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Xg Blood Groups of 78 Patients with Klinefelter's Syndrome and of Some of their Parents

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X-linked characters, such as the Xg blood groups (Mann *et al.*, 1962), can sometimes trace the origin of the X chromosomes in patients with too many or too few of them. Lindsten *et al.* (1963) applied Xg to a series of patients with Turner's syndrome with a 45,XO karyotype, as did Frøland *et al.* (1963) and Ferguson-Smith *et al.* (1964) to some cases of Klinefelter's syndrome. In this paper similar observations on a series of Danish patients with Klinefelter's syndrome are reported.

Subjects and Methods

The propositi are 78 males with sex chromosome abnormalities whose karyotypes were all determined by one of us (A.F.): 70 of the patients were referred because of endocrinological complaints, such as gynaecomastia, sterility, and hypogonadism. The remainder were found during sex chromatin screening of various groups of patients. The ages of the patients varied from 1 to 80 years; most of them were between 15 and 45.

In 41 patients chromosome studies were done on cultured blood cells only (Moorhead *et al.*, 1960), in 6 on fibroblast cultures only (Frøland, 1961), and in 31 both types of cell were cultured. Generally not less than 50 cells were examined from each culture.

All the patients had a normal number of autosomes. The sex chromosome complements were as follows: XXY: 68 patients, including a pair of monozygotic twins; XXXY: 2 patients; XXXXY: 4 patients; XX: 2 patients; XY/XXY: 1 patient; and XY/XX/XXY: 1 patient.

The Xg groups were done at the M.R.C. Blood Group Research Unit. The tests included both parents of 30 propositi, the father only of 4, the mother only of 12, and neither parent of 32; 19 other relatives were also tested.

The family relationships were checked by a number of autosomal blood groups (ABO, Rh, P, Lewis, Kell, and Duffy); these tests were usually done at the Statens Seruminstitut but sometimes by Dr. Mogens Hauge or at the Institute of Forensic Medicine, all in Copenhagen; haptoglobin tests were done by one of us (A.F.).

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Results

Six of the present patients have already been briefly reported (four by Frøland *et al.*, 1963, and two by Lewis *et al.*, 1964). Cytogenetical, endocrinological, and clinical investigations, as well as blood group tests, on 41 of the patients will be described in detail (Frøland, 1968). The Xg groups of relatives other than parents are not included in the present report for they give no additional information.

XXY Propositi. In Table I the distribution of the Xg groups in XXY patients and their parents is set out. Ten families give information about the parental origin of the X chromosomes of the patients.

(1) In two families the fathers and the Klinefelter sons are Xg(a+) and the mothers are Xg(a−), so the patients must have received an Xg^a gene, and therefore an X chromosome, from their fathers. This means non-disjunction had happened during spermatogenesis, with the production of an XY bearing spermatozoon. The formula for such persons is X^MX^PY (the superscript M indicating a maternal and P a paternal origin of the X chromosome). In two families the fathers are dead but the mothers are Xg(a−) and their XXY sons are Xg(a+): again this means that the sons have received an X chromosome from their father, who must have been Xg(a+). Thus, four X^MX^PY persons were demonstrated.

(2) Three other XXY propositi also have Xg(a+) fathers and Xg(a−) mothers, but the patients are Xg(a−) and cannot have received an X chromosome from their fathers. Patients having two maternal X chromosomes are designated X^MX^MY. The mothers of two Xg(a−) patients are dead, but the fathers are Xg(a+). Again the

TABLE I

Xg GROUPS OF 67 XXY KLINEFELTER PATIENTS* AND OF SOME OF THEIR PARENTS

Mating Type		Patients		Total
Father	Mother	Xg(a+)	Xg(a−)	
Xg(a+)	Xg(a+)	9	1 ($X^{M1}X^{M1}Y$)	10
Xg(a+)	Xg(a−)	2 ($X^{M}X^{P}Y$)	3 ($X^{M}X^{M}Y$)	5
Xg(a+)	Not tested	—	2*($X^{M}X^{M}Y$)	2
Xg(a−)	Xg(a+)	10	—	10
Xg(a−)	Xg(a−)	—	—	—
Xg(a−)	Not tested	1	—	1
Not tested	Xg(a+)	7	—	7
Not tested	Xg(a−)	2 ($X^{M}X^{P}Y$)	2	4
Not tested	Not tested	24	4	28
	Total	55	12	67*

* A monozygotic $X^{M}X^{M}Y$ twin has been deducted.

fathers cannot have transmitted an X chromosome to their sons. So five $X^{M}X^{M}Y$ patients were found (the pair of monozygotic twins is counted as one propositus).

(3) In one family both parents are Xg(a+), whereas the son is Xg(a−). The mother must be heterozygous, *Xg*^{a}*Xg*, and her son must have inherited her *Xg* locus *in duplo*. This can be described by the formula $X^{M1}X^{M1}Y$ (or $X^{M2}X^{M2}Y$).

Propositi of Other Karyotypes. Table II shows the Xg groups of Klinefelter patients with other types of sex chromosome anomalies and the groups of some of their parents. The parental groups of one of the four XXXXY patients are informative (previously mentioned by Lewis *et al.*, 1964): the father is Xg(a+), the mother Xg(a−), and the son Xg(a−). Evidently the four X chromosomes of the son are all of maternal origin.

Discussion

The abnormal sex chromosome constitutions in Klinefelter's syndrome are produced by non-disjunction, either during spermatogenesis or oogenesis, or at an early cleavage division of the fertilized ovum.

TABLE II

Xg GROUPS OF 10 KLINEFELTER PATIENTS WITH KARYOTYPES OTHER THAN XXY, AND OF SOME OF THEIR PARENTS

Karyotype	No. of Patients	Father	Mother	Patient
XXXY	2	Not tested	Not tested	Xg(a+)
XXXXY	1	Xg(a+)	Xg(a+)	Xg(a+)
XXXXY	1	Xg(a+)	Xg(a−)	Xg(a−)
XXXXY	2	Xg(a−)	Xg(a+)	Xg(a+)
XX	1	Xg(a+)	Xg(a+)	Xg(a+)
XX	1	Not tested	Xg(a+)	Xg(a+)
XY/XXY	1	Not tested	Not tested	Xg(a+)
XY/XX/XXY	1	Not tested	Not tested	Xg(a+)

(1) $X^{M}X^{P}Y$. This formula reflects non-disjunction during spermatogenesis. As the two X chromatids normally separate from the Y chromatids during the first meiotic division, the error can be traced to that stage. This mechanism is demonstrated when an XXY son is Xg(a+) and the mother is Xg(a−).

(2) $X^{M}X^{M}Y$. This formula is less discriminating than $X^{M}X^{P}Y$, as it may result from any of several abnormal mechanisms: non-disjunction during the first meiotic division, or non-disjunction during the second meiotic division, or mitotic non-disjunction during an early cleavage division of a normal XY zygote with subsequent loss of YO cells. One or another of these types of maternal origin of the two X chromosomes is demonstrated when fathers are Xg(a+) and XXY sons are Xg(a−).

(3) $X^{M1}X^{M1}Y$. This formula means that the *Xg* loci of both maternally derived X chromosomes are identical. It can be produced in the following ways: (a) non-disjunction during the first meiotic division of oogenesis accompanied by crossing-over between the *Xg* locus and the centromere; (b) non-disjunction during the second meiotic division without preceding crossing-over; (c) mitotic non-disjunction of an XY zygote. When both parents are Xg(a+) and the XXY son is Xg(a−) the $X^{M1}X^{M1}Y$ type is demonstrated.

(4) $X^{M}X^{M}X^{M}X^{M}Y$. Several explanations are possible: four maternal X chromosomes could have resulted from two sequent non-disjunctions during oogenesis, or from two sequent mitotic misdivisions, or from a combination of meiotic and mitotic errors. The $X^{M}X^{M}X^{M}X^{M}Y$ type is demonstrated when the patient is Xg(a−) and his father Xg(a+).

The literature records how the source of the X chromosome has been determined by the Xg groups in two other types of sex chromosome anomalies associated with Klinefelter's syndrome as follows.

(5) $X^{M}X^{P}YY$. De la Chapelle *et al.* (1964b) and Pfeiffer *et al.* (1966) each record a family in which an XXYY patient and his father were Xg(a+) and the mother Xg(a−). The most likely explanation is non-disjunction at the first and the second meiotic division of paternal spermatogenesis, giving rise to an XYY sperm which fertilized a normal ovum. Mitotic misdivision of the Y chromosome in an $X^{M}X^{P}Y$ zygote is, however, also possible.

(6) $X^{M}X^{M}$. An apparently normal female karyotype is sometimes found in Klinefelter's syndrome. De la Chapelle *et al.* (1964a) observed an Xg(a−) phenotype in a patient and his mother. The father was Xg(a+). Both X chromosomes must be of

maternal origin. A second such family is recorded by De la Chapelle *et al.* (1965). It is possible that an X^MX^MY zygote was originally assembled, and that the Y chromosome was subsequently lost due to mitotic anaphase lagging. There is, however, another possibility which at present seems the more likely. During the past six years the Blood Group Research Unit has been sent samples of blood from 17 XX males whose Xg distribution fits that expected of males and differs significantly from that expected of people with two Xs ($p = 1$ in 200). If this proves not to be a chance departure it will strongly indicate that XX males have a single *Xg* locus. This would fit well with Ferguson-Smith's (1966) thesis that XX males have one normal X and one X which carries some genetic material interchanged from a Y chromosome. (The complementary arrangement, X material on a Y, has been invoked by Sanger *et al.*, 1964 and by Noades *et al.*, 1966, to explain certain rare families in which the gene for the antigen Xg^a appears to be carried on the Y.)

Of the 67 XXY propositi, 55 are Xg(a+) and 12 Xg(a−). According to Noades *et al.* (1966), among 67 males of the mainland of northern Europe 40·2 would be expected to be Xg(a+) and 26·8 Xg(a−). The two distributions are significantly different ($p = 1$ in 4,000). On the other hand, 67 women would be expected to be divided into 56·3 Xg(a+) and 10·7 Xg(a−), which is very close to the distribution found in the patients.

This similarity between the Xg distribution of the XXY patients and normal females must mean that the frequency of $X^{M1}X^{M1}Y$ (or $X^{M2}X^{M2}Y$) is rather low, as the Xg blood group distribution in such individuals should be that expected of males.

Relative Frequencies of X^MX^PY and X^MX^MY. One of the four possible Xg mating types is always informative, and this parental combination, Xg(a+) father and Xg(a−) mother, is found in nearly 10% of the matings of northern Europeans (Noades *et al.*, 1966): from it the Xg(a+) XXY sons are of the X^MX^PY type and the Xg(a−) XXY sons are X^MX^MY. The only other combination which is informative is an Xg(a−) XXY son of an Xg(a+) father and a heterozygous Xg^aXg mother; he is $X^{M2}X^{M2}Y$.

Mathematical formulae, based on the method of maximum likelihood, have been worked out to estimate, from the Xg results, the proportion of X^MX^PY, $X^{M1}X^{M2}Y$, and $X^{M1}X^{M1}Y$ plus $X^{M2}X^{M2}Y$ (Fraser, 1963). The application of these tests to a series of 190 XXY men and both parents of 50 of them was reported by Edwards *et al.* (1966); in 7 of these families the Xg groups had given direct information of the origin of the X chromosomes. It was calculated that the proportion of X^MX^PY patients was 0·36, of $X^{M1}X^{M2}Y$ 0·42, and of $X^{M1}X^{M1}Y$ (or $X^{M2}X^{M2}Y$) 0·22: these proportions are tentative only, for the mathematical tests require larger numbers.

The proportion of patients with X^MX^PY and X^MX^MY constitutions in the present material is four to five, if the four cases are included where the Xg groups of a missing parent can be deduced from those of the other parent and the patient (see Table I).

Parental Age and Source of X Chromosomes in Klinefelter's Syndrome. It is generally found that the maternal age at the birth of patients with Klinefelter's syndrome is significantly higher than in the average population (Ferguson-Smith, 1958; Lenz, 1959).

Table III gives the parental ages for those patients in the present series where the Xg groups have given information about the origin of the X chromosomes: the ages in the class due to paternal non-disjunction do not differ very much from the ages in the class with two maternal X chromosomes.

From official records the average age of 62 fathers at the birth of XXY patients in the present series was 34·9 years and of 64 mothers 31·6 years. It is interesting to note that the average maternal age at the birth of the patients, both in the X^MX^PY class and in the X^MX^MY class, is closer to that of the Danish population (29·4 years) than to that found in the patient series as a whole. This may be so partly because the number of patients having a

TABLE III

PARENTAL AGE AT BIRTH OF 11 KLINEFELTER PATIENTS THE ORIGIN OF WHOSE X CHROMOSOMES WAS MADE CLEAR BY Xg GROUPS

Patient	Age at Birth of Patient	
	Father	Mother
X^MX^PY	22	16
X^MX^PY	30	32
X^MX^PY	44·	36
X^MX^PY	30	27
Mean	31·5	27·8
X^MX^MY	39	41
X^MX^MY	36	31
X^MX^MY	30	19
X^MX^MY	32	30
X^MX^MY	36	30
$X^{M1}X^{M1}Y$	30	27
Mean	33·8	29·7
$X^MX^MX^MX^MY$	40	32

known origin of the X chromosomes is rather small, and partly because the probability of the parents being alive, and so available for grouping, is decreasing with increasing parental age. The average age of 39 mothers at the birth of the XXY in the present series was 31·0 years when one or both parents had been tested; when neither parent had been tested the average age of 25 mothers was 32·5 years. The corresponding paternal ages showed no difference: 35·3 years and 34·7 years, respectively, in the two groups.

The possibility of introducing a sampling error of this type demands great caution when estimating the influence of paternal or maternal age on the aetiology of the XXY syndrome, except when the propositi are very young.

Summary

The Xg blood groups of a series of 78 Danish patients with Klinefelter's syndrome are reported together with those of their available parents. In 11 patients the origin of the X chromosomes could be traced. In four patients with a 47,XXY karyotype the X chromosomes were derived from both parents (X^MX^PY), showing that the non-disjunction had happened during spermatogenesis. In six patients both X chromosomes were of maternal origin (X^MX^MY), due to non-disjunction either during oogenesis or at an early postzygotic division. In one patient with a 49,XXXXY karyotype all four X chromosomes were evidently of maternal origin.

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