Ovarian Dysgenesis and Presumed Isochromosome of the Long Arm of X

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Turner's syndrome has long been associated with primary amenorrhoea, webbing of the neck, cubitus valgus, and short stature (Turner, 1938). The majority of these cases are chromatin negative and have an XO chromosome complement. Ovarian maldevelopment is frequently a feature of this condition (Albright, Smith, and Fraser, 1942), as is the presence of multiple somatic abnormalities. Chromatin positive cases have been reported (De la Chapelle, 1962; Lindsten, 1963; Williams, Engel, and Forbes, 1964) bearing some of the features of the classical Turner's syndrome. Stunting of stature is nearly always evident, but webbing of the neck and other somatic abnormalities are less commonly found. Most of these subjects have been shown to be chromosomal mosaics XO/XX. A much rarer group is thought to be associated with an X iso X chromosome complement.

The isochromosome is metacentric and may be formed by replication of the long arm of the X chromosome. The exact mechanism is not known though there are several theories (Jacobs, Harnden, Buckton, Court Brown, King, McBride, MacGregor, and Maclean, 1961; Lindsten, Fraccaro, Ikkos, Kaijser, Klinger, and Luft, 1963). The latter authors suggest that the isochromosome may result from a misdivision of the centromere which divides transversely instead of longitudinally. They postulate that this is more likely to happen at the first reduction division of meiosis in spermatogonia owing to the possible difficulties in pairing up the morphologically dissimilar X and Y chromosomes.

Published material records 16 cases of X iso X chromosome complement in which there was no evidence of mosaicism (Jacobs *et al.*, 1961; Hamerton, Jagiello, and Kirman, 1962; Forbes and Engel, 1963; Lindsten *et al.*, 1963; Lindsten, 1963; Sparkes and Molutsky, 1963; Williams *et al.*, 1964). This paper records two further cases of primary ovarian failure due to presumed isochromosome of the long arm of X.

Case Reports

Case 1. The second child of unrelated parents born in November 1948, birthweight 6½ lb. (2.9 kg.), was first seen by me in February 1964. Although a scanty menstrual discharge had occurred some six months previously a regular cycle had never been established. Her mother was 37 and her father 41 years old when she was born. She had a brother who was three years her senior. Both parents and her brother were phenotypically normal. Her mother's height was 5 ft. 5 in. (165 cm.), and her father's height was 6 ft. (183 cm.). Her brother was 6 ft. 2 in. (188 cm.).

Detailed inquiry into the maternal and paternal family history revealed no evidence of thyroid disease or diabetes mellitus. Her father suffered from intermittent claudication and on one occasion his serum cholesterol level had been 305 mg./100 ml. Both his parents were stated to have died from coronary thrombosis, as did his eldest brother at the age of 50 years.

The patient (Fig. 1) was short (4 ft. 8½ in. (144.2 cm.))

FIG. 1. The patient aged 15. Note diminutive stature, short neck, cubitus valgus, and scanty pubic hair.

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FIG. 2. Photograph demonstrating normal breast contour, infantile nipples, and absent axillary hair. Some pigmented naevi are evident.

and rather obese. The neck was short but there was no webbing. The external genitalia was infantile: the labia majora and clitoris were absent, and the labia minora was very poorly developed. Vaginal examination was not possible but a probe could be passed for a distance of 1 in. (2.54 cm.) into the poorly developed vagina. On rectal examination a small uterus was palpable. There was some scanty pubic hair but no axillary hair. The lower jaw tended to recede, and the palate was high and arched. There were multiple pigmented naevi particularly evident on the face. The breast contour was normal, and there was slight pigmentation of the areolar, but Montgomery's tubercles were absent. The nipples (Fig. 2) were poorly-developed infantile struc-

FIG. 3. Neutrophil polymorph with large drumstick. ($\times 1500$.)

tures. There was a well-marked cubitus valgus. The fingers were rather short, but the nail development was normal. A transverse palmar crease was present on the left hand but not on the right. No evidence of colour blindness was found (Ishihara plates) and the visual acuity in both eyes was 6/12. The visual fields were normal. Both femoral pulses were palpable. The blood pressure was 145/85 mm. Hg. The electrocardiogram was normal.

Intelligence was apparently average. She attended a Secondary Modern School and was in a 'C' stream. In a recent terminal examination she was placed 5th in a class of 29 children. In certain subjects she was reported to be above average ability.

RADIOGRAPHIC AND LABORATORY INVESTIGATIONS

Radiographs of the hands, wrists, and knees showed epiphysal development to be within normal limits. The skull and sella turcica were normal.

Fifty oral cells were examined from buccal smears, 54% of which showed a single nuclear sex chromatin mass. Examination of stained neutrophil leucocytes was made for nuclear appendages. 400 cells were counted of which 64 (16%) contained 'large drumsticks' (Fig. 3) and 69 (17%) contained 'small clubs'.

The 24-hour urinary excretion of 17-oxosteroids and 17-hydroxycorticosteroids was 4.75 mg. and 8.85 mg. respectively. Serum protein electrophoresis showed a normal pattern. The serum sodium, potassium, chloride, calcium, and inorganic phosphorus levels were within normal limits, as was the blood urea. The serum alkaline phosphatase was 19 King Armstrong units (normal range 2-12 units per 100 ml.). The serum cholesterol content estimated on two occasions was 260 mg./100 ml. and 263 mg./100 ml., respectively. Repeated estimations of blood sugar levels ranged between 65 mg. and 80 mg./100 ml., and routine analysis of the urine revealed no abnormality. The haemoglobin content, total white cell count, and differential count were normal, and haemoglobin electrophoresis showed normal adult type haemoglobin.

Dr. Ruth Sanger examined samples of blood from the subject and her parents for the sex-linked Xg^a blood group. All were Xg (a+). The erythrocyte glucose phosphate dehydrogenase (G6PD) activity was 2400 units/ml. of red cell mass.

No thyroid auto-antibodies could be detected in the subject's serum or in that of her parents using the latex particle technique, but a weak positive titre (1/25) was found when the subject's serum was examined by the tanned red cell haemagglutination test. The 4-hour uptake of 25 microcuries of ¹³¹I was 35% of the dose and the 48-hour uptake was 52%. The 48-hour protein bound iodine (PBI) was 0.7% of the dose per litre of plasma. (Normal range 0.03 to 0.3% of the dose per litre of plasma.)

CHROMOSOME STUDIES. This work together with the autoradiographic findings was undertaken by Dr. F. Lewis of Southmead Hospital, Bristol. The chromosomal pattern was studied on growing cells of the per-

FIG. 4. A cell in metaphase with karyotype. The isochromosome is indicated by an arrow.

TABLE I

	Subject						Mother			Father			
No. of chromosomes	42	43	44	45	46	Total	45	46	Total	44	45	46	Total
Cells counted	1	—	1	—	28	30	4	26	30	1	—	29	30
Number of oral cells	50						50			50			
Percentage having Barr body	54						52			—			

pheral blood by a modification of the method of Moorhead, Nowell, Mellman, Battipps, and Hungerford (1960). The results, together with the buccal smear analysis, are summarized in Table I.

Subject. The modal cell showed 22 pairs of autosomes and an abnormal sex chromosome in all the spreads examined. In place of the second X chromosome was a large chromosome which paired in size with chromosome number 3 (Denver Classification, 1960). The two apparently equal arms were similar in size to the long arm of the other X. This abnormal chromosome was present also in the two hypomodal cells (Fig. 4). Autoradiographic studies on cultured leucocytes labelled

with H.3 thymidine in the S phase of DNA synthesis (Muldal, Gilbert, Lajtha, Lindsten, Rowley, and Fracaro, 1963; Giannelli, 1963) were performed on 20 cells in which one heavily labelled chromosome could be demonstrated. In each case this was morphologically consistent with a presumptive isochromosome of X.

Mother. 30 cells in metaphase were examined and 13 cells fully analysed by photography. There was no significant abnormality in the cells fully analysed.

Father. A sample of 30 cells in metaphase was examined. Of these 29 contained 46 chromosomes, the karyotype being consistent with the normal male of 22 pairs of autosomes and sex chromosomes X and Y.

FIG. 5. Main lines of dermal ridge patterns on finger-tips, palms, and soles.

DERMATOGLYPHIC ANALYSIS. Dermatoglyphic patterns (Fig. 5) were examined by Prof. L. S. Penrose of The Galton Laboratory, University College, London. Finger-tip patterns show fairly high intensity. The total ridge count (that is the sum of the highest count on each finger) was 182. Comparison with counts obtained on the average for Turner's syndrome and related conditions, and for normal female controls is shown in Table II where measurements on the palms are also given. There were strong hallucal patterns on the feet, which were of the normal type.

TABLE II

Subject	Total Ridge Count (right and left)	a-b Ridge Count (right and left)	Maximal atd Angle (right and left)
Subject	182	83	95°
Turner's syndrome and related conditions	167	96	107°
Female controls	130	84	88°

TREATMENT. Intermittent treatment with 0.01 mg. ethinyl oestradiol per day resulted in withdrawal bleeds which simulated a normal menstrual flow. As yet there has been no improvement in the development of the external genitalia.

Case 2. This patient was seen in April 1964, at the age of 30, by Mrs. M. Bennett at Southmead Hospital, Bristol, for investigation of amenorrhoea. Some 17 years previously three or four scanty periods had occurred. Most of her life had been spent abroad, and she had received, on occasions, various forms of medication with resultant withdrawal bleeding. She had two brothers aged 45 and 33 years, and two sisters 36 and 34 years old, respectively.

The patient was of diminutive stature (Fig. 6), height 4 ft. 7½ in. (141 cm.), and weighed 5 st. 11 lb. (36.8 kg.). There was a wide carrying angle of the arm. There was no axillary hair but some pubic hair of pubertal distribution. Some pigmented naevi were present on the skin of her chest and face. The breasts were reasonably well developed, but the nipples were infantile in appearance. The labia were poorly developed and the vagina was small. Examination under anaesthetic revealed a small atrophic uterus from which no curettings could be obtained. The ovaries were not palpable. The cardiovascular system was normal and the blood pressure was 130/70 mm. Hg.

RADIOGRAPHIC AND LABORATORY INVESTIGATIONS. Radiography showed a normal sella turcica. Epiphyses in the wrist were fused. The pelvis was normal as was a chest radiograph. Gynaecography failed to reveal ovarian shadows.

Vaginal smears showed that 90% of the cells had large well-formed nuclei, and 10% showed a variable degree of pyknosis.

The haemoglobin was 13.9 g./100 ml. (94%). Routine

urinalysis was normal. The urinary 17-oxosteroid excretion was 6.4 mg./24 hours, and the 17-hydroxy-corticosteroid excretion was 6.8 mg./24 hours. The urinary 24-hour excretion of creatinine was 0.8 g.

CHROMOSOME STUDIES. Chromosomal analysis (Dr. F. Lewis) was performed on cultured leucocytes. The patient had a modal cell line of 46 chromosomes, comprising 22 pairs of autosomes, a single X chromosome, and a large medial chromosome resembling an isochromosome of the long arm of X.

FIG. 6. The second patient aged 28. Short stature, cubitus valgus, and multiple pigmented naevi are evident.

Discussion and Conclusions

The two patients described show the same clinical features as subjects with a presumed isochromosome of the long arm of X described by previous authors. Stunting of stature is invariable, as is sexual infantilism. The majority of cases so far reported may be classified with the type of ovarian dysgenesis, without webbing of the neck, and of small stature, as suggested by Polani (1961), but a patient described by Hamerton *et al.* (1962) was a low-grade mental defective, had severe somatic abnormalities, and had webbing of the neck.

In view of the report by Forbes and Engel (1963) on the high incidence of diabetes mellitus in patients with gonadal dysgenesis and in their close relatives, particular care was taken to find evidence of diabetes mellitus in the paternal and maternal relatives of the first subject; with negative results. Repeated examinations for sugar content were made on

samples of urine and blood obtained both from the subject and from her parents. No abnormal findings were reported.

Autoradiographic studies performed on cultured leucocytes from subjects with an X iso X chromosome complement show that it is invariably the isochromosome that is inactivated (Muldal *et al.*, 1963). This apparent contradiction of the Lyon (1961) hypothesis has been explained by Gartler and Sparkes (1963) by assuming that at some stage of early embryonic life the cells whose normal X chromosomes are inactivated will die, the survivors being those cells in which the isochromosome is inactivated. The final result, therefore, should be similar to those individuals having an XO chromosome composition, in which the single X chromosome is either inactivated, in which case the cell dies, or remains extended and genetically active, ensuing clones being normal.

There is some evidence (Reed, Simpson, and Chown, 1963) suggesting that in normal individuals inactivation of the X chromosome may not be complete, and it is of interest to speculate if inactivation of the isochromosome may be likewise incomplete.

Some authorities consider that abnormal mitosis may be produced by abnormal immunological mechanisms (Falikow, 1964). The finding of a high frequency of thyroid auto-antibodies (Engel, Forbes, Mantooth, and Socolow, 1963) in patients with the more usual form of ovarian dysgenesis (XO, and XO/XX mosaics) supports this view. In patients having an X iso X chromosome complement, there is a high incidence of thyroiditis (Williams *et al.*, 1964; Sparkes and Motulsky, 1963). Although there is as yet no evidence of thyroiditis in the first patient reported in this communication, she does have a weak thyroglobulin antibody titre, though no thyroid auto-antibodies could be demonstrated in her parents' sera. In Hashimoto's thyroiditis there may be a normal ^{131}I uptake, but PBI is often raised (Murray, 1964). This is due to (a) impaired iodine binding resulting in a release of unbound iodide from the thyroid cells, the so-called discharge phenomenon; (b) the leakage of abnormal, butanol insoluble, iodoproteins or iodopeptides directly into the circulation; and (c) a destructive reduction of the total thyroid iodine pool. The disorder usually becomes manifest in middle-aged women when hypothyroidism is associated with a rapidly enlarging goitre. Some cases of autoimmune thyroiditis remain euthyroid. It is of interest to note that the subject had a normal ^{131}I uptake but a raised PBI. Her age was 15 years when these investigations were carried out

compared with 23 and 24 years, respectively, in the subjects reported by Sparkes and Motulsky, and 34 and 31 years in the subjects described by Williams *et al.*

Other workers suggested that abnormalities of the X chromosome were directly responsible for abnormal antibody formation. According to Burch (1963), cell bound antibody may be synthesized via messenger RNA coded from an autosomal locus and a locus on one X chromosome; the former synthesizing the 'light' polypeptide, and the latter the 'heavy' polypeptide chains. On theoretical grounds it was suggested (Burch and Rowell, 1963) that as well as somatic autosomal mutations, four mutant inherited genes on the X chromosome may feature in the aetiology of Hashimoto's thyroiditis. If one assumes that either the isochromosome of the long arm of X is not completely inactivated, or that in stem cells of the lymphoid series inactivation does not occur, and that the isochromosome is isolellic (i.e. the arms are genetically identical), it would be necessary to postulate the inheritance of only two mutant genes on one parental X chromosome before isochromosome formation during meiosis. If this were correct, and as yet there is no experimental evidence, it may help to explain the high incidence of active thyroiditis in subjects with an X iso X chromosome complement. It would also be necessary to postulate that the isochromosome is not completely genetically inert.

Perhaps both theories are acceptable. A high circulating level of auto-antibodies may produce chromosomal abnormalities, but if by chance abnormalities of the X chromosome occur, abnormal antibody production may result. The study and follow-up of similar patients and their relatives may shed more light on this problem.

Summary

Reports are given of 2 patients with gonadal dysgenesis due to presumed isochromosome formation of the long arm of X. The clinical features agree with those described by previous authors. The association between abnormalities of the X chromosome and antibody production is discussed.

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