Intersex

I.A. HUGHES

Department of Paediatrics, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

Introduction

The term intersex conventionally refers to the appearance of the external genitalia being at variance with normal development for either sex and to present a problem of sex assignment. Generally the problem is evident at birth, manifesting as ambiguous genitalia of the newborn. Puberty is another stage when an intersex disorder may arise by the masculinizing effects on the external genitalia of a child hitherto raised as a female. How to establish a cause for intersex and thereby formulate a management plan is probably one of the most challenging clinical conditions for the practising paediatric endocrinologist and appropriate surgical colleague. Management in the longer term needs the skills of counsellors trained and experienced in psychosexual issues affecting children and adolescents. Genetic issues dominate the pathophysiology of intersex and genetic counselling should be available to families needing advice about recurrence risks, carrier detection and options for prenatal diagnosis, and perhaps prenatal treatment. Clearly, the management of intersex is complex and a multidisciplinary team network of appropriate professionals is mandatory.

The approach to investigating and managing intersex requires an understanding of normal prenatal sexual development. The complexity of intersex management has been compounded by the use of confusing terminology and lack of clarity in the use of definitions. For the purpose of this review, *sex determination* refers to the formation of the gonad (testis or ovary) and *sex differentiation* refers to the formation of the genital phenotype (internal and external) consequent upon hormonal effects. The composite of these two processes defines sex development; further hormone-induced amplification of genital phenotype occurs at puberty, followed by the acquisition of reproductive potential. Sex outcome at birth is the result of a co-ordinated and sequential series of developmental events controlled by a network of temporally expressed genes and hormones. How an intersex disorder may occur can be ascertained logically when normal sex determination and differentiation is understood. When the classification and causes of intersex are considered in this review, the terminology will refer to the masculinized female, the under-masculinized male and the true hermaphrodite as three principal classes of disorders. It is recommended that the terminology 'pseudohermaphrodite' with male or female prefixes currently has no place in an enlightened era of intersex management involving closer collaboration with the patient and family. Geneticists, particularly those working on identifying sex-determining genes, prefer the terms 'complete' and 'partial' sex reversal, prefixed by the sex chromosomes (XY or XX).

Embryology of prenatal sex development

There is compelling evidence that internal and external prenatal sex development is constitutively female. The studies by Jost [1] in rabbit embryos showed female phenotypic development when the ovary or testis was excised, whereas a male phenotype could be restored with testis engrafting. The imprint of his seminal work on the understanding mammalian sex development is summarized in Fig. 1. The dual formation of internal genital ducts in both sexes is described in standard texts [2–4]; they comprise the Wolffian and Müllerian ducts. The former develop from the mesonephric tubules and differentiate into the vas deferens, epididymis and seminal vesicles under the influence of high concentrations of androgens (testosterone) acting locally. The Müllerian ducts stabilize in the absence of anti-Müllerian hormone (AMH, secreted by Sertoli cells) and form the uterus, Fallopian tubes and upper part of the vagina. While not generally regarded as a problem of intersex, the persistent Müllerian duct syndrome in otherwise normally developed males is caused by mutation of either the *AMH* or the *AMH* receptor gene [5]. External genital development is also common to both sexes initially, the genital tubercle only enlarging from 10 weeks of gestation onwards in the male as a result of androgen action. The genital swellings later develop either into the scrotum or labia majora, depending on exposure to androgens. In the developing male, the urethral folds fuse ventrally to form the urethra, with the urogenital orifice finally sited at the tip of the glans penis. Failure in this developmental pathway results in hypospadias, the commonest congenital anomaly in males.

Formation of the gonad precedes the events of internal and external genital development by several weeks. A thickening of the coelomic mesoderm on the medial aspect of the urogenital ridge at around 5–6 weeks of gestation is the first indication of a gonad which, at this stage, is
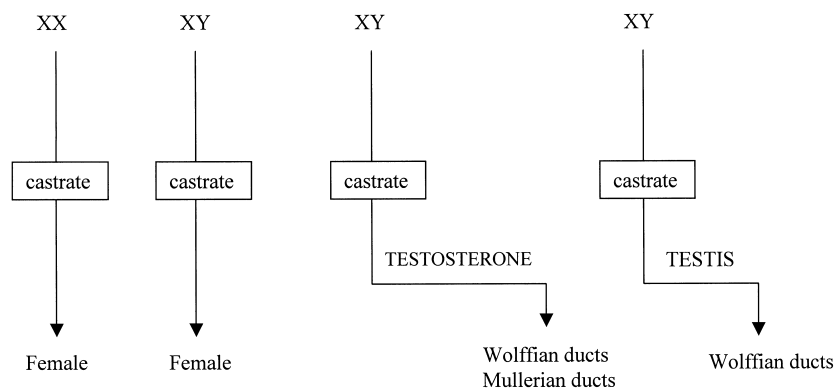
Fig. 1. Embryology of internal genital development, as based on experiments of Jost [1].
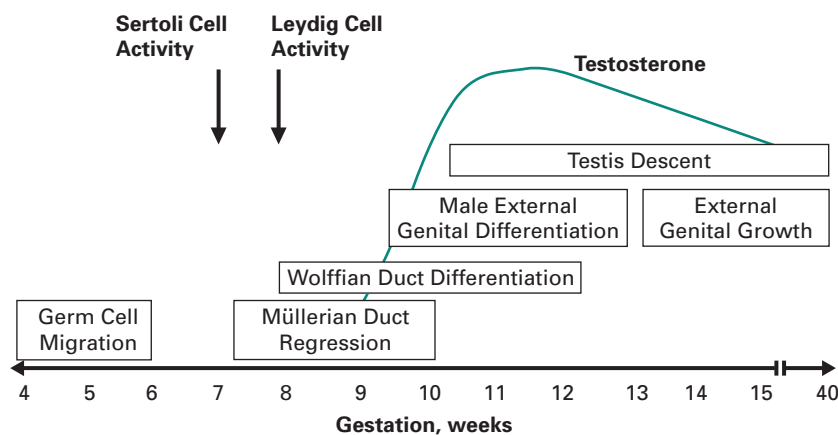

Fig. 2. Schematic of male fetal sex development. The solid line represents the rise in fetal serum testosterone concentrations; maximum levels ≈ 10 nmol/L.

histologically indifferent. The development is juxtaposed with mesonephric cells of the primitive kidney. The close interdependence between gonad and kidney development is illustrated by the absence of gonads and kidneys in mice with targeted disruption of the *wt-1* gene which prevents thickening of the mesoderm in the urogenital ridge [6]. The human Denys-Drash and Frasier syndromes resulting from mutations in *WT-1* are characterized by renal disorders and varying degrees of gonadal dysgenesis [7,8].

The first histological evidence for testis development is the appearance of seminiferous cords from the condensation of primary sex cords together with Sertoli cells. This occurs at ≈ 7 weeks' gestation; by contrast, the ovary is not defined for about another 4 weeks. Interstitial cells derived from the mesenchyme are present by 9 weeks and differentiate as the steroid-secreting Leydig cells. Primordial germ cells migrate to the site of gonad formation from the wall of the yolk sac, via the mesentery of the hindgut. The migratory pathway is dependent on chemoattractants and cell adhesion molecules, a process still to be fully explained [9,10]. While gonad determination will take place even if germ cells fail to migrate, there may be some sex-dependence to the pattern of germ cell migration.

Prenatal male sex development is completed by the migration of the testis into the scrotum; this is a two-stage process of transabdominal migration and inguinoscrotal descent [11]. The first phase of descent, at least in the mouse, is controlled by an insulin-like peptide relaxin produced by the testis; when the gene encoding this protein is disrupted, bilateral cryptorchidism occurs [12]. However, studies of the human homologue of this gene in boys with undescended testes have only rarely reported mutations [13,14]. Inguinoscrotal descent is androgen-dependent, as exemplified by abnormal gonadal positions in hypogonadotrophic hypogonadism and syndromes of androgen insensitivity [15,16]. Sex determination and sex differentiation is dependent in the male on a coordinated interplay of temporally related genetic and hormonal control events. The embryological events are schematically summarized in Fig. 2; the following section briefly describes the key genes and hormones involved in prenatal male sex development.

Genetic and hormonal control of fetal sex development

Sex development is constitutively female so that the active imposition of male development requires factors to deter-

mine the testis, regress the Müllerian ducts and differentiate the internal and external genitalia as male. There is a panoply of genes involved in testis determination, many yet to be identified. Syndromes of sex reversal and studies on mouse embryos have been critical in identifying key genes (see [17–19] for detailed reviews). The *SRY* gene (*sex-r*elated gene on the *Y* chromosome) is central to testis formation, as exemplified by the presence of testes in XX males and the male outcome after transgenic insertion of the *sry* gene into XX mouse embryos. Pairing of X and Y chromosomes at their pseudoautosomal regions occurs during paternal meiosis. As *SRY* is located close to the pseudoautosomal boundary, the gene can become translocated to the X chromosome if X-Y interchange of genetic material extends beyond the pseudoautosomal boundary. *SRY* encodes a protein which contains a central region of 80 amino acids and is homologous with a large family of high mobility group (HMG) nuclear proteins. The SRY protein functions as a transcription factor by bending DNA, thereby promoting protein-protein interactions. Mutations in *SRY* are associated with gonadal dysgenesis and complete XY sex reversal (Swyer's syndrome). However, only 15–20% of such patients have an *SRY* mutation, thereby indicating that other genes must be involved in testis determination. One such gene is *SOX9*, which also encodes a protein containing an HMG-related motif of amino acids, and is a transcription factor. Mutations in *SOX9* lead to a syndrome of campomelic dysplasia (severe thoracic and limb skeletal defects) with gonadal and genital abnormalities in most. *SOX9* is probably activated by *SRY* as both genes are expressed in fetal Sertoli cells in a temporally related fashion. However, other genes must be regulated by these two key transcription factors and remain to be identified. Notably, *SOX9* up-regulates *AMH* gene expression.

Have any ovarian-determining genes been identified? So far, no genes whose products direct the development of the ovary have been identified. However, genes which appear to operate in an 'anti-testis' fashion are described. Duplication of the short arm of the X chromosome can lead to complete XY sex reversal. A gene identified in this region, *DAX1*, is a member of the nuclear hormone receptor family. It is postulated that over-expression of *DAX1* either inhibits *SRY* directly or inhibits its action as an up-regulator of *SOX9*. Another gene, *WNT4*, located on chromosome 1p34 appears to be an anti-testis gene based on sex reversal in an XY female with duplication of 1p31-p35. Both *DAX1* and *WNT4* are expressed in the fetal testis and ovary initially, but persist only in the ovary. A simplified summary of the key factors involved in gonad determination is shown in Fig. 3.

Fetal development of the female phenotype does not require oestrogens. In contrast, male sex differentiation requires the production of high concentrations of andro-
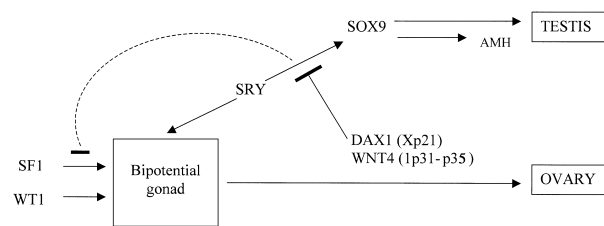

Fig. 3. Main genetic factors controlling testis or ovary determination from a bipotential gonad.

gens during a critical period (Fig. 2). Furthermore, the principal androgens, testosterone and DHT, mediate their developmental effects through binding to a specific androgen receptor (AR) in target tissues. Androgens are synthesized by the Leydig cells, initially autonomously, but then dependent on placental hCG secretion. Later in gestation with declining hCG concentrations, androgen synthesis is controlled by LH secretion from the fetal pituitary gland. Growth of the phallus occurs during this later stage of gestation, hence the occurrence of micropenis typically observed in a male infant with congenital hypopituitarism. Optimal androgen production and action sufficient to differentiate the internal and external genitalia is dependent on an intact membrane bound LH/hCG receptor on Leydig cells, a sequence of enzyme steps to synthesise testosterone from cholesterol, the conversion of testosterone to its more potent metabolite DHT, and finally androgen-induced (testosterone and DHT) activation of the AR transcription factor. Logic dictates that a defect in any one of these components would result in an XY intersex phenotype.
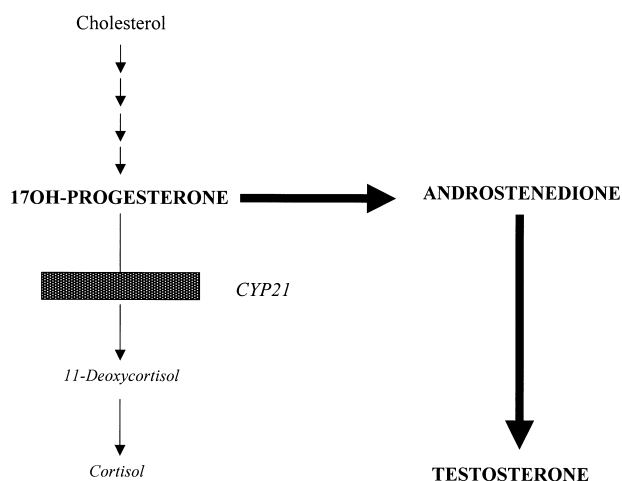
Intersex disorders – a pragmatic classification

Table 1 shows a simple classification of intersex which is both descriptive and includes broad aetiological categories. Congenital adrenal hyperplasia (CAH) and the other rare causes of a masculinized female are not strictly disorders of sex determination and sex differentiation. However, CAH is the commonest cause of ambiguous genitalia of the newborn and hence must be included in this review.

CAH

More than 90% of cases of CAH are caused by 21-hydroxylase deficiency [20,21]. The degree of virilization of the external genitalia can be so marked as to present as a newborn male with bilateral impalpable testes. Hence it is mandatory that infants born with such findings are investigated early. Generally in CAH, there is clitoromegaly, rugose and pigmented labioscrotal folds and a single perineal opening representing a urogenital sinus.

Table 1 Simple classification of intersex

Type/cause	Result
Masculinized female	
Fetal androgens	CAH, placental aromatase deficiency
Maternal androgens	adrenal tumours, ovarian tumours
Under-masculinized male	
Abnormal testis determination	gonadal dysgenesis, XO/XY mosaicism
Defects in androgen biosynthesis and metabolism	17β-OH -dehydrogenase deficiency, 5α-reductase deficiency
Resistance to androgens	partial AIS
True hermaphroditism	
Presence of both testicular and ovarian (with follicles) tissue	XX, XY, XX/XY

Cholesterol

↓
↓
↓
↓

17OH-PROGESTERONE ➡ **ANDROSTENEDIONE**

CYP21

↓

11-Deoxycortisol

↓

Cortisol **TESTOSTERONE**

Fig. 4. Biochemical consequences of 21-hydroxylase deficiency. The hatched bar denotes the blockage in steroid synthesis.

Establishing a diagnosis of 21-hydroxylase deficiency is relatively straightforward, as plasma 17OH-progesterone concentrations, the steroid proximal to the enzyme block, are markedly elevated (Fig. 4). There is placental production of the steroid, so the blood sample collection should be delayed 24–48 h after birth. Most affected infants are salt-losers but salt-wasting does not usually manifest until the second or third week of life. The external genitalia in affected males are generally normal at birth, thereby exposing them to a life-threatening adrenal crisis. It is for this reason that many countries now screen newborns for CAH using filter-paper blood-spot 17OH-progesterone measurements [22]. No screening is currently undertaken in the UK.

The fetal adrenal produces steroids as early as 8 weeks of gestation; signs of clitoromegaly and labial fusion are well established by the second trimester in a female with CAH. Prenatal treatment to prevent virilization is now possible with the use of dexamethasone administered to the mother [23,24]. Treatment needs to start as soon as an at-risk pregnancy is confirmed; prenatal tests are undertaken on a sample of chorionic villi. These include the karyotype and mutational analysis of the 21-hydroxylase gene, *CYP21*. Only mothers carrying an affected female need continue dexamethasone treatment. As CAH is an autosomal recessive condition and only affected females require treatment, seven of eight fetuses are unavoidably exposed to dexamethasone for several weeks until there is a definitive decision about the need for continued treatment. There is understandable concern about the longer term effects of such exposure to dexamethasone treatment; shorter term outcome studies are reassuring [24,25]. Virilization of the external genitalia is completely prevented in many instances and the remainder require no surgery or minimal intervention. Maternal weight gain can be excessive, but side-effects of glucocorticoid therapy, e.g. hypertension and glucose intolerance, are not evident. Growth and development of infants exposed to dexamethasone, either temporarily or throughout pregnancy, has been normal over the current follow-up of 10–12 years. Nevertheless, it is recommended that this treatment programme, unique in preventing a major congenital malformation, should only be undertaken in centres which participate in national and international clinical studies using uniform diagnostic and treatment protocols, and long-term outcome monitoring with appropriate controls.

The question of when surgery should be used in an infant with CAH and ambiguous genitalia, and which procedure to be adopted, is the subject of ongoing and unresolved debates [26–29]. There is no unanimity on whether surgery should be early (2–6 months) or later (1–2 years). There appears to be even more debate about whether girls needing clitoroplasty and vaginoplasty should have this performed as a one-stage procedure in infancy, or as a two-stage procedure with the vaginoplasty delayed until late childhood or early adolescence. There is agreement on the need to preserve the glans clitoris and the neurovascular bundle as far as is possible. There is also a move towards not operating, at least on the clitoris, in infants with lesser degrees of clitoromegaly.

The medical management of CAH in infancy is relatively simple; glucocorticoid replacement with hydrocortisone, 12–15 mg/m²/day in divided doses, and mineralocorticoid replacement with 9α-fludrocortisone 50–150 μg/day, together with salt supplements to the oral feeds initially. There is a danger of over-treatment with glucocorticoids during infancy, a period characterized by rapid growth. This may underlie some of the problems

evident in later life, e.g. obesity, menstrual irregularities, reduced adult stature and infertility.

Transplacental exposure to androgens

Rarely, a female fetus may be virilized as a result of a maternal androgen-secreting tumour involving the adrenals or ovaries. The types of ovarian tumours include luteoma, hyperreactio luteinalis (both benign) and primary malignancy. Worsening of polycystic ovarian disease during pregnancy may sometimes cause virilization.

An even rarer cause of transplacental transport of excess androgens to the fetus is aromatase deficiency [30]. The cytochrome P450 aromatase enzyme catalyses the conversion of androgens (C19 steroids) to oestrogens (C18 steroids). The enzyme is widely expressed, including the ovary and testis, brain, adipose tissue and the placental syncytiotrophoblast. The fetal adrenal gland produces large quantities of weak androgens (dehydroepiandrosterone and hydroxylated products) which form substrates for the production of more potent androgens, such as testosterone. Normally, these androgens are converted to oestrogens by placental aromatase and pass to the maternal circulation. Measurement of plasma and urinary maternal estriol levels was formerly used as a test of placental function. Mutations in the CYP19 aromatase gene can result in profound virilization of a female fetus and her mother during pregnancy. Maternal virilization resolves postnatally, but will recur in subsequent pregnancies. This rare condition should be considered in the investigation of a virilized female newborn, in whom CAH and maternal androgen-secreting tumours have been excluded.

Intersex with an XY karyotype

An infant with ambiguous genitalia in whom the karyotype is 46,XY is a problem for both diagnosis and management. Typically, there is micropenis with chordee, perineo-scrotal hypospadias and a bifid scrotum. Gonads may or may not be palpable. It has been reported that the presence of impalpable gonads and severe hypospadias makes an intersex state more likely, and warrants a karyotype test [31,32]. Intersex is not a diagnosis; even when XY infants with the signs previously ascribed to intersex are investigated in detail, an underlying specific cause is established only in a few. In general, failure of masculinization of the external genitalia can result from either inadequate production of, or response to, androgens.

Defects in androgen biosynthesis

Testosterone is synthesized in Leydig cells under the trophic control of hCG in early gestation and later, fetal pituitary LH secretion. Both peptides bind to a seven-transmembrane LH receptor to activate intracellular steroidogenesis. The starting substrate is cholesterol which is converted via a series of enzymatic steps to testosterone. This androgen is also further metabolized to the more potent androgen DHT, via the 5α-reductase type 2 enzyme. Key steps necessary for optimal androgen production are shown in Fig. 5, together with the genes which, when mutated, cause varying degrees of XY sex reversal.

Inactivating mutations of the LH receptor can give rise to a range of phenotypes which includes complete sex reversal, ambiguous genitalia or isolated hypospadias [33]. How a single mutation type can cause such a range of sex reversal is not currently known. Histology of the gonads shows Leydig cell hypoplasia; serum LH levels are elevated and there is no testosterone response to stimulation with hCG.

An intriguing phenomenon of 'double sex reversal' is observed in the androgen biosynthetic disorders, 17β-hydroxysteroid dehydrogenase deficiency and 5α-
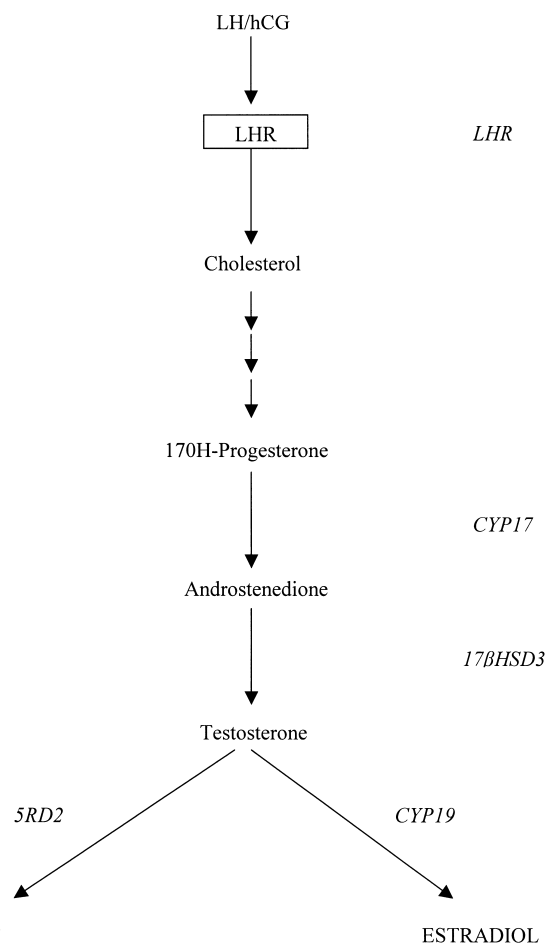
Fig. 5. Key regulatory steps in the synthesis and metabolism of androgens. The relevant genes are shown in italics.

reductase deficiency [34,35]. In both disorders, the external genitalia at birth are either female or partially masculinized, yet there is marked virilization at puberty with intact testes. It is not clear why masculinization does not occur prenatally in response to hCG/LH stimulation. However, each of these two enzymes operate as isoenzymes and mutations in only one isoenzyme cause androgen biosynthetic disorders (type 3 and type 2 in the case of 17β-hydroxysteroid dehydrogenase and 5α-reductase, respectively. It is postulated that substrate usage by alternative isoenzymes results in sufficient androgen production at puberty to cause virilization. These are considerations which need to be borne in mind in the context of the sex of rearing if a diagnosis is established soon after birth. Figure 5 illustrates that measuring plasma concentrations of androstenedione, testosterone and DHT before and after hCG stimulation is essential to the biochemical diagnosis of these enzyme deficiencies. Chromatographic analysis of androgen metabolites in urine is also helpful, particularly for 5α-reductase deficiency. Once the clinical and biochemical parameters for diagnosis have been established, mutation analysis of the *17βHSD3* or the *5αRD2* genes is available in certain specialized centres.

Defects in androgen action

The androgen insensitivity syndrome (AIS) is a paradigm of resistance to the action of a hormone, in this case androgens. The complete form (complete AIS) is manifest as complete XY sex reversal and is not a problem of intersex. However, the partial form of the syndrome (partial AIS) is the commonest cause of fetal male undermasculinization [19]. Partial AIS is defined as a defect in androgen action of variable degree in an XY male with normal testis determination, testosterone biosynthesis and metabolism of DHT. The phenotype can range widely to include ambiguous genitalia, simple hypospadias and even normal genital development in a male with infertility.

Androgen action in target tissues, including the genitalia, is mediated by ligand activation of the nuclear AR. This transcription factor up-regulates androgen-responsive genes to elicit the biological actions typical of androgens, i.e. muscle and skeletal development, linear growth, genital development and voice changes at puberty, stimulation of skin integuments, erythopoiesis and in adults, spermatogenesis. These pleiotropic effects are assumed to occur as a result of androgen binding to a single AR. The AR gene is located on Xq11–12; complete AIS is invariably the result of mutations in this gene [36]. Only a minority of patients who fulfil the criteria to define partial AIS have identifiable mutations in the gene [37]. As the AR is a transcription factor it is possible that the same

phenotype can arise from abnormalities in androgen-responsive genes. These genes remain to be identified.

Once a mutation has been identified it is possible to recreate the abnormal receptor by site-directed mutagenesis to study function *in vitro*. While such work is of considerable value to analyse structure-function relationships, the correlation between genotype and phenotype in partial AIS is extremely variable. Nevertheless, the presence of an AR gene mutation does not necessarily imply no response to high doses of androgens given *in vivo* to a patient with partial AIS.

Intersex: chromosome abnormalities and gonadal dysgenesis

Ambiguous genitalia of the newborn may occur as a result of chromosome abnormalities or from a partial form of XY gonadal dysgenesis. The commonest chromosome abnormality is 45XO/46XY mosaicism associated with mixed gonadal dysgenesis. The phenotype may include signs consistent with features of Turner syndrome (particularly short stature) as well as the abnormal genital development. Sometimes there may be normal male development; this is consistent with the observation that most instances of XO/XY karyotypes established prenatally are found in normal phenotypic males [38]. The paediatric endocrinologist and surgeon therefore are consulted on a skewed population of XO/XY patients. One gonad, at least, can produce adequate testosterone levels after hCG stimulation. The concomitant streak gonad should be removed because of the risk of malignancy. A 46XX/46XY karyotype can be associated with a variable phenotype, depending on the nature of the gonads. True hermaphroditism is a histological diagnosis based on the presence of ovarian (including follicles) and testicular tissue in the individual. Internal and external genital development can be variable. The most frequent karyotype is 46,XX.

Syndromes and intersex

The number of syndromes which include a genital anomaly, usually hypospadias, is legion. The genetic abnormality has been identified in some, e.g. Smith-Lemli-Opitz, Robinow and Rubinstein–Taybi syndromes. Rarely are mutations identified in these genes when analysed in patients with isolated genital anomalies.

Hypospadias is not an intersex disorder but familial forms of hypospadias may occur from mutations in known genes such as the AR gene. There are families with non-X-linked forms of hypospadias, including affected sibling pairs and father-son pairs. Further studies are needed to identify possible autosomal gene loci for this common congenital anomaly.

Intersex and the environment

In recent years it has become necessary to consider the role of environmental factors in the causes of male reproductive tract disorders [39]. There is evidence from examining wildlife and epidemiological studies in man that chemicals in the environment may act as endocrine disrupters during critical stages of genital development. A testicular dysgenesis syndrome has been hypothesized to occur in males to account for the fall in sperm count and quality, coupled with an increase in testis cancer, hypospadias and undescended testes. The causal agent(s) inducing dysgenesis of the testis have oestrogen-like or anti-androgenic properties; there is abundant experimental evidence *in vitro* and in animals to show that numerous compounds to which the population is exposed have these properties. The effects are not so severe as to elicit a state of intersex; nevertheless there was a clear association between the occurrence of hypospadias in a cohort of 7000 male births in the South-west of England and the frequency of mothers who were vegetarian during their pregnancy [40].

Intersex – the initial investigation

There are numerous investigations which may need to be undertaken, subsequent to an initial screen for the infant with ambiguous genitalia. There is no substitute for an initial adequate history and physical examination. Family history and information about exposure to potential teratogenic compounds are particularly relevant. The examination must also consider the relevance of any extra-genital anomalies.

The initial investigations which should be undertaken are:

- Chromosomes: fluorescent *in situ* hybridisation on interphase spreads with X,Y specific probes; full karyotype.
- Hormones: serum 17OH-progesterone at 24–48 h; serum testosterone; plasma renin; save serum, DNA, urine.
- Biochemistry: serum electrolytes, plasma glucose.
- Imaging: pelvic ultrasonography for uterus/cervix; renal ultrasonography.

It is mandatory to undertake a karyotype in an apparently normal male with bilateral impalpable testes; it is also the author's view that the karyotype should be assessed in a male with hypospadias and impalpable testes. Further investigations may be required, consequent on the karyotype result and 17OH-progesterone level:

- Chromosomes: high-resolution karyotype (mosaicism, e.g. XO/XY).

- Fluorescent *in situ* hybridisation studies (mosaicism).
- Possible karyotype in fibroblasts.
- Hormones: serum gonadotrophins, serum AMH; hCG stimulation test (1500 U daily for 3 days); androstenedione, testosterone, DHT; urinary steroid profile.
- Imaging: pelvic ultrasonography; MRI (locate gonads); urogenital sinogram (also urethroscopy).
- Genital: skin biopsy (at the time of surgery); androgen binding studies; DNA/mRNA for mutation analysis; gonadal biopsy (at the time of surgery).

The most difficult is the XY intersex infant; the hCG stimulation test is useful as a marker of the presence of testes and whether they produce androgens normally. The information may also predict what is likely to occur at puberty. Serum AMH measurement has been proposed as a reliable marker of the presence of testes [41,42], but the test is not yet universally available.

Conclusion

Intersex or ambiguous genitalia of the newborn is a rare disorder which needs prompt investigation, logically based on a sound knowledge of normal sex determination and differentiation. The complexity of the problem requires a multidisciplinary team working in specialist centres. Longer-term management was not the focus of this review. Nevertheless, the personnel involved in decisions on the sex of rearing must be aware of changes in practice which continue to develop, particularly in the timing and nature of any surgical intervention [43].

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Correspondence: I.A. Hughes, Department of Paediatrics, University of Cambridge, Addenbrooke's Hospital, Box116, Hills Road, Cambridge CB2 2QQ
e-mail: iah1000@cam.ac.uk

Abbreviations: AMH, anti-Müllerian hormone; **HMG**, high mobility group; **AR**, androgen receptor; **CAH**, congenital adrenal hyperplasia; **AIS**, androgen insensitivity syndrome.