Intersex

Robert H. WHITAKER

The management of children with intersex anomalies is both medically and surgically complex and should be left to those who specialise in these problems. To understand developmental abnormalities that lead to intersex problems, an appreciation of normal development is essential. Figure 1 illustrates the main steps that provide normal gonads, internal and external sexual appearances and function.

Genetic sex is determined at conception as 46XX or 46XY and a primitive bipotential gonad appears on the genital ridge. Beyond this time development of the male fetus is active and rapid whilst female determination is slower and passive.

Male development

Testis Determining Factor (TDF), from the SRY gene on the short arm of the Y chromosome, first influences the developing gonad by 6-7 weeks to differentiate. Sertoli cells first appear and, together with germ cells that migrate from the yolk sac, become enclosed as testicular cords. Leydig cells soon differentiate outside the cords and under the influence of tropic factors, produce testosterone which, together with Müllerian Inhibiting Substance (MIS) from the Sertoli cells, control development of external and internal genitalia.

MIS, the production of which is under the control of a gene on the short arm of chromosome 19 [12], diffuses to cause local involution of all the ipsilateral Müllerian (paramesonephric) duct except for two small parts that remain as the appendix testis and prostatic utricle.

Testosterone diffuses into the androgen sensitive cells of the developing genitalia in which it may be converted by 5α reductase to dihydrotestosterone (DHT), a potent metabolite. Both testosterone and DHT combine with intracellular androgen receptor. These complexes enter the nucleus and bind to chromosomal DNA target sites to influence gene expression. Human chorionic gonadotropin (hCG) and fetal luteinising hormone (LH) control the production of testosterone which in turn influences the maturation and growth of the penis and scrotum, and the descent of the testes. Testosterone itself, by direct action, causes differentiation of the Wolffian ducts to vas, seminal vesicle and epididymis.

Female development

Without TDF, the 46XX zygote develops an ovary and, under the influence of fetal pituitary follicular stimulating hormone (FSH), follicles develop within it. Without testosterone the Wolffian ducts involute and in the absence of MIS the Müllerian ducts, urogenital sinus and primitive genitalia differentiate passively. The cloaca splits posteriorly into the rectum and anteriorly into urogenital sinus (UGS) which in turn becomes part of the bladder, urethra and lower two thirds of the vagina.

Abnormal sexual development

There are 3 categories of abnormal development:

- Chromosomal anomalies (often leading to gonadal dysgenesis).
- Virilisation of female
- Inadequate virilisation of male.

Some of these are not associated with ambiguous genitalia, instead patients may present at puberty or later with infertility, gynecomastia or lumps in the groins.

Chromosomal anomalies and gonadal dysgenesis

During meiosis or mitosis chromosomes may suffer non-disjunction, deletion, translocation, rearrangement or breakage. These are summarised as missing, additions and apparently normal:

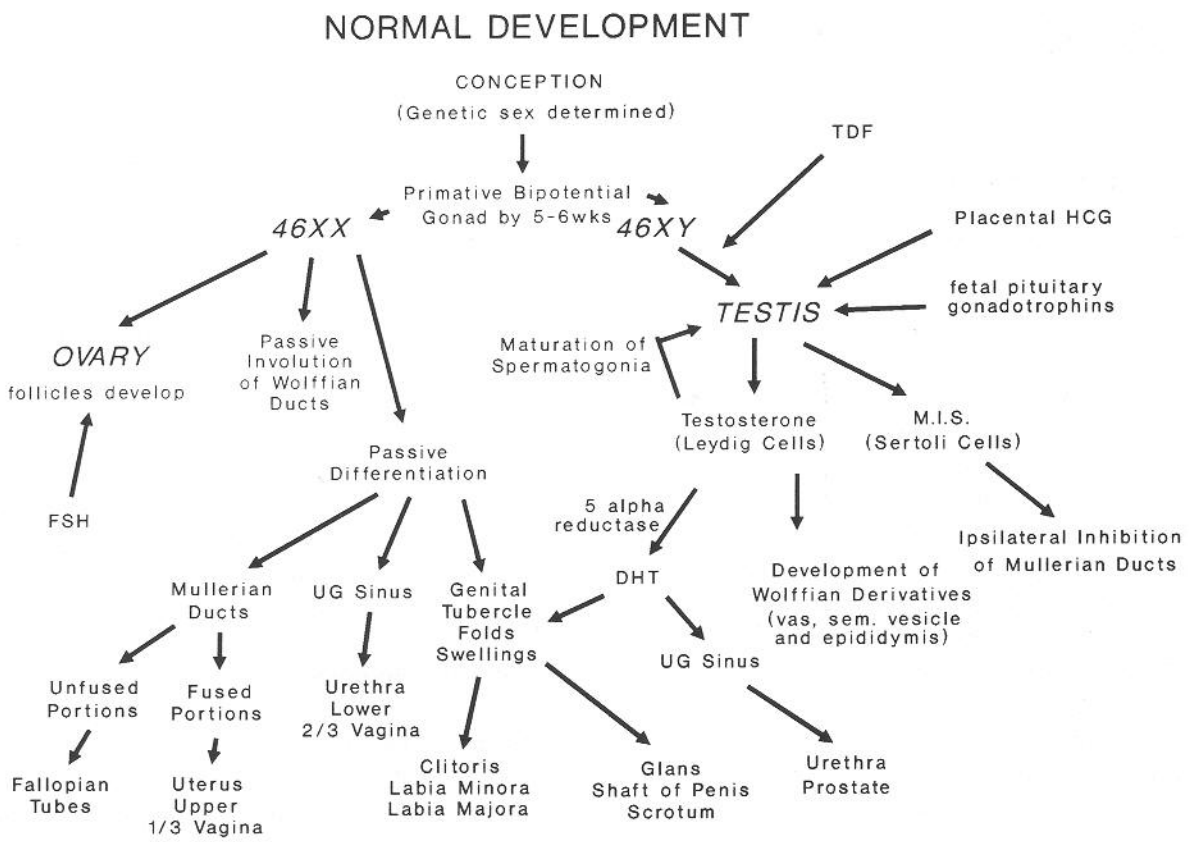

Figure 1. Map of normal development.

Missing (or mosaic)

1. Turner's syndrome (45X0 or variant)

Without a Y chromosome these patients are female with unambiguous genitalia and streak gonads. Clinical features include webbing of the neck, increased carrying angle, short stature, sexual infantilism and primary amenorrhoea.

2. Mixed gonadal dysgenesis (mostly mosaic 45X0/46XY)

These patients with ambiguous genitalia are usually more female than male. A testis is often palpable in the groin or scrotum on one side but contralaterally the gonad is a streak with ipsilateral persistence of Müllerian structures (fallopian tube and abnormal uterus) due to lack of MIS. There is a short hypospadiac phallus and infertility as the testis has only Sertoli and Leydig cells. Virilisation and gynecomastia develop at puberty.

Male rearing is possible when the phallus is adequate or responds well to testosterone stimulation but most patients are reared as female. Gonads have an increased risk of malignancy [11].

Female rearing requires genitoplasty whilst males require removal of dysgenetic tissue and a hypospadias repair.

3. True hermaphrodite (46XX/46XY)

Approximately a third of true hermaphrodites have this karyotype [7] (see below).

Additions

1. Klinefelter's syndrome (47XXY-49XXXXY or mosaic 46XX/47XXY)

The Y chromosome leads to a male patient with no ambiguity of the genitalia. Testes are small and firm with tubular dysgenesis. Patients are tall, infertilite and often develop gynecomastia.

Apparently normal

1. 46XX male

These rare, phenotypically male patients have short stature, hypospadias and usually undescended testes which are firm, small and lead to infertility. Wolffian structures are normal but Müllerian structures are suppressed suggesting normal early production of MIS and testosterone. Gynecomastia and hyalinisation of the testes occurs at puberty. DNA from the short arm of the Y chromosome, containing the recently recognised SRY gene, is transposed to the distal end of the short arm of the X chromosome [12].

2. True hermaphroditism (46XX or 46XY)

True hermaphrodites have both ovarian and testicular tissue either separately or as an ovotestis. 13 % are reported to have a 46XY karyotype, 57-80 % have 46XX and the rest have a mosaic karyotype. They have ambiguous external genitalia and variable, usually asymmetrical internal organs as influenced by the ipsilateral gonad. A hypoplastic or unicornuate uterus is common.

Sex of rearing is based mainly on the adequacy of the phallus. Choice of surgery lies between genitoplasty and hypospadias repair. The presence of a Y chromosome dictates removal of dysgenetic tissue to avoid malignancy [11]. Rearing as female is logical as a 46XX karyotype is compatible with menses and the occasional pregnancy.

3. Pure gonadal dysgenesis (46XX or 46XY)

These patients with bilateral streak gonads and either a 46XX or 46XY karyotype have normal female appearance and do not present in the neonatal period. They have hypoplastic Müllerian internal organs but no Wolffian structures. Patients with a 46XX karyotype are tall with sexual infantilism and amenorrhea but only those with 46XY, have clitorial hypertrophy and gonadal cancer risk.

Virilisation of female

Female pseudohermaphroditism - 46XX

In Congenital Adrenal Hyperplasia (CAH), the commonest form of female virilisation, there is a block in steroid synthesis resulting in an accumulation of metabolic precursors which act as substrates for increased androgen production. Excess androgen may rarely be due to maternal exogenous intake or androgen secreting tumours that reach the fetus transplacentally.

Congenital adrenal hyperplasia

CAH is autosomally recessive, occurs in 1:13,000 births and accounts for approximately 70 % of all children with ambiguous genitalia. Females present with ambiguous genitalia and salt loss in 75 %; in boys it usually presents in the neonatal period with salt loss.

The adrenal gland normally produces glucocorticoids, mineralocorticoids and sex hormones by a series of enzymatic steps from cholesterol. Deficiency of any of these enzymes leads to a lack of cortisol production. Lack of normal feedback leads to excess ACTH production and thus hypertrophy of the adrenal glands.

21 hydroxylase deficiency accounts for more than 95 % of cases of CAH. This enzyme is encoded by 2 genes located on the short arm of chromosome 6, one of which has become inactive due to a series of deleterious mutations [17]. The excess production of the steroid precursor, 17 hydroxy-progesterone, is diagnostic (Fig. 2). This precursor is metabolised to androstenedione, a weak androgen, and then to testosterone.

Three-quarters of patients also have a block in the production of aldosterone resulting in increased urinary sodium excretion and potassium retention.

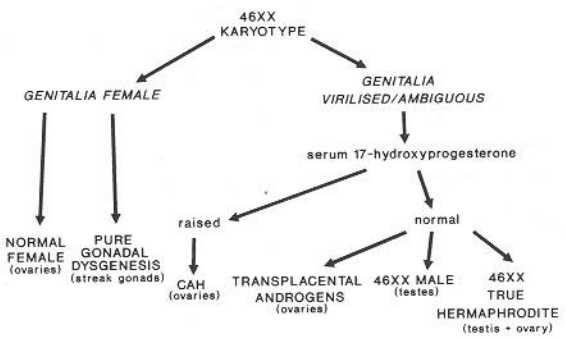

Figure 2. Diagnostic pathway for 46XX karyotype based on appearances of genitalia and biochemistry.

Clinical features

The spectrum of virilisation ranges from mild clitoromegaly to an almost male appearance with hypospadias and an empty scrotum. The internal genitalia are normal female. Hyperpigmentation may result from excess ACTH production. Affected children commonly present early in life from the effects of salt loss with vomiting, shock, hyponatremia and hyperkalemia.

In **11 beta hydroxylase deficiency**, a rarer form of CAH, the enzyme block is further down the pathway and this results in not only a raised 17 hydroxyprogesterone, but also elevation of 11 deoxycortisol and 11 deoxycorticosterone. The mineralocorticoid activity of the latter leads to develop hypertension and hypokalemia in addition to the virilisation.

Treatment of CAH

Urgent replacement of the missing cortisol and aldosterone with hydrocortisone and fludrocortisone respectively is needed. Beyond that, treatment should provide minimal dosages to inhibit excess ACTH and renin/angiotensin stimulation so that normal growth and development is attained.

The results of clitoral reduction and vaginoplasty in these girls are excellent. Adequate medical and surgical management can lead to normal sexual function and fertility.

Inadequate virilisation in males

Male Pseudohermaphroditism - 46XY

Most anomalies are due to either inadequate production of testosterone and/or DHT or failure of these hormones to act peripherally. An additional rare cause is failure of the Sertoli cells to produce MIS, leading to ipsilateral persistence of Müllerian duct derivates (fallopian tube and uterus) in a 46XY fetus (hernia uteri inguinale or Persistent Müllerian Duct Syndrome) [4]. These children have a normal phallus but suffer poor sperm and hormone production from testes that have a gonadal cancer risk.

Normal virilisation requires adequate production of testosterone from the testis, androgen sensitive cells which are able to convert it to DHT under the influence of 5α reductase and an intracellular ability in the end organ to bind both testosterone and DHT to androgen receptor. The **testosterone-receptor complex** regulates gonadotropin production from the hypothalamus/pituitary axis, stimulates spermatogenesis and continues to have a tropic effect on the derivatives of the Wolffian ducts. The **DHT-receptor complex** virilises the external genitalia and urogenital sinus and helps with maturation at male puberty. Defects of any of these 3 activities can produce intersex with undervirilisation.

1. Decreased production of testosterone

Defects in testosterone biosynthesis are due to deficiencies of enzymes in the androgen production pathway, such as 17-β hydroxysteroid dehydrogenase and 3-β steroid dehydrogenase. MIS is produced normally hence no Müllerian structures remain. Such affected patients have ambiguous genitalia, normal male internal organs, severe hypospadias and variable virilisation with breast development at puberty. Measurement of the plasma and urinary androgen precursors as well as testosterone levels before and after hCG stimulation leads to the diagnosis (Fig. 3) [6]. Sex of rearing and appropriate surgery are discussed below.

2. Abnormal testosterone metabolism

5α reductase deficiency, an autosomal recessive condition, leads to a failure of conversion of testosterone to DHT. Without DHT virilisation is minimal but at puberty an increase in androgen production causes marked virilisation. In the Dominican Republic where the condition was prevalent it was accepted that affected children were raised as girls and that at puberty virilisation led to the adoption of the male role. Diagnostic tests include measurement of the testosterone/DHT ratio in plasma and the ratio of 5α to 5β androgen metabolites in urine after stimulation with hCG [10] (Fig. 3). Sex of rearing determines the type of surgery that is needed.

3. Defective end organ response (Androgen insensitivity)

In two thirds of patients with the androgen insensitivity syndrome there is an X-linked pattern of familial inheritance. The gene for the androgen receptor is located on the long arm of the X chro-

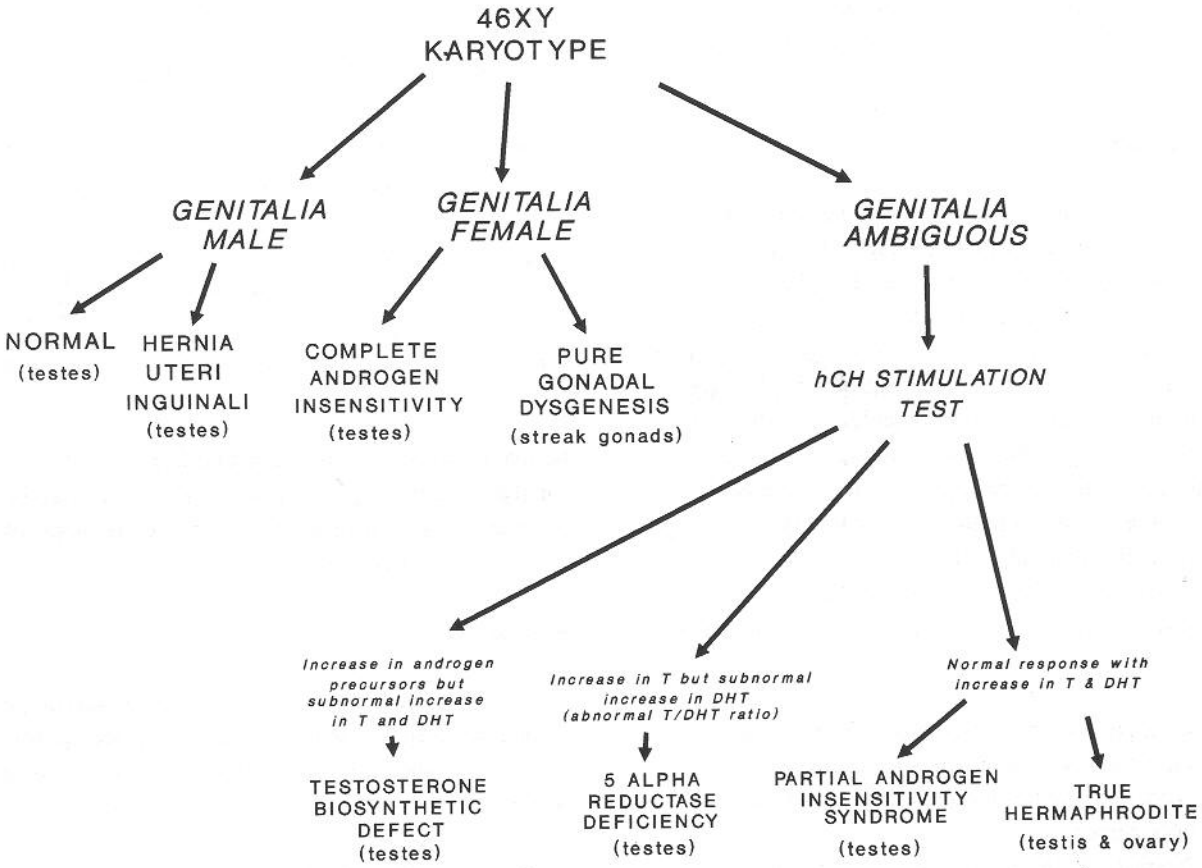

Figure 3. Diagnostic pathway for 46XY karyotype based on appearances of genitalia and result of hCG stimulation test.

mosome, Xq11-12 [2], and mutations within this gene have now been identified in patients with androgen insensitivity [1].

In **Complete Androgen Insensitivity Syndrome** (CAIS) tissues are totally resistant to androgens and thus there are normal female external genitalia (Fig. 3). Presentation is in infancy or childhood with labial swellings or herniae which contain testes. Alternatively, presentation is primary amenorrhea in adolescence. Ultrasound or laparoscopy fail to show female internal genitalia and testicular biopsy shows Leydig cells and no spermatogenesis. Plasma testosterone levels are either normal for age or may be higher because of increased stimulation by LH.

Patients with CAIS are reared as female. Breast development occurs normally and no genital reconstruction is needed. The gonads must be removed because of the malignancy risk. This is now performed early as it is no longer believed that the testes should be left until puberty.

Partial Androgen Insensitivity Syndrome (PAIS) leads to variable genital ambiguity with a small phallus, degrees of labioscrotal fusion, undescended testes and gynecomastia at puberty. PAIS is diagnosed by showing normal testosterone and testosterone precursors, and a normal DHT response to hCG stimulation, contrasting with testosterone biosynthetic defects (Fig. 3). No female internal genitalia are seen on ultrasound, but vaginal remnants may be present. Histologically, testis shows immaturity with prominent Leydig cells.

PAIS can be a diagnostic challenge and difficult to manage despite early assessment of the external genitalia. Prediction of the degree of virilisation that may occur at puberty if the child is reared as male, is unreliable although a trial of androgen treatment with, for example, 2 or 3 monthly injections of Testosterone enanthate, 25 mg can be helpful. Birth registration and naming of the child should be delayed until a final decision has been made.

If the sex of rearing is female, appropriate genital surgery and gonadectomy are performed early, and

oestrogen replacement is commenced at the time of puberty. Males need orchiopexy and repair of the severe hypospadias.

Diagnosis

Because of the life threatening metabolic disturbances in neonates with CAH early diagnosis and medical treatment is essential. For the sake of the parents of all children with ambiguous genitalia a fast and accurate assignment of sex of rearing is important. In CAH there is no problem but in some conditions delay is unavoidable. A combined approach by pediatric endocrinologist, urologist and genetic counselor produces the most satisfactory decision as to sex of rearing and best helps the parents during this difficult period.

Delayed registration and naming of the child is preferable to an untimely guess at the likely sex of the child.

Investigation for intersex anomalies is needed in:
- Any child with ambiguous genitalia
- Apparent females with a degree of clitoral enlargement
- Neonates with inguinal masses or herniae
- Apparent males with micropenis
- Apparent males with severe hypospadias particularly if associated with undescended testes
- Apparent males with bilateral impalpable undescended testes.

A history of parental consanguinity, neonatal vomiting or deaths, intersex, genital anomalies, primary amenorrhoea or infertility should be sought together with details of maternal exposure to drugs. Physical examination should seek dysmorphic features, abnormal pigmentation and details of the genitalia and gonads.

Blood analysis reveals the karyotype and the levels of electrolytes, LH, FSH, testosterone, cortisol, ACTH and 17-hydroxyprogesterone. Urine analysis of hormones and their precursors with or without stimulation with hCG has been referred to above.

Other investigations, such as ultrasound, laparoscopy, genitography and androgen binding studies are needed as appropriate. Early laparotomy or laparoscopy is reserved for suspected true

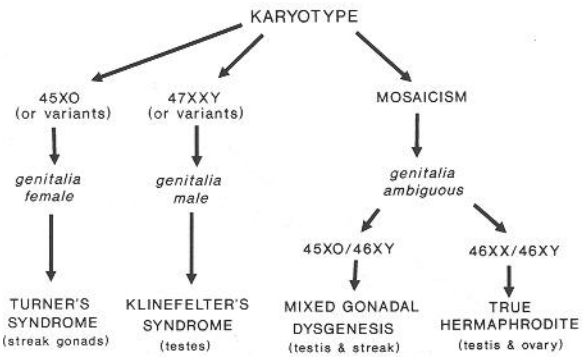

Figure 4. Diagnostic pathway for gonadal dysgenesis based on karyotype and appearances of the genitalia.

hermaphroditism where a gonadal biopsy is needed for the correct diagnosis. Later exploration may be required in several other conditions for removal of inappropriate internal organs.

Karyotype

Chromosomal analysis to determine the karyotype should be available within 48 hours. Based on this result 3 groups emerge: Abnormal, 46XX and 46XY.

1. Karyotype abnormal

The 4 conditions in the gonadal dysgenetic group are shown in Figure 4. The appearance of the external genitalia together with the abnormal karyotype give a firm diagnosis of Turner's and Klinefelter's syndromes and mixed gonadal dysgenesis. The latter is the only one likely to present neonatally. True hermaphroditism with a mosaic pattern is more difficult to diagnose and may need a laparotomy to identify the nature of the gonads.

2. Karyotype 46XX

Figure 2 shows the possible diagnoses for a 46XX karyotype. CAH is diagnosed biochemically. The genital appearances of the 46XX male may be somewhat ambiguous but there should be two testes. Transplacental androgenic activity is now rare. A true hermaphrodite with 46XX karyotype requires a laparotomy to identify both testicular and ovarian tissue.

Some patients with pure gonadal dysgenesis have a 46XX karyotype and normal female genitalia.

3. Karyotype 46XY

In this group (Fig. 3) there is undervirilisation in all but the child with Persistent Müllerian Duct Syndrome [4] who presents with a hernia in which there are Müllerian derived structures (uterus and tube) or such remnants may be found at the time of an orchiopexy. 46XY associated with normal female external genitalia is typical of the complete androgen insensitivity syndrome; testes may be palpable in the inguinal region. Patients with 46XY pure gonadal dysgenesis have impalpable streak gonads and present later as previously discussed.

Neonates with a 46XY karyotype and ambiguous genitalia have either a testosterone biosynthetic defect, an inability to convert testosterone to DHT or resistance in the androgen sensitive cells to the action of androgens. They are distinguished by an hCG stimulation test [6] (Fig. 3). Laparotomy is needed to make the final diagnosis in the 15 % of true hermaphrodites with a 46XY karyotype [7].

Difficult diagnoses include the undervirilised males with a small phallus in whom the predictive response to a trial of testosterone is uncertain. Typically, these patients have partial androgen resistance or a testosterone biosynthetic block.

Sex of rearing

All CAH patients should be reared as female, as should all patients with complete androgen insensitivity, Turner's syndrome and pure gonadal dysgenesis. 46XX males and patients with Klinefelter's syndrome should remain male as they both have male external genitalia.

Varying degrees of under or over virilisation are associated with mixed gonadal dysgenesis, true hermaphroditism, testosterone biosynthetic defects and partial androgen insensitivity. In these patients the decision for sex of rearing is based on the size of the phallus and its potential for enlargement under androgen stimulation, and the presence and accessibility of a structure to provide a vagina. Lesser factors are the prospect for fertility, applicable only in CAH and the very occasional patient with true hermaphroditism, and the potential for malignant change in the gonads. The risk of malignancy in dysgenetic gonads is associated with a Y chromosome but this will rarely influence the sex of rearing.

Gonadal tumour risk

The association between gonadal neoplasia and intersex disorders is well established [11,18]. Such tumours, which are of germ cell origin, range from the more common benign gonadoblastomas to malignant dysgerminomas/seminomas. A combination of a Y chromosome and gonadal dysgenesis gives the highest risk but dysgenesis is not essential as true hermaphrodites with a Y chromosome and patients with androgen insensitivity, particularly the complete variety, are also at risk. Undescent of the testis probably adds to the risk but the intrinsic gonadal abnormality is probably more important. The exact increased risk of malignancy is unknown but it is considerably higher than for the adult male population [14].

Between a quarter and a third of patients with 46XY pure gonadal dysgenesis or mixed gonadal dysgenesis (45X0/46XY) develop a gonadoblastoma in streak gonads and some of these progress to malignant dysgerminoma/seminoma [11]. Dysgenetic conditions with no Y chromosome (Turner's syndrome (45X0) or pure gonadal dysgenesis (46XX)) are not associated with risk.

Surgery for patients with ambiguous genitalia

1. Genitoplasty

The genitalia are reconstructed by clitoral reduction and vaginoplasty to give female appearances and function [13,15,16]. The glans is preserved with its blood supply and sensation but most of the corpora cavernosa are removed. The vagina is opened onto the perineum in the correct position and the labia are reconstructed from excess phallic skin which may also be used for vaginal reconstruction.

Details of the operation are fully described elsewhere [16] but essentially the dorsal nerve of the phallus with its associated blood vessels are preserved and, ventrally, the vascular mucosal strip (urethral plate) is also kept.

For social and psychological reasons the operation for low lesions is best performed at 3-6 months of age when the child's general medical and metabolic state is satisfactory. However, if there is a high confluence of vagina and urethra the operation

is more difficult and best delayed until 2-3 years of age.

Previous to the currently used techniques the clitoris was either completely removed [3], which was too radical, or recessed under the pubic arch which led to discomfort during sexual stimulation [8].

When the confluence of the vagina and urethra is high there are alternatives for vaginal reconstruction. Hendren [5] located the small upper segment of the vagina suprapubically and then brought it down to meet the exposed perineal dissection. Recently, Passerini [9] has described a method of tubularising the phallic skin. The small vaginal segment is most easily identified at operation by placing a Fogarty balloon catheter in it endoscopically.

Late follow up before puberty with an examination under anesthetic is needed in all children so that any vaginal inadequacy can be dealt with by a revision operation. If the glans remains too large, which is unlikely, its lateral edges can be excised at any stage.

2. Hypospadias repair

Children with hypospadias who are to be reared as males need repair with similar techniques that are used as for any boy with severe hypospadias. Skin loss, fistula or stenosis, may occur in 5-25 % of patients and require correction. If a small utricle is present it can be left alone but a larger one may need excision if complicated by infection or stone formation.

3. Orchiopexy

All boys with undescended testes need orchiopexy by the age of 2 years unless the gonad is severely hypoplastic or a streak; in which case it should be excised. If extensive dissection is needed it can accompany a laparotomy for excision of inappropriate internal genitalia.

4. Laparotomy/laparoscopy

In true hermaphrodites and some other groups of patients it is necessary to inspect and biopsy the internal genitalia at an early stage to complete the diagnosis. In others inappropriate organs need excision.

5. Mastectomy

Inappropriate breast development at puberty is an indication for mastectomy in patients reared as males.

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Robert H. Whitaker, MD MChir FRCS
Associate Lecturer
University of Cambridge