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13.7.3 Normal and abnormal sexual differentiation 2435 to trigger puberty and ensure its continuation after treatment is stopped. Permanent hypogonadism however needs continuation of treatment to ensure pubertal progression. In hypergonadotropic hypogonadism, baseline and GnRH tests show high levels of LH and FSH (FSH >LH) with low sex steroid levels due to gonadal failure. Here, chromosomal disorders need to be considered and a karyotype would be important. Treatment In constitutional delay, treatment is not necessary and often reassur- ance, understanding, and regular review is sufficient. However, due to the distress for boys in particular, treatment may be initiated to induce puberty although if stated at too young an age may affect final height achieved Treatment for constitutional delay is in the form of low dose sex steroids as detailed earlier. The aim of this is to stimulate puberty by activating the pituitary to release gonadotropins and spontaneously continue pubertal progression. Oxandrolone (1.25–2.5 mg daily) is a mild anabolic steroid and another option. It may augment height but has no effect on puberty. However, as Oxandrolone is not aroma- tized, unlike testosterone it does not produce gynaecomastia. For those with permanent hypogonadotropic hypogonadism, treatment needs to continue into adult life. The main aim is start at a low dose with slow increments over a 2–3-year period. In males, this entails starting testosterone esters, IM at 50 mg every 4 weeks. This is slowly increased over 2–3 years to an adult dose of 250 mg every 3–4 weeks. Other preparations of testosterone such as transdermal gels or longer acting IM testosterone are available and can be substituted as adult replacement. Treatment in females follows a similar approach. For prolonged treatment, transdermal patches are recommended to induce secondary sexual changes. Initially 25 μg 17 β-oestradiol ma- trix patch ¼ patch for 3 days per week building up over 2–3 years depending on response and age at initiation to adult replacement. Low dose oral ethinyloestradiol (2 μg/day) is a potential alternative. Progestogens should be introduced only after a suitable duration of unopposed oestrogen (usually 2–3 years) or if more than one episode of significant breakthrough bleeding occurs. For Turner syndrome and other congenital causes of short stature, there was a tendency to delay pubertal induction in an attempt to allow optimal growth, but recent evidence has demonstrated that growth hormone plus Oxandrolone results in maximal final adult height and therefore puberty can be induced at an appropriate age. Gonadal stimulants may be

appropriate for young males with hypogonadotropic hypogonadism to induce testicular growth. This is in the form of HCG injections twice a week but has variable re- sults. Normal testicular function is required to commence HCG treatment. FURTHER READING Alikasifoglu A, et al. (2015). Changing etiological trends in male pre- cocious puberty: evaluation of 100 cases with central precocious puberty over the last decade. Horm Res Paediatr, 83, 340–4. Heger S, et al. (2006). Kisspeptin-GPR54 signaling in the neuroendo- crine reproductive axis. Mol Cell Endocrinol, 25, 91–6. Howard SR (2018). Genes underlying delayed puberty. Mol Cell Endocrinol, 476, 119–28. Hughes IA, Kumanan M (2006). Long-term GnRH agonist treatment for female central precocious puberty does not impair reproductive function. Mol Cell Endocrinol, 25, 217–20. Nathan BM, Palmert MR (2005). Do all girls with apparent idio- pathic precocious puberty require gonadotropin-releasing hormone agonist treatment? J Pediatr, 137, 819–25. Wales J (2012). Disordered pubertal development. Arch Dis Child Educ Prac Ed, 97, 9–16. Wei C, et al. (2015). Recent advances in the understanding and man- agement of delayed puberty. Arch Dis Child, 101, 481–8. Wei C, et al. (2017). The investigation of children and adolescents with abnormalities of pubertal timing. Ann Clin Biochem, 54, 20–32. Wolfenden H, Ryan F (2014). Delayed puberty. Paediatric and Child Health, 24, 124–30.

13.7.3 Normal and abnormal sexual differentiation S. Faisal Ahmed and Angela K. Lucas-Herald

ESSENTIALS Human sex development follows an orderly sequence of embryo- logical events coordinated by a cascade of gene expression and hor- mone production in a time- and concentration-dependent manner. Underpinning the entire process of fetal sex development is the simple mantra: sex chromosomes (XX or XY) dictate the gonadotype (ovary or testis), which then dictates the somatotype (female or male phenotype). The constitutive sex in fetal development is female. The develop- ment of the gonad into a testis or an ovary and the development of the internal reproductive organs and external genitalia is due to (1) critical genes involved in gonadal development, such as SRY (sex chromosome related gene on the Y chromosome), which is first ex- pressed in the XY gonad at 6 to 7 weeks of gestation and leads to the development of the testis; and (2) production by the testis of hor- mones such as anti-Müllerian hormone—to repress Müllerian ducts forming the uterus and fallopian tubes, and androgens (testosterone and dihydrotestosterone) that stabilize the Wölffian ducts and pro- mote the masculinization of the external genitalia. An understanding of these basic principles is essential to formulate a logical approach to the diagnosis of disorders of sex development. Disorders of sex development (DSD) can be classified into three broad categories based on the knowledge of the karyotype: (1) sex chromosome abnormality (e.g. X/XY, mixed gonadal dysgenesis); (2) XX DSD (e.g. congenital adrenal hyperplasia); (3) XY DSD (e.g. partial androgen insensitivity syndrome). Clinical features—the commonest presentations of DSD are (1) atypical genitalia of the newborn; and (2) abnormalities of pu- bertal development in the adolescent. Investigation and management—an extensive repertoire is avail- able, but the choice of genetic, endocrine, and imaging tests should

 be based on the DSD classification and aimed at reaching a con- sensus about the choice of sex assignment. Any surgical procedure required to alter the external phenotype consonant with sex assign- ment need not be undertaken urgently. It is essential that families of children with DSD have the benefit of support and counselling by a multidisciplinary team that comprises at a minimum an endocrin- ologist, urologist/gynaecologist, geneticist, and psychologist. Normal fetal sex development The following key facts underpin the mechanism of dimorphic sex development: • The constitutive sex is female. • Typical male development requires the presence of a Y chromo- some, a testis, and the production and action of testosterone during a critical time in early gestation. • Fetal experiments in mammals indicate that

early castration leads to a female phenotype, despite the presence of the Y chromosome (Jost's hypothesis). • Absence of an ovary does not affect the female phenotype at birth (e.g. Turner syndrome). • Oestrogen is not required for fetal female development, but an- drogen is essential for fetal male development. The link between sex chromosomes, gonad determination and the expression of the phenotype (somatotype) is illustrated in simple configuration in Fig. 13.7.3.1. The events that occur during fetal male development are depicted in Fig. 13.7.3.2. The indifferent gonad develops in the urogenital ridge, where the kidney and ad- renals also have their origins. This is germane to the frequent as- sociation of urinary tract anomalies with atypical genitalia and the occasional occurrence of nests of adrenal remnants found in the testis of males with congenital adrenal hyperplasia (see Chapter 13.5.2). Germ cells migrate from the yolk sac to take their position within the developing gonad. The testis is histologically defined initially by its seminiferous tubules and predates com- parable maturation of the ovary by a few weeks. Three products of the somatic component of this testis are key to development of the male phenotype (i.e. sex differentiation). Anti-Müllerian hor- mone (AMH), a product of Sertoli cells, acts on its type II receptor to repress Müllerian ducts forming the uterus and fallopian tubes. This process is permitted to occur in the female because of the ab- sence of AMH at this stage in gestation. Testosterone produced by Leydig cells under the control initially of placental human chori- onic gonadotrophin (hCG) acts locally in high concentration to sta- bilize the Wölffian ducts. These form the vas deferens, epididymis, and seminal vesicles. A further product of the Leydig cells, insulin-like factor 3 (INSL3), is required for the transabdominal phase of migration of the testis from the urogenital ridge to its site at birth within the scrotum. The inguinoscrotal phase of testis descent in late gestation is under the control of androgens. All these events take place in a specific chronological order and are controlled by genes and hormones expressed at critical dosage thresholds and timing. The genetic and hormonal control of the events in male sex development is shown in Fig. 13.7.3.3. Not all the genes charac- terized for mammalian development are shown, but those identi- fied in the human and relevant to disorders of sex development are emphasized. The formation of the urogenital ridge is dependent on factors such as WT1 and SF1, their roles vividly illustrated by mouse gene knockout studies (absence of gonads and kidneys or adrenals, respectively) and syndromes of urogenital anomalies (WAGR, Denys–Drash, Frasier syndromes) and combined gonadal dysgenesis/adrenal insufficiency in humans with inactivating mu- tations of WT1 and SF1 genes, respectively. The master regulator of testis development (sex determination) is SRY (sex chromosome related gene on the Y chromosome) which is first expressed in the XY gonad at 6 to 7 weeks of gestation, just before the indifferent gonad differentiates as a testis. SRY is a 204 amino acid protein functioning as a high mobility group (HMG) box transcription factor. The HMG box of 79 amino acids is related to similar proteins such as SOX (SRY-like HMG box) which is also a key protein in the regulation of testis development. That SRY is essential for testis development is supported by the following ob- servations: (1) translocation of SRY to the X chromosome during paternal meiosis is present in 90% of XX males; (2) mutations in SRY in 15 to 20% of XY females with complete gonadal dysgenesis leads to complete sex reversal; (3) induction of a male phenotype occurs by transgenic insertion of Sry in XX mice. However, the observation that 10% of XX males lack SRY and the vast majority of XY gonadal dysgenic females have a normal SRY indicates that other genes must also be involved in testis determination. Candidates such as SOX9 and SF1 play some role, but their inactivation in humans leads to various syndromes of which gonadal dysgenesis is only one compo- nent. The role of clinicians recording detailed phenotypes in cases of disordered sex development is essential to continue the search for the multitude of genes that must be involved in testis determination. What is known in the human is

summarized in Fig. 13.7.3.3. The ovary is devoid of known factors, although genes such as WNT4, RSPO1, and DAX1 may act in female development by suppressing testis-determining genes. The differentiation of the internal genital ducts and external genitalia into male structures is entirely androgen dependent. Chromosomal sex (genotype) Phenotypic sex (somatotype) XY XX Gonadal sex Fig. 13.7.3.1 The basic components of fetal sex development.

13.7.3 Normal and abnormal sexual differentiation 2437 For this to occur, an intact pathway of gonadotrophin-induced steroidogenesis is required to produce the potent androgens, testos- terone and dihydrotestosterone (DHT), which in turn promote an- drogen signalling by ligand activating the nuclear androgen receptor (AR) in target cells. The pathways of testicular steroidogenesis and the ligand-activated AR signalling are shown in Figs. 13.7.3.4 and 13.7.3.5, respectively. The enzymatic steps in androgen produc- tion are encoded by genes, each of which, when mutated, results in syndromes of undermasculinization. Some of the more proximal enzyme defects also involve adrenal steroidogenesis and lead to syn- dromes that include adrenal insufficiency (see Chapter 13.5.1). Androgen signalling is mediated by a single AR encoded by an X-linked gene at Xq11–12. The AR is a member of a large family of nuclear receptors that comprise four general functional do- mains: an N-terminal transactivation domain, a central DNA- binding domain, a hinge region, and a C-terminal domain to which the ligand binds. Subdomains are involved in dimerization, nuclear localization, and transcriptional regulation. Circulating andro- gens are bound to carrier proteins such as sex hormone binding globulin (SHBG) and albumin but diffuse freely into target cells where the ligand binds to cytoplasmic AR complexed to heat shock proteins. The hormone–receptor complex translocates to the nu- cleus where it binds to DNA response elements as a homodimer. An added refinement in androgen signalling is provided by inter- action with several coactivator proteins to enhance upregulation of androgen-responsive genes via the general transcriptional Mesoderm Sertoli cells Leydig cells Germ cell migration Testosterone Testis descent External genital growth Male external genital differentiation Mullerian duct regression Wolffian duct stabilization Gestation (weeks) 4 5 6 7 8 9 10 11 12 13 14 15 40 10 nmol/litre Fig. 13.7.3.2 The embryology of fetal sex development in the male. Mesoderm represents the tissue source for the somatic components of testis development. The solid line denotes the rise in fetal serum testosterone levels (nmol/litre). Gonadal development Genital development Intermediate mesoderm Testis WT1, NR5A1 Bipotential gonad Ovary Leydig cells Sertoli cells Testosterone AMH Male internal genitalia Regression of Müllerian ducts Male external genitalia NR5A1, SRY FGF9, SOX9, DAX1 DHT WT1, NR5A1 AMH-R LHR Steroid synthesis: SRD5A2 HSD17B3 AR WNT4, RSPO1, SOX3 FOXL2 Ovary (mature) MAMLD1 Fig. 13.7.3.3 Genetic and hormonal control of fetal sex development. Emphasis is placed on genes relevant to human development.

section 13 Endocrine disorders 2438 machinery. Androgens have pleiotropic effects beyond fetal male development. These include the development of secondary sexual characteristics at puberty, muscle and skeletal growth, stimulation of sebaceous glands, and elongation and thickening of the vocal cords that give rise to the ‘voice breaking’ characteristic of the later stages of male puberty. Prostate development and growth is also androgen dependent, a feature that is countered by antiandrogenic forms of treatment for prostate cancer. It is also clear that androgens have effects on brain development, with prenatal influences being especially relevant to the sex dimorphism in gender role behaviour. Much of the evidence for the role of androgens in psychosocial functioning has come from studies of females exposed to excess androgens (e.g. congenital adrenal hyperplasia) and syndromes associated with defects in androgen signalling (e.g. complete an-

drogen insensitivity syndrome). Definitions, terminology, and nomenclature Clarity of thought is required when faced with a newborn infant whose external genitalia are so unusual in appearance that sex as- signment is not instantaneously possible. The problem does not need compounding by the use of unclear, ambiguous terms such as ‘true hermaphroditism’ and ‘pseudohermaphroditism’. The following LH/hCG Dihydrotestosterone Oestradiol 17–20 lyase 17βHSD Androstenedione 17α-hydroxylase CYP17 Pregnenolone 17-OH-Pregnenolone Dehydroepiandrosterone Androstenediol Testosterone HSD17B3 17-OH-Progesterone Progesterone CYP17 CYP17 CYP17 17α-hydroxylase 17–20 lyase 17βHSD HSD17B3 SRD5A2 5αRD CYP19 P450arom LHR P450scc Cholesterol StAR CYP11A1 HSD3B2/ 3βHSD Fig. 13.7.3.4 Pathway of androgen biosynthesis, including aromatase conversion to oestrogen. 3βHSD, 3β-hydroxysteroid dehydrogenase; 5αRD, 5α-reductase; 17βHSD, 17β-hydroxysteroid dehydrogenase; P450arom, P450 aromatase. The cognate genes are depicted in italics.

13.7.3 Normal and abnormal sexual differentiation 2439 definitions are relevant to the understanding of normal sex develop- ment, both somatic and psychosexual: • development of the gonads—transformation of the indifferent gonad into a testis or an ovary, also referred to as sex determination • development of the genitalia—development of the phenotype as an expression of hormones produced by the gonad • sex assignment—allocation of male or female at birth, usually instantaneous • gender identity—the sense of self as being male or female • gender role—sex-typical behaviours and preferences in which males and females differ (e.g. toy preferences, aggression) • sexual orientation—refers to the target of sexual arousal • gender attribution—assigning as male or female on first encounter with an individual ‘Gender dysphoria’ is a condition characterized by a mismatch be- tween the body habitus (phenotype as male or female) and gender identity as perceived by the affected individual (‘I feel like a woman trapped in a man’s body’). The desire to ‘convert’ from male to fe- male, or vice versa, is a state of transsexualism. In most cases, there is no clear evidence of a genetic or endocrine explanation for gender dysphoria. However, rarely, gender dysphoria may be encountered in older individuals with a disorder of sex development such as an androgen biosynthetic defect. The term ‘intersex’ has traditionally been applied to the clinical scenario of an infant born with ambiguous genitalia and in whom the sex is indeterminate. That allocation has often strayed beyond this defined scenario to include conditions such as severe hypo- spadias, milder forms of congenital adrenal hyperplasia, and the complete androgen insensitivity syndrome, where intersex is an in- appropriate term. In response, a consensus statement produced by a faculty of experts in genetics, endocrinology, surgery, and psych- ology redefined the terminology in 2006. While the new phrase ‘disorders of sex development’ is nowadays used commonly, many members of the affected community feel that the term ‘disorders’ may lead to unnecessary pathologizing of their condition. Disorders of sex development (DSD) classification The DSD nomenclature is simple, reflecting procedures followed during initial investigation and is flexible enough to be adapted as new conditions are recognized and defined. Subtending the entire process is knowledge of the karyotype, a starting point which is now routine for the investigation of DSD. Since fertilization of the ovum with an X- or Y-bearing spermatozoon and sex chromosome aneu- ploidy or mosaicism are so fundamental to normal fetal sex devel- opment, it is logical to consider the causes of DSD in three broad categories. Table 13.7.3.1 contains a fairly comprehensive list of the causes of DSD; Table 13.7.3.2 focuses on the causes of ambiguous genitalia from a functional standpoint. Sex chromosome DSD It can be argued that Klinefelter’s and Turner’s syndromes are not ex- amples of DSD in the context of abnormalities of the external geni- talia. Klinefelter syndrome (47,XXY) has a genital component that typically

comprises small, soft testes with oligo- or azoospermia, and infertility. The syndrome affects 1 in 500 to 1 in 1000 live births. The problem of infertility is not absolute now that advances in artificial reproductive technologies have enabled some men with Klinefelter's syndrome to father children through testicular sperm extraction combined with intracytoplasmic sperm injection. Small testes are al- ready evident in childhood and the penis may be small. Hypospadias may also occur and genitalia can be sufficiently undermasculinized to lead to ambiguity and even a sex-reversed phenotype. Klinefelter variants such as 48,XXXY, and 49,XXXXY may have associated genital anomalies. Turner's syndrome without evidence of Y chromosomal material is seldom associated with external genital abnormalities. The DSD classification has included this syndrome since it is a congenital disorder characterized by gonadal (ovarian) dysgenesis and a sex chromosome aneuploidy. Turner's syndrome has an incidence of 1 in 2500. The classic form is associated with a 45,X karyotype, which accounts for more than 50% of cases. Genital anomalies are more evidenced in mosaic forms of Turner's syndrome characterized by a 45,X/46,XY karyotype. The external genitalia can range in ap- pearance from normal female or mild clitoromegaly, through overt ambiguous genitalia to a male spectrum of simple hypospadias or normal male genitalia. Indeed, most individuals with this karyotype appear to be normal males based on amniocentesis analyses that have revealed fetuses with a 45,X/46,XY karyotype. The minority with ambiguous genitalia are detected at birth and can pose difficul- ties for assignment of gender. A multitude of factors need to be con- sidered, including the genital appearance and urogenital anatomy,
Fig. 13.7.3.5 Schematic of androgen action in a target cell. ARA70, an androgen receptor specific coactivator; GTA, general transcriptional apparatus; HSP, heat shock protein; P, phosphorylation; SHBG, sex hormone binding globulin.

section 13 Endocrine disorders 2440 risk of gonadal tumour, fertility and reproductive options, gender identity, and psychosexual function. Those infants who are severely undermasculinized and have a uterine remnant are likely to be as- signed female and any dysgenetic gonad should be removed. Infants assigned male will require several hypospadia procedures and re- moval of any dysgenetic gonads. Long-term outcome studies are not available. Furthermore, finding a 45,X/46,XY karyotype during in- vestigations for male infertility or in men presenting with a tumour of the testis is rare. Ovotesticular DSD is a rare cause of abnormal genital develop- ment and is characterized by the presence of testicular and follicle- containing ovarian tissue. The external genitalia can be variable, comprising ambiguous genitalia or just severe hypospadias. The gonadal distribution may be a testis on one side and an ovary on the other, bilateral ovotestes, or, most frequently, an ovotestis on one side and a testis or ovary on the other. An ovary will be sited in its normal pelvic position whereas a testis or an ovotestis can be located anywhere along the migratory path to the scrotum, but usually in the inguinal region. The pattern of internal genital ducts can also be variable, but generally follows that of the ipsilateral gonad. A ru- dimentary uterus is often found adjacent to an ovary or ovotestis. The karyotype is 46,XX in most cases, but only in about 30% is the SRY gene X-translocated. Familial cases are reported, with evidence for both autosomal recessive and sex-limited autosomal dominant transmission. The syndrome of XX male is similar to Klinefelter's syndrome where the external genitalia usually differentiate normally as male but the testes are small. Hypospadias may occur infrequently. The in- cidence is 1 in 20 000 male births and, unlike Klinefelter's syndrome, height is below average for normal males. Infertility is invariable; around 90% of XX males are SRY positive. However, the coexistence of ovotesticular DSD and XX male within families and the reported occurrence of 46,XX SRY-negative monozygotic twins with genital anomalies suggests that these two forms of DSD are manifestations

of the same underlying disorder in gonad determination. Mutations in RSPO1, an ovarian-specific determining gene, results in female- to-male XX sex reversal and hence may explain the phenotype in some SRY-negative XX males. The phenotype is also replicated in an Rspo1 (–/–) XX mouse model. SOX3 is another SRY-related HMG box-containing gene which is postulated to have evolved from SRY. Table 13.7.3.2 Causes of atypical genitalia: a functional classification Type/cause Illustrative examples Masculinized female (46,XX DSD) Fetal androgens CAH, placental aromatase deficiency Maternal androgens Ovarian and adrenal tumours Undermasculinized male (46,XYDSD) Abnormal testis determination Partial (XY) and mixed (XO/XY) gonadal dysgenesis Androgen biosynthetic defects LH receptor inactivating mutations 17β-OH-steroid dehydrogenase deficiency 5α-reductase deficiency Resistance to androgens Androgen insensitivity syndrome variants Ovotesticular DSD Presence of testicular and ovarian tissue Karyotypes XX,XY,XX/XY Syndromal Denys–Drash, Frasier's Smith–Lemli–Opitz Table 13.7.3.1 Classification of disorders of sex development (DSD) Sex chromosome DSD 46,XY DSD 46,XX DSD A: 47,XXY (Klinefelter's syndrome and variants) B: 45,X (Turner's syndrome and variants) C: 45,X/46,XY (mixed gonadal dysgenesis) D: 46,XX/46,XY (chimerism) A: Disorders of gonadal (testicular) development

1. Complete or partial gonadal dysgenesis (e.g. SRY, SOX9, SF1, WT1, DHH, and so on)
2. Ovotesticular DSD
3. Testis regression A: Disorders of gonadal (ovary) development
4. Gonadal dysgenesis
5. Ovotesticular DSD
6. Testicular DSD (e.g. SRY+, dup SOX9, RSP01) B: Disorders in androgen synthesis or action
7. Disorders of androgen synthesis LH receptor mutations Smith–Lemli–Opitz syndrome Steroidogenic acute regulatory protein mutations Cholesterol side chain cleavage (CYP11A1) 3β-hydroxysteroid dehydrogenase 2 (HSD3B2) 17α-hydroxylase/17,20-lyase (CYP17) P450 oxidoreductase (POR) 17β-OH steroid dehydrogenase (HSD17B3) 5α-reductase 2 (SRD5A2)
8. Disorders of androgen action Androgen insensitivity syndrome Drugs and environmental modulators B: Androgen excess 1. Fetal 3β-hydroxysteroid dehydrogenase 2 (HSD3B2) 21-hydroxylase (CYP21A2) P450 oxidoreductase (POR) 11β-hydroxylase (CYP11B1) Glucocorticoid receptor mutations
9. Fetoplacental Aromatase (CYP19) deficiency Oxidoreductase (POR) deficiency
10. Maternal Maternal virilizing tumours (e.g. luteomas) Androgenic drugs C: Other
11. Syndromic associations of male genital development (e.g. cloacal anomalies, Robinow, Aarskog, hand–foot–genital, popliteal pterygium)
12. Persistent Müllerian duct syndrome
13. Vanishing testis syndrome
14. Isolated hypospadias (CXorf6, MAMDL1)
15. Congenital hypogonadotropic hypogonadism
16. Cryptorchidism (INSL3, GREAT)
17. Environmental influences C: Other
18. Syndromic associations (e.g. cloacal anomalies)
19. Müllerian agenesis/hypoplasia (e.g. MURCS)
20. Uterine abnormalities (e.g. MODY5)
21. Vaginal atresias (e.g. McKusick–Kaufman)

22. Labial adhesions

13.7.3 Normal and abnormal sexual differentiation 2441 When overexpressed in XX mice, it induces testis development and male sex reversal. Furthermore, genomic rearrangements of the SOX3 regulatory region were identified in three XX males who were SRY negative. It is likely that the mechanism of testis determination in the absence of SRY is via ectopic gonadal expression of SOX3 and subsequent activation of the male pathway of development. SOX3 rearrangements are quite frequent in XX males and the observation goes some way to explain the paradox of XX male development in the absence of an SRY gene. 46,XX DSD In most instances, this broad category of DSD is characterized by a list of conditions where the effect of an abnormal excess of endogenous or exogenous androgens is superimposed on the constitutive female sex development. Thus, internal genital devel- opment is normal (ovaries, uterus, and fallopian tubes; absence of Wölffian ducts) whereas the external genitalia are virilized to a vari- able degree. The prime example of XX, DSD is congenital adrenal hyperplasia. Congenital adrenal hyperplasia (CAH) This autosomal recessive disorder of adrenal steroidogenesis is covered in detail in Chapter 13.5.2. CAH is the commonest cause of ambiguous genitalia of the newborn, and mutations affecting the CYP21A2 gene account for more than 90% of cases. The degree of external masculinization can be so extreme as to resemble a new- born male. However, routine newborn examination should reveal the absence of palpable testes, a sign that must prompt urgent inves- tigation. The diagnosis is straightforward and must be undertaken promptly in view of the potential life-threatening consequences for the infant from glucocorticoid and mineralocorticoid deficiency. The much rarer enzyme deficiencies affecting early steps in adrenal steroidogenesis are also potentially life-threatening and are de- scribed in Chapter 13.5.2. Other causes of endogenous fetal androgen excess The fetal adrenals are unique in containing a large fetal zone which involutes after birth. Its peculiar role in producing large amounts of androgens does not become established again until late childhood when the zona reticularis differentiates functionally and is mani- fest as adrenarche. Large quantities of dehydroepiandrosterone (DHA) and its sulphated moiety (DHAS) are produced greatly in excess of the principle adrenal glucocorticoid, cortisol, yet the pre- cise function of this steroid in both fetal and postnatal life remains a mystery. DHEAS is 16-hydroxylated in the adrenals and liver before transfer to the placenta where the sulphate is cleaved by placental sulphatase. The substrates DHEA and 16-OH-DHEA are converted to more potent androgens such as androstenedione and testosterone and all three androgens are converted to oestrogens (oestrone, oes- tradiol, oestriol). This reaction is mediated by a single gene, CYP19, which encodes for the placental aromatase enzyme via a tissue- specific promoter. Measurement of maternal serum or urinary oestriol was previously used as a nonspecific marker of placental dysfunction. However, the levels are specifically low in CAH, in pla- cental sulphatase deficiency (as the sulphate moiety is uncleaved to allow substrate for aromatization), and as a marker of suppression of fetal adrenal steroidogenesis during prenatal treatment for CAH. The effects of placental aromatase deficiency are profound on both the fetus and the mother. Exposing a female fetus to large amounts of adrenal-derived androgens leads to virilization of the external genitalia as severe as in CAH. Salt wasting is not an asso- ciated feature. The mother is also virilized during pregnancy, with evidence of hirsutism, acne, and sometimes clitoromegaly. These signs resolve postnatally but can recur in subsequent pregnancies. Affected girls in later life can present with delayed puberty due to primary gonadal failure where there are cystic ovarian changes and hypergonadotropism. A male fetus exposed to excess adrenal androgens, as with CAH, is not affected at birth. However, males with aromatase deficiency are very tall in young

adulthood be- cause of failure in oestrogen-induced closure of the growth plate. The aromatase enzyme has a massive capacity to convert androgens to oestrogens as the mother escapes virilization if only 1 to 2% ac- tivity of mutant enzyme remains. The mechanism of virilization in a 46,XX infant with P450 oxidoreductase (POR) deficiency may also be partly related to a disturbance in aromatase deficiency as POR is also an electron donor for P450 aromatase. It has also been suggested that an alternative fetal pathway to DHT synthesis is adopted secondary to POR deficiency (see Chapter 13.5.2). Maternal androgen excess The fetus is protected against excess androgens because of a highly efficient placental aromatase enzyme that converts androgens to oestrogens. Women with CAH who become pregnant have relatively high serum androgen levels during pregnancy, yet virilization of a female fetus has not been observed. However, the enzyme can become overwhelmed by excess androgens originating from maternal adrenal or ovarian androgen-secreting tumours. Ovarian luteomas can be recurrent in pregnancies and are most common in multip- arous women of Afro-Caribbean descent. Other virilizing ovarian tumours include hyperreactio luteinalis, arrhenoblastoma, hilar cell tumour, and a Krukenberg tumour. Occasionally, polycystic ovarian syndrome may result in fetal virilization. Danazol, a synthetic de- rivative of 17β-ethinyltestosterone with androgenic, antioestrogenic, and antiprogestogenic properties, readily crosses the placenta. It is used for several conditions as diverse as endometriosis, benign fibrocystic breast disease, for unexplained female infertility, and in hereditary angioedema.
A female fetus can become virilized and the use of danazol is contraindicated in pregnancy.
46,XY DSD A lengthy list of causes is included in this category of DSD and the frequency is quite high if more common conditions such as hypo- spadias and cryptorchidism are included. Although the problem of XY DSD is more complex than XX DSD in terms of diagnosis and management, knowledge of the normal process of male fetal sex de- velopment allows the causes to be subdivided into (1) disorders of gonadal development or gonadal dysgenesis, (2) defects in androgen biosynthesis, and (3) resistance to androgens. Gonadal dysgenesis The pivotal role of SRY in human testis development is vividly illus- trated by the phenotype of XY sex reversal as a manifestation of an inactivating mutation of the SRY gene. There is complete gonadal dysgenesis with the histological appearance of streak gonads and no discernible testis development in the form of seminiferous tubules

 or Leydig cells. Lack of Sertoli cells and hence no AMH production leads to a uterine remnant and the external genitalia are female as a result of no androgen production. Presentation does not usually occur until adolescence on account of delayed puberty and primary amenorrhoea. The condition when presenting in adulthood has often been referred to as Swyer’s associated with a gonadal tumour, typic- ally as a dysgerminoma. Other tumours include gonadoblastoma, teratoma, and embryonal carcinoma. The risk of gonadal tumours is in the range of 15 to 35%. Müllerian structures are preserved and the uterus increases in size when oestrogen replacement is started. Bone mineral density is reduced in the majority. Adult height in a United Kingdom series was 174 cm as compared with 164 cm in the normal female population. Successful pregnancies have been achieved fol- lowing egg donation. Mutations in the SRY gene are found in only 15 to 20% of cases of complete XY gonadal dysgenesis. The majority are located within the HMG box, a DNA-binding domain which appears to function by modulating local chromatin structure in order to transcribe adjacent target genes. Most mutations are de novo, yet there is a curious subset of cases where the mutation is familial and found in the phenotypically normal and fertile father. Explanations for this paradox include expression of the gene to a sufficient threshold against a particular genetic background that is not present in the

46,XY daughter. Alternatively, paternal gonadal mosaicism may be an explanation for familial cases. Partial gonadal dysgenesis refers to evidence of some virilization in the form of clitoromegaly and partial labial fusion or a more male pattern of external genital devel- opment with severe hypospadias, bifid scrotum, and undescended gonads. This phenotype is common to many other causes of 46,XY DSD. Mutations in SRY are not identified in this form of gonadal dysgenesis but mutations in NR5A1 are increasingly being found in partial gonadal dysgenesis without adrenal insufficiency as well as in phenotypes as diverse as hypospadias, anorchia, and male infertility. Several syndromes are described in association with XY gonadal dysgenesis where the genital anomalies can constitute complete sex reversal or varying degrees of external genital ambiguity. Denys– Drash syndrome (OMIM 194080) is characterized by gonadal dysgenesis, an early-onset nephropathy due to diffuse mesangial sclerosis and a high risk of Wilms' tumour.
A related condition is Frasier's syndrome (OMIM 136680) which is also characterized by gonadal dysgenesis but of a greater severity (streak gonads), a later-onset nephropathy due to focal segmental glomerulosclerosis, and a high risk of gonadal tumours. The two syndromes may rep- resent a continuum of phenotypes. Both have in common muta- tions in the Wilms' tumour-related gene, WT1, as the underlying cause. The gene encodes for a four-zinc-finger transcription factor expressed in the developing urogenital ridge, kidney, and gonads. A macrodeletion affecting the region on chromosome 11p13 where WT1 resides causes the WAGR syndrome (Wilms' tumour, aniridia, genitourinary abnormalities, and mental retardation; OMIM 194072). Denys–Drash and Frasier's syndromes are caused by heterozygous point mutations in WT1 that have a dominant-negative effect on the wild-type protein. In the former syndrome, these affect the DNA-binding zinc-finger region of WT1. Most mu- tations causing Frasier's syndrome involve the donor splice site of exon 9. The use of an alternative splice donor site for exon 9 re- sults in the addition of three amino acids—lysine (K), threonine (T), and serine (S)—between the third and fourth zinc fingers. The +KTS and –KTS isoforms are thought to have differential effects on gonad and renal development and an imbalance in the ratio of these isoforms may be the explanation for the phenotype of Frasier's syndrome. It has been proposed that all cases of XY DSD with ex- ternal genital anomalies should have routine urinalysis for protein- uria and renal ultrasound to exclude a Wilms' tumour. Rarely, a WT1 mutation has been identified in a case of isolated hypospadias but this does not justify routine WT1 screening for this common genital anomaly. Another syndrome associated with gonadal dysgenesis and genital anomalies is caused by mutations in SOX9 which encodes for an SRY-related HMG box protein of 509 amino acids. The protein is expressed in the developing testis shortly after SRY ex- pression. The protein is also expressed in cartilage; heterozygous mutations in SOX9 (chromosome 17q24–25) cause campomelic dysplasia (long bone bowing, hypoplastic scapula and rib cage, de- formed pelvis, cleft palate, macrocephaly, cardiac and renal defects) as well as the associated genital anomalies. SOX9 mutations do not occur without the skeletal abnormalities. There are rare examples of gonadal dysgenesis occurring in association with mutations in desert hedgehog (DHH) and testis-specific protein-like-1 (TSPYL1) genes and with chromosomal deletions at 9p24-pter, 10q26-pter, and Xq13.3. Chromosomal duplications at Xp21.2 (DAX1 candi- date gene implicated) and 1p35 (WNT4 and RSP01 candidate genes implicated). Another gene now found to be associated with XY sex reversal is CBX2, the human homologue of M33, which, when ablated in mice, leads to XY sex reversal. A loss-of-function CBX mutation in a girl whose prenatal karyotype was 46,XY was shown to be the cause of her sex reversal based on detailed functional studies. This gene appears to actively repress ovarian development in XY gonads. Remarkably, a uterus and histologically normal ovaries were present in this girl. A linkage analysis study successfully identified mutations in MAP3K1 in a familial form of

XY gonadal dysgenesis as well as in some sporadic cases. The mutations cause alterations downstream in the MAP kinase signalling pathway, possibly by altering SOX9 or β-catenin activities. Genes implicated in XY gonadal dysgenesis can cause other comorbidities, in particular in the cardiovascular system. GATA- binding protein 4 (GATA4) mutations result in a variable pheno- type ranging from 46,XY partial gonadal dysgenesis to a more severe phenotype with ambiguous genitalia and azoospermia. Systolic car- diac murmurs have been reported in the milder cases of atypical genitalia. XX carriers of these mutations may have congenital heart disease. Similarly, zinc finger protein multitype 2 (FOG2/ZFPM2) mutations have been reported in cases with XY partial gonadal dysgenesis and atrial septal defects. Neurodevelopmental disorders are also associated with XY go- nadal dysgenesis. Aristaless-related homeobox (ARX) mutations result in a varied phenotype of X-linked developmental disorders associated with anomalies of the male genitalia. Arx-/- knockout mice have also been shown to lack fetal Leydig cell differentiation. Finally, 80% of individuals with α-thalassaemia/mental retardation syndrome X-linked (ATRX) demonstrate gonadal abnormalities ranging from gonadal dysgenesis to hypospadias. Analysis of rare mutations in candidate gonad-determining genes is chipping away at the current low frequency of determination of the molecular mechanism of XY gonadal dysgenesis.

13.7.3 Normal and abnormal sexual differentiation 2443 Defects in androgen biosynthesis The steps in androgen production from LH-induced steroidogenesis via cholesterol through to testosterone and DHT are illustrated in Fig. 13.7.3.4. Many of the early steps in androgen biosynthesis are also essential for adrenal steroid biosynthesis and are described in Chapter 13.5.1. The same G-protein-coupled gonadotrophin re- ceptor (LHR) on Leydig cells binds both placental hCG and later, fetal pituitary luteinizing hormone (LH) for the initiation of an- drogen biosynthesis. Inactivating mutations in the LHR gene in 46,XY DSD lead to a variety of phenotypes that include complete sex reversal, ambiguous genitalia, severe hypospadias, or even just isolated micropenis. Why the phenotype should be so heteroge- neous is not entirely clear, although partial loss-of-function muta- tions that result in a milder phenotype such as micropenis tend to localize within the seventh transmembrane domain of the receptor. The biochemical profile comprises elevated LHRH-stimulated LH and follicular stimulating hormone (FSH) levels and low androgen concentrations which do not respond to prolonged hCG stimula- tion. Leydig cells are absent or decreased in number on histological examination. Sertoli cells and seminiferous tubules are present but spermatogenic arrest attests to the importance of intracellular androgen concentrations in mediating the final stages of sperm- atogenesis. A range of homozygous or compound heterozygous mutations of the LHR gene are reported in this syndrome of Leydig cell hypoplasia. Their pathogenicity can be confirmed in vitro by demonstrating impaired hCG stimulation of intracellular cAMP due to disturbances in hCG binding, receptor stability, or receptor trafficking. The extracellular N-terminal ligand-binding domain of LHR consists of nine leucine-rich repeats flanked by cysteine-rich regions. The C-terminal cysteine-rich region is referred to as a hinge region of the receptor within which amino acid residues Asp330 and Tyr331 are key components of LH/hCG signalling. An XX female has been reported with an LHR-inactivating mutation. The pheno- type comprised normal onset of puberty but associated primary amenorrhoea and elevated LH levels. Studies on a rare gene muta- tion in this context provides information to indicate that while the LHR is not required for oestrogen synthesis, it is necessary for the induction of ovulation and fertility, although some affected females may still have regular cycles. Two penultimate steps in androgen biosynthesis essential for normal male sex differentiation are shown in Fig. 13.7.3.4. Both conversion steps are characterized by the

respective substrates being the subject of catalysis by different isoenzymes and not involving steroid biosynthesis in the adrenals. The forms of XY DSD resulting from deficiencies in the two enzymatic steps have in common a se- vere degree of undermasculinization at birth but profound viriliza- tion at puberty. Thus, if unrecognized at birth and the affected infant is assigned female, the clinical presentation occurs at puberty with distressing signs of clitoromegaly, hirsutism, and deepening of the voice in a pubertal girl. There are 14 known 17β-hydroxysteroid dehydrogenase (17HSD) isoenzymes, of which 12 are present in humans. They belong to a family of oxidoreductases involved in the metabolism of steroids, prostaglandins, and retinols. Of most relevance to XY DSD is 17HSD type 3, which is predominantly expressed in the testis and converts androstenedione to testosterone. The reaction is reversible and util- izes nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. The cognate gene, HSD17B3, is located on chromosome 9q22. A spectrum of mutations in this gene generally results in com- plete XY sex reversal at birth and can be mistaken for complete an- drogen insensitivity syndrome. Presentation in infancy may be in the form of an inguinal hernia or labial swelling where investigation reveals the presence of a testis. If sex assigned female, gonadectomy must be undertaken before puberty to avoid a pubertal girl be- coming virilized. The mechanism of such profound androgenic ef- fects is postulated to be the result of extraglandular conversion to androgens utilizing other isoenzymes such as types 1, 2, and 5. Some 17HSDB3 mutations are associated with retention of 15 to 20% of normal 17βHSD3 activity that leads to sufficient virilization of the external genitalia at puberty for sex reassignment. The biochemical profile shows elevated androstenedione and decreased testosterone levels so that the ratio of testosterone to androstenedione is typic- ally 0.8 or less in this disorder. However, there are cases that are de- scribed with a higher ratio than this. Wölffian ducts are stabilized to form the vas deferens, epididymis, and seminal vesicle which is pre- sumably the result of high local concentrations of androstenedione. About 20 mutations in the 17HSDB3 gene are now reported, most being homozygous or compound heterozygous missense mutations. Females with 17βHSD type 3 deficiency are asymptomatic. Testosterone is converted irreversibly to dihydrotestosterone (DHT) by the 5α-reductase type 2 enzyme which is expressed in the primordium of the prostate and external genitalia, but not in the Wölffian ducts until after their differentiation to male internal genital ducts. As with 17βHSD deficiency, the male internal genital ducts develop normally in 5α-reductase deficiency. The phenotype is as- sociated with some external virilization so that presentation is more frequent at birth because of ambiguous genitalia or severe hypospa- dias. This cause of XY DSD became well characterized through de- tailed descriptions of a genetic isolate in the Dominican Republic where males were born with severely undermasculinized external genitalia but then virilized to varying degrees at puberty. The testes enlarge appropriately at this stage, but the prostate gland remains hypoplastic, indicative of the DHT-dependent growth of this organ. Histology of the testes shows Leydig cell hyperplasia and decreased spermatogenesis due to maldescent of the testes. However, there are reports of male fertility either following artificial reproductive tech- niques or even spontaneously after hypospadias repairs had been completed. Gender role changes occur frequently in this condition. The biochemical profile is classically an elevated ratio of serum tes- tosterone to DHT of more than 25:1 after puberty (or following hCG stimulation in a prepubertal child) and a reduced ratio of urinary 5α- to 5β-reduced C19 steroids. However, cases of 5α-reductase de- ficiency without a marked abnormality in these ratios have been de- scribed. The 5α-reductase enzyme is also utilized in the metabolism of glucocorticoids, so C21 5α/5β steroids can usefully be analysed even when gonadectomy has already taken place. There are two iso- enzymes of 5α-reductase, the type 2 enzyme being affected in this condition. SRD5A2 is located on chromosome 2p23 and encodes

for a 254 amino acid protein. The type 1 enzyme is expressed in skin and may contribute to the virilization which takes place at puberty. More than 40 mutations have been detected in the SRD5A2 gene. The ma- jority are missense mutations, including the Gly183Ser substitution observed in the Dominican Republic population. A complete gene deletion is found in an affected New Guinea population.

section 13 Endocrine disorders 2444 Defects in androgen action Androgen resistance is defined as a failure in complete male sex differentiation despite the presence of a normal 46,XY karyotype in association with testes that produce age-appropriate circulating concentrations of androgens. The androgen insensitivity syndromes (AIS) are subdivided into complete (CAIS) and partial (PAIS) forms as defined by complete XY sex reversal (female phenotype) and par- tial virilization of the external genitalia. The degree of virilization in the latter category can vary from mild, isolated clitoromegaly to normal male development with oligospermia. Total resistance to androgens leading to CAIS is the sine qua non of a hormone resistance syndrome and this condition typically pre- sents in adolescence with primary amenorrhoea. There is normal breast development as male-typical androgen levels are aromatized to oestrogens, but there is absent or scanty pubic and axillary hair growth. The external genitalia are female, and a shortened vagina is blind-ending. The upper part of the vagina, together with the uterus and fallopian tubes, are structures derived from the Müllerian duct, hence these are absent in CAIS as a result of normal AMH action by the testes. CAIS may also present in infancy because of the ap- pearance of inguinal herniae which, at surgical repair, are found to contain testes. Thus, a karyotype check in all female infants with an inguinal hernia is highly recommended. The increasing trend towards prenatal tests that reveal the karyotype is also a mode of presentation when the phenotype at birth is realized to be a mis-match with the prenatal genotype. A defect in any one of the steps in androgen signalling may underlie the pathophysiology of CAIS. The problem is generally located with the AR where numerous mu- tations have been identified that result in CAIS or PAIS. These are recorded on an international database (http://androgendb.mcgill. ca/). Mutations are distributed throughout the coding region of the AR gene and include deletions, insertions, premature stop codons, and splice site, as well as missense mutations. The majority are lo- cated within the ligand-binding domain and codons such as Arg840 and Arg855 appear to be relative ‘hotspots’ for mutagenesis. AIS is an X-linked disorder and approximately 30% of AR gene muta- tions are spontaneous. The same mutation may manifest as different phenotypes, between and within affected families and to the extent of different sex assignments. The reasons for phenotypic variability are unclear but may include somatic mosaicism and differences in the lengths of the two AR trinucleotide repeats in the N-terminal domain, glutamine, and glycine. Hyperexpansion of the triplet re- peat (>50) underlies the pathogenesis of spinal and bulbar muscular atrophy (Kennedy’s disease). Males affected with this neurological disorder display signs of mild androgen insensitivity. Certain asso- ciations are also described for the glycine (GGN) repeat, either alone or in combination with variations in the CAG repeat. Gender assignment and sex of rearing in CAIS is female, as is later gender identity. There is a reported 5% risk of gonadal tumours. A precursor lesion to tumour development is intratubular germ-cell neoplasia unclassified (ITGNU), also referred to as carcinoma in situ. This may subsequently lead to a gonadoblastoma, an occurrence which is rare before puberty. The timing of gonadectomy is vari- able but there is merit in delaying until young adulthood to enable spontaneous puberty to occur. There is no evidence that the slightly reduced bone mineral density in CAIS is ameliorated by this man- agement approach, suggesting that androgens have a direct role in normal bone architecture. Oestrogen replacement needs to be started at about 11 years of

age when gonadectomy is performed early. Final height rests between the average height of adult males and females. Mutations are also distributed throughout the AR gene in PAIS, but are predominantly missense in nature. The partial androgenic effect can be verified by functional assays in vitro which demonstrate reporter gene responses close to the normal AR, but usually only after induction with very high concentrations of androgens. Such in- formation can be valuable for predicting outcome at puberty in PAIS patients assigned male. Establishing a precise diagnosis in PAIS can be difficult as so many other disorders can be associated with the typical phenotype of severe hypospadias, micropenis, bifid scrotum, and undescended testes. These include androgen biosynthetic de- fects, partial gonadal dysgenesis, and mixed gonadal dysgenesis. In many instances, no single genetic cause can be found for a PAIS-like phenotype: external genitalia as described and normal androgen production. There is a strong association with low birth weight for gestational age in PAIS cases that have no AR gene mutation. This suggests placental dysfunction being a common link between early fetal growth restriction and inadequate placental hCG-induced early Leydig cell steroidogenesis. The infant with PAIS assigned male may require several surgical procedures to correct hypospa- dias, orchidopexy for undescended testes, and high supplemental androgen treatment to induce puberty. The risk of gonadal tu- mours is probably higher than in CAIS, but once in the scrotum, the testes can be monitored by self-examination and periodic tes- ticular ultrasonography. Outcome data in adult males with PAIS are sparse but sexual function is reported to be impaired; fertility is rare. Those assigned female require genitoplasty procedures in infancy, gonadectomy before puberty, and oestrogen treatment to induce fe- male secondary characteristics. Other conditions within the XY DSD category Some disorders are associated with incomplete male development but do not raise any doubt that sex assignment at birth should be male. These include hypospadias, undescended testes, and the per- sistent Müllerian duct syndrome (PMDS). Isolated hypospadias has a birth prevalence of 3 to 4 per 1000 live births. Although the cause in most cases is unknown, it is possible that more comprehensive endocrine and genetic analysis of a wider panel of genes involved in testis development, androgen synthesis, and action will reveal more abnormalities. Familial cases occur with a 7% incidence of one or more additional family members being af- fected with hypospadias. There is an association with increased ma- ternal age, paternal subfertility, maternal vegetarian diet, maternal smoking, assisted reproductive techniques, exposure to pesticides, and twinning. The aforementioned low birth weight is also a further association, which is strong. Hypospadias is generally classified as mild to severe based on the site of the urethral meatus being distal, mid-shaft, or proximal (severe). There is often an associated chordee in the severe form. Numerous surgical techniques are described to resite the urethral opening on to the glans penis and may require several procedures. The initial procedure is usually undertaken in infancy. Complications include fistulas, meatal stenosis, and ur- ethral strictures and may occur in about 25 of the cases and espe- cially in the more severe forms of hypospadias. Undescended testes or cryptorchidism is the commonest birth defect in boys, affecting 2 to 9% of male live births. Again, there is a strong association with low birth weight as well as disorders

13.7.3 Normal and abnormal sexual differentiation 2445 that affect pituitary–gonadal function and androgen action. These observations emphasize the importance of androgens in mediating complete descent of the testes into the scrotum by their action during the inguinoscrotal phase of descent. Other associations include maternal smoking or use of nicotine substitutes, alcohol use, and gestational diabetes. There is an association with intra- uterine insemination, but not with other forms of artificial repro- ductive technology. Genetic factors also play a part, particularly for

first-degree relatives among brothers and maternal half-brothers. Cryptorchidism can be unilateral or bilateral with the testis sited in the abdomen (nonpalpable), inguinal canal, suprascrotal, or high scrotal (where it is not possible to manipulate the testis to the bottom of the scrotum). Undescended testis must be distinguished from a retractile testis which ascends in response to a pronounced cremasteric reflex but can be manipulated completely into the scrotum. A testis may be descended at birth but found to be un- descended at a later age. This has been termed the ‘ascending’ testis or an acquired form of cryptorchidism. Studies indicate that the phenomenon is more likely with a history of retractile testis, the processus vaginalis may be patent, and the testis is usually located in the inguinal region. Ascending testis accounts for nearly one- half of the cases of undescended testis and mostly explains why late orchidopexies occur around 7 years of age. It is recommended that orchidopexy for congenital cryptorchidism is undertaken between 6 to 12 months of age. Early surgery is associated with improved growth of the testis, less evidence of abnormal germ-cell devel- opment and a lower risk of developing a seminoma in adulthood. Hormonal treatment has low efficacy. A short 3-week period of hCG stimulation that is performed as a means of assessing testes function is associated with a 25% chance of testes decent but pri- marily in those with inguinal testes. Despite evidence for the role of INSL3 and its receptor in testis descent, mutations in the genes that encode these proteins are found only in a minority of boys with cryptorchidism. The components of a quartet of male reproductive tract disorders—hypospadias, cryptorchidism, abnormal spermatogenesis, testis cancer—are each interlinked, for which there is some epidemio- logical evidence to suggest an increase in frequency. Environmental factors have been proposed to explain the observation through the development of a testicular dysgenesis syndrome which has its origin in fetal life. Humans are exposed to more than 80 000 chemicals in the environment with any adverse effects assumed to be more pro- found on the developing fetus. Evidence that chemicals such as pes- ticides and phthalates can disrupt the androgen/oestrogen balance critical for normal fetal sex development is present in wildlife and in animal experiments. It is more difficult to prove similar effects in humans. However, such chemicals labelled as endocrine disruptors are reported to be present in higher concentrations in cord blood, placentas, and breast milk samples of mothers having male offspring with hypospadias or cryptorchidism, compared with normal control offspring. Furthermore, the anogenital distance, which is a sensitive index of androgen action used in rodent reproductive studies, is re- duced in male infants of mothers who had higher prenatal exposure to phthalates. Bilateral anorchia, also referred to as the vanishing testis syn- drome, in an otherwise normal male infant indicates that testes were present and functioning normally in early gestation in order to programme normal male sex differentiation. It is hypothesized that interruption of the vascular supply to the testes must have oc- curred in later gestation (akin to bilateral torsion). This is supported by surgical findings which show a preserved vas deferens entering the internal inguinal ring at the end of which is only a nubbin of fi- brous tissue containing haemosiderin-laden macrophages and dys- trophic calcification. The diagnosis is confirmed by demonstrating elevated LH and FSH concentrations, no testosterone response to hCG stimulation, and an undetectable serum AMH. Even with this endocrine scenario, surgeons generally still perform a laparoscopy to ensure that any gonadal remnant is removed to avoid the risk of malignancy. PMDS is a very rare phenomenon but given that it will be as- sociated with a phenotypic spectrum, its exact prevalence will be difficult to determine. It is associated with testis maldescent but in this instance normal testes are prevented from descending to the scrotum because of being attached to a fallopian tube. The uterus and tubes in this syndrome are retained from early fetal develop- ment because of the lack of AMH action. This can either be the result of a mutation in the AMH gene with low or undetectable

serum AMH, or serum AMH concentrations may be normal but the protein is unable to bind to its receptor because of a mutation in the gene coding for the AMH type II receptor. A mutation is found in the majority of cases with equal distribution between the two causative mutant genes. The phenotypes are identical. The external genitalia are otherwise normal; both testes may be descended to one hemiscrotum. Such transverse testicular ectopia is diagnostic of PMDS. The diagnosis is usually made at orchidopexy or for an inguinal hernia repair where the sac is found to contain a uterus or a fallopian tube. Care must be taken to resite the testis to its normal position as such mobilization may damage the vas deferens. The uterus is often left in place. Aphallia is a condition with clear urological and psychological consequences which is reported to be as rare as 1 in 30 million births. It is believed to be as a result of a failure of development of the genital tubercle but is otherwise associated with normal testes function and virilization. Over 50% of cases have associated genitourinary malformations and no genetic abnormality has yet been identified. Mortality is reported to be higher in those cases with an associ- ated malformation and those where the urethral opening is in the rectum and proximal to the anal sphincter as opposed to distal to the sphincter. Communication The initial contact with the parents of a child with a DSD is im- portant as first impressions from these encounters often persist. A key point to emphasize is that the child with a DSD has the po- tential to become a well-adjusted, functional member of society. The use of the phrase ‘differences or variations in sex development’ may be useful in introducing the concepts of the extent of variation in sex development. An 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 con- sequence 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 coexisting health issues and, thus, requires thorough clinical evaluation. While it is likely DSD may be a more complex and challenging group of conditions, discussions that use the aforementioned approach as the first step may reduce the stigma that is often experienced by families. 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 as the parents’ perception of their own child’s condition may be quite different from the clinician’s perception of the severity of illness. In those cases where there is true genital ambiguity, it should be ex- plained to the parents that the best course of action may not initially be clear, but the healthcare team will work with the family to reach the best possible set of decisions in the circumstances. The healthcare team should discuss with the parents the information to be shared in the early stages with family members and friends. In the case of an affected adolescent, the initial assessment should not only start the process of diagnosis but should also be used to develop a rapport with the patient. In adolescents with an existing DSD, conversation should always start with a review of the patient’s own understanding of their condition. The process of transferring to adult services is also an opportunity to review the diagnosis and consider more novel investigations that may not have been available earlier. Assessment of a DSD Atypical genitalia of the newborn and concerns about secondary sexual characteristics at puberty are the two key stages in life when a problem of DSD requires careful assessment based on clinical examination followed by a focused and logical investigation plan. It must also be recognized that while a definitive diagnosis may not be possible in some cases, this must not delay a decision on sex assign- ment unduly and lessen the importance for a management plan. Examination It is

important that the affected person undergoes a full systematic examination. Approximately 25% of cases of DSD may have an asso- ciated malformation. Assessment of blood glucose and urinalysis for proteinuria should be routine. For the infant with atypical genitalia, the following details need to be recorded: the size of the phallus, presence of chordee, and whether the appearance is indicative of clitoromegaly or a micropenis; site of urethral opening; single or dual openings on the perineum; development of labioscrotal folds or a bifid scrotum; whether gonads are palpable and their site. Allied to the examination are salient points in the clinical history such as family history and exposure to potential reproductive tract terato- gens. Problems arising only at the time of puberty include signs of virilization occurring in a girl, delayed pubertal development, and primary amenorrhoea. Although scoring systems such as the Prader scoring system for XX DSD and modifications of this system for XY DSD may provide an integrated summary description of the geni- talia, these scoring systems are not sufficiently discriminate to por- tray the full spectrum of the variation encountered in the external genitalia. The external masculinization score (EMS), which indi- vidually scores 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. Investigations Infants with suspected DSD who require further clinical evalu- ation and need to be considered for investigation by a specialist should include those with isolated proximal hypospadias, isolated micropenis, isolated clitoromegaly, any form of familial hypospadias and those who have a combination of genital anomalies with an EMS of less than 11 (Box 13.7.3.1). This will avoid unnecessary detailed investigations of boys with isolated glandular or mid-shaft hypo- spadias and boys with unilateral inguinal testis. In approximately 25% of affected cases, DSD is part of a complex condition and the coexistence of a systemic metabolic disorder, other malformations, or dysmorphic features, would lower the threshold for investiga- tion as would a family history of consanguinity, stillbirths, multiple miscarriages, fertility problems, genital abnormalities, hernias, de- layed puberty, genital surgery, unexplained deaths, and the need for steroid replacement. In addition, maternal health, and pregnancy history itself may hold key information. In those with ambiguous genitalia and/or bilateral impalpable gonads, a first tier of investiga- tions should be undertaken to define the sex chromosomes and de- lineate, by pelvic ultrasound, the internal genitalia and exclude CAH (Box 13.7.3.2). This first tier should, therefore, also include plasma Box 13.7.3.1 Newborn problems that merit DSD investigation • Genitalia that are so unusual that sex assignment cannot be performed (i.e. ambiguous genitalia) • Apparent female genitalia with: — Enlarged clitoris — Posterior labial fusion — Inguinal/labial mass • Apparent male genitalia with: — Nonpalpable testes — Isolated perineoscrotal hypospadias — Severe hypospadias, undescended testes, micropenis • Genital anomalies associated with syndromes • Family history of DSD, such as CAIS • Discordance between genital appearance and prenatal karyotype Box 13.7.3.2 Investigating an affected infant • Genetics — FISH (X centromeric and SRY probes) — Karyotype (high resolution; abundant mitoses) — Save DNA with consent • Endocrine — 17-OH progesterone, 11-deoxycortisol (plus routine biochemistry; save serum), renin — ACTH, 24-h urinary steroids (also check proteinuria) — Testosterone, androstenedione, DHT — LH, FSH, AMH, inhibin B — hCG stimulation test (define dose, timing) • Imaging — Pelvic, adrenal, renal ultrasound — MRI — Cystourethroscopy and sinogram • Surgical — Laparoscopy — Gonadal biopsies — Genital skin biopsy (extract DNA and RNA)

 glucose, serum 17OH-progesterone (17OHP) and serum electro- lytes. Serum 17OHP is usually unreliable before the age of 36 hours, and in the salt-losing form of CAH, serum electrolytes usually do not become abnormal before day

4 of life. The results of polymerase chain reaction or FISH analysis using Y- and X-specific markers should be available within one to two working days, and labs should attempt to report 17OHP results in such a circumstance within two working days. In situations where the level of suspicion of CAH is very high and the infant needs immediate steroid replacement therapy, further serum samples should be collected and stored be- fore starting therapy. These should be of a sufficient volume to assess 17OHP, testosterone, androstenedione and, possibly, renin activity or concentration, in that order of priority. At least one spot or 24-h urine sample (at least 5 ml) for a urine steroid profile should be col- lected before starting therapy. The results of these initial investiga- tions shall often dictate the second tier of investigations. In an infant with impalpable gonads, a karyotype of 46,XX, a significantly elevated serum 17OHP and the presence of a uterus makes congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency very likely. A urine steroid profile can confirm this diag- nosis and can also identify other rare forms of CAH, which may also be associated with a raised 17OHP in the newborn such as 11β- hydroxylase deficiency and 3β-hydroxysteroid dehydrogenase defi- ciency. For the XY or X/XY infant with DSD, AMH and testosterone measurement will provide information about the presence of func- tioning testes. Depending on the age of the child, an hCG stimula- tion test may be required. Confirmation of a specific diagnosis will often require further biochemical identification of a defect in the an- drogen biosynthesis pathway and detailed genetic analysis. Imaging studies (ultrasonography and MRI) may locate the site of gonads but often laparoscopy is the only reliable method to identify gonads. This also provides the opportunity to obtain biopsies for histology, the only sure way to establish a diagnosis of ovotesticular DSD. Management Only the principles of DSD management can be described since each cause of DSD has specific requirements, some of which have been covered for CAH in Chapter 13.5.2. The greatest challenges for the clinician are to manage the newborn with ambiguous geni- talia and the pubertal child who develops physical signs incongruent with the sex of rearing. It is axiomatic that management should only be undertaken by a multidisciplinary team that comprises, at a min- imum, a paediatric endocrinologist, urologist, gynaecologist, a gen- eticist, and a clinical psychologist. There is consensus that all infants with DSD should have a gender assignment, but this may have to be delayed until the results of relevant investigations are available. Surgery required to make the genitalia concordant with gender as- signed may be deferred, even to an age where the child is of suf- ficient cognitive development to be involved with the discussions. Psychological support is required for the family from the outset, as misinformation given early can impact adversely in the longer term. As the child grows older, explanation of the diagnosis must be care- fully planned with the parents. In a girl with XY DSD (CAIS, for example), this will entail explanation concerning the nature of the gonads, the presence of a Y chromosome, absence of a uterus, lack of menses, and future infertility. Such explanation requires skilled counselling delivered as appropriate to the child’s development. Transitional care from adolescence to young adulthood is a further level of complexity that requires the recruitment of adult specialists relevant to whether sex assignment has remained male or female. Longer-term studies in women with CAH are now being conducted and provide valuable information on surgical, endocrine, and psy- chosexual outcomes. In terms of XY DSD, outcome data are reason- ably robust for miscellaneous conditions such as cryptorchidism, hypospadias, mixed gonadal dysgenesis (XO/XY), and CAIS. In contrast, data remain sparse in PAIS and some androgen biosyn- thetic defects, conditions where sex reassignment may arise in later childhood and adolescence. FURTHER READING Achermann JC, et al. (2015). Disorders of sex development: effects of molecular diagnostics. Nat Rev Endo crinol, 11, 478–88. Ahmed SF, et al. (2013). Understanding the genetic aetiology in pa- tients with XY DSD. Br Med Bull, 106, 67–89. Ahmed SF, Bryce J, Hiort O (2014).

International networks for sup- porting research and clinical care in the field of disorders of sex development. In: Hiort O, Ahmed SF (eds) Understanding differences and disorders of sex development, 27, pp. 284–92. Karger, Basel. Ahmed SF, et al. (2004). Prevalence of hypospadias and other genital anomalies among singleton births, 1988–1997, in Scotland. Arch Dis Child, 89, F149–51. Ahmed SF, Gardner M, Sandberg DE (2013). Management of children with disorders of sex development: new care standards explained. Psychology & Sexuality, 5, 5–14. Ahmed SF, Iqbal A, Hughes IA (2000). The testosterone: andro- stenedione ratio in male undermasculinisation. Clin Endocrinol, 53, 697–702. Ahmed SF, Khwaja O, Hughes IA (2000). The role of a clinical score in the assessment of ambiguous genitalia. BJU Int, 85, 120–4. Ahmed SF, O'Toole S (2012). Management of boys and men with dis- orders of sex development. Curr Opin Endocrinol Diabetes Obes, 19, 190–6. Ahmed SF, Rodie M (2010). Investigation and initial management of ambiguous genitalia. Best Pract Res Clin Endocrinol Metab, 24, 197–218. Ahmed SF, et al. (2000). Phenotypic features, androgen receptor binding, and mutational analysis in 278 clinical cases reported as androgen insensitivity syndrome. J Clin Endocrinol Metab, 85, 658–65. Ahmed SF, et al. (2011). UK guidance on the initial evaluation of an in- fant or an adolescent with a suspected disorder of sex development. Clin Endocrinol, 75, 12–26. Ahmed SF, et al. (2015). Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015). Clin Endocrinol (Oxf), 84, 771–88. Barseghyan H, Délot E, Vilain E (2015). New genomic technologies: an aid for diagnosis of disorders of sex development. Horm Metab Res, 47, 312–20. Bashamboo A, McElreavey K (2015). Human sex-determination and disorders of sex-development (DSD). Semin Cell Dev Biol, 45, 77–83. Baskin LS, Ebbers MB (2006). Hypospadias: anatomy, etiology, and technique. J Pediatr Surg, 41, 463–72. Blackless M, et al. (2000). How sexually dimorphic are we? Review and synthesis. Am J Hum Biol, 12, 151–66. Brain CE, et al. (2010). Holistic management of DSD. Best Pract Res Clin Endocrinol Metab, 24, 335–54.

 Cools M, et al. (2018). Caring for individuals with a difference of sex development (DSD): a consensus statement. Nat Rev Endocrinol, 14, 415–29. Cox K, et al. (2014). Novel associations in disorders of sex develop- ment: findings from the I-DSD Registry. J Clin Endocrinol Metab, 99, e348–55. di Clemente N, Belville C (2006). Anti-Müllerian hormone receptor defect. Best Pract Res Clin Endocrinol Metab, 20, 599–610. Duguid A, et al. (2007). The psychological impact of genital anomalies on the parents of affected children. Acta Paediatr, 96, 348–52. Hughes IA (2010). The quiet revolution. Best Pract Res Clin Endocrinol Metab, 24, 159–62. Hughes IA, Acerini C (2008). Factors controlling testis descent. Eur J Endocrinol, 159 Suppl 1, S75–82. Hughes IA, et al. (2006). Consensus statement on management of intersex disorders. Arch Dis Child, 91, 554–63. Josso N, Rey RA, Picard J-Y (2013). Anti-müllerian hormone: a valu- able addition to the toolbox of the pediatric endocrinologist. Int J Endocrinol, 2013, Article ID 674105. Knarston I, Ayers K, Sinclair A (2016). Molecular mechanisms associ- ated with 46,XX disorders of sex development. Clin Sci, 130, 421–32. Kolesinska Z, et al. (2014). Changes over time in sex assignment for disorders of sex development. Pediatrics, 134, e710–15. Lee PA, et al. (2015). Global disorders of sex development update since 2005: perceptions, approach and care. Horm Res Paediatr, 85, 158–80. Leon NY, Reyes AP, Harley VR (2019). A clinical algorithm to diagnose differences of sex development. Lancet Diabetes Endocrinol, 7, 560–74. Looijenga LH, et al. (2011). Dissecting the molecular pathways of (tes- ticular) germ cell tumour pathogenesis; from initiation to treatment- resistance. Int J Androl, 34(4 Pt 2), e234–51. Main KM, et al. (2010). Genital anomalies in boys and the environ- ment. Best Pract Res Clin Endocrinol Metab, 24, 279–89. Meyer-Bahlburg HFL, et al. (2016). Gender assignment, reassignment and outcome in disorders of sex development: update of the 2005 consensus conference. Horm Res Paediatr, 85, 112–18. Paterski

V, et al. (2010). Consequences of the Chicago consensus on disorders of sex development (DSD): current practices in Europe. Arch Dis Child, 95, 618–23. Rodie M, et al. (2011). Factors that influence the decision to perform a karyotype in suspected disorders of sex development: lessons from the Scottish Genital Anomaly Network Register. Sex Dev, 5, 103–8. Schneuer FJ, et al. (2015). Prevalence, repairs and complications of hypospadias: an Australian population-based study. Arch Dis Child, 100, 1038–43. Sim H, et al. (2008). Boys, girls and shuttling of SRY and SOX9. Trends Endocrinol Metab, 19, 213–22. Thankamony A, et al. (2009). Anogenital distance from birth to 2 years: a population study. Environ Health Perspect, 117, 1786–90. Verkauskas G, et al. (2007). The long-term follow up of 33 cases of true hermaphroditism: a 40 year experience with conservative gonadal surgery. J Urol, 177, 726–31. Vidal I, et al. (2010). Surgical options in disorders of sex development (DSD) with ambiguous genitalia. Best Pract Res Clin Endocrinol Metab, 24, 311–24. Vorona E, et al. (2007). Clinical, endocrinological, and epigenetic features of the 46,XX male syndrome, compared with 47,XXY Klinefelter patients. J Clin Endocrinol Metab, 92, 3458–65. Wang MH, Baskin LS (2008). Endocrine disruptors, genital develop- ment and hypospadias. J Androl, 29, 499–505.

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