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SEX CHROMOSOMES AND ABNORMAL SEX DEVELOPMENT*

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In the last decade interest has been revived in the chromosomal constitution of man, following the demonstration of its relation to various clinical syndromes, congenital abnormalities and malignancy. The chromosome number, which had been accepted as 48, was shown in 1956 by workers[1a,b] from different laboratories to be 46, i.e. 22 pairs of autosomes plus 2 sex chromosomes.

These recent advances have resulted from the recognition of the sex chromatin body, the application of tissue-culture methods, and improvements in the technique of making squash preparations. This field is still in its infancy and the evaluation and interpretation of the findings at this stage is somewhat involved and difficult, with many apparent contradictions.

SEXUAL DEVELOPMENT

This depends on the initial processes of determination and subsequent differentiation.

Sex Determination

Sexual development in the embryo is primarily dependent on the chromosomal constitution of the zygote, i.e. it is genetically determined by the sex chromosomes and probably by specific autosomes on which the genes which transmit the sex characters are located ('primary inductors').

Sex Differentiation

The zygotic sex dictates organ development along either the male or the female pathway.

1. *Foetal Differentiation—Primary Sex Development*

(*a*) *Gonad.* The differentiation of the gonad is determined by the zygotic sex of the embryo. During embryogenesis the germ cells migrate from the yolk sac to the undifferentiated gonad. Failure to do so may be a factor in abnormal sexual development. In man it is possible that the testis produces morphogenetic substances ('secondary inductors') which cause male differentiation, and that female differentiation results from the absence of these inductors and not from the presence of ovarian substances. Jost[2] showed this in the embryos of lower animals in castration experiments.

(*b*) *Internal accessory sex organs.* In the male these are differentiated from the Wolffian system and in the female from the Mullerian system.

(*c*) *External genitalia.* The differentiation of the external genitalia is probably hormone dependent, the hormones being the androgens produced by the foetal testis and adrenal. It appears to be independent of internal differentiation.

2. *Pubertal Differentiation—Secondary Sex Development*

This is hormone dependent ('tertiary inductors'), the source of the hormones being both gonadal and extragenital endocrine glands. Both the male and female hormones are important.

Sex abnormalities can occur at any of the above stages of sexual development.

THE SEX OF THE INDIVIDUAL

This can be assessed at different levels.

Morphological Level

This includes the following:

1. *Chromosomal sex* at the nuclear level. Chromatin positive implies the presence of the sex chromatin body in a high proportion of nuclei, as in normal females. If it is absent the individual is chromatin negative, as in normal males.

2. *Gonadal sex.* This includes: (*a*) ovaries, (*b*) testes, (*c*) mixed—true hermaphrodite, and (*d*) neuter—undifferentiated gonad.

3. *Internal accessory sex organs.*

4. *External sex organs.*

In (3) and (4) these may be normal male or female, or may be mixed or ambiguous as in the true hermaphrodite or pseudohermaphrodite.

Physiological Level

The adult endocrine pattern responsible for the development and maintenance of secondary sex characteristics.

Psychological Level

1. Assigned sex at birth.
2. Sex of rearing, which is determined by assigned sex.
3. Psychological influences of the environment.

Synchronization

When all the above components are compatible and synchronize, the sexual development is normal male or female. If any single one is conflicting, the result is abnormal, and different clinical syndromes may present.

This discussion will be chiefly at the cellular level, i.e. the karyotype and chromosome number associated with recognized clinical abnormalities.

KARYOTYPES

Sex Chromatin

Barr and his co-workers in 1949[3] identified this structure in the nuclei of female inter-phase somatic cells as a more densely staining chromatin body approximately 1 μ in diameter. It is most easily recognized when closely applied to

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TABLE I. RECENT INVESTIGATIONS IN PATIENTS WITH CONFLICTING SEX COMPONENTS

Clinical syndrome	*Phenotype*	*Gonad*	*Internal genital organs*	*External genitalia*	*Secondary sex characteristics*	*Congenital anomalies*	*Nuclear sex*	*Chromosome number*	*Karyotype*
Klinefelter's syndrome[4,5]	Male—sterile	Testis, hypospermatogenesis and hyalinization of tubules	Male	Male	(1) Gynaecomastia + or —. (2) Absent or deficient facial hair	—	Chromatin +ve	47	XXY
Klinefelter double male[6]							Chromatin +ve	48	XXYY
Klinefelter mosaic[7,8]						Psychopath	Chromatin +ve, some cells 2 sex chromatin bodies	47/46	XXY/XX mosaic
Klinefelter variant[9]						Primary amentia	Chromatin +ve. Double chromatin bodies	48	XXXY
Turner's syndrome[10,11]	Female—usually sterile	(*a*) Rudimentary undifferentiated gonad, (*b*) ovarian stroma, + or — mesonephric ducts	Female—infantile	Female	Sexual infantilism	++	Chromatin —ve	45	XO
Pure gonadal agenesis[12]		? Primitive gonadal ridges			Sexual infantilism	—	Chromatin —ve	46	XY
Turner mosaic[13]							Chromatin —ve or weakly +ve.	45/46	XO/XX mosaic
Triplo-X female 'super-female'[14,15]	Female—(*a*) sterile, (*b*) fertile	Ovary	Female—(*a*) normal, (*b*) underdeveloped	Female—normal	(*a*) Normal, (*b*) underdeveloped	(*a*) Absent, (*b*) mental retardation	Chromatin +ve, some cells 2 chromatin bodies	47	XXX
Triplo-X mosaic[16]							Chromatin +ve, some cells 2 chromatin bodies	47/45	XXX/XO mosaic
Testicular feminization[17]	Female—sterile	Testis—undescended	Mainly male	Female	Breasts present. Pubic and axillary hair scanty. Primary amenorrhoea		Chromatin —ve	46	XY
True hermaphrodite[18,19]	Mixed—(*a*) predominantly male, (*b*) predominantly female	(*a*) Ovary + ovotestis, (*b*) testis + ovotestis, (*c*) testis + ovary, (*d*) ? bilateral ovotestis*	Variable, both male and female differentiation. Testicular tissue ——> male differentiation on that side	Ambiguous from imperfect masculinization	Mixed male and female		Majority chromatin +ve	46 in 5 cases who are chromatin +ve	XX
Hermaphrodite mosaic[20]								46/45	XY/XO mosaic
Chromosomal attenuation[18]	Female	Rudimentary streaks, one of which consisted of ovarian tissue plus a few primitive follicles	Female—underdeveloped	Female—underdeveloped	Sexual infantilism	Low normal intelligence	7% cells chromatin +ve, the chromatin body being smaller than normal	46	Xs, reduction in length of 1 X chromosome
Female pseudohermaphrodite, the adreno-genital syndrome	Female—(*a*) pseudohermaphrodite (congenital), (*b*) with virilization (adult)	Ovary	Female—(*a*) normal, (*b*) underdeveloped, (*c*) with or without prostate	Imperfect masculinization	Female with evidence of virilism	—	Chromatin +ve	46	XX

*Recently, in our laboratory, a true hermaphrodite was shown to have bilateral ovotestes, both gonads being in the scrotum.

the nuclear membrane, and is then usually planoconvex in shape. If it is present in a high percentage of cells, the individual is classed as chromatin positive (as in normal females). A low percentage of sex chromatin is classed as chromatin negative (as in normal males). It was then postulated that the sex chromatin results from the presence of the XX chromosomes in female nuclei.

When the test is applied to individuals with abnormal sex development it is found that:

1. In the majority of patients with Turner's syndrome who present as phenotypic females the cells are chromatin negative.
2. In Klinefelter's syndrome, although the individual is a phenotypic male, the cells are chromatin positive.
3. Patients with the testicular feminization syndrome who are phenotypic females are chromatin negative.
4. The majority of true hermaphrodites, who are phenotypically mixed male and female, are chromatin positive.

Since abnormal karyotypes had been shown to be associated with intersexes in Drosophila, it was suggested that these could occur similarly in man, and it became imperative to investigate his chromosomal constitution.

Technique of Investigation

Various tissues, e.g. bone marrow, skin, or leucocytes are cultured *in vitro* for short periods. Colchicine is introduced into the culture. This inhibits spindle formation and the cells are therefore arrested in the metaphase stage of mitosis. The addition of hypotonic sodium citrate produces swelling and divergence of the chromatids at their centromeres. The cells are then stained, e.g. with feulgen, and squash preparations are made.

Chromosomal counts are performed, and the karyotype is determined by matching the chromosomes in order of size, position of the centromere (metacentric, submetacentric or acrocentric), and length of the chromatid arms. They are accordingly arranged in pairs in 7 groups from No. 1 to No. 22 (Denver system). The X chromosome is identified as a medium-sized metacentric chromosome and the Y as a small acrocentric chromosome. In the female the sex chromosomes are XX, and in the male XY.

Correlation of Some Abnormal Sex-chromosome Karyotypes with Clinical Syndromes

Table I is a summary of some of the recent investigations of patients in whom one or other component of sex is conflicting.

The female pseudohermaphrodite of the adreno-genital syndrome has been included for clinical completeness, although no abnormal karyotype has been found. This syndrome may occur in:

(*a*) patients with adrenal cortical hyperplasia and dysfunction,

(*b*) patients with tumours of the adrenal cortex, or

(*c*) the offspring of mothers who have been receiving synthetic progesterones over a prolonged period during the first three months of pregnancy.

It is essential that this syndrome be recognized early and treated effectively.

Theory of Mechanism of Formation of the Abnormal Karyotype

A. Normal disjunction in meiosis — (Fig. 1). During parental gametogenesis the diploid number (2n) is reduced

Fig 1

to the haploid number (n) in the germ cells. The separation of like chromosomes at anaphase is referred to as disjunction. Normal ova therefore all contain one X chromosome and normal sperms either X or Y. Fertilization results in a zygote which may be either XX (a normal female) or XY (a normal male).

B. Non-disjunction of the sex chromosomes. This implies that during the first (Fig. 2) or second divisions of meiosis both sex chromosomes migrate to the same pole. This will lead to ova with XX or O chromosomes. The resultant zygote may then be one of the following combinations:

Fig 2

(*a*) XXX—triplo-X ('super-female'), (*b*) XXY—Klinefelter's syndrome, (*c*) XO—Turner's syndrome, or (*d*) YO—non-viable. Similarly, non-disjunction may take place in either meiotic division in the sperm, producing abnormal sperm karyotypes.

C. Non-disjunction in the zygote—(Fig. 3). Abnormal karyotypes may presumably also result from this phenomenon in mitosis in the zygote. If this occurs in a normal zygote, a mosaic karyotype of the order XXX/XO, i.e. triplo-X/Turner's syndrome may be produced; if in an

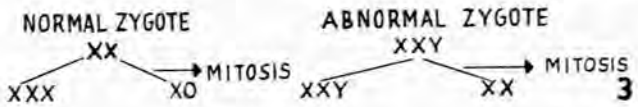

Fig 3

abnormal zygote, a mosaic XXY/XX, i.e. Klinefelter's syndrome mosaic may result.

Comparison with Lower Animals

As stated previously, abnormal karyotypes and their associated anomalies had been noted originally in Drosophila. In Table II, which compares the two species, it becomes apparent that the manifestations of the abnormal karyotypes differ, and therefore the localization of the sex genes on the chromosomes are not identical.

TABLE II. COMPARISON OF ABNORMAL KARYOTYPES IN DROSOPHILA AND MAN

Abnormal karyotype	*Drosophila*	*Homo sapiens*
XXY	Fertile, apparently normal female	Sterile male
XO	Sterile, apparently normal male	Sterile female
XXX	Super-female with accentuated secondary sex characters	(*a*) Sterile female, (*b*) fertile female, (*c*) mental defects
YO	Non-viable	Non-viable

Recently, studies in the mouse have shown the occurrence of the XO karyotype and also the presence of masculinizing genes on the Y chromosome.

DISCUSSION

Investigation of the chromosomal constitution in man has shown a correlation between the karyotype and the nuclear chromatin pattern. The presence of the sex chromatin body in the interphase nucleus indicates XX chromosomes. Double sex chromatin bodies suggest at least XXX chromosomes, e.g. triplo-X, 'super-female'. The explanation of the formation of the sex chromatin is unknown. A theory has been proposed that the X chromosomes display differential behaviour and that it is the heteropyknotic X chromosome that accounts for the sex chromatin. Nuclear sex determination is an important laboratory procedure in patients who present with clinical syndromes involving sexual development. In chromatin-negative individuals with Turner's syndrome it can be deduced that the karyotype is XO and in chromatin-positive patients with Klinefelter's syndrome the karyotype is XXY.

Abnormal sex differentiation may occur despite apparently normal sex determination. In the testicular feminization syndrome the karyotype is male, the gonads are testes, but the phenotype is female. In this condition the testes may be physiologically abnormal. Similarly, in the syndrome of pure gonadal dysgenesis, the karyotype is XY, but the gonad remains in an undifferentiated state and the phenotype is female. Perhaps in both these syndromes the individual target organs do not respond to their respective inductors. Conversely, in 5 true hermaphrodites the karyotype was found to be XX, in spite of which testicular differentiation occurred with accompanying male differentiation on the corresponding side.* The possibility of translocation of a portion of the Y chromosome bearing masculinizing genes must be considered here.

Abnormal sex determination is usually, but not inevitably, accompanied by abnormal sex differentiation, e.g. Turner's syndrome and Klinefelter's syndrome. However, there is a recorded case in which the karyotype is XO with completely normal sexual development.[21]

The rôle of the sex chromosomes is therefore incompletely understood. Certain deductions may, however, be drawn:

1. In man the Y chromosome carries masculinizing genes (cf. Drosophila in which the Y appears to be inert). Its presence is linked with the development of the testis.

2. Testicular development may occur in the absence of the Y chromosome.

3. The Y chromosome does not ensure the development of a testis.

4. XX chromosomes are not essential for normal female sex development.

5. The sex chromosomes are not solely responsible for sexual development in a given direction, and there are probably masculinizing and feminizing genes on the autosomes. Coordination of both may be required for normal sex development.

6. In the presence of vestigial gonads female development invariably occurs.

* In a recent report[22] a boy of 8 who is a phenotypic male was shown to have an XO chromosomal constitution and a rudimentary testis. This provides further evidence of testicular differentiation in the absence of the Y chromosome.

SUMMARY

1. Sexual development depends on the process of sex determination and sex differentiation.

2. The sex of the individual should be assessed at different levels.

3. Nuclear sex determination is an important laboratory investigation in patients with abnormal sex development. The mode of the formation of the sex chromatin is unknown.

4. Abnormal sex-chromosome karyotypes may be associated with certain clinical syndromes.

5. Abnormal karyotypes are at present explained on non-disjunction of chromosomes at cell division.

6. The rôle of the sex chromosomes in sex development is incompletely understood, but the Y chromosome appears to be more important than was previously suspected. The possibility of autosomal sex determination in conjunction with the sex chromosomes must be postulated.

7. Abnormal sexual development can occur at any stage of differentiation. An abnormal sex-chromosome karyotype is only one of the known factors which is associated with, and may account for, sexual anomalies.

8. Several factors in sexual differentiation remain to be evaluated. These include the response of target organs to inductors, the possibility of abnormal physiologic function of the gonad, and the possible influence of the germ cells on sex-organ differentiation and behaviour.

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