THE TRIPLO-X SYNDROME: CLINICAL AND CYTOLOGIC FEATURES

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Sex is not what it used to be. The present view reveals a number of interrelated components:

1. Morphologic sex, comprising 3 elements: (*a*) Chromosomal or genetic, (*b*) Histologic, and (*c*) Macroscopic or anatomic.
2. Physiologic sex—the endocrine patterns underlying the secondary sex characters.
3. Psychologic sex.

The discovery of sexual dimorphism in interphase nuclei, and improved cytogenetic techniques in recent years, have focused attention particularly on the chromosomal basis of sex. Purely notional concepts of events at the nuclear level have become crystallized and almost tangible for the physician with the demonstration of the relationship between phenotypic abnormalities and aberrations of the sex chromosomes.

Investigation of patients in mental institutions and at endocrine and infertility clinics has been especially fruitful in bringing to light such anomalies of the genetic sex. In a recent survey on a population of 1,662 mental defectives in South Africa, 10 cases of sex chromosome aneuploidy were reported.[1] Of these, 4 were phenotypic females with 2 Barr bodies in their buccal mucosal cells, modal counts of 47 and karyotypes compatible with the triplo-X (XXX) status. These 4 'super' females form the basis of this report.

CASE REPORTS

Case 1

The patient was a female born in 1932. Her father was 51 and her mother 29 years of age at that time. She is the youngest in a family of 8 (7 sisters and a brother), all of whom are said to be normal. The nuclear sex of one available sister was normal. The patient was admitted to the mental institution in 1938 because of mental retardation—rated as a high-grade defective.

Sexual development. External genitalia and breast development were normal. Axillary and pubic hair were normal. Menarche occurred at the age of 13 years. Regular menses followed (2 - 3/28 days). She gave birth to a female baby 1½ years ago: the child appears normal and is chromatin positive.

Measurements. The following measurements were recorded: height 67½ in., crown—pubis 33½ in., pubis—sole 34 in., arm span 68 in., and the head circumference 21½ in.

Other features. Colour vision was normal (Ishihara chart). No physical abnormalities were detected.

Nuclear sex. The buccal mucosal smear was chromatin positive (44% double bodies: 36% single bodies).

Chromosome counts.

Blood	< 45	46	47	48 >	Total
	1	—	19	—	20

Sex chromosome complement XXX

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Case 2

The patient was a female born in 1952. Her father was 40 and her mother 34 years old at that time. She was admitted to the institution in 1961 because of severe mental retardation and epilepsy.

Family history.

Sib number	1	2	3	4-9	**10**	11
Sex	M	F	M	?	**F**	F
Maternal age	19	24	32		**34**	38
Paternal age	25	30	38		**40**	44

Deaths: 6 miscarriages (4-9) all towards the end of the first trimester

One of the patient's male cousins on the father's side has severe mental deficiency and one on the mother's side has ectrodactyly. Neither could be interviewed.

Sexual development. The child was just beginning to show signs of pubescence. External genitalia were normal.

Measurements. The following measurements were recorded: height 49 in., crown—pubis 27 in., pubis—sole 22 in., span 49 in., and the head circumference 20 in.

Other features. Gross mental retardation. Occasional right-sided Jacksonian seizures. Shortened left leg as a result of 'femoral head pathology'.

Nuclear sex. The buccal mucosal smear was chromatin positive (36% double positive, 48% single).

Chromosome counts.

Blood	< 44	44	45	46	47	48 >	Total
	—	1	2	1	26	—	30

Sex chromosome complement XXX

Case 3

The patient was a female born in 1922 and admitted to the institution in 1953 with a diagnosis of catatonic schizophrenia. No record was available of family history or of their whereabouts.

Sexual development. Menarche ? Irregular menses up till menopause in 1959 at the age of 37 years. Pubic and axillary hair scant but external genitalia and breasts normal.

Measurements. Height 69 in., crown—pubis 37 in., pubis—sole 32 in., span 70 in., and the head circumference 21 in.

Other features. A simian palmar crease on both hands. There was a right-sided club-foot. Has been violent at times. Does not talk but continuously mutters to herself.

Nuclear sex. The buccal mucosal smear was chromatin positive (30% double positive, 45% single).

The blood showed 21 drumsticks and 15 sessile appendages in 1,000 polymorphs.

Chromosome counts.

Blood	< 46	46	47	48 >	Total
	—	1	24	—	25

Sex chromosome complement XXX

Case 4

The patient was a female born in July 1935. She was admitted to the institution in 1962 with the diagnosis of non-specific schizophrenia. Her family and family history were not available for study.

Sexual development. Scant axillary and pubic hair were present. The external genitalia and breasts were normal. No history about the menarche was obtained. The menses were regular 2 - 3/28 - 30 days.

Measurements. The following measurements were recorded: Height 67 in., crown—pubis 32 in., pubis—sole 35 in., span 67 in., and the head circumference 21 in.

Other features. Severe mental retardation was noted. Her palate was high and arched. She was virtually mute.

Nuclear sex. The buccal mucosal smear was chromatin positive (40% double bodies, 41% single). The blood showed 7 drumsticks, 9 sessile appendages and 1 double drumstick in 500 polymorphs.

Chromosome counts.

Blood	< 44	44	45	46	47	48	48 >	Total
	—	1	—	1	26	2	—	30

Sex chromosome complement XXX

A typical triplo-X karyotype from one of the patients is shown in Fig. 1. Figs. 2, 3 and 4 show respectively a chromatin-negative nucleus, a chromatin-positive nucleus and a chromatin double-positive nucleus (the latter being typical of the triplo-X status).

DISCUSSION

The first triplo-X (or super) female was described in Edinburgh in 1959[2] and from this same group the most extensive report on sex chromosome abnormalities has recently been published.[3] A preliminary survey of the nuclear sex of live-born females in Edinburgh showed 4 out of 3,000 (or 1·33/thousand births) to have double sex-chromatin bodies.[4] The final results of this survey have shown 12 such females in 10,000 consecutive births (or 1·2/1,000).[3]

This figure contrasts with the relatively high proportion of triplo-X females found in mentally defective populations. The present series (4 out of 899 mentally retarded cases, or 4·4/1,000) is similar to the findings in 4 other large surveys—Fraser *et al.*[5] (4 in 595 or 6·7/1,000), Johnston *et al.*[6] (3 in 827 or 3·6/1,000), Maclean *et al.*[7] (8 in 1,907 or 4·2/1,000) and Day *et al.*[8] (3 in 1,088 or 2·75/1,000). When pooled, these data give a frequency of 22 out of 5,316 or 4·1/1,000. Assuming that there is no differential mortality between triplo-X and XX females at any stage of life, then a significant association between oligophrenia and the increased complement of X chromosomes is seen to exist. However, not all such cases are mentally retarded. Those that are (as exemplified by the present series) exhibit wide variation in the degree of retardation, from mild feeblemindedness as in case 1 to gross idiocy as in case 2. Furthermore, the triplo-X status seems to occur more frequently in individuals with schizophrenia[9] and it is noteworthy that the diagnosis appended to case 3 on admission was catatonic schizophrenia.

Clinically, these cases do not show any characteristic abnormalities and in phenotype resemble normal females.[3] In some instances there is underdevelopment of the secondary sex characters and secondary amenorrhoea may bring them to the attention of the clinician. There is, however, no consistent menstrual or ovarian change and hormone assays have been equivocal.[6,8] The body proportions are within normal limits and most of these women in fact develop normally and are fertile.[3] Several births have been recorded and all those studied, as with the child of case 1, have been found to have a normal nuclear sex[5,9-13] On theoretical grounds it would be expected that half the children of an XXX female would have an abnormal sex-chromosome complement due to secondary non-disjunction. It is likely that selection against abnormal ova occurs in these cases.

Mechanisms of Sex Chromosome Aberration

The sex chromosome aberration might arise in a number of ways:

1. Because of non-disjunction in gametogenesis in either of the 2 stages of meiosis in either parent;
2. By chromosome loss due to anaphase lag;
3. By chromosome loss occurring between fertilization and first cleavage; and
4. By chromosome loss in early mitosis (mitotic non-disjunction) with elimination of the XO cell line.

Meiotic non-disjunction is probably the most usual mechanism. Simply stated, this may be considered to take place in the developing ovum, where during reduction division the two X-chromosomes, instead of separating and each migrating to its own pole and ultimately to separate daughter cells, they remain together and move into the same cell. An ovum with 24 chromosomes (22 autosomes and two X-chromosomes) finally results, and upon fertilization by an X-bearing sperm (22 autosomes plus X), a zygote is produced with a chromosome number of 47 - 44 autosomes and three X-chromosomes. Of course the number of triplo-X females detected in the population does not necessarily reflect the incidence of non-disjunction, since intra-uterine survival may be low.

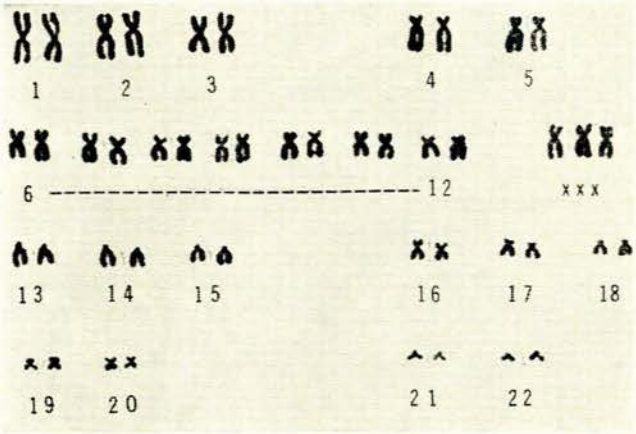

Fig. 1. Typical karyotype of the triplo-X female. There are 47 chromosomes present: there being 3 instead of 2 X-chromosomes.

The aetiologic factors underlying these mechanical events remain speculative. Parental age (so important in mongolism) does not seem to be significant in triplo-X cases but sufficient data is not yet at hand for adequate assessment.[8] The concentration of aneuploid conditions within certain families suggests that the aberrations are not random events.[14-16] The frequency of diabetes mellitus among the family members of some cases with chromosome abnormalities had led to the postulate that the pre-diabetic state may lead to meiotic or mitotic faults.[17] The association of auto-immune conditions and chromosome

aberrations is impressive but their exact relationship is not clear.[18,19]

Nothing is known of the genetic information carried by the autosomes. On the other hand, because of the XX/XY sex-determining mechanism in man, the study of sex-linked traits in appropriate pedigrees has paved the way for the spatial construction of a genetic map of the X-chromosome. Thus it is known that the genes for the Xg^a blood group, glucose-6-phosphate dehydrogenase (G6PD), deutan colour blindness and haemophilia A, lie along a segment of the X-chromosome in that order.[20] A given female receives one of her X-chromosomes from her mother (X^m) and one from her father (X^p). It is apparent, therefore, that in patients with abnormalities in the number of X-chromosomes, the use of one of the X-linked genetic

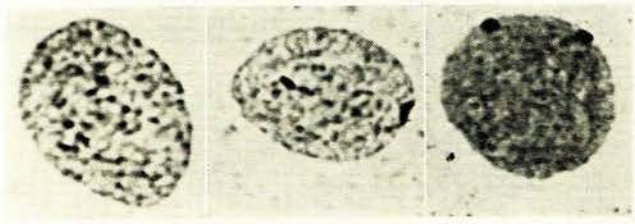

Fig. 2 *Fig. 3* *Fig. 4*

Fig. 2. Chromatin-negative nucleus seen in a buccal smear, stained with lacto-aceto-orcein (× 900).
Fig. 3. Chromatin-positive nucleus from a normal female (× 900).
Fig. 4. Chromatin double-positive nucleus as seen typically in a buccal smear from a triplo-X female (× 900).

markers may be useful in ascertaining in which parent the meiotic accident responsible for the aberration occurred. As an example, a case of XO Turner's syndrome was found to be G6PD deficient. The father was normal and the mother heterozygous normal (i.e. her one X-chromosome carried the G6PD deficiency gene) indicating that the offspring had received her only X-chromosome from her mother (i.e. an example of X^mO Turner's syndrome).[21] No cogent information has been forthcoming regarding the origin of the additional X in triplo-X females. G6PD levels were normal in the cases studied[22] and the use of the Xg^a blood group to chart the sequence of non-disjunction was not helpful, since all the offspring tested were $Xg^{a(+)}$.[23]

In somatic cells during mitosis, the XX bivalent is heteropyknotic. One X-chromosome is highly coiled and deeply staining. It is late reduplicating, as shown by tritiated thymidine studies, preferentially takes up a position against the nuclear membrane and, in part or whole, forms the sex-chromatin (Barr) body. The number of Barr bodies found in the nucleus is always one less than the number of X-chromosomes present. Thus, the normal XY male and XO Turner individuals have no chromatin body; the normal XX female and XXY Klinefelter cases have one; the XXX and XXXY individuals have two, and so on.

Lyon Hypothesis

The Lyon hypothesis suggests that part or all of one X-chromosome in the cells of the normal female become genetically inactive before the 16th day of embryogenesis, so that in some cells it is the paternal and in others the maternal X-chromosome which is suppressed. The normal female is thus a mosaic (a mixture of X^p and X^m) with regard to her active X-chromosome. Furthermore, it is the inactive X which constitutes the Barr body and all X-chromosomes in excess of one are similarly inactivated and appear as Barr bodies. The female, in terms of this hypothesis, has no greater number of functional X-chromosomes than does the male. In addition, the characteristics of X-linked recessive inheritance are in accord with the theory: although the heterozygous female has in all no more active X-chromosomes than the hemizygous male, only about 50% of her active X-chromosomes carry the peccant gene, rather than all of them, as in the affected male. Dosage compensation, implying that the normal female with two X-chromosomes has not more of certain proteins produced by sex-linked genes than does the normal male with only one X-chromosome,[24] can likewise be reconciled with the Lyon hypothesis. Such has in fact been shown to be the case for several gene products, one example being G6PD; here enzyme activity in females is the same as in males[20] and also the same in triplo-X females.[22] However, if Lyonization beyond the first X was total, then the number of X-chromosomes present should make no difference to phenotype; and XO, XX, XXX and XXXX should all (but do not) present the same clinical picture. The two possible theories to explain this—(1) that a part of the one X is not inactivated; and (2) that there is a small paired active segment on X and Y—are not entirely satisfactory.[25] Furthermore, Xg^a blood-group studies have not come out in support of the hypothesis.[31,32]

'Super' Females

With regard to the diagnosis of 'super' females, some degree of clinical suspicion may be aroused by oligophrenia or secondary amenorrhoea. Examination for Barr bodies in buccal mucosal smears is simple and rapid and may be carried out by the physician with a minimum of equipment.[1] Inspection of the polymorphonuclear leucocytes in a blood film provides useful ancillary evidence, since both the Barr body and the drumstick of mammals are true sex-chromatin and represent heteropyknotic X-chromosomes that are present in excess of one.[26] Finally, chromosome analysis using peripheral blood leucocytes, skin or bone marrow, is diagnostic. A method for leucocyte culture as used in the present cases by Dr. Clive Wallace, has been outlined previously.[1] Alternatively, a micro-method using only a drop of blood may be employed.[27] The mitotic X-chromosome is medium-sized with a submedian centromere. It is about the 6th or 7th longest chromosome and is placed in group C with autosomes 6 - 12, from which it cannot readily be differentiated. Chromosome 6 (with which the X is most easily confused) can often be positively identified by the presence of a narrow secondary constriction on its short arm, while the X often has a pale gap in its long arm adjacent to the centromere.[28] Marked secondary constrictions characterize autosomes 6 and 7, when cells are incubated in a calcium-free medium for the last 2 hours of culture.[29] The heteropyknotic X can be identified on autoradiograms using labelled thymidine and it also develops a 'fuzzy' outline if the slide is flamed before staining.[30] Accurate identifica-

tion of the X-chromosomes is not a crucial matter when one is purely concerned with the number of Xs in a cell. Correlation between the sex-chromatin findings and the karyotype is, however, essential.

SUMMARY

The genetic basis of sex has attracted particular attention in the last 5 years. Sex-chromatin surveys of mentally retarded populations have been especially fruitful in bringing to light aberrations of the sex-chromosome complement.

The first triplo-X females to be described in South Africa were discovered in a survey of mental defectives at a mental institution. The clinical and cytologic features of the syndrome are outlined.

Consideration is given to the genetic significance of the X-chromosome of man, the Lyon hypothesis and diagnostic methods.

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