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1963

Reprinted from
NATURE
Vol. 197, No. 4 868 - 1963

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(*Reprinted from Nature, Vol.* 197, *No.* 4868, *pp.* 648–649, *February* 16, 1963)

EVIDENCE THAT THE Xg BLOOD GROUP GENES ARE ON THE SHORT ARM OF THE X CHROMOSOME

By J. LINDSTEN

Department of Endocrinology and Metabolism, Karolinska Sjukhuset, Stockholm 60

DR. M. FRACCARO

Biology Division, Euratom, c/o Istituto di Genetica, Università di Pavia

PROF. P. E. POLANI and J. L. HAMERTON

Paediatric Research Unit, Guy's Hospital Medical School, London, S.E.1

AND

DR. RUTH SANGER and DR. R. R. RACE, F.R.S.

Medical Research Council Blood Group Research Unit, Lister Institute, London, S.W.1

FROM an examination of two women with ovarian dysgenesis, and of their families, evidence has emerged which suggests that the locus for Xg, the X-linked blood group system[1], is on the short arm of the X chromosome.

The two patients were consistently sex chromatin positive and each displayed, in the tissues sampled for cytological study, chromosome mosaicism of two cell lines: one, the minority, had 45 chromosomes including a single X and the other, the majority, had 46 chromosomes. In the cells of the latter line there was one normal X chromosome and a metacentric chromosome resembling No. 3 of the set; this abnormal chromosome has been interpreted[2], in these and in other patients with similar karyotype, as an isochromosome for the long arm of the X chromosome. While other interpretations are possible the suggestion that these metacentric chromosomes are isochromosomes for the long arm of the X seems likely from the combined cytological and clinical evidence. A recent observation[3] supports this interpretation: the sex chromatin masses of five such patients have a greater amount of DNA than do those of normal females. Furthermore, the presumptive isochromosomes have been shown[4,5] to be symmetrically 'hot' along their whole length when labelled with ^{3}H-thymidine late in the period of DNA synthesis.

The argument to follow concerning the site of the Xg locus hinges on this interpretation—that the structurally altered chromosome consists of two long arms of the X chromosome without a short arm.

Family J. The pedigree of this family is shown in Fig. 1. The proposita, III-3, briefly reported by Lindsten[6], is aged eighteen; she has primary amenorrhœa and the output of pituitary gonadotrophin in her urine is increased while that of œstrogens is decreased. She is of short stature (4 ft. 6 in. or 138 cm). She has neither an unusual number of pigmented nævi nor webbing of the neck. Her father was thirty-three and her mother twenty-five at the time of her birth.

Cytological investigations showed that the proposita was sex chromatin positive in cells from buccal mucosa smears, skin sections and in cells from two skin biopsies grown *in vitro*. Cells were cultured from one blood sample and from two different skin biopsies and chromosome mosaicism was observed: the majority of cells had 46 chromosomes including an apparently normal X and a presumptive isochromosome for the long arm of the X, the minority of cells had 45 chromosomes with an XO karyotype. The parents and sibs of the proposita have sex chromatin corresponding to their phenotypic sex.

From the family history it is clear that the maternal grandfather, I-2, was colour blind. By Ishihara and anomaloscope tests III-2 and III-5 are deuteranopic: the proposita, III-3, is deuteranopic according to Ishihara but has a normal Raleigh equation with an increased contrast. By both tests II-3 and II-4 and III-1-4-6 were normal, and by Ishihara II-1 and II-2 were normal.

The Xg groups of the family are shown in the pedigree. The proposita lacks her father's Xg^a antigen and this indicates that her normal X is from her mother, as does the fact that she has her mother's colour-blindness gene but not her father's gene for normal colour vision: the isochromosome would therefore have to be of paternal origin. (The family illustrates crossing-over between the loci for Xg and for deutan colour vision; this is to be expected for the cross-over rate between the two genes is known to be high[7].)

Family R. The pedigree of this family is shown in Fig. 1. The proposita, II-2, is aged twenty-two years, has primary amenorrhœa with markedly increased output of pituitary gonadotrophin, is of short stature (4 ft. $9\frac{1}{2}$ in. or 146 cm) and has numerous pigmented nævi but no webbing of the neck. At the time of her birth her father was thirty-one and her mother twenty-six. She has a normal elder brother.

The patient is sex chromatin positive in cells from the oral mucosa, from skin biopsy grown *in vitro* and in the leucocytes of the blood smear; some of the sex chromatin masses in the oral mucosa cells appeared to be unusually large. The patient's chromosomes were examined in cells from two blood cultures and one skin culture and showed her to be a chromosome mosaic: the majority of her cells had 46 chromosomes, including an apparently normal X

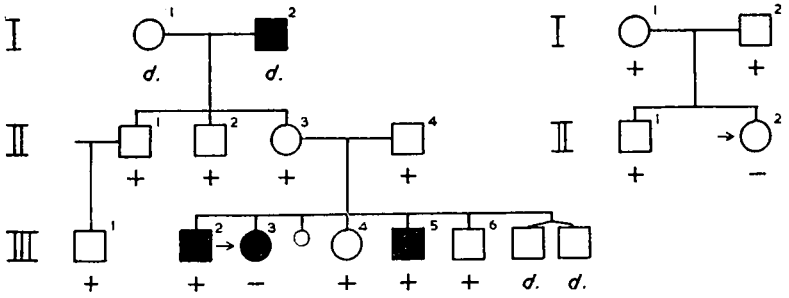

Fig. 1. The family J. (left) and the family R. (right). Arrow = proposita. Xg groups: + = Xg(a+), − = Xg(a−). Colour vision: solid black = colour-blind (deuteranopic), hollow = normal (for details see text). *d.* = dead and not tested for Xg or for colour vision

and a presumptive isochromosome for the long arm of the X, and the minority of cells had 45 chromosomes with an XO karyotype. The presumptive isochromosome in the cells with 46 chromosomes was found[5] to be symmetrically 'hot' and to label with ^{3}H-thymidine later than the other chromosomes in the course of DNA synthesis. The father and mother had a normal chromosome complement in cells from blood cultures.

The proposita, her mother and father have normal colour vision; the first two were tested on the anomaloscope and the Ishihara, the father only on the latter.

The Xg groups of the family are shown in the pedigree: because the proposita is Xg(a−) while her father is Xg(a+) it must be considered that the morphologically normal X which she has in both her cell lines is from her mother (who must be heterozygous Xg^aXg). It follows that the presumptive isochromosome present in the cells with 46 chromosomes is of paternal origin—yet the patient is Xg(a−).

Other cases. The families of 12 other patients who have presumptive isochromosomes for the long arm of the X have been tested, but the Xg groups did not segregate in the informative way they did in the J. and R. families. The groups of these other families are recorded in Table 1: in four of the 12 patients the presumptive isochromosome appears to be of paternal origin (families Fra., All., And., Eri.; see discussion).

Discussion. Analysis of the two families, J. and R., indicates that the presumptive isochromosome of both patients is of paternal origin. If this abnormal chromosome found in the Xg(a−) daughters consists of two long arms of the X chromosome from the Xg(a+) father then these arms do not contain the Xg locus, which should therefore be located on the short arm. (The evidence that family J. gives for the deutan locus being on the short arm has already been discussed[8,9].)

Table 1. TWELVE FAMILIES IN WHICH THE PROPOSITA HAS A PRESUMPTIVE ISOCHROMOSOME FOR THE LONG ARM OF THE X BUT IN WHICH THE Xg GROUPS ARE LESS INFORMATIVE THAN THOSE OF THE FAMILIES J. AND R.

Investigator	Identification	Xg groups of Father	Mother	Proposita	Brothers	Sisters
Court Brown and Whyte	Hig.*	+	+	+	−	
	Tho.*	+ c.b.	+	+ c.b.		+
Motulsky	Sea.	+	+	+		
Polani and Hamerton	Fra.	−	+	+		
	Gib.*	+	+	+	+	+
	Rog.	+	+	+	−	
Lindsten	Las.*	−	+ ‡	−	. + + +	
	All.*	−	+	+		+
	And.	−	+	+	+ +	
	Eri.	−	+	+		+
	Sjo.	−	+	−		+
	Sve.	+	+	+		

+, Xg(a+). −, Xg(a−). c.b., colour blind.
* not found to be a mosaic (XO/X iso-X).
‡ Her mother, sister and 2 brothers are all +.

Two possibilities could upset this argument. The hypothesis of Lyon[10], which postulates that in the mouse and possibly in all mammals only one X chromosome is 'active' in any one cell, makes it theoretically possible that the isochromosomes of our two patients are 'inactive' in all cells. If this were so the Xg(a −) reactions of the two patients could merely reflect lack of gene action and would not afford evidence of the arm on which Xg is located. The fact that the isochromosome is 'hot' when labelled with ^{3}H-thymidine late in the period of DNA synthesis[4,5] indicates that it synthesizes later than the other chromosomes: whether this means that it is also 'inactive' remains to be demonstrated.

The second possibility is that the two different cell lines found in both patients might be so distributed that those producing the Xg groups have 45 chromosomes including the single, maternal, X carrying the silent *Xg* gene. In this event again the Xg locus could be on either arm of the X chromosome. However, an identical, non-random distribution of two cell lines in two unrelated individuals seems most unlikely.

Thus, though the evidence is suggestive, definite proof that the Xg locus is on the short arm of the X chromosome must await clarification of these problems.

In some of the patients, the Xg findings can help in tracing the origin of the presumptive isochromosomes, but there are certain limitations and two sets of factors have to be taken into consideration: the 'activity' or otherwise of the presumptive isochromosomes and the situation of the Xg locus on the short or long arm of X chromosomes generally. If the presumptive isochromosomes of the patients were 'inactive' in all cells, a paternal origin could be assumed in six of the 14 patients (R., J., Fra., All., And., Eri.) irrespective of the situation of the Xg locus: on the other hand, if they were 'active', one could conclude a paternal origin for the six only on the assumption that the Xg locus is on the short arm. In the

two patients J. and R., 'activity' of the isochromosomes would imply that the Xg locus is on the short arm (if we disregard mosaicism), and indeed this is the object of the present article.

We thank Dr. P. M. F. Bishop, London, who referred the proposita of family R. for investigation, Dr. M. Svenmar, Rävemåla, for help in obtaining blood samples from the family J., and Prof. I. Kugelberg, Stockholm, for the colour vision investigation of that family. We also thank Dr. W. M. Court Brown, Edinburgh, Dr. E. G. Oastler and Dr. W. G. Whyte, Glasgow, and Dr. A. Motulsky, Seattle, for samples from three of the families listed in Table 1. We thank Dr. J. D. Mann, Grand Rapids, and Dr. A. Cahan, New York, for generous supplies of the anti-Xg^a serum.

One of us (J. L.) gratefully acknowledges a grant from Nordisk Insulinfond.

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Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.

CDNA00311ENC