A Marker X Chromosome

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Descriptive human cytogenetics is entering a new and important phase. The major trisomies and monosomies are well described but generally appear as isolated, unpredictable, and hence unpreventable events, except when a large translocation is known. New syndromes due to smaller cytogenetic changes continue to emerge. In addition, from 3% to 5% of the population had an identifiable chromosomal variant in the Edinburgh survey (Court Brown *et al.*, 1966), and the number and percentages of variants can be expected to increase as preparative techniques continue to improve, quantitative karyotyping becomes routine, and large numbers of persons are studied.

The process of determining the clinical significance of these variants is complicated and variable. The finding of the same cytogenetic variant in both normal and abnormal phenotypes does not establish normality, since neither duplication-deficiency nor small translocations leading to an increased frequency of aneuploidy in the next generation can be ruled out without enough clinical data to treat statistically.

The marker X chromosome described in this paper offers an additional mechanism by which the expression of the effects of a chromosomal variant may vary from generation to generation or individual to individual and permits several basic observations about the behavior and identification of the human X chromosome. Such heritable variants of the human karyotype may prove to be the most important group of cytogenetic abnormalities both because they are common and because they may permit prevention of clinical disease by identifying high-risk marriages and allowing subsequent amniocentesis and abortion of abnormal fetuses if requested by the family.

TECHNIQUES

Metaphase preparations were prepared from cultured leukocytes by a microtechnique (Hungerford, 1965) using colcemide (5×10^{-4} mg/cc) for two hours, 0.075 M KCl, and 1.5% orcein staining. The high quality of the metaphases produced by this technique undoubtedly was instrumental in detecting the chromosome variant in the present family. Four hours prior to harvesting, H^3-thymidine was added to one bottle from each culture. The slides were dipped in NTB-3 emulsion and exposed for three to seven days. Appropriate metaphases were photographed both by bright-

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field and phase-contrast illumination. The latter was used for the illustrations because it afforded a better representation of the chromosomes under the exposed emulsion.

A Leitz 6× magnifying lens with a scale marked to 0.1 mm and 8 × 10-inch enlargements were used for quantitative measurement of arm lengths. Each of the 46 chromosomes in 20 cells was measured and individual measurements were normalized by expressing each arm as a per cent of the total length of the 46 chromosomes. The mean diameter of the C-chromosome satellite was added to the centromere-secondary constriction measurement to determine the length of the long arm.

Colorblindness was tested by the H-R-R pseudoisochromatic plates and the Dvorine color plates. Glucose-6-phosphate dehydrogenase (G6PD) typing was carried

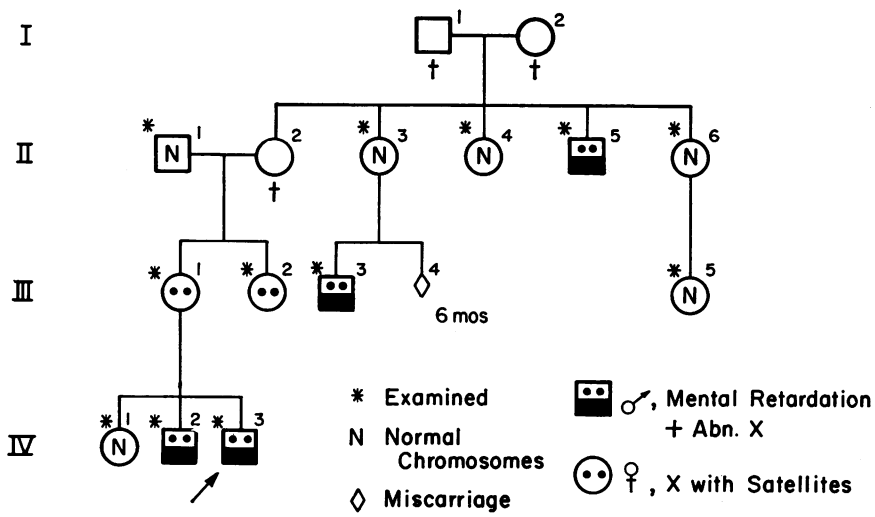


FIG. 1.—Pedigree showing that each of the four retarded males had the marker X chromosome. Two females with the chromosome were normal. A third (II-3) must have transmitted the chromosome to her son, but the secondary constriction was not observed in 250 cells.

out by Dr. T. Shows (Department of Biology, Yale University) and Xm determination by Dr. A. G. Bearn (Department of Medicine, the New York Hospital–Cornell Medical Center). Dr. F. Allen (New York Blood Center) carried out typing studies for Xg^a and other blood groups.

FAMILY DATA

The proband (Fig. 1, IV-3) was referred for chromosome study because of mental retardation and multiple minor anomalies. Because of the finding of a previously undescribed chromosome variation in the proband, a family study was undertaken to determine the possible clinical and genetic significance of this observation. Four male members of the family were found to have borderline-to-severe mental retardation and each of the four studied had the same variant chromosome. In two of these, however, there were additional possible environmental causes for retardation. None of the three females with this chromosome was retarded. There was no consanguinity.

Only the proband had congenital anomalies, and there was only one known miscarriage. None of the affected males was married. All living family members were examined, but only the details of the examinations of the four affected males will be presented since the examinations of other family members were unremarkable. Developmental evaluations of the proband and both siblings were carried out by Drs. S. Provence, M. McGarry, and M. Thomas (Yale Child Study Center).

(1) *II-5. Age 56 years.* Each member of the family questioned reported him to be "slow" mentally. He completed eighth grade and has held only menial jobs. Each of his sisters graduated from high school and holds a responsible job. His I.Q. in 1965 was 70 (Wechsler). There was little scatter in the tests, although his best scores were in tests involving information and comprehension, the lowest in tests requiring generalizations. In the digit symbol tests, many symbols were reversed when copied. His medical history was unremarkable and there were no congenital anomalies.

(2) *III-3. Age 29 years.* He was severely retarded but able to speak a few intelligible words and care for himself. His mother stated that the retardation followed encephalitis at five years of age and that subsequent X-ray studies had shown severe brain damage. The records of the physicians caring for him, however, showed only that he was found unconscious and had seizures. No encephalitis was recorded. The hospital where the studies were said to have been carried out had no record of his admission. Examination revealed prominent maxillae, low-set large ears, cogwheeling arm movements, and rigidity with no other anomalies. As judged from the family photograph album, his unusual appearance was not evident until he was eight years old. It is unclear whether he simply had a seizure during a febrile illness and that retardation was only noticed subsequently or whether he indeed had superimposed damage from encephalitis, but it seems reasonable to attribute at least part of his retardation to the presence of the marker X chromosome.

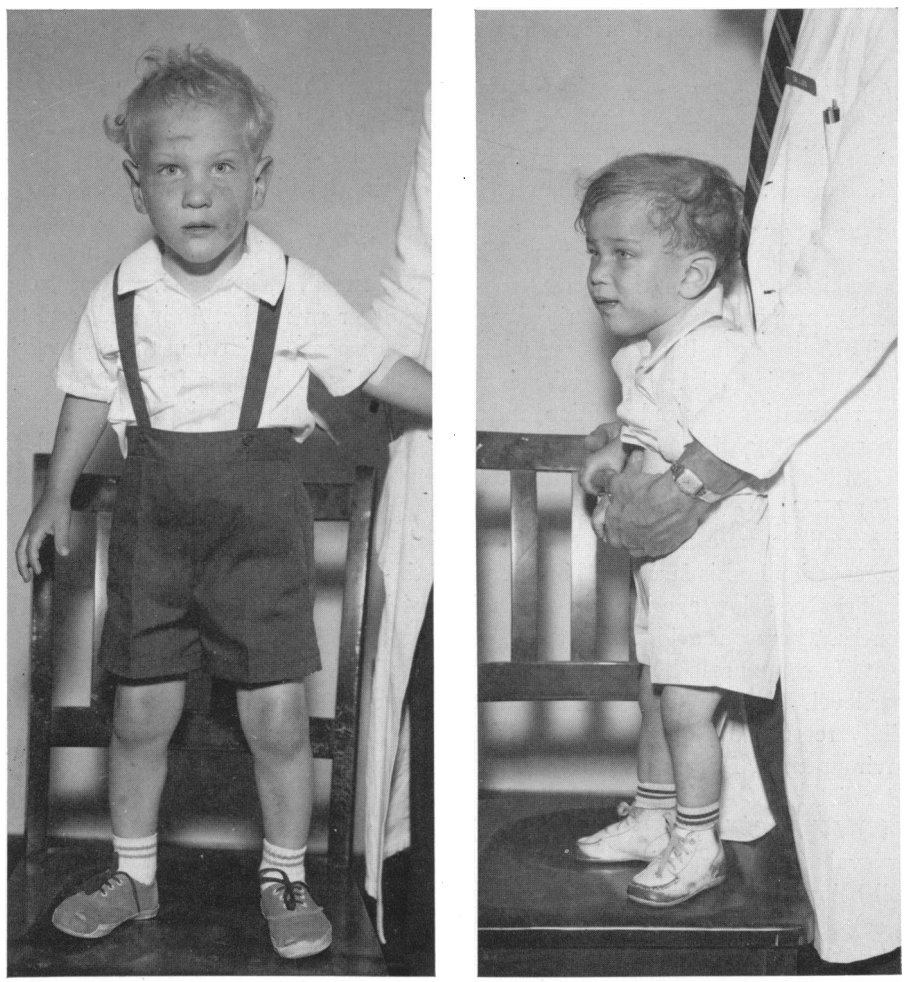
(3) *IV-2. Age 3 years.* Older brother of the proband (Fig. 2*a*). No anomalies or abnormalities were noted on examination, although there was an area of brown pigmentation on his face, and his gait was "stiff-legged" with a broad base. He was significantly below age level in all sections of his developmental evaluation at 33 months. There was evidence of considerable emotional and intellectual impoverishment. In some areas he functioned at a borderline level and in others at the two-year level. His vocabulary was limited to five words. He was considered to be only mildly retarded and to have a significant organic component to his retardation. Environmental factors also may have contributed to his poor performance. His older sister (IV-1) performed well within her age range at three years, 10 months.

(4) *IV-3 (Proband, YNHH 65-91-42).* He was first admitted at one year of age for evaluation of physical and mental retardation. His height and weight were well below the third percentile, although his birth weight had been normal (7 lb, 4½ oz). At that time the following anomalies were noted: unusual appearance characterized by microcephaly (43 cm, < third percentile), flat nasal bridge with wide, flared nostrils and a left esotropia; frenulum attached to the anterior portion of the tongue; right inguinal hernia; and limited motion at the elbows, knees, and hips (Fig. 2*b*). There was slight spasticity which remained present at two years

of age. The developmental examination showed relatively greater retardation: his motor development was at the 36–40 week level and adaptive behavior at the 40–44 week level, with a wide scatter from 28 to 54 weeks. He did not talk and had a limited language comprehension. Laboratory evaluation revealed only a generalized aminoaciduria and an unusual secondary constriction on a group C chromosome.

CHROMOSOME STUDIES

In one-third of the proband's metaphase figures, an unusual secondary constriction was seen at the ends of the long arm of a group C chromosome (Fig. 3). This gave the appearance of large satellites, but the chromosome was never seen in association with the acrocentric chromosomes. The diameter of these satellites was less



a *b*

FIG. 2*a*.—Proband's older brother.
FIG. 2*b*.—Proband.

than the width of the adjacent chromatid but greater than the largest acrocentric satellite. Although most easily recognized when it appeared as in Figure 3, it also appeared as a single centrally placed satellite (probably only apparent fusion caused by lack of resolution of two very close satellites) or with the displacement of one satellite to the other chromatid (Fig. 4). Whether the latter appearance represents a break or displacement due to uncoiling is not certain, but a pair of double fragments the size of these satellites which was not adjacent to the C chromosome was seen in only two cells of several thousand examined. Similar fragments are occasionally seen and cannot be taken as proof of deletion. In a few cells, tandem satellites were present (Fig. 4).

The frequency with which this secondary constriction or any of its various forms described above were found in family members is shown in Table 1. None were observed in II-3, whose son had the secondary constriction, although 250 excellent cells from three cultures over a four-month period were scored and many more were observed during scanning. She must have had the same unusual X chromosome since she transmitted it to her son, unless a factor not located on the X chromosome is responsible for the presence of the constriction. The secondary constriction, however, persisted over a six-month period in the proband (IV-3) and his mother (III-1). Exact counts of the frequency of the distribution of this constriction were made in

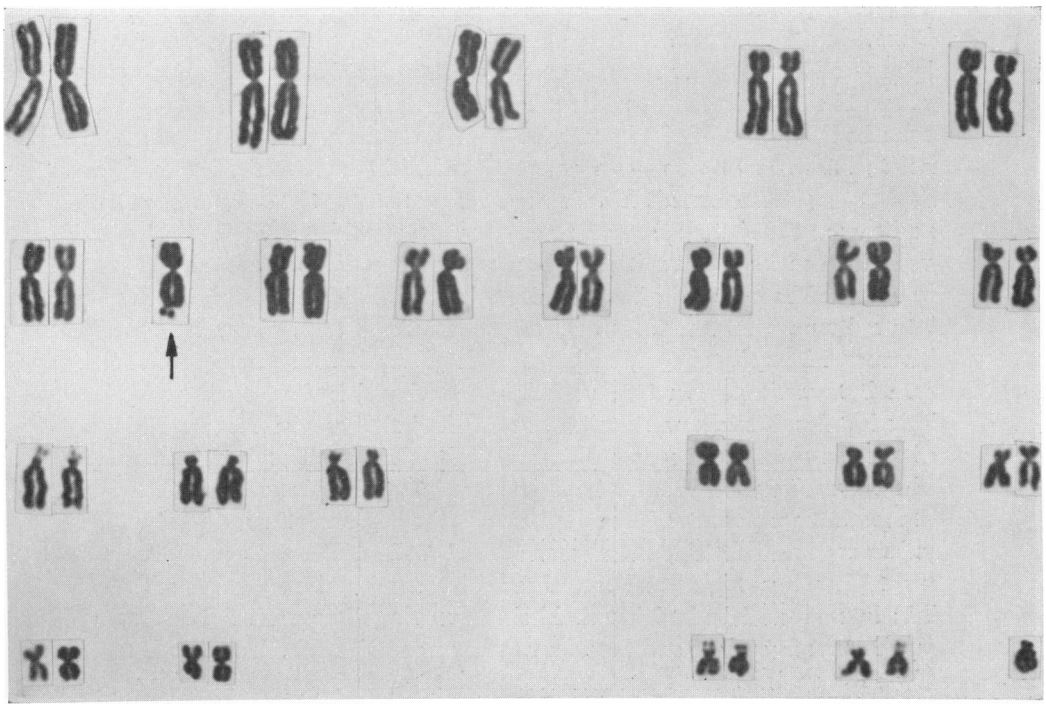


FIG. 3.—Karyotype from the proband showing the marked secondary constriction present in one-third of his cells. The resulting satellites are smaller in diameter than the chromatid but larger than any of the satellites on the acrocentric chromosomes.

only one culture from each of these individuals, however. The lowest observed frequencies were 1% and 3%, respectively, in two cultures from III-2. With this exception, the frequency of the constriction varied between 12% and 33%. There was no apparent correlation between sex or age and the frequency of the marker X. The reason for the low frequency of detectable marker X chromosomes in some family members is unclear, but it is important in studying similar families to be

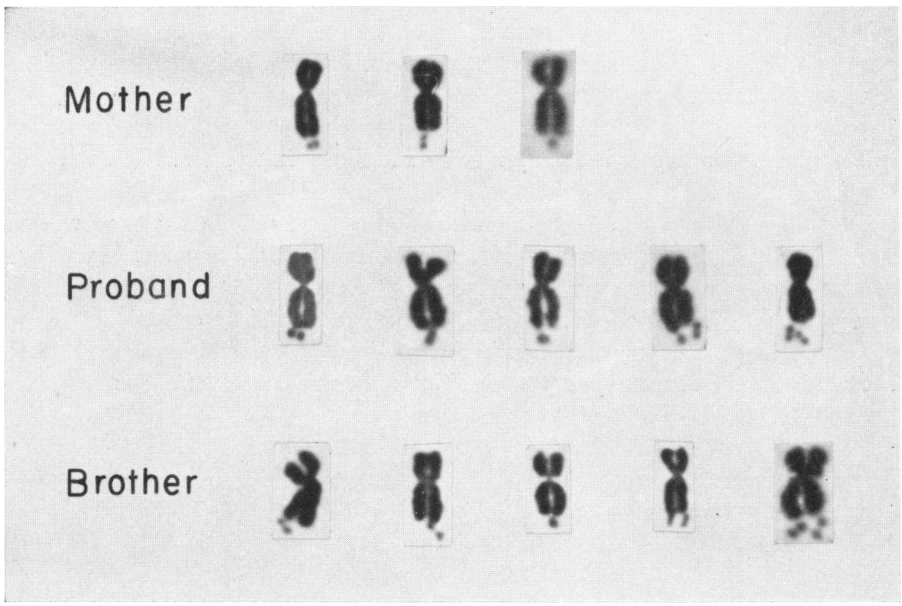


FIG. 4.—The satellites appeared symmetrically at the end of the chromatids (*left*), with displacement of one satellite to the other chromatid (*left center*), as a single centrally placed satellite (*center*) or rarely as tandem satellites (*right*).

TABLE 1

NUMBER OF CELLS WITH MARKER X CHROMOSOME

Pedigree Number	Age	Sex	Number of Cells Studied	Number with Secondary Constriction
II-1	53	♂	50	0
3a	59	♀	100	0
b	.....	.....	100	0
c	.....	.....	50	0
4	57	♀	100	0
5	56	♂	81	12
6	41	♀	100	0
III-1	24	♀	100	28
2a	22	♀	100	3
b	.....	.....	100	1
3	29	♂	100	18
5	14	♀	50	0
IV-1	4	♀	100	0
2	3	♂	100	13
3	2	♂	100	33

aware that a negative study does not rule out the presence of the marker chromosome. This low frequency may have contributed to the relatively long delay in detection of this variant, and it is of interest that it was first observed in the individual culture which had the highest frequency of secondary constrictions. The frequency of this variant, however, is probably very low since it has not been seen in 4,000 consecutive newborns, and was seen only once in 1,500 routine diagnostic cultures (Lubs, unpublished data). Only two cells have been karyotyped in each of the newborns, however, and this constriction may be detected subsequently when additional cells are studied.

Labeling Studies

The size and arm ratio of the chromosome (Table 2) as well as the presence of retardation only in males with the secondary constriction suggested that the chromosome might be the X chromosome. Twenty cells from III-1 were observed in which both the secondary constriction and a single late replicating C chromosome were present. In six of these 20 cells, the C chromosome with the secondary constriction was the most heavily labeled chromosome (Fig. 5). This was felt to be additional strong evidence that the secondary constriction was on the X chromosome.

Eighteen cells from the proband and his brother, in which both the secondary constriction and differential labeling of the chromosome with the constriction were present, were also examined. In nine of these, the long arm was heavily labeled but the short arms and satellites were unlabeled (Fig. 6). In eight cells a similar pattern was found with the short arms being only lightly labeled and the long arms being more heavily labeled, but the differential pattern of labeling was not as marked. Only one cell was observed in which the short arm and satellites were heavily labeled and the long arm was unlabeled. Unfortunately, the usefulness of these data may be limited by the occasional occurrence of a similar pattern of labeling in No. 6. A similar pattern of labeling was noted in III-1 when the marker X was not the most heavily labeled C chromosome.

It was possible to compare the length of the earlier replicating, satellited X chromosome with the late replicating, unsatellited X chromosome in 10 cells. No evidence of differential contraction was found: the heavily labeled X was the shorter homologue in six cells and the longer in four.

Arm-Length Measurements

Since the secondary constriction in the long arm of this chromosome presented difficulties in measurement, an attempt was made to determine the usefulness of the short-arm measurements in identifiying the X chromosome. Twenty metaphases with the constriction and comparable in quality to that in Figure 3 were measured from the proband and his brother and were normalized as described above under Techniques. The mean normalized values for the C group are shown in Table 2. As can be seen in Figure 3 and Table 2, only Nos. 6, 7, and 11 have short arms comparable in length to the chromosome with the secondary constriction. Several standard arm-ratio methods of analysis were utilized, but the best discrimination resulted from ranking the short arms of the marker X in relation to the three pairs of chromosomes with the longest short arms in each metaphase (Fig. 7). The short

TABLE 2

SIZE AND ARM RATIOS OF THE GROUP C CHROMOSOMES

GROUP	TOTAL LENGTH				LONG ARM				SHORT ARM				ARM RATIO			
C	M	SD	CV	SE	M	SD	CV	SE	M	SD	CV	SE	M	SD	CV	SE
6.....	2.827	0.084	0.030	0.013	1.746	0.079	0.045	0.012	1.084	0.053	0.049	0.008	0.617	0.017	0.028	0.003
X.....	2.610	0.143	0.055	0.032	1.596	0.119	0.074	0.027	1.018	0.063	0.062	0.014	0.610	0.021	0.035	0.005
7.....	2.570	0.102	0.040	0.016	1.559	0.090	0.058	0.014	1.016	0.056	0.055	0.009	0.605	0.020	0.033	0.003
8.....	2.356	0.115	0.049	0.018	1.521	0.098	0.064	0.015	0.838	0.069	0.083	0.011	0.644	0.024	0.038	0.004
9.....	2.406	0.114	0.048	0.018	1.579	0.097	0.061	0.015	0.832	0.068	0.081	0.011	0.655	0.023	0.036	0.004
10.....	2.260	0.091	0.040	0.014	1.540	0.085	0.055	0.013	0.724	0.068	0.094	0.011	0.680	0.026	0.039	0.004
11.....	2.220	0.102	0.046	0.016	1.315	0.082	0.062	0.013	0.909	0.072	0.079	0.011	0.591	0.025	0.043	0.004
12.....	2.195	0.071	0.032	0.011	1.450	0.074	0.051	0.012	0.750	0.064	0.086	0.010	0.660	0.027	0.041	0.004

NOTE.—M = mean of 20 measurements; SD = standard deviation; CV = coefficient of variation of mean; SE = standard error of mean.

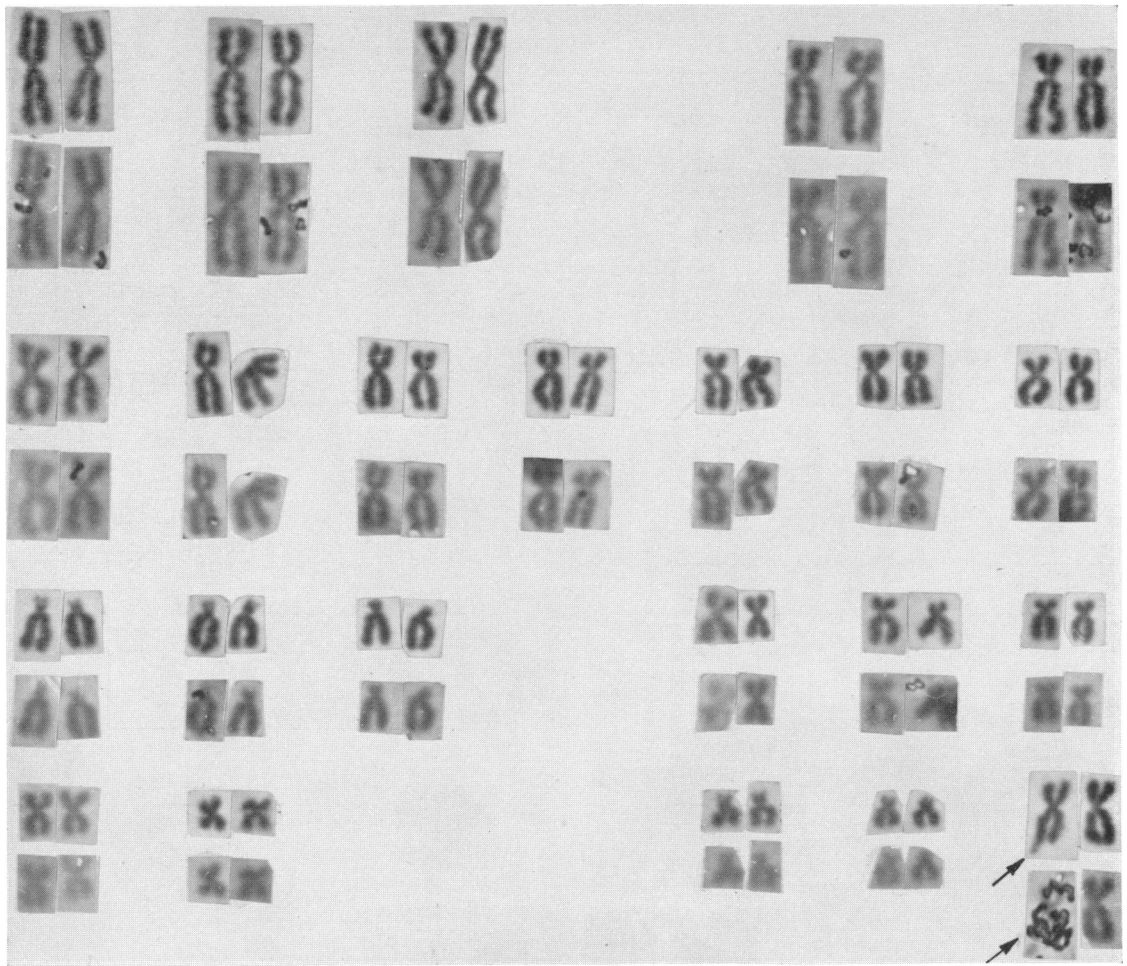


FIG. 5.—Autoradiograph from III-1. The karyotype (photographed by phase microscopy) with the emulsion in place is shown below the usual karyotype. The only chromosome with heavy labeling also was the chromosome with satellites (*lower right*).

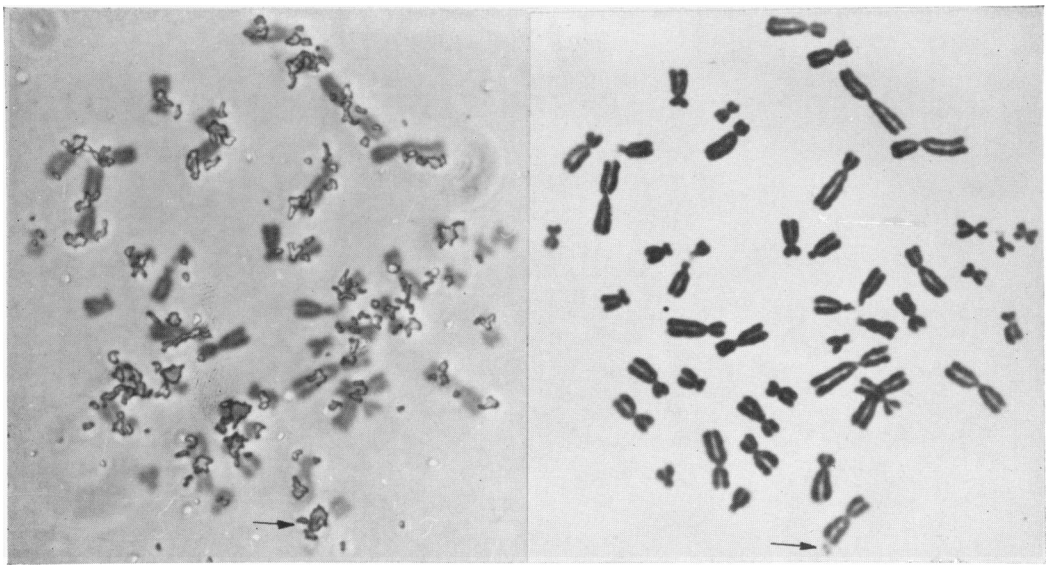


FIG. 6.—Autoradiograph and metaphase cell from a male family member (III-2) showing heavy labeling over the long arm of the C chromosome with satellites, and absence of label over the short arms and satellites of the same chromosome.

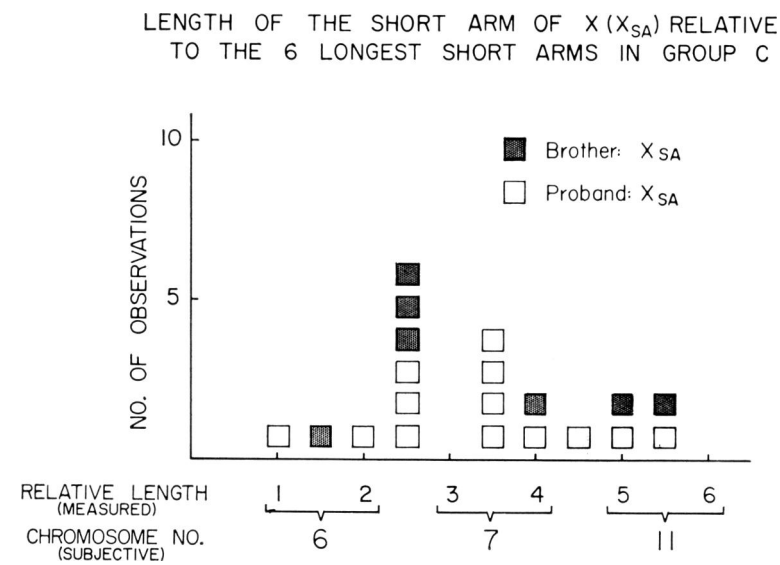


FIG. 7.—Comparison of short-arm measurements of the marker X chromosome with the six longest short arms in group C. As shown on the bottom line, these six chromosomes were subjectively karyotyped as the No. 6, 7, and 11 pairs. The short arm of the marker X chromosomes was ranked most frequently between the second and third of these chromosomes.

arm of the X fell between the second and third of these chromosomes (karyotyped as Nos. 6 and 7) in six of 20 cells and was as large as the No. 7 short arm in 15 of 20 cells. The variation in short-arm size was great, but both the ranking technique and the means of the short arms (Table 2) demonstrated that the X chromosome was very similar in size to No. 7. The reason for the cell-to-cell variation was not apparent on inspection of the cells. The relative size of the satellited C chromosome did not vary consistently with either the extended or the contracted state of the metaphase chromosomes, and peripheral distortion due to air drying appeared to account for only part of this variation.

LINKAGE STUDIES

The family data were uninformative with respect to colorblindness and G6PD, since no variants were found in either case. All family members tested were Xg(a+). Some information can be gained by considering the *Xm* locus, since two family members were Xm(a+) (Table 3). Certain assumptions or deductions, however, must

TABLE 3

RESULTS OF XM TYPING

Member	Xm	Member	Xm
II-1	−	III-2	+
II-3	−	III-3	−
II-4	+	III-5	−
II-5	−	IV-1	−
II-6	−	IV-2	−
III-1	−	IV-3	−

be made with respect to two family members who were no longer living (I-2 and II-2), including the presence of the marker X, an Xm^a/Xm genotype, and paternity as shown in the pedigree. Both of their husbands were Xm(a−) and contributed morphologically normal X chromosomes with an *Xm* allele to their daughters. (This was demonstrated in II-1, but must be assumed in I-1, who was not retarded and had Xm[a−] daughters.) In both generations II and III, therefore, one daughter must have received an Xm^a marker X (II-2 and III-2) and one an *Xm* marker X (II-3 and II-1) from these mothers. The family data are consistent with these assumptions. I-2, II-2 (and II-3), for example, must have had the marker X since they transmitted it. Taking into account the uncertainties mentioned above, the best interpretation of these data is that crossing over occurred in both generations, and it is unlikely that the *Xm* locus is closely linked to the marker secondary constriction at the end of the long arm of the X chromosome.

DISCUSSION

Initially, it appeared that the secondary constriction was not clinically significant since it was present both in a normal mother and her abnormal son. Family studies, however, demonstrated a pattern of mental retardation consistent with X-linked

recessive inheritance in three generations. Each of the four retarded males had the unusual chromosome, which was shown to be the X chromosome by H^3-thymidine labeling studies, and this chromosomal variant may have caused the pattern of X-linked retardation. There are three possible explanations for the pattern of retardation: a recessive gene producing retardation which is closely linked to the secondary constriction, an effect of the secondary constriction itself, or variable deletion at the site of the constriction during development. A clear distinction between these possibilities cannot be made at present. Several large pedigrees consistent with X-linked recessive mental retardation have been published (Allan and Herndon, 1944; Allan *et al.*, 1944; Martin and Bell, 1943). There is, however, no characteristic clinical picture, although muscular wasting was also present in the family reported by Allan *et al.* (1944). None of these families has had chromosome studies. Retardation was less severe in the present family and no muscle wasting was present. The syndrome described by Lesch and Nyhan (1964), and recently by Seegmiller *et al.* (1967), is a different syndrome clinically. If the retardation is due to the secondary constriction itself, it would represent a new type of cytogenetic abnormality in man. The observation of the same constriction in other families with retarded males in several generations would support this hypothesis, but a number of such families will have to be studied since the constriction is uncommon. Precedent for deletion at the site of a secondary constriction has been provided by two families with definite deletion at sites of similar constriction in chromosomes Nos. 8 and 16 (Lubs, 1969), and by the recent report of Day *et al.* (1967) of a similar deletion of No. 18 in lymphocytes. Fragments consistent in appearance with an origin by deletion distal to the constriction were seen in only two of several thousand cells examined in this family, and the deletion itself would be too small to detect directly by inspection of measurement. While deletion remains a possible explanation, it is unproven.

Several investigators have described a similar appearance in a C chromosome in cells derived from Burkitt's lymphoma cells and mononucleosis cells (Kohn *et al.*, 1967, 1968; Miles and O'Neill, 1967). The constriction was placed on chromosome No. 10, however, not the X chromosome. In the present family, the presence of the constrictions in only one homologue and the X-linked pattern of transmission of mental retardation argue against a persisting viral infection, but the possibility that the chromosomal constrictions are in some way related to a viral infection remains to be investigated.

The difficulty in evolving a quantitative system permitting identification of a particular chromosome in every cell or even in the majority of cells is well demonstrated by the standard deviations and other results shown in Figure 7 and Table 2. The data can, however, be used to make some statement about the size of the X chromosome. In no case was the short arm of X longer than No. 6, although in three of 20 instances it was equal to No. 6. Similarly, the mean total length, including the satellites, was less than No. 6, and there seems little justification for placing the largest C chromosome as the X in karyotypes. The data agree with previous labeling studies (Bishop *et al.*, 1965; German, 1964) in identifying the X chromosome as being similar to No. 7. The observation that the late replicating and earlier replicating X's were similar in size supplements German's data and permits generalization

of his findings to both X chromosomes. There was, however, no obvious correlation between the total measured length of the chromosomes in a particular cell, that is, the degree of compaction, and the relative position of the X chromosome in group C, although German showed that with longer time in colcemide the X chromosome was occasionally similar to No. 11 in size. The smaller number of cells in the present study probably accounts for this difference. It is also of interest that there was no preferential late labeling of this possibly abnormal X chromosome as occurs with ring and isochromosome X's.

The finding of a marker X chromosome has permitted observations which have added to the knowledge of the biology of human X chromosomes and some information about X linkage. The most immediate medical consequence of these studies has been the opportunity to offer amniocentesis and therapeutic abortion to two women in this family if the marker chromosome is found in a male fetus.

SUMMARY

A marker X chromosome with a secondary constriction near the end of the long arm giving the appearance of large satellites was described. This marker chromosome permitted the identification of the X chromosome in males and both X chromosomes in heterozygous females for the first time. Quantitative studies demonstrated that the marker X chromosome was similar to No. 7 in size.

Four males in three generations had the marker X chromosome in 10%–33% of cells and also had mild to severe mental retardation. Either the secondary constriction itself or a closely linked recessive gene may account for the pattern of X-linked inheritance. Detection of this marker chromosome *in utero* in this family would permit identification of future male offspring with a high probability of mental retardation.

The marker chromosome permitted the pattern of replication of the non-late replicating X chromosome to be studied in both males and females and for certain comparisons to be made between the two X chromosomes in female cells. In male cells, the short arm of the X chromosome was significantly less heavily labeled than the long arm. In female cells, the normal X and marker X were randomly late replicating, and there was no evidence of differential contraction of the late replicating X.

Linkage studies demonstrated that the *Xm* locus was probably not near the end of the long arm of the X chromosome.

REFERENCES

ALLAN, W., and HERNDON, C. N. 1944. Retinitis pigmentosa and apparently sex-linked idiocy. *J. Hered.* **35**:41–43.

ALLAN, W., HERNDON, C. N., and DUDLEY, F. C. 1944. Some examples of the inheritance of mental deficiency: apparently sex-linked idiocy and microcephaly. *Amer. J. Ment. Defic.* **68**:325–334.

BISHOP, A., LEESE, M., and BLANK, C. E. 1965. The relative length and arm ratio of the human late-replicating X chromosome. *J. Med. Genet.* **2**:107–111.

COURT BROWN, W. M., BUCKTON, K. E., JACOBS, P. A., TOUGH, I. M., KUENSSBERG, E. V., and KNOX, J. D. E. 1966. *Chromosome studies on adults.* Cambridge Univ. Press, London.

DAY, E. J., MARSHALL, R., MACDONALD, P. A. C., and DAVIDSON, W. M. 1967. Deleted chromosome 18 with paternal mosaicism. *Lancet* **19**:1307.

GERMAN, J. 1964. Identification and characterization of human chromosomes by DNA replication sequence. Pp. 191–207 in R. J. C. Harris (ed.), *Cytogenetics of cells in culture.* Academic, New York.

HUNGERFORD, D. A. 1965. Leukocytes cultured from small inocula of whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl. *Stain Techn.* **40**:333–338.

KOHN, G., DIEHL, V., MELLMAN, W. J., HENLE, W., and HENLE, G. 1968. C-group chromosome marker in long-term leukocyte cultures. *J. Nat. Cancer Inst.* **41**:795–804.

KOHN, G., MELLMAN, W. J., MOORHEAD, P. S., LOFTUS, J., and HENLE, G. 1967. Involvement of C group chromosomes in five Burkitt lymphoma cell lines. *J. Nat. Cancer Inst.* **38**:209–222.

LESCH, M., and NYHAN, W. L. 1964. A familial disorder of uric acid metabolism and central nervous system function. *Amer. J. Med.* **36**:561.

LUBS, H. A. 1969. Gap-deletion: A new class of cytogenetic abnormalities. (In preparation.)

MARTIN, J., and BELL, J. 1943. A pedigree of mental defect showing sex-linkage. *Arch. Neurol. Psychiat.* **6**:154–157.

MILES, C. P., and O'NEILL, F. 1967. Chromosome studies of 8 in vitro lines of Burkitt's lymphoma. *Cancer Res.* **27**:392–402.

SEEGMILLER, J. E., ROSENBLOOM, F. M., and KELLY, W. N. 1967. Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. *Science* **155**:1682–1684.