <a href="http://www.hypospadias.co.uk">www.hypospadias.co.uk</a>
Clinical Networks
• The Scottish Genital Anomaly Network – <a href="http://www.sgan.nhsscotland.com">www.sgan.nhsscotland.com</a>
• Netzwerk Intersexualitat – <a href="http://www.netzwerk-dsd.uk-sh.de">www.netzwerk-dsd.uk-sh.de</a>
Research Networks
• EuroDSD – <a href="http://www.eurodsd.eu/index.php">www.eurodsd.eu/index.php</a>
• European DSD Registry – <a href="https://tethys.nesc.gla.ac.uk/">https://tethys.nesc.gla.ac.uk/</a>
Consensus Views
• Consensus Statement on 21-Hydroxylase Deficiency from The European Society for Paediatric Endocrinology and The Lawson Wilkins Pediatric Endocrine Society – <a href="http://www.eurospe.org/clinical/Docs/CAH.pdf">http://www.eurospe.org/clinical/Docs/CAH.pdf</a>
• Consensus Statement on management of intersex disorders – <a href="http://adc.bmj.com/content/91/7/554.full">adc.bmj.com/content/91/7/554.full</a>
Medical & Genetic Overview
• Medline Plus– <a href="http://www.nlm.nih.gov/">www.nlm.nih.gov/</a>
• Genecard

educate other health-care staff in the appropriate initial management of affected newborns and their families and should also have the ability to review and discuss its own performance through audit of clinical activity and attendance at joint clinics and educational events. For new infants with a DSD, the team should develop a plan for clinical management with respect to diagnosis, gender assignment and treatment options prior to making any recommendations. Ideally, discussions with the family are conducted by one professional with appropriate communication skills. Transitional care should be organised with the multidisciplinary team operating in an environment comprising specialists with experience both in paediatric and adult practice. Whilst support groups have an important role to play and their contact details should be supplied to the parents, it is possible that affected parents may prefer to talk to local families affected in a similar way. The availability of such a local pool of voluntary helpers who had some support from the specialists would complete the composition of the multi-disciplinary team.

**Clinical evaluation of the infant with a suspected DSD**

The infant with a suspected DSD may need evaluation for four broad reasons. First, there may be a need to determine the sex of rearing. Second, there may be concerns about immediate, life-threatening metabolic conditions that are more likely to be associated to certain diagnoses that are, for instance, associated with adrenal insufficiency. Third, an improved knowledge of the aetiology of the underlying condition may allow the development of a long-term management plan. Finally, continued evaluation over the longer term will allow the affected individuals and their care-providers to better understand issues such as fertility, sexual function and the risk of tumour development and help with informed disclosure of the diagnosis itself.

## Initial approach

It is very likely that the clinician from whom a specialist opinion is sought will encounter the infant and the parents after the family have already been seen by other health professionals. Their anticipation of meeting someone who can answer all their questions, provide them with reassurance and solve all the problems can be a daunting and impossible task for a single clinician, irrespective of his/her level of expertise. It is likely that this clinician will form a long-lasting relationship with this family and, over time, with the help of the multidisciplinary team, will be able to address most of the issues above. It is, therefore, very important to have a positive and systematic approach that starts the first encounter with the family with emphasising the general well-being of the child.

## History

An adequate history should concentrate particularly on:

- Parental consanguinity, history of salt-losing, unexplained infant deaths or DSD in relatives. These elements may indicate autosomal recessive genetic disorders associated with disturbed steroidogenesis. By contrast, an X-linked recessive mode of inheritance is suggestive of androgen insensitivity syndrome (AIS).
- Maternal ingestion of drugs or exposure to specific environmental factors capable of inhibiting virilisation of the foetus during the pregnancy.
- Whether the pregnancy was planned.
- Some assisted-conception techniques including some of the progestagen-containing drugs that are used for some of these methods have a higher likelihood of male offspring with genital anomalies.
- In cases where parents have had some prenatal advice and discussion, it is useful to have access to these previous discussions and parent's recollection of these discussions which may have occurred during the state of pregnancy.
- Results of prenatal tests.
- Social history with an enquiry about the family's social network.
- Parents' general understanding of DSD and their current concerns.
- Knowledge of what has already been discussed with the parents by health professionals is essential.

## General examination

The general physical examination should determine whether there are any dysmorphic features and the general health of the baby.<sup>9–11</sup> Affected infants, particularly those who have XY DSD, are more likely to be small for gestational age and may display other developmental anomalies.<sup>1</sup> Examples of some known syndromes that are associated with genital anomalies and their characteristic features in early infancy are listed in [Table 3](#). In addition to a systematic examination, the affected infant should be examined for mid-line defects which may point towards an abnormality of the hypothalamic–pituitary axis. The state of hydration and blood pressure should be assessed as various forms of adrenal steroid biosynthetic defects can be associated with differing degrees of salt loss, varying degrees of masculinisation in girls or under-masculinisation in boys, or hypertension. Although the cardiovascular collapse with salt loss and hyperkalaemia in congenital adrenal hyperplasia (CAH) does not usually occur until the second week of life and so will not be apparent at birth in a healthy neonate, it should be anticipated in a suspected case. Jaundice (both conjugated and unconjugated) may be observed in cases of hypopituitarism or cortisol deficiency. The urine should be checked for protein as a screen for any associated renal anomaly (e.g., Denys–Drash/Frasier syndromes) and a pre-feed blood glucose level should be checked for hypoglycaemia. Urinary tract anomalies such as ureteropelvic junction obstruction, vesicoureteric reflux, pelvic or horseshoe kidney, crossed renal ectopia and renal agenesis may occur in as many as 1% and 5% of cases with isolated distal and proximal hypospadias, respectively.<sup>12</sup>

**Table 3**

Characteristics of 46, XY disorders of sex development.

	Inheritance & Gene	Genitalia	Wolffian duct derivatives	Mullerian duct derivatives	Gonads	Outlook	Hormone profile
Gonadal dysgenesis	Several single gene disorders & chromosomal rearrangements	Female, ambiguous or male	Absent or hypoplastic	Present	Streak, ovotestes	Infants with female phenotype may present in adolescence with primary amenorrhoea	Variable reduction in AMH and T response to hCG stimulation
Lipoid CAH	Autosomal Recessive, StAR	Female, rarely ambiguous 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 & sex steroids
P450SCC def	Autosomal Recessive, CYP11A1	Female, rarely ambiguous	Hypoplastic or normal	Absent	Testes or absent	Severe adrenal insufficiency in infancy with salt loss ranging to milder adrenal insufficiency with onset in childhood	Usually deficient of glucocorticoids, mineralocorticoids & sex steroids
3 $\beta$ -hydroxysteroid dehydrogenase II def	Autosomal Recessive, HSD3B2	Ambiguous, hypospadias	Normal	Absent	Testes	Severe adrenal insufficiency in infancy, poor virilisation at puberty with gynaecomastia	Increased concentrations of $\Delta^5$ C <sub>21</sub> - & C <sub>19</sub> - steroids, 17 hydroxypregnenolone and DHEA suppressible by dexamethasone
Combined 17 $\alpha$ -hydroxylase/17,20-lyase def	Autosomal Recessive, CYP17	Female, ambiguous or hypospadias	Absent or hypoplastic	Absent	Testes	Absent or poor virilisation at puberty, gynaecomastia, hypertension	Decreased T, increased LH & FSH, increased plasma deoxycorticosterone, corticosterone & progesterone, decreased plasma renin activity, low renin hypertension with hypokalaemic alkalosis
Isolated 17,20-lyase def	Autosomal Recessive, CYP17, usually affecting key redox domains	Female, ambiguous or hypospadias	Absent or hypoplastic	Absent	Testes	Absent or poor virilisation at puberty, gynaecomastia	Decreased T, DHEA, androstenedione & oestradiol, abnormal increase in plasma 17-hydroxyprogesterone & 17-hydroxypregnenolone, increased LH & FSH, increased ratio of C <sub>21</sub> -deoxysteroids to C <sub>19</sub> -steroids after hCG stim

17 $\beta$ -hydroxysteroid dehydrogenase type 3 def	Autosomal Recessive HSD17B3	Female, ambiguous, blind vaginal pouch	Present	Absent	Testes	Virilisation at puberty, gynaecomastia variable	Increased plasma estrone, decreased ratio of testosterone/ androstenedione and oestradiol after hCG stim, increased FSH & LH
5 $\alpha$ -reductase-2 def	Autosomal Recessive SRD5A2	Ambiguous, micropenis, hypospadias, phallus, blind vaginal pouch	Normal	Absent	Testes	Decreased facial and body hair, no temporal hair recession, prostate not palpable	Decreased ratio of 5 $\alpha$ /5 $\beta$ C <sub>21</sub> - & C <sub>19</sub> -steroids in urine, increased T/DHT ratio before & after hCG stim, modest increase in LH, decreased conversion of T to DHT in vitro
PAIS	X-linked recessive AR	Ambiguous with blind vaginal pouch, isolated hypospadias, normal male with infertility (mild)	Often normal	Absent	Testes	Decreased to normal axillary & pubic hair, beard growth & 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
CAIS	X-linked recessive AR	Female with blind vaginal pouch	Often present depending on mutation type	Absent or vestigial	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
P450 oxidoreductase def	Autosomal Recessive, POR	Ambiguous, hypospadias or normal male	Absent or hypoplastic	Absent	Testes	Variable virilisation at birth and puberty, glucocorticoid deficiency, features of Antley-Bixler Syndrome in some cases	Combined P450c17 & P450c21 insuff, normal or low cortisol with poor response to ACTH stim, elevated 17-hydroxyprogesterone, T low
Leydig cell hypoplasia	Autosomal Recessive, LH/HCGR	Female, hypospadias or micropenis	Hypoplastic	Absent	Testes	Undermasculinisation with variable failure of sex hormone production at puberty	Low T & DHT, elevated LH & FSH, exaggerated LH response to LHRH, poor T & DHT response to hCG stimulation
Persistent Mullerian Duct Syndrome	AMH gene or receptor mutation	Male	Present	Present	Testes	Cervix, fallopian tube, uterus may be present. Fertility can be affected	Normal T response to hCG stimulation

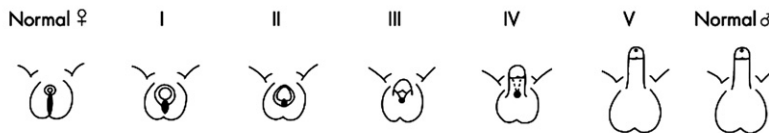
DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinising hormone; T, testosterone; DHEA, dehydroepiandrosterone; ACTH, adrenocorticotropin hormone.

### Examination of the external genitalia

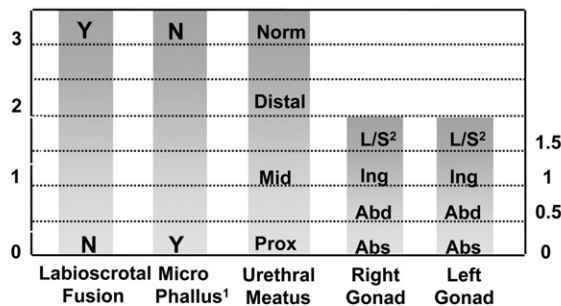
A detailed physical examination and documentation of the genitalia is necessary to evaluate the degree of genital anomaly. The first step is a careful inspection and palpation. In a number of infants, gonads or swellings may be visible in the labioscrotal folds or the inguinal regions but they may disappear on palpation. In those presenting with apparently normal female external genitalia, bilateral hernias containing testes (and, rarely, uterus or fallopian tubes) should be sought by palpation. In any case, if gonads are palpated externally, these will invariably be testes (ovaries tend to remain in the pelvic position) or, occasionally, ovotestes. A careful measurement of the phallus (stretched dorsal length) and comparison to published normative data is recommended to assess the extent of deviation of the appearance from normal and to explain this difference to the parents. The presence or absence of a chordee should be noted; the location of two (urethral and vaginal) or one orifice (urethral or urogenital sinus) that opens on the dorsal (epispadias) or ventral surface (hypospadias) of the phallic structure should be noted. An epispadias is a very rare condition and is usually part of a spectrum of conditions (bladder and cloacal extrophy) where there can be a failure of fusion of a number of lower abdominal and pelvic organs including external genitalia. Hypospadias is a much more common condition where the location of the urethral orifice may be proximal and close to the perineum, mid-shaft or distal and close to the coronal sulcus or the glans (Fig. 1). The description of the degree of labioscrotal fold fusion, that is, complete absence of scrotal fusion, a posterior fusion of labia majora, a partially fused hemiscrotum or completely fused scrotum, is also very important. Finally, the nature of the skin of the genitalia and labioscrotal folds (texture and pigmentation) and the shape of the folds and whether they are sac-like provides helpful information on androgenisation and the possibility of finding testes.

Although scoring systems such as the Prader scoring system for XX DSD and modifications of this system for XY DSD<sup>13,14</sup> may provide an integrated summary description of the genitalia, these scoring systems are not sufficiently discriminate to portray the full spectrum of the variation encountered in the external genitalia (Fig. 1). The external masculinisation score (EMS), which individually scores

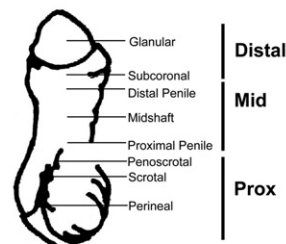
#### A Prader Stage



#### B External Masculinisation Score



#### C Hypospadias Descriptions



**Fig. 1.** Scoring External Genitalia. A. The external genitalia can be objectively scored using the Prader staging system which provides an overall score for the appearance of the external genitalia. B. Alternatively, each individual feature of the genitalia (phallus size, labioscrotal fusion, site of the gonads and location of urethral meatus) can be individually scored to obtain the External Masculinisation Score (EMS). Adapted from Ahmed et al., *BJU Int.* 2000;**85**:120–4. For the EMS, the site of the urethral meatus is based on C. <sup>1</sup>Microphallus refers to a phallus below the male reference range. <sup>2</sup> L/S – labioscrotal.



external genitalia for scrotal fusion, microphallus, location of urethral meatus and location of each gonad, may be a more discriminate and objective method of describing the external appearance (Fig. 1).<sup>15</sup>

## Why investigate

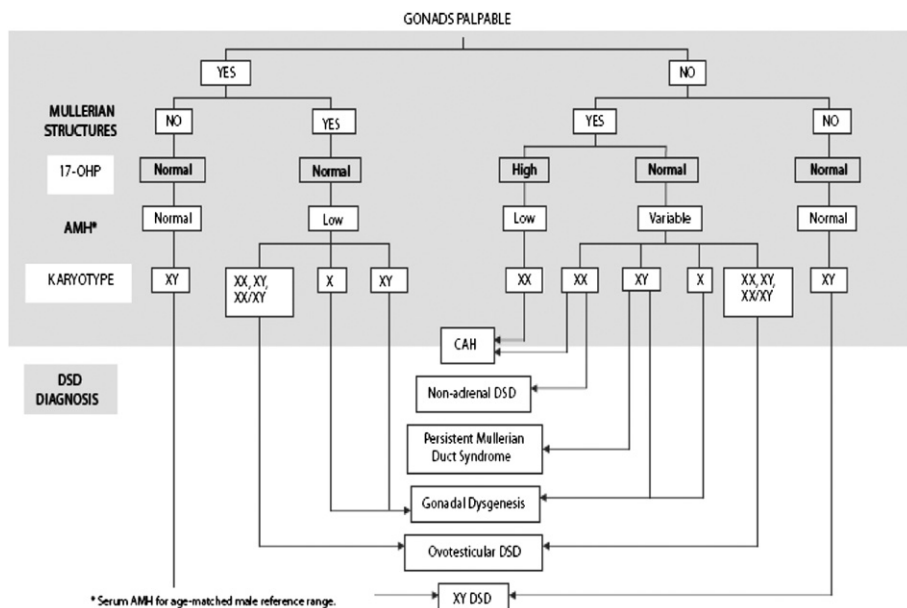
There are clear reasons for investigating an infant with genital anomalies and these include determination of sex of rearing, concerns about early medical problems, concerns about medical and surgical problems in later childhood and development of a long-term plan that anticipates future health issues, such as sexual development and function, tumour risk and fertility. A clear knowledge of the underlying aetiology may also facilitate explanation of the condition to the parent and the older child. Thus, investigations should be performed with these different objectives in mind and should be split into first-line and second-line investigations. First-line investigations should, in most cases, be sufficient to guide sex of rearing, exclude early medical problems and provide an idea of the nature of the problem. In the newborn infant, detailed dynamic endocrine investigations should only be performed if they can alter the management plan of the child; in most cases these investigations can be performed after 3 months when many reproductive and adrenal-related hormones have reached a *status quo* and the results are easier to interpret. Furthermore, collecting blood samples maybe simpler in the older child and collection of multiple blood samples from an otherwise-healthy infant may exert unnecessary stress on the child's parents.

## Which infant should be investigated

Most infants with a suspected DSD will present with:-

- overt genital ambiguity
- a family history of DSD, such as complete androgen insensitivity syndrome (CAIS)
- a discordance between genital appearance and a prenatal karyotype
- apparent female genitalia with an enlarged clitoris and posterior labial fusion
- apparent female genitalia with an inguinal/labial mass
- apparent male genitalia with bilateral undescended testes
- apparent male genitalia with a microphallus
- apparent male genitalia with proximal hypospadias
- apparent male genitalia with distal or mid-shaft hypospadias with undescended testis

The greatest amount of debate regarding the need for investigation involves the case of the boy presenting with hypospadias and/or cryptorchidism, that is, the under-masculinised boy. Considerable variation exists about the extent to which these infants should be investigated.<sup>16</sup> Routine systematic examination of 423 consecutive newborn boys in one hospital revealed that 412 (98%) had an EMS of 12. The median (10th centile) EMS for the group of 11 infants with an EMS of less than 12 was 11 (10). One infant with isolated micropenis had an EMS of 9; three infants with isolated glandular hypospadias had an EMS of 11; three infants with absent unilateral testis also had an EMS of 11; four infants with a unilateral inguinal testis had an EMS of 11.5. Thus, an EMS of <11 was only encountered in one out of 423 boys.<sup>15</sup> These data are similar to population data suggesting that genital anomalies occur in about 1:300 total births and 75% of these patients have an associated hypospadias.<sup>1</sup> Population studies also suggest that approximately 50% of hypospadias cases affect the distal penis (glandular or coronal).<sup>17</sup> The largest study to date of karyotype analysis in children with isolated cryptorchidism, isolated hypospadias or a combination of the two anomalies revealed chromosomal anomalies in 27 of the 916 patients with cryptorchidism (2.94%) and in seven of the 100 with hypospadias (7%) as well as in four of the 32 with a combination of cryptorchidism and hypospadias (12.5%).<sup>18</sup> The incidence of chromosome aberrations was 1.8% in cases of isolated cryptorchidism and 6.7% in those of other associated anomalies. In patients with hypospadias, abnormal karyotypes were only detected when there were additional congenital abnormalities. In one specialist centre, out of 63 unselected cases with proximal



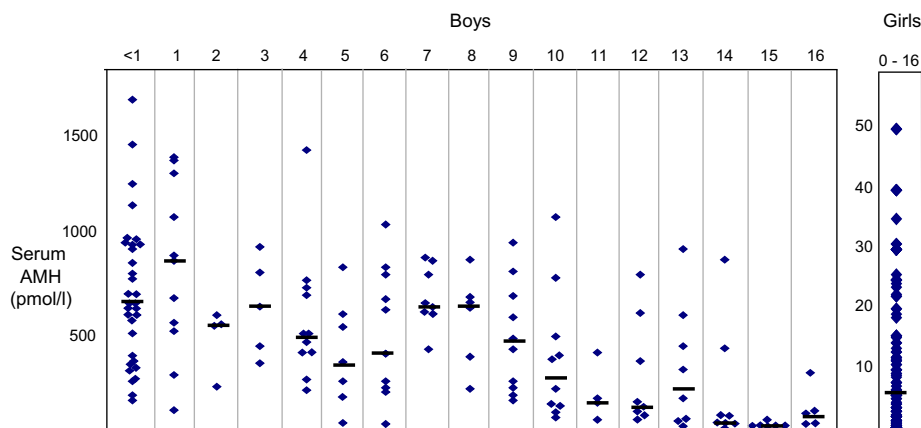
**Fig. 2.** The use of first line investigations in the newborn suspected of a DSD. 17-OHP- Hydroxyprogesterone; AMH - anti-Mullerian hormone; CAH - congenital adrenal hyperplasia.

hypospadias (penoscrotal, scrotal, perineal, etc.), who were studied for all known causes of hypospadias with clinical as well as molecular biological techniques, in 31% of cases an underlying aetiology was identified and this included complex genetic syndromes in 17%, chromosomal anomalies in 9.5%, vanishing testes syndrome in one, the AIS in one and 5 $\alpha$ -reductase type 2 deficiency in one, respectively.<sup>19</sup> Thus, infants who require further evaluation and investigation should include all children with EMS of less than 11 and all children with familial hypospadias. This will avoid detailed investigations of boys with isolated glandular hypospadias and boys with isolated inguinal testes.

### First-line investigations

Typically, in the young infant with DSD, gonadal palpability combined with karyotyping, ultrasound examination for mullerian structures, a serum anti-mullerian hormone (AMH) level and determination of 17-hydroxyprogesterone (17OH-progesterone) level should provide a reasonable guide for the initial practical management of the newborn with a DSD (Fig. 2). The results of the karyotype and the ultrasound should be available within 48 h of presentation.<sup>20</sup> Whilst fluorescence *in situ* hybridisation (FISH) or polymerase chain reaction (PCR) analysis using X- and Y-specific probes is sufficient for initial management, it is recommended that these tests are confirmed by a formal karyotype. It also needs to be borne in mind that any mosaicism that is evident may be tissue dependent. Finally, karyotype should be repeated in cases of prenatal karyotype mismatch. Whilst ultrasound examination is the most common modality that is used for imaging of the internal sex organs, there are occasions when it may provide misleading results, especially when the infant is unwell, does not have a full bladder or the operator lacks experience. In such situations, there may be a need to consider other methods of imaging including magnetic resonance imaging (MRI), a genitogram or a laparoscopy.<sup>21,22</sup>

In addition to 17OH-progesterone, biochemistry tests that should be included in the first-line investigations should include serum testosterone, AMH, cortisol, androstenedione, gonadotrophins and urinalysis. It is likely that these results shall be available within a week. Any spare sample should be stored for analysis at a latter time point. The clinician needs to have an intimate knowledge of assays and normal values for age and should establish a close liaison with the specialist biochemistry



**Fig. 3.** Distribution of serum AMH concentrations (pmol/l) per year age band for boys ( $n$ , 154) and for the whole cohort of girls upto the age of 16 yrs ( $n$ , 130). Horizontal line denotes median value for that age band. Courtesy of Prof AM Wallace.

laboratory. Given that steroid hormones and gonadotrophins fluctuate over the first few weeks of life, serial measurements are particularly valuable to perform. This also applies to urea and electrolyte estimations; infants with salt-losing forms of CAH do not usually have any abnormalities of their electrolytes until the second to third week of life.

Serum testosterone estimation has often been used as a marker of functioning testes as well as a sign of intact pathways for the synthesis of testosterone. However, given that many commercially available testosterone assays are non-specific in the early neonatal period<sup>23</sup> and can cross-react with other conjugated steroids, it is possible that, for the newborn infant, serum AMH level is a more diagnostically reliable marker of testes than serum testosterone.<sup>24</sup> The use of serum AMH has been widely advocated as a method of assessment of genital anomalies especially as it can be a clear discriminator in cases of anorchia, 46 XY complete gonadal dysgenesis and cases of persistent müllerian duct syndrome (PMDS) with a defect of the AMH gene. There is a clear difference between AMH concentration in boys and girls, especially in early childhood. Our data suggest that, in boys under the age of 8 years, a serum AMH concentration of approximately  $200 \text{ pmol l}^{-1}$  may be an appropriate cut-off mark to denote normality, given that it was the approximate 5th centile for this age range. However, AMH concentrations are higher in the young boy before they fall in late childhood (Fig. 3). It should also be noted that AMH concentrations tend to rise over the first 3 months in some young infants<sup>25</sup> and may, in some cases, be lower than  $200 \text{ pmol l}^{-1}$  at initial evaluation, although still above  $25 \text{ pmol l}^{-1}$  which approximately represents the 95th centile for girls.<sup>26</sup>

### Second-line investigations

Second-line investigations may include a number of investigations that will be guided by the results of the first-line investigations. In most cases, but not all, these tests shall be performed to investigate the underlying aetiology and are usually not necessary to determine the sex of rearing. In most cases, the results of the first-line investigations would generally suffice.

These investigations could include:-

Biochemistry to assess the gonadal and adrenal axes – human chorionic gonadotrophin (hCG) stimulation to assess production of testosterone, androstenedione, dihydrotestosterone (DHT), 11-deoxycortisol and 17OH-pregnenolone. To detect abnormalities of the last three steroids, it may be more effective to analyse a urine steroid profile (spot or 24 h) by gas chromatography-mass spectrometry (GC-MS). Other biochemical investigations that may need to be considered include luteinising hormone-releasing hormone (LHRH), adrenocorticotrophic hormone (ACTH) stimulation, renin and aldosterone. Measurement of serum cholesterol and 7-dehydrocholesterol are indicated in the child who has features consistent with Smith–Lemli–Opitz syndrome.

- Imaging – Ultrasound scan, MRI, genitogram, cystourethroscopy, laparoscopy, etc.
- Pathology – Gonadal biopsy; there are, however, unresolved questions as to whether one biopsy represents the whole gonad. In addition, it is unclear as to what is the minimum amount of ovarian or testicular tissue that should be present to classify the gonad as an ovotestis.
- Genetics – High-resolution karyotype, karyotype from different tissues (blood, skin, gonads, etc.), DNA for storage and analysis in the clinical genetics department.
- Functional studies of androgen sensitivity – A functional assessment of androgen sensitivity can be performed by assessing the clinical response of testosterone on the phallus. However, there is no consensus on dosage, method of administration, timing, duration of androgen treatment and the definition of a satisfactory response in the size of the phallus. Second, androgen sensitivity can be assessed by measuring change in an androgen-responsive circulating protein such as sex hormone-binding globulin (SHBG); SHBG levels should fall following androgen exposure and a failure to show this reduction may be indicative of androgen insensitivity.<sup>27</sup> The utility of this test in the young infant is unclear given that circulating SHBG is very variable in the young infant. Androgen-binding studies involve the evaluation of the concentration of androgen receptors (ARs), the number of receptors and their affinity for testosterone are measured on cultured genital skin fibroblasts. However, the results may depend on the site from which the skin is originally collected. Over 80% of cases with a phenotype consistent with CAIS and abnormal androgen binding may have a mutation in the AR gene.<sup>28</sup> However, in cases consistent with a partial androgen insensitivity syndrome (PAIS) phenotype, only 50% of cases with abnormal binding may have a mutation in the AR gene. Given that AR gene analysis may reveal a mutation in over 80% of cases with a CAIS phenotype anyway, there probably is no need to perform androgen-binding studies in this group of infants. As the yield of AR mutations in the case with the PAIS phenotype is lower at about 30%, androgen-binding studies, as well as other functional measures of androgen sensitivity may be more helpful in informing the case for mutational analysis.

### The hCG stimulation test

Although controversy exists regarding the optimal regimen, stimulation with hCG has been used to assess the presence of functioning testicular tissue and the detection of defects in testosterone biosynthesis and action for over 40 years. In the UK, a number of different protocols are used for hCG stimulation, but most use intramuscular hCG 1000–1500 units on 3 consecutive days for a standard test.<sup>16</sup> If there is a poor response, this test can be followed by prolonged hCG stimulation 1500 units on 3 consecutive days for the first week followed by 1500 units on 2 days a week for the 2 following weeks. The combined regimen that is employed in our unit is outlined in Fig. 4. The definition of a normal response may depend on the age of the child and the regimen itself. In infants and older children, who have a more active gonadotrophin axis, the Leydig cells may be more responsive to hCG stimulation and the shorter duration of hCG stimulation may be sufficient.<sup>29</sup> Besides testosterone, other androgens that should be assessed include DHT and androstenedione. For these two metabolites, the day-4 sample is more important than the day-1 sample. There does not seem to be any additional benefit of collecting a sample for these two metabolites on day 22. In combination with the urinary steroid profile, the results of the hCG stimulation test can be very informative and direct the clinician towards the underlying diagnosis in XY DSD (Fig. 5).

### Aetiology of XX DSD

46XX DSD can be divided into disorders of ovarian development, disorders of androgen synthesis, disorders of mullerian development and other conditions affecting sex development.

#### *Disorders of gonadal development*

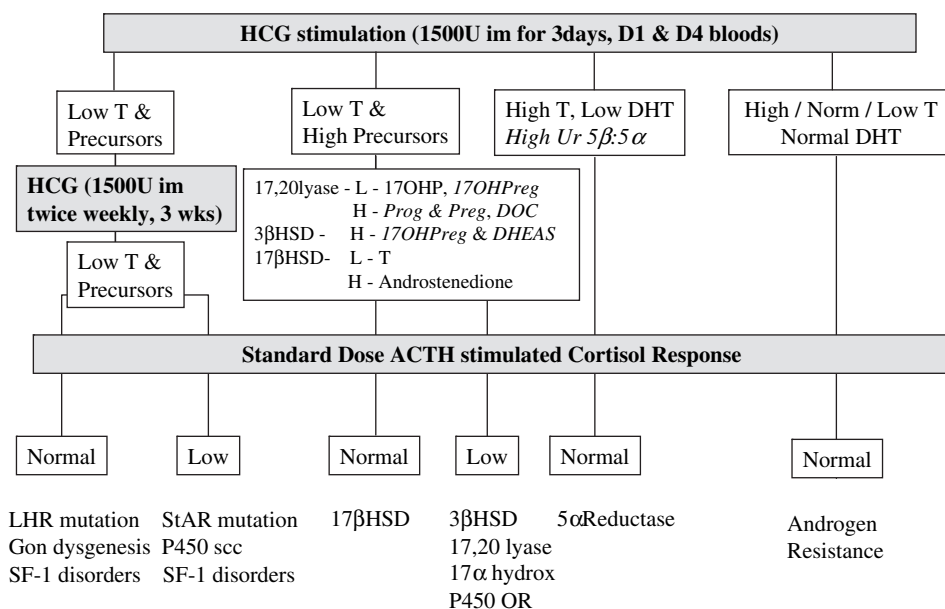
##### *46XX ovotesticular DSD ('true hermaphrodites') and 46XX testicular DSD ('46XX males')*

Rarely, the developing ovary may contain some testicular tissue (ovotesticular DSD) or may develop as a functioning testis that secretes adequate amounts of testosterone for adequate virilisation and AMH for regression of the mullerian ducts (testicular DSD). Ovotesticular DSD can be sub-classified according

Week	Wk1				Wk2		Wk3		Wk4	>Wk8
Date										
Day	Mon	Tue	Wed	Thu	Mon	Thu	Mon	Thu	Mon	
HCG 1500 im	*	*	*		*	*	*	*		
Serum Testos, SHBG <sup>1</sup>	*			*					*	
Salivary Testosterone (optional) <sup>2</sup>	*	*	*	*					*	
Serum Androstenedione, DHT, DHEAS, Store <sup>3</sup>	*			*						
Serum AMH <sup>4</sup>	*									
Urine Steroid Profile <sup>4</sup>	*									
LHRH Stim Test (0,20,60min)	*									
Karyotype & DNA <sup>4</sup>	*									
Ultrasound scan of Testes & Renal Tracts <sup>5</sup>									*	
Stretched Penile length	*			*					*	
Examine for Testes (Scrotal, Ing, Abdo, Absent)	*			*					*	
Endocrine Follow-up										*

**Fig. 4.** A clinical protocol for hCG Stimulation in childhood. This protocol is extended to a prolonged period of hCG stimulation. 1- Serum for Testosterone is very important; SHBG is much less important, particularly in infants. 2- Saliva could be used as an alternative in those cases where venepuncture is difficult; need clear instructions for collection. 3- These androgens are listed in order of priority with Androstenedione being most important. 4- These samples should preferably be collected on the first day but can be collected on any visit.

to the type and location of the gonads. Lateral cases (20%) have a testis on one side and an ovary on the other. Bilateral cases (3%) have testicular and ovarian tissues present bilaterally as ovotestes. Unilateral cases (50%) have an ovotestis present on one side and a normal ovary or testis present on the other side. In ovotesticular DSD, the initial manifestations are ambiguous genitalia in almost all cases and the internal duct structures display gradations between male and female and there is often a urogenital sinus and a uterus or a hemi- or a rudimentary uterus on the side of the ovary or ovotestis. Breast development will occur in puberty and even menses may occur in a significant proportion when ovarian



**Fig. 5.** Combining the use of the ACTH stimulation test and the hCG test for investigating XY DSD.

tissue is present. However, without removal of testicular tissue, these children will also proceed to virilisation at puberty. Presence of functional testicular tissue can be investigated by checking AMH or testosterone levels following hCG stimulation. Assessment of functioning ovaries by biochemical markers has not been thoroughly explored and the utility of measuring oestradiol after repeat FSH stimulation or measurement of an ovarian specific marker such as inhibin A requires further study. Nearly two-thirds of affected children are raised as boys. If the testicular components are removed, serial AMH levels may allow adequate confirmation of complete removal of functioning testicular tissue. In contrast, 46XX testicular DSD is usually associated with a normal male phenotype or a relatively mild abnormality of the male genitalia, such as distal or mid-shaft hypospadias. In adulthood, although testosterone synthesis is not affected, spermatogenesis is usually severely affected.

*Ovarian dysgenesis.* Ovarian dysgenesis is most frequently seen in association with sex chromosome aneuploidy such as Turner syndrome and related variants. However, these conditions do not present in infancy with physical abnormalities of sex development.

### *Disorders of androgen excess*

#### *21 $\alpha$ -hydroxylase (CYP21) deficiency*

CAH due to 21-hydroxylase deficiency is the most common cause of 46XX DSD and consensus guidelines exist for management of this condition in infancy as well as in the older child.<sup>30</sup> The newborn girl with this condition can be virilised to a varying extent. High serum concentration of 17OH-progesterone ( $>300 \text{ nmol l}^{-1}$ ) after the first 48 h of birth and high androstenedione and testosterone in the early neonatal period are the biochemical hallmarks of this condition. More than 75% of these infants will also be salt-losers because of a deficiency of mineralocorticoid synthesis and the affected child will present with a salt-losing crisis in the second or third week of life.

#### *3 $\beta$ -hydroxysteroid dehydrogenase (HSD3B2) deficiency*

3 $\beta$ -Hydroxysteroid dehydrogenase (3 $\beta$ HSD) type 2 catalyses the conversion of  $\Delta^5$  steroids to  $\Delta^4$  steroids and a deficiency of this enzyme results in adrenal insufficiency as well as accumulation of pregnenolone, dehydroepiandrosterone (DHEA) and androstenediol. In peripheral tissues, as well as the placenta, the accumulating steroids, and particularly DHEA, can be converted to more potent androgens, such as testosterone, by the Type 1 isoenzyme. Most girls with this condition present with relatively mild signs of virilisation such as clitoromegaly, associated with adrenal deficiency.

#### *P450 oxidoreductase (POR) deficiency*

Defects in P450 oxidoreductase can cause combined deficiencies of 21 $\alpha$ -hydroxylase, 17 $\alpha$ -hydroxylase and aromatase enzymes and this can be associated with abnormal genital development in both girls and boys. Children with this condition usually have cortisol deficiency but have normal mineralocorticoid function.

#### *11 $\beta$ -hydroxylase (CYP11B1) deficiency*

This is the second most common cause of virilising CAH accounting for approximately 5% of all cases. Apart from a DSD, this condition may also be associated with hypertension and hypokalaemia, but these abnormalities are not universally present and, particularly, not in infancy. These abnormalities are due to the accumulation of 11-deoxycorticosterone, which is a weak mineralocorticoid. They may be associated with low renin level. Children with this condition usually have cortisol deficiency.

#### *Familial glucocorticoid resistance*

This is a rare condition, usually, due to a heterozygous mutation in the glucocorticoid receptor gene. The partial end-organ insensitivity leads to high ACTH, cortisol, mineralocorticoids and androgens. A case of a girl with a homozygous mutation in this gene and a co-existing heterozygous mutation in CYP21 has been described to be associated with marked virilisation at birth. Isolated

heterozygous gene abnormalities of CYP21 are not generally associated with virilisation. This condition's biochemical picture is quite unique and appropriate investigations should facilitate its identification.

#### *Aromatase (CYP19) deficiency*

Aromatase deficiency is inherited as an autosomal recessive condition and has been described in approximately 10 girls with a variable extent of virilisation. There is often a history of maternal virilisation after the second trimester of pregnancy coupled with elevated maternal androgen levels which resolve after the pregnancy. In infancy and subsequently during puberty, these girls shall have high serum androgens and low oestrogen concentrations, show no signs of feminisation and shall progressively virilise. In addition, inadequate oestrogen supplementation may be associated with osteoporosis and a failure of timely epiphyseal fusion.

#### *Maternal androgen excess*

Any maternal source of elevated androgens can induce virilisation of the female foetus. Ovarian tumours include luteoma of pregnancy, arrhenoblastoma, hilar-cell tumour, masculinising ovarian stromal cell tumour and Krukenberg tumour. Discrepancy between the marked virilisation of the mother and the minimal androgen effect in female offspring can be explained by the placental aromatase activity, which converts androgens to oestrogens, or to the metabolism of androgen, which thus becomes less active. Apart from untreated maternal virilising CAH, androgen-secreting adrenal tumour in the mother is rare. In both cases, investigation of abnormal androgen production by the mother must be performed immediately after delivery. Maternal ingestion of androgens, progestagens or other drugs is another cause of foetal virilisation. Exogenous steroids administered during the pregnancy may cause posterior fusion of the labia, clitoral enlargement and even increased degrees of androgenisation. In the past, several oral progestational compounds, given because of threatened abortion, have been implicated, such as 19-nor testosterone. Other drugs, such as danazol or stilboestrol that is used in pregnancy, have also been associated with abnormalities of the genitalia.

#### *Disorders of mullerian development*

Abnormalities in uterine development can result in bicornuate uterus, uterine hemiagenesis, hypoplasia or agenesis. These can be associated with renal, cardiac or spine abnormalities as part of the Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome or mullerian, renal, cervical spine syndrome (MURCS). On rare occasions, absence of mullerian structures, and the presence of co-existing hyperandrogenaemia, has been associated with a mutation in the WNT4 gene.<sup>31</sup> Other conditions, such as maturity-onset diabetes of the young, the hand-foot-genital syndrome, Laurence-Moon-Biedl syndrome have also been associated with abnormalities of mullerian development.

#### *Other 46XX DSD*

Complex urogenital abnormalities such as cloacal anomalies can affect both sexes and require major reconstructive surgery.

#### *Variations that may present as DSD*

Clitoral lengths are variable and when in doubt should be compared to published norms.<sup>4</sup> In addition, the clitoris may be enlarged in conditions such as neurofibromatosis. In any newborn girl, the labial folds may be very swollen and oedematous immediately after birth and may look like scrotal sacs. In premature babies, the lack of labial adipose tissue may pronounce the relative size of the clitoris so that it is mistaken for clitoromegaly. Labial adhesions and vaginal bleeding in the newborn are signs of the normal oestrogen surge in the newborn period.



## Aetiology of XY DSD

46XY DSD can be divided into disorders of testis development, disorders of androgen synthesis, disorders of androgen action and other conditions affecting sex development, and are summarised in Table 3. Biochemically, based on AMH and the hCG stimulation test, these disorders can also be divided into conditions where (i) AMH levels are low and testosterone levels do not rise following hCG stimulation – abnormalities of testes development or maintenance, (ii) AMH levels are normal and testosterone levels do not rise following hCG stimulation – abnormalities of testosterone synthesis, (iii) AMH levels are normal and testosterone levels do rise following hCG stimulation – abnormalities of testosterone action, DHT synthesis, PMDS or non-specific disorder of masculinisation, (iv) AMH levels are low and testosterone levels do rise following hCG stimulation – PMDS. However, in many cases, the biochemical assessment shall not clearly delineate the case into any of these four categories.

### *Disorders of testis development*

These disorders can have a spectrum of phenotypes and presentations. In the most extreme case, complete testicular dysgenesis, infants raised as girls do not present until adolescence with primary amenorrhoea. These girls will have normal external female genitalia and müllerian structures, and this condition is also often called Swyer syndrome. Partial gonadal dysgenesis may be associated with a variable and internal phenotype even extending to a phenotype of simply male infertility. Accordingly, there will be a variable reduction in AMH and testosterone response to hCG stimulation. Given that several single-gene disorders, as well as chromosomal rearrangements, have been described to be associated to the clinical picture of gonadal dysgenesis, the latter should not necessarily be considered the final diagnosis. These disorders are often associated with abnormalities in other systems and a thorough clinical evaluation of the affected infant will prove very useful in directing appropriate genetic analysis that can lead to the correct diagnosis. Currently, a genetic diagnosis is only reached in approximately 30% of cases of gonadal dysgenesis. The importance of reaching a genetic diagnosis in these cases is highlighted by conditions such as those associated with a mutation in the steroidogenic factor (SF1) gene, which may occasionally be associated with adrenal deficiency or a mutation of the Wilms' tumour-related gene-1 (WT1), where the DSD may be the first sign of conditions such as WAGR syndrome, Denys-Drash syndrome and Frasier Syndrome.

### *Disorders of androgen synthesis*

Defects anywhere along the pathway of androgen synthesis and target organ action can result in an XY DSD.

#### *Cholesterol synthesis defects*

A deficiency of 7-dehydrocholesterol reductase (DHCR7) results in a failure of cholesterol synthesis and results in the Smith–Lemli–Opitz syndrome is associated with a wide range of clinical features including microcephaly, cardiac defects, micrognathia, cleft palate, polydactyly and syndactyly. In childhood, these children may display mental retardation and growth failure. The genitalia in the affected XY infant may range from hypospadias to completely normal female external genitalia with no müllerian ducts. The condition is diagnosed by low levels of cholesterol and elevated levels of its precursor, 7-dehydrocholesterol, as well as an androgen deficiency and a normal AMH level. Adrenal insufficiency may occur in some cases and needs evaluation. Mutational analysis of the *DHCR7* gene will provide further confirmation of the diagnosis.

#### *Leydig cell hypoplasia*

A defect of the LH receptor leads to impaired sensitivity to hCG and LH leading to Leydig cell agenesis or hypoplasia. The genitalia in the affected XY infant may range from isolated hypospadias or micropenis to completely normal female external genitalia with no müllerian ducts. The biochemical picture may include high basal and LHRH-stimulated LH and follicle-stimulating hormone (FSH) levels. There is a poor response to hCG stimulation and the AMH levels should be normal. Histology of the



testes in the prepubertal child will show a marked lack of Leydig cells. Mutational analysis of the LH/hCG receptor gene will provide further confirmation of the diagnosis.

#### *Congenital lipoid adrenal hyperplasia*

Defects in the steroidogenic acute regulatory protein (StAR) lead to deranged intracellular transport of cholesterol and abnormalities of steroid biosynthesis. Affected XY infants have severe adrenal failure and the external genitalia are unambiguously female with no müllerian structures. The testes may be palpable in the labioscrotal folds but are usually undescended. Computed tomography (CT) or MRI imaging of the adrenal glands, as well as histology, may reveal lipid accumulation. This condition is more common in Japan and Korea. A non-classical form of this condition also exists and is associated with progressive adrenal insufficiency in early childhood but without any overt abnormalities of androgen synthesis. Mutational analysis of the StAR gene will provide further confirmation of the diagnosis.

#### *P450 side-chain cleavage deficiency*

Defects in the P450<sub>scc</sub> enzyme result in a failure of conversion of cholesterol to pregnenolone which is the first common step in steroid biosynthesis. Affected XY infants have a phenotype which is very similar to congenital lipoid adrenal hyperplasia due to defect of StAR protein. Mutational analysis of the P450<sub>scc</sub> gene (also called *CYP11A1*) will provide further confirmation of the diagnosis.

#### *3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD) Type 2 deficiency*

Defects in 3 $\beta$ HSD Type 2 results in a failure to convert  $\Delta^5$ - to  $\Delta^4$ -steroids. The genitalia in the affected XY infant may range from isolated hypospadias or micropenis to more severe under-masculinisation but not completely normal female external genitalia. There are no müllerian ducts. Besides a poor androgen response to hCG stimulation, affected infants will have adrenal deficiency which may not necessarily include salt wasting. A urine steroid profile that shows high concentrations of  $\Delta^5$ -steroids (e.g., 17OH-pregnenolone, pregnenolone and DHEA) and low concentrations of  $\Delta^4$ -steroids (e.g., progesterone and cortisol) is helpful. However, there is a need for careful analysis and interpretation of the steroid profile as extra-adrenal/gonadal 3 $\beta$ HSD Type 1 may raise the levels of some  $\Delta^4$ -steroids such as androstenedione and 17OH-progesterone. Mutational analysis of the 3 $\beta$ HSD Type 2 gene (also called *HSD3B2*) will provide confirmation of the diagnosis.

#### *17 $\alpha$ -hydroxylase/17,20-lyase deficiency*

Defects of the P450<sub>c17</sub> enzyme can lead to a variable extent of a combined deficiency of 17 $\alpha$ -hydroxylase and 17,20-lyase activity. In the affected XY infant, this will be associated with a variable degree of under-masculinisation ranging from mild abnormalities of the genitalia to unambiguously female external genitalia. Besides a poor androgen response to hCG stimulation, affected infants will have a poor cortisol response to adrenal stimulation but may not display adrenal insufficiency as they have highly elevated deoxycorticosterone levels, which may lead to state of low renin hypertension and hypokalaemic alkalosis in the older child. Mutational analysis of the P450<sub>c17</sub> gene (also called *CYP17*) will provide confirmation of the diagnosis.

#### *P450 oxidoreductase deficiency*

The P450 oxidoreductase enzyme is necessary for electron transfer from nicotinamide adenine dinucleotide phosphate (NADP) to many P450 enzymes and its deficiency can affect the activity of a number of P450 enzymes. Infants with XY DSD and an abnormality of this enzyme tend to present with a clinical picture consistent with combined deficiency of 21 $\alpha$ -hydroxylase deficiency and 17,20-lyase deficiency. The genitalia in the affected XY infant may range from isolated hypospadias or micropenis to more severe under-masculinisation but not completely normal female external genitalia. There are no müllerian ducts. Besides a poor testosterone response to hCG stimulation, affected infants will have adrenal deficiency which is usually restricted to glucocorticoid deficiency. The deficiency of this enzyme may be associated with a condition called Antley–Bixler syndrome, which is a skeletal dysplasia classically characterised by radiohumeral stenosis and craniosynostosis. This syndrome is not universally associated with abnormalities of the P450 oxidoreductase enzyme. Mutational analysis of the P450 oxidoreductase gene (also called *POR*) may provide confirmation of the diagnosis.

### *17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ HSD) Type 3 deficiency*

17 $\beta$ HSD has six isoenzymes which convert androstenedione, DHEA and oestrone to testosterone. Deficiency of 17 $\beta$ HSD Type 3 is associated with XY DSD and affected infants often present with female external genitalia or, sometimes, ambiguous genitalia. However, these children can undergo spontaneous virilisation during puberty with a rise in testosterone levels, possibly due to increased activity of the other isoenzymes. Thus, early accurate diagnosis of this condition is important as the affected infant may need sex reassignment if initially raised as a girl. These infants shall have a poor testosterone response to hCG but may have a relatively high level of serum androstenedione such that the testosterone:androstenedione ratio may be less than 0.8. However, this is not an invariable finding in this condition and, furthermore a low ratio may also be found in poorly functioning. Mutational analysis of the 17 $\beta$ HSD Type 3 gene (also called *HSD17B3*) will provide confirmation of the diagnosis.

### *Steroid 5 $\alpha$ -reductase (5 $\alpha$ -RD) Type 2 deficiency*

5 $\alpha$ -RD exists as two isoenzymes. Type 1 is expressed in skin and Type 2 in the genitalia and converts 5 $\alpha$ - to 5 $\beta$ -steroids. In XY DSD infants may present with a variable phenotype ranging from micropenis or hypospadias to female external genitalia. This phenotype is due to reduced activity of 5 $\alpha$ -RD Type 2 and a failure to convert testosterone to DHT. The classical biochemical profile normally includes a high testosterone:DHT ratio following hCG stimulation and which usually exceeds 30:1. An additional diagnostic feature is a urinary steroid profile which shows a decreased ratio for 5 $\alpha$ :5 $\beta$ -reduced C<sub>21</sub> and C<sub>19</sub> steroids. It may not be possible to detect this abnormality in the urine until late infancy. Like 17 $\beta$ HSD Type 3 deficiency, these children can undergo spontaneous virilisation during puberty with a rise in testosterone levels, possibly due to increased activity of the Type 1 isoenzymes. Thus, early accurate diagnosis of this condition is important as the affected infant may need sex reassignment if initially raised as a girl. Application of topical DHT cream may be a useful test of virilisation as well as help explain the condition to the family.

### *Disorders of androgen action*

In XY DSD, a disorder of the AR leads to a phenotype that can range from a man with infertility through to a range of abnormalities of the genitalia in the newborn boy (PAIS) to completely female external genitalia (CAIS). Children with AIS should have normal testosterone and DHT response to hCG stimulation and should have a normal urinary steroid profile. However, a number of children with a confirmed genetic diagnosis of AIS may have a poor response to hCG stimulation and this may be related to associated abnormalities of the testes or the test, itself. The AMH level should be normal; sometimes, it has been shown to be somewhat high for age-matched standards. Similarly, LH levels may be high especially following LHRH stimulation. There are no mullerian ducts. In the older infant, fixed treatment with testosterone may not be accompanied by changes in testosterone-responsive effects, such as a fall in SHBG or change in the size of the phallus. Mutational analysis of the AR gene (also called AR) will provide confirmation of the diagnosis. Androgen-binding studies may be helpful in directing mutational analysis, particularly in cases of PAIS. As the condition is inherited in an X-linked pattern, a consistent family history is very helpful. Furthermore, exploration of X-linked markers in affected and non-affected family members can examine the likelihood of the condition. A number of cases of XY DSD are incorrectly labelled as 'PAIS' when no firm biochemical or genetic abnormalities are identified in gonadal function, androgen synthesis or androgen action. Strictly speaking, the term PAIS should be reserved for those children who have XY DSD and a genetic abnormality of the AR gene. The children without a genetic abnormality may be better described as 'XY DSD with a non-specific disorder of under-masculinisation'.

### *Persistent mullerian duct syndrome (PMDS)*

AMH is secreted by the Sertoli cells from ~7 weeks of gestation and subsequently acts through the AMH type 2 receptor to lead to regression of the mullerian ducts. PMDS occurs due to a mutation of the AMH gene or its receptor. In XY infants with PMDS, boys are born with male external genitalia but have

persistence of internal müllerian structures. The diagnosis is usually suspected when a child has a repair of an inguinal hernia, orchidopexy or coincidental intra-abdominal surgery. There are two anatomic forms. In the commoner type, there is one inguinal hernia which contains the ipsilateral testis and the ipsilateral fallopian tube and the uterus. In some of these herniae, the contralateral testis may also be present. In the less common form, all the structures including the testes are present in the pelvis. Affected children have normal testosterone response to hCG but fertility and, sometimes, Leydig cell function may be compromised in adulthood due to unsuccessful attempts at orchidopexy and anatomical abnormalities of the epididymis and the vas deferens. Surgical opinion about the timing of salpingectomy and hysterectomy vary.

### *Disorders of testes maintenance*

A number of different terminologies (bilateral vanishing testes, embryonic testicular regression, rudimentary testes, congenital anorchia, etc.) are used to describe a group of conditions which are characterized in infants with a XY karyotype and absent or rudimentary testes. The syndrome entails the presence of testes which vanish during embryogenesis. The aetiology of this syndrome is unclear: regression of the testes *in utero* may be due to a genetic mutation, a teratogen factor or a bilateral torsion. Clinically, the syndrome encompasses a spectrum of phenotypes, ranging in severity from genital ambiguity to a male phenotype with an empty scrotum. The management of patients with defect of testes maintenance is dictated by their position in the clinical spectrum of the disorder. Patients with *rudimentary testes* have a male phenotype with micropenis, small atrophic testis with pre-Sertoli and Leydig cells. Some patients present with perineal hypospadias and persistent müllerian derivatives. 'Congenital anorchia' is characterised by the complete absence of testicular tissue at birth, but normal male sexual differentiation without müllerian derivatives.

### *Sex assignment in the affected newborn*

Initial gender uncertainty is unsettling and stressful for families. Expediting a thorough assessment and decision is required. Factors that influence gender assignment include the diagnosis, genital appearance, surgical options, need for life-long replacement therapy, the potential for fertility, views of the family and, sometimes, circumstances relating to cultural practices. More than 90% of 46XX CAH patients and all 46XY CAIS assigned female in infancy identify as females. Evidence supports the current recommendation to raise markedly virilised 46XX infants with CAH as female.<sup>32–34</sup> Approximately 60% of 5 $\alpha$ -reductase (5 $\alpha$ RD2)-deficient patients assigned as female in infancy and virilising at puberty (and all assigned male) live as males.<sup>35</sup> In 5 $\alpha$ RD2 and possibly 17 $\beta$ HSD3 deficiencies, where the diagnosis is made in infancy, the combination of a male gender identity in the majority and the potential for fertility (documented in 5 $\alpha$ RD2, but unknown in 17 $\beta$ HSD3) should be discussed when providing evidence for gender assignment. Amongst patients with PAIS, androgen biosynthetic defects, and incomplete gonadal dysgenesis, there is dissatisfaction with the sex of rearing in ~25% of individuals whether raised as male or female.<sup>36</sup> Available data support male rearing in all patients with micropenis, taking into account equal satisfaction with assigned gender in those raised as male or female, but no necessity of surgery and the potential for fertility in patients reared as male. The decision on sex of rearing in ovotesticular DSD should consider the potential for fertility based on gonadal differentiation and genital development, and assuming that the genitalia are, or can be made, consistent with the chosen sex. In the case of mixed gonadal dysgenesis (MGD), factors to consider include prenatal androgen exposure, testicular function at and after puberty, phallic development and gonadal location. Individuals with cloacal exstrophy reared as female show variability in gender-identity outcome, but >65% appear to live as women.

### **Surgical management**

The surgeon has a responsibility to outline the surgical sequence and subsequent consequences from infancy to adulthood. Only surgeons with expertise in the care of children and specific training in the surgery of DSD should perform these procedures. Parents now appear to be less inclined to choose

surgery.<sup>3</sup> As orgasmic function and erectile sensation may be disturbed by clitoral surgery, the surgical procedure should be anatomically based to preserve erectile function and the innervation of the clitoris.<sup>37</sup> Emphasis is on functional outcome, rather than a strictly cosmetic appearance. It is generally felt that surgery that is performed for cosmetic reasons in the first year of life relieves parental distress and improves attachment between the child and the parents. However, systematic evidence for this belief is lacking. It is anticipated that surgical reconstruction in infancy will need to be refined at the time of puberty. Vaginal dilatation should not be undertaken prior to puberty.<sup>38</sup> The surgeon must be familiar with a number of operative techniques in order to reconstruct the spectrum of urogenital sinus disorders. An absent or inadequate vagina (with rare exceptions) requires a vaginoplasty in adolescence when the patient is psychologically motivated and a full partner in the procedure.<sup>39</sup> In the case of a DSD associated with hypospadias, standard techniques for surgical repair include chordee correction, urethral reconstruction and the judicious use of testosterone supplementation applies. The magnitude and complexity of phalloplasty in adulthood should be taken into account during the initial counselling period. Parents should also be explained that sexual contentment is not only dependent on penetrative sex. Parents must not be given unrealistic expectations about penile reconstruction, including the use of tissue engineering. The testes in patients with CAIS and those with PAIS, raised as female, need to be removed to prevent malignancy in adulthood but this can be deferred until adolescence allowing spontaneous feminisation and the development of a partnership with the patient. The streak gonad in a patient with MGD raised as a male should be removed in early childhood. Bilateral gonadectomy is performed in early childhood in females (bilateral streak gonads) with gonadal dysgenesis and Y-chromosome material. In patients with androgen biosynthetic defects raised female, gonadectomy should be performed before puberty. A scrotal testis in patients with gonadal dysgenesis remains at risk for malignancy and there is little consensus on screening besides regular palpation in adolescence and adulthood.

## **Psychosocial management**

Psychosocial care should be an integral part of management in order to promote positive adaptation and allow parents to express and resolve their concerns.<sup>40</sup> Whilst the mental health-care staff should have some knowledge about DSD, in most cases, the early concerns of parents may be less to do with the long-term implications of the condition and more to do with coping and adjustment of the parents during early infancy and some of these issues are generic to many stressful neonatal situations. Health-care staff with this experience and who work as part of a clinical network where they have access to others with more specialist knowledge and experience may be particularly valuable in providing generic psychosocial support. A common issue appears to be related to how the condition should be explained to friends and relatives.<sup>8</sup> This expertise can facilitate team decisions with regard to gender assignment/reassignment, timing of surgery and sex hormone replacement. Psychosocial screening tools that identify families at risk for maladaptive coping with a child's medical condition should be considered. The new parents should be explained that it is routine practice to involve mental health staff and that they will have access to these staff throughout the child's development. Once the child is sufficiently developed for a psychological assessment of gender identity, such an evaluation must be included in discussions regarding gender reassignment. Gender-identity development begins prior to the age of 3 years, but the earliest age at which it can be reliably assessed remains unclear. The generalisation that the age of 18 months is the upper limit of imposed gender reassignment should be treated with caution and viewed conservatively. Atypical gender-role behaviour is more common in children with DSD than in the general population, but should not be taken as an indicator for gender reassignment. The parents should be explained that the process of disclosure concerning facts about karyotype, gonadal status and prospects for future fertility is a collaborative ongoing action which requires a flexible individual-based approach; it should be planned with the parents from the time of diagnosis. Medical education and counselling for children as well as the parents shall be a recurrent gradual process of increasing sophistication, which is commensurate with changing cognitive and psychological development.

## Conclusion

Infants with a DSD and who present with truly ambiguous genitalia is a rare occurrence. These infants require multidisciplinary specialist input and a paediatric endocrinologist who has a sound knowledge of the underlying pathophysiology and the strengths and weaknesses of the battery of investigations that can be performed in the infant plays a vital role in the clinical team. Often, the paediatric endocrinologist is also the main clinician who communicates on behalf of the team with the family and the need for excellent communication skills, an empathic attitude and an awareness of the rights of the infant and the views of the family, as well as the whole multidisciplinary team, are essential characteristics of this clinician. Finally, whilst this clinician shall be basing management plans on past experience and knowledge, it has to be acknowledged that there will be major advances in our knowledge of these conditions in the future and, coupled with continuing changes in societal attitudes, it is likely that controversies and uncertainties will continue to exist. National and international clinical and research networks that share information and experiences will collectively improve our knowledge of these rare conditions and provide a platform for future research and audit of clinical practice.

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