Demonstration of the fra(X) in lymphocytes, fibroblasts, and bone marrow in a patient with a testicular tumour

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SUMMARY A man aged 34, whose mental retardation and appearance were consistent with the fra(X)(q28) syndrome, had a craggy lump in his left testis. The fragile X was found in lymphocytes, fibroblasts, and bone marrow. The testicular tumour was benign and inflammatory in nature and within the testis spermatogenesis was absent.

Clinical features

The proband was ascertained during a screening programme of mentally defective patients. He was 34 years old with large testes, a prominent jaw, and a fairly severe kyphosis without scoliosis. Associated with the left testis was a craggy mass which was considered malignant and therefore removed intact with this testis.

Cytogenetic investigations

Lymphocytes taken at the time of the operation were cultured in medium 199 supplemented with 2% fetal calf serum (FCS) and methionine (100 mg/l); slides were prepared after 72 hours' culture. Fibroblast cultures were set up in Ham's F10 with 10% FCS; methotrexate (10^{-7} mol/l) was added 24 hours before harvesting. Bone marrow was cultured for 48 hours in MEM Eagle's supplemented with 20% human serum. Cells were harvested after being synchronised.¹ A hundred cells were scored for C group chromosomes showing q telomeric gaps or breaks; the same slide was then destained and G banded. The fragile X was clearly present in 38% of lymphocytes. In spite of this proportion, only 2% were expressed in bone marrow and 10% in fibroblasts (T Webb, 1982, personal communication).

Histopathology

Fragments of testis for light microscopy were fixed in Bouin's fluid and the rest in 10% formol saline. Paraffin wax sections were stained with haematoxylin

and eosin and selected sections with the elastic Van Gieson, Perl's method for ferric iron, periodic Schiff method, Ziehl-Neelsen method, and Gram's stain. Specimens for electron microscopy were fixed in 4% glutaraldehyde with post-fixation in osmium tetroxide. Resin sections were stained with uranyl acetate and lead citrate.

The testis was received bisected and fixed. It was enlarged ($9.0 \times 6.5 \times 2.0$ cm) with a shiny white surface and dull grey parenchyma. Within the epididymis was a blood filled cyst (2.5 cm diameter) with a firm tough wall. The tumour in the epididymis was formed from chronic inflammatory tissue surrounding partly organised blood clot. No giant cell granulomas, evidence of malignancy, or micro-organisms were found.

The lining to the seminiferous tubules included tall Sertoli cells and only random spermatogonia with no mitoses. The average external diameter of the tubules was 127 μ m and most were patent, but a few included some degenerate desquamated cells. The

FIG 1 Testicular biopsy appearance. (Elastic Van Gieson, original magnification $\times 200$.)

basement membrane was of normal thickness and there were no interstitial changes. Small clumps of Leydig cells were present (fig 1). Electron microscopy in addition revealed fat and lipofuscin in the Sertoli cells. Leydig cells had Reinke crystalloids and a well developed smooth endoplasmic reticulum (figs 2, 3).

FIG 2 Central spermatogonium with Sertoli cell on either side. (Lead citrate, original magnification $\times 5000$.)

FIG 3 Leydig cell and Reinke crystalloid. (Lead citrate, original magnification $\times 4000$. Insert, original magnification $\times 12\,450$.)

Discussion

Since the recognition of the association of the fragile X with mental deficiency the syndrome has also been related, although inconsistently, with macro-orchidism.²⁻⁴ The chromosome marker is usually found in peripheral lymphocytes but Sutherland⁵ attempted to demonstrate it in bone marrow from a male who had previously shown the marker in lymphocytes. The bone marrow cells, however, did not show a single fragile site in 200 metaphases and no other similar reports have been found. The results from the marrow culture of our patient demonstrate that the technique can be successful although producing a lower yield than circulating lymphocytes.

We know of no reports of patients with the fra(X)(q28) syndrome associated with testicular tumours. The tumour in this case was benign and similar to a sperm granuloma in its location and its morphology but without spermatozoa. The absence of any active spermatogenesis in this testis would make the development of such a granuloma most unlikely. The organising blood clot within the area of inflammation suggests the possibility that the tumour was associated with trauma but there was no clinical support for this conclusion.

Previous reports of this syndrome which included histological data from comparably aged patients^{6,7} all record normal spermatogenesis in some parts of the biopsy specimens. Our findings of tubules lined by Sertoli cells and including only a few spermatogonia are therefore unusual. The presence of elastic tissue in the interstitial tissue implies that puberty had been reached. The possible role of an outlet block produced by the epididymal tumour on the testicular morphology is difficult to evaluate since there are no striking features associated with this phenomenon.⁸ The diameter of the tubules can be slightly reduced, as in this case, but all stages of spermatogenesis are present although without the usual orderly pattern. Sertoli cell increase is not a feature. These cells are seen in increased numbers, together with occasional germ cells, in some examples of Klinefelter's syndrome where there are also Leydig cell changes.⁹ The findings of similar epithelial features in this testis, but without Leydig cell changes, suggests that with greater experience a spectrum of changes within the testis can be anticipated in the fragile X syndrome.

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testicular tissue culture, and to Mrs Veronica Buckle for her help with the bone marrow preparations.

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Additional manifestations of the Neu-Laxova syndrome

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SUMMARY A newborn female with intrauterine growth retardation, bilateral cleft lip and palate, absent external nares and eyelids, low set ears, short contracted limbs, webbed digits, intestinal malrotation, and unilateral renal agenesis is reported. These multiple malformations are considered part of the Neu-Laxova syndrome.

The Neu-Laxova syndrome is a rare, autosomal recessive disorder associated with severe malformations and neonatal death. Fourteen cases have been reported so far,^{1–5} and the purpose of our report is to describe another case with additional anomalies.

Case report

A severely malformed female infant was born to apparently non-consanguineous Mormon parents. The father, aged 24, is adopted and knows nothing of his background. The mother, aged 23, has one brother, one full sister, and one paternal half-sister. Her full sister has one normal son, and her half-sister has had one miscarriage and three normal children. The maternal grandmother had 11 pregnancies, eight of which resulted in spontaneous abortion. A great-aunt is said to have had 'mongolism'. The

mother, her mother, her uncle, and a cousin have psoriasis. The parents divorced soon after the birth of this child. The mother has remarried and has had a normal infant with her second husband.

Both parents had a history of drug abuse with hashish, marijuana, lysergic acid diethylamide, mescaline, amphetamines, and phencyclidine several years before the birth of their child. When the mother first became pregnant, she was taking Robaxin and Norgescic, but she discontinued them when pregnancy was confirmed. She spotted briefly early in pregnancy, in the fourth month took Gantrisin for a urinary tract infection, and in the sixth month took aspirin, Actifed, penicillin, and cough syrup for a 'flu-like' illness. On thyroid tablets intermittently for several years, the mother took thyroid replacement and antenatal vitamins daily throughout pregnancy. She states that she never felt strong fetal movements during the pregnancy.

At approximately 36 weeks' gestation, a female infant was born by spontaneous vaginal delivery. A heart beat was detected for a brief period. After death the body was preserved intact in formalin and later sent to the Los Angeles County-University of Southern California Medical Center for necropsy.

The body weighed 940 g. Body measurements included: head circumference 23.5 cm, chest circumference 24.0 cm, crown-heel length 34.0 cm, and crown-rump length 21.0 cm. The infant had