THE INVESTIGATION OF DEVELOPMENTAL SEXUAL ABNORMALITIES*

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Since the introduction of cytogenetics for the investigation of individuals manifesting abnormal sexual development, some of the problems have been elucidated, many remain unsolved, and new ones have emerged. Assessment of developmental sexual abnormalities is incomplete without nuclear sex determination and chromosomal analysis, which must be correlated with the clinical findings.

CLASSIFICATION (*Table I*)

Abnormal sexual development exists when any single component of sex is incompatible with the others.

The classification is based on the histology of the gonads. The foetal undifferentiated gonad normally develops into either a testis or an ovary.

TABLE I. CLASSIFICATION

Female and male hermaphroditism	*Normal*	*Gonadal dysgenesis*
A. FEMALE HERMAPHRODITISM	OVARY	I. GONADAL APLASIA
(1) Adrenogenital syndrome	↑	(1) Turner's syndrome
(2) Maternal or exogenous androgens	|	(2) Gonadal dysplasia with Leydig-cell component
(3) Unknown cause	|	(3) Pure gonadal aplasia
	UNDIFFERENTIATED GONAD	II. TRUE HERMAPHRODITISM
B. MALE HERMAPHRODITISM	↓	III. ASYMMETRICAL GONADAL DIFFERENTIATION
	TESTIS	
(1) Femininizing testes		IV. TESTICULAR DYSGENESIS (Klinefelter's syndrome)
(2) Ambiguous type		

Female and Male Hermaphroditism

Female hermaphrodites have normal ovaries and internal female organs but ambiguous external genitalia. This is usually due to abnormal adrenal metabolism of steroids, or androgenic steroids of maternal or exogenous origin. In rare cases no cause is found.

Male hermaphrodites have bilateral testicular tissue with Sertoli cells and spermatogonia. Two groups are recognized. In the one the external genitalia are ambiguous. The Mullerian system may be absent or show varying degrees of differentiation. In the second group (the femininizing testes syndrome) the individual has a female phenotype (external appearance) and Mullerian development is usually absent. The testes may be intra-abdominal or in the inguinal region.

Generally neither male nor female hermaphrodites have abnormalities of the nuclear sex or chromosomes.

Gonadal Dysgenesis

Gonadal aplasia. This syndrome presents if the foetal gonad persists in the undifferentiated state. It consists of Turner's syndrome and its variants. The gonads are usually 'white streaks' composed of wavy connective tissue simulating ovarian stroma without ovarian follicles. The individual has the female phenotype and is reared as a female.

In these conditions abnormalities of the chromosome number (aneuploidy) or morphology occur. In Turner's syndrome one sex chromosome is missing and only 45 chromosomes are present, instead of the normal number 46. In accordance, the cells are chromatin negative. In some cases the chromosomal complement is complete but one X chromosome is abnormal, having either a deletion of one of the arms X^x, or a duplication xX (isochromosome). In the former the sex chromatin bodies are reduced in size and in the latter they appear larger than normal. Mosaicism, i.e. the identification of two cell populations, one of which may be a normal cell line 46/XX, is being more frequently reported. In these patients the percentage of sex chromatin bodies may be decreased as compared with a normal female.

In gonadal dysplasia the external genitalia are female but the phallus is enlarged. The gonad shows the presence of well-differentiated Leydig cells. Two patients who have been investigated were chromatin positive. Mosaicism, 45XO/46XX, was present in one; chromosomal analysis was not performed in the second case.

Tall patients in whom the abnormality is confined to sexual development are classified as pure gonadal aplasia. The cells are chromatin negative and chromosomal analysis shows the presence of 46 chromosomes with the sex-chromosomal complement XY.

True hermaphrodites have both ovarian and testicular tissue either in one gonad or both. The ovotestis may lie either intra-abdominally, in the inguinal canal, or in the scrotum. The patient presents with ambiguous external genitalia and, in many, breast development occurs at puberty. There is invariably some differentiation of the Mullerian system. The majority of true hermaphrodites are chromatin positive, with the karyotype 46/XX (the normal female pattern). Some are chromatin negative 46/XY (the normal male pattern), and a few have been reported to be mosaics with two cell lines.

Asymmetrical gonadal differentiation refers to apparent males in whom there is unilateral testicular development and an absent or streak gonad on the opposite side. The internal sex organs are female and the external genitalia are ambiguous. All these individuals were shown to be chromatin negative 46/XY. In recent reports two such patients have been described, one with 45/XO karyotype, and the other with a mosaic 45XO/46XY.

Testicular dysgenesis (Klinefelter's syndrome). The patient presents as a male with apparently normal external genitalia and is reared accordingly. Although the testes are scrotal there is defective development of the seminiferous tubules, with morphologically normal Leydig-cell differentiation. These phenotypic males may or may not have gynaecomastia. The nuclear sex is chromatin positive,

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with one, two, or occasionally three, sex chromatin bodies. The sex chromosomes are XXY, XXXY, XXXXY, or XXYY, with a corresponding increase in the total number of chromosomes. Mosaicism has been described.

SCHEME OF INVESTIGATIONS (*Table II*)

Clinical Examination

Certain features may suggest the presence of sexual abnormality and indicate its type:

History. Male hermaphroditism is known to occur in families. Masculinization of the foetus (i.e. female hermaphroditism) may be due to synthetic progesterones taken during the first 3 months of pregnancy. We have studied two such children.

Examination at birth may demonstrate ambiguous external genitalia—apparently normal female genitalia with an accompanying inguinal tumour, or cryptorchidism occurring with male genitalia.

After puberty the combination of infertility and small, firm testes suggests a Klinefelter's syndrome.

Associated anomalies. Congenital abnormalities (e.g. cardiac or renal, or mental retardation, or absent secondary sex characteristics) may be the first indication of associated abnormal sexual development.

Investigations

Nuclear sex determination is the most important examination, and is performed on every patient. Correlation of the clinical syndrome, history, and nuclear sex, provides the initial information as to the type of sexual abnormality. The report 'chromatin positive' indicates the presence of at least XX chromosomes. In the presence of a single X chromosome the result is 'chromatin negative': the sex-chromosome constitution is not necessarily XY, for XO occurs and XYY has also been described. The number of sex chromatin bodies is one less than the number of X chromosomes (e.g. in XXX karyotypes the nuclei contain 2 sex chromatin bodies). From this test the differential diagnosis is confined to the following:

TABLE II. SCHEME OF INVESTIGATIONS

A. HISTORY
B. CLINICAL EXAMINATION } *In all cases*
C. NUCLEAR SEX DETERMINATION
D. SPECIAL PROCEDURES

Group	*Nuclear sex*	*Hormone studies*	*Endoscopy*	*Laparotomy gonadal biopsy*
FEMALE HERMAPHRODITES				
(1) Adrenogenital syndrome	Positive	17-Ketosteroids+ Pregnanetriol+	No	No
(2) Exogenous androgens	Positive	17-Ketosteroids, Pregnanetriol } normal	No	No
MALE HERMAPHRODITES				
(1) Testicular femininization	Negative	Not essential	No	Yes
(2) Ambiguous genitalia	Negative	Not essential	Yes	Yes
GONADAL APLASIA	Negative	FSH+(usually)	No	Not essential
TRUE HERMAPHRODITES	Positive	17-Ketosteroids, Pregnanetriol } normal	Yes	Yes
	Negative	Not essential	Yes	Yes
ASYMMETRICAL GONADAL DIFFERENTIATION	Negative	Not essential	Yes	Yes
TESTICULAR DYSGENESIS	Positive (one or more chromatin bodies)	FSH+(usually)	No	Not essential

E. CHROMOSOMAL ANALYSIS

Chromatin Positive	*Chromatin Negative*
1. Female hermaphrodites	1. Male hermaphrodites
2. True hermaphrodites	2. True hermaphrodites
3. Klinefelter's syndrome	3. Asymmetrical gonadal differentiation
	4. Gonadal aplasia

Further procedures depend on the group to which the patient is assigned.

Chromatin-Positive Group

A. Female Hermaphrodites

(1) *Adrenogenital syndrome.* Raised urinary 17-ketosteroids and pregnanetriol due to abnormal adrenal metabolism supply conclusive evidence in these patients. The immediate recognition and treatment of this syndrome is vital for the future of the individual.

(2) *Exogenous androgen* intake during pregnancy will form part of the history elicited from the mother.

B. Klinefelter's syndrome will be diagnosed from the history and clinical examination. Biopsy of the testis is not essential but may be performed if required.

C. True hermaphrodites are dealt with in the chromatin-negative group, for the nuclear sex determination may give either result.

Chromatin-Negative Group

A. (1) *Male hermaphrodites with ambiguous genitalia,* (2) *true hermaphrodites,* and (3) *patients with asymmetrical gonadal differentiation,* are subjected to further investigations.

(a) *Endoscopic examination* is performed to demonstrate the presence of internal female organs. On urethroscopy a vaginal opening into the posterior wall of the urethra and the absence of a verumontanum may be noted. Urethrogram will outline the vagina and uterus. However, if the vagina terminates blindly these examinations will prove fruitless. These studies are of value for surgical correction.

(b) *Laparotomy and gonadal biopsy.* The final diagnosis can only be made by exposing the internal sex organs, and on the histology of the gonads. Macroscopic identification of an ovotestis is often impossible and histological confirmation by wedge biopsy is essential.

B. Male hermaphrodites (femininizing testes syndrome). These individuals have the female phenotype and hence require only laparotomy and gonadal biopsy.

C. Gonadal aplasia. This syndrome is diagnosed on the history, clinical examination, and nuclear sex. Further investigations are not essential.

Chromosomal Constitution

Analysis of the chromosomes is primarily of academic importance. It confirms the nuclear sex and supplies the details of the sex-chromosomal complement. Therefore it completes the investigation of the patient.

PATIENTS INVESTIGATED BY THE UNIT (*Table III*)

Four male hermaphrodites, 2 European and 2 Bantu, diagnosed as the femininizing testes syndrome were found to

be chromatin negative, with the expected karyotype 46/XY. In 3 of these patients gonadal biopsy showed testicular adenomatous hyperplasia (this term is preferred to that of Sertoli-cell adenoma used by other authors). The 4th patient has still to be proved by gonadal biopsy. One of these patients has 2 cousins on the maternal side with male hermaphroditism.

TABLE III. PATIENTS INVESTIGATED BY THE UNIT FROM JUNE 1962 TO JULY 1963

Group	*No. of cases*	*Karyotype (blood)*	*White*	*Bantu*
FEMALE HERMAPHRODITES				
(1) Adrenogenital syndrome	1		1	
(2) Exogenous androgens	2		2	
MALE HERMAPHRODITES				
(1) Femininizing testes	4	46XY	2	2
(2) Ambiguous genitalia	2	46XY	1	1
GONADAL APLASIA	5	45XO; 45XO/46XY; 45XO/46XX; Twins 45X+fragment	4	1
TRUE HERMAPHRODITE	7	Six 46XX One 46XX/46XY*		7
ASYMMETRICAL GONADS	1	46XY		1
TESTICULAR DYSGENESIS	1	47XXY	1	

*See text.

Two male hermaphrodites of the ambiguous variety, one White and one Bantu, were chromatin negative 46/XY. In the White patient only one gonad was biopsied; hence true hermaphroditism cannot be excluded.

Seven Bantu patients were true hermaphrodites. Of these, 2 were of the alternating variety, with one testis and one ovary, 2 had bilateral ovotestes, and 2 were of the unilateral variety with one ovotestis and one ovary. In the 7th case a scrotal ovotestis and an ovary were present (Mrs. Friedberg, of the Physiology Department of the Durban Medical School, performed the chromosomal analysis and kindly permitted us to include the case in this series). All 7 patients were chromatin positive, 6 were 46/XX and the 7th patient had two cell lines.* (A single White child and 2 Bantu children, suspected of being true hermaphrodites, with the karyotype 46/XX have not yet been subjected to laparotomy. In another true hermaphrodite, who has been lost to follow-up examinations, we have diagnosed bilateral scrotal ovotestes.)

In the group of gonadal aplasia there were 5 patients. One of them, a Bantu infant clinically diagnosed as Bonnevie-Ullrich syndrome, was chromatin negative 45/XO. Three, White patients, had the stigmata of Turner's syndrome. (One was chromatin negative and had a mosaic pattern 46XY/45XO, the other two were chromatin-negative identical twins with the karyotype 45/X+a fragment. The origin of the fragment is open to conjecture, being derived from either the X or the Y chromosome with loss of the remaining portion. The accident may have occurred during spermatogenesis or oogenesis, or during the first division after formation of the zygote.) The 5th patient, a White, had 5% sex chromatin bodies in the cells and two cell lines, viz. 46XX/45XO. Initially she was suspected of being a variant of Turner's syndrome. She has, however, since developed secondary sex characteristics and is menstruating.

A single White patient with Klinefelter's syndrome was chromatin positive and had the chromosomal constitution 47/XXY, as anticipated.

The only patient classified as asymmetrical gonadal differentiation was a Bantu child with the karyotype 46/XY.

*One of these cell lines was 46/XX and the other either 46/XY or 46/X^{x} (deletion of the long arm).

DISCUSSION

Although this series of patients is small it appears that the commonest variety of congenital sexual abnormality in the Bantu patient is true hermaphroditism. Since the inception of the cytogenetics unit we have not had an adult case of Turner's syndrome or a patient with Klinefelter's syndrome referred for chromosomal analysis. A further observation is the absence of the congenital adrenogenital syndrome in the chromatin-positive group of cases we investigated. These impressions have been confirmed by the medical staff of the Bantu hospitals.

Abnormal sexual development associated with other congenital abnormalities, including mental retardation, suggests that the karyotype will show abnormalities of the sex chromosomes. A visible chromosome abnormality will affect a large number of genes and this can be expected to manifest itself by multiple anomalies. Klinefelter's syndrome, Turner's syndrome and the triplo-X syndrome support this opinion.

It is our experience that in those individuals in whom there is no apparent sex-chromosomal aberration (e.g. cases of true hermaphroditism and of femininizing testis syndrome) the abnormality is localized to sexual development, as anticipated. Possibly, with present methods minor alterations of the chromosome cannot be observed. An alternative suggestion is that mutant genes either on a sex chromosome or on an autosome, may be responsible. Perhaps in the same way that enzyme defects have been identified in the adrenogenital syndrome, so in other syndromes abnormal or deficient enzyme systems may eventually be recognized.

Chromosomal analysis has contributed much to the understanding of abnormal sexual development. Two, and even three, cell populations have been shown to exist in an individual. Further information will accumulate when the gonadal chromosomal constitution is investigated.

SUMMARY

A classification of abnormal sexual development is presented. Features suggesting the possible diagnosis and the methods of investigation are discussed. The results in patients studied by this unit are submitted. It is concluded that true hermaphroditism is the commonest form in the Bantu. A combination of abnormal sexual development with other congenital anomalies, including mental retardation, is often associated with an abnormality of the sex chromosomes.

A Turner's syndrome in identical twins is reported, in whom the karyotype is 45/X+a fragment.

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