A comparison of the clinical and cytogenetic findings in nine patients with a ring (X) cell line and 16 45,X patients

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Abstract

In this study, the clinical, IQ, and cytogenetic findings in nine Turner's syndrome patients with a ring (X) cell line are compared with those in 16 patients in whom only a 45,X cell line could be found. The ring (X) patients lacked many of the "classic" Turner's syndrome features and the majority were not karyotyped until after the age of 11, usually because of pubertal failure. They also showed a reduction in IQ of 11 points compared with the 45,X group. Some ring (X) patients show characteristic facial features including a broad nose with anteverted nostrils, prominent philtrum, long palpebral fissures, and a wide mouth with a thin upper lip. Neither the physical features nor the IQ are related to the parental origin of the chromosome error. In the majority of cases the ring (X) chromosome was late replicating but XIST activity is being studied further.

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Several authors[1–5] have drawn attention to the severe phenotype sometimes seen in patients who have a cell line containing a ring (X) chromosome. Most of these patients also have a 45,X cell line and until recently would have been expected to show some or all of the features of Turner's syndrome. The clinical and cytogenetic findings in a series of females with a 45,X cell line and a cell line containing one or more ring X chromosomes are reported and compared with a group of females in whom only a 45,X cell line was identified, to clarify further the relationship between the ring (X) chromosome and various phenotypic features which are atypical of Turner's syndrome.

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Methods

In 1990, The Wessex Regional Genetics Laboratory reinvestigated a series of Turner's syndrome patients with regard to karyotype and parental origin of the chromosome error.[6] All 31 45,X patients and 12 ring (X) patients in that study known to be resident in Wessex were recontacted together with a further two 45,X and three ring (X) patients who had been karyotyped since 1990. One 45,X patient had died of status epilepticus aged 26 and one ring X patient (patient 1[5]) had died aged 35 months. Patients were visited at home by one of the authors (ALC) and details of the past medical history, treatment, and education obtained. A general clinical examination was performed and recognised features of Turner's syndrome[7] were noted systematically. In patients under 16 years old IQ was measured using the short form of the British Ability Scales[8] and for those 16 years or over the English version of the Wechsler Adult Intelligence Scale (WAIS)[9] was used. At the time of the examination ALC was unaware of the karyotype or the parental origin of the chromosome abnormality.

CYTOGENETICS AND MOLECULAR ANALYSIS

The details of the cytogenetic results and the molecular methods used to identify the ring chromosome and determine the parental origin of the chromosome error have been included in a previous publication.[6] All patients had at least 100 cells analysed. Patients with a 46,XX cell line were excluded from the study and none of the 45,X patients had evidence of a second cell line. All nine patients with a ring (X) cell line had the mosaic karyotype 45,X/46,X,r(X), with the cell line containing the ring (X) present in blood in proportions varying from 1% to 71%.

Results

The clinical, IQ, cytogenetic, and parental origin findings in the two groups are summarised in table 1 and fig 1.

TURNER'S SYNDROME FEATURES

Both groups showed almost universal short stature and hypogonadism. In the 45,X group three patients had heights on or above the 3rd centile. One patient was on the 3rd to 10th centile for height aged 4 years 1 month; another aged 9 years was on the 10th centile for height having been on growth hormone injections for 18 months. A 22 year old in this group had a height above the 25th centile, but both her parents were extremely tall, with heights above the 97th centile. In the ring (X) patients one was on the 3rd centile aged 10 years and a girl aged 11 years 3 months was on 3rd to 10th centile. Both had had or were receiving growth hormone therapy. Hypogonadism was evident in all patients who had reached the age of puberty. Apart from a few pubic hairs there had been no spontaneous development of secondary sexual characteristics in any patient.

Many of the physical features considered "classical" in Turner's syndrome were seen much less frequently in the ring (X) group. Most striking is a history of congenital lymph-

Table 1 Features in nine patients with a ring (X) cell line compared with 16 45,X patients

	r(X) patient										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*Total r(X)*	*Total 45,X*
Lab ID	891693	882273	872889	891531	903634	840941	902485	853194	892043		
Short stature	+	+	−	+	+	+	+	−	+	7/9	13/16
Gonadal dysgenesis	+			+	+	+	+		+	6/6	10/10
Widely spaced nipples	+	−	−	+	−	+	+	+	−	5/9	9/16
Webbed neck	−	−	−	−	−	+	−	−	−	1/9	6/16
Short neck	−	+	+	+	−	+	+	+	−	6/9	10/16
Pectus excavatum	−	−	+	−	−	−	−	−	−	1/9	4/16
Congenital lymphoedema	−	−	−	−	−	d/k	−	−	−	0/8	12/16
Epicanthic folds	−	+	−	−	−	−	+	−	−	2/9	10/16
Forward facing ears	−	+	−	−	−	−	+	−	−	2/9	8/16
Low posterior hairline	−	−	+	+	+	−	−	+	−	4/9	12/16
Narrow/hyperconvex nails	+	−	−	−	−	−	−	+	−	2/9	7/16
Cubitus valgus	−	−	−	−	−	−	−	−	−	0/9	3/16
Short 4th metacarpal	+	+	−	+	−	−	+	−	+	5/9	9/16
Narrow palate	−	+	+	+	−	−	+	−	−	4/9	11/16
Small jaw	−	−	−	−	−	+	−	−	−	1/9	5/16
Excess pigmented naevi	+	−	−	−	+	+	−	+	−	4/9	6/16
Glue ear/hearing loss	+	+	+	+	+	d/k	+	+	−	7/8	10/16
Squint ever	−	−	+	+	−	+	−	+	+	5/9	7/16
Cardiac anomaly	−	−	+	−	−	−	−	−	−	1/9	1/16
Renal anomaly	−	−	−	−	−	−	−	+	−	1/9	4/16
Head circumference (centile)	>90	>50	50	>25	>3	>75	<3	>10	d/k		
General IQ	110	106	<70	72	80	70	67	104	81	m 84	m 95
Parental error*	P	M	P	M	P	M	M	M	P	5M 4P	8M 7P
% r(X) cells	14	20	50	1	10	6	71	11	1		
Size of r(X)†	med	sm	sm	med	med	med	sm	min	lge		
Replication of r(X)	5 late 2 early	d/k	17 late 9 equiv 1 early	late	late	late	late	late	late		

* M = maternal error, P = paternal error (1 unknown in 45,X group).
d/k = don't know.
† Size of ring: min = minute, smaller than G group chromosome
sm = small, size of G group chromosome
med = medium, size of F group chromosome
lge = size of C group chromosome

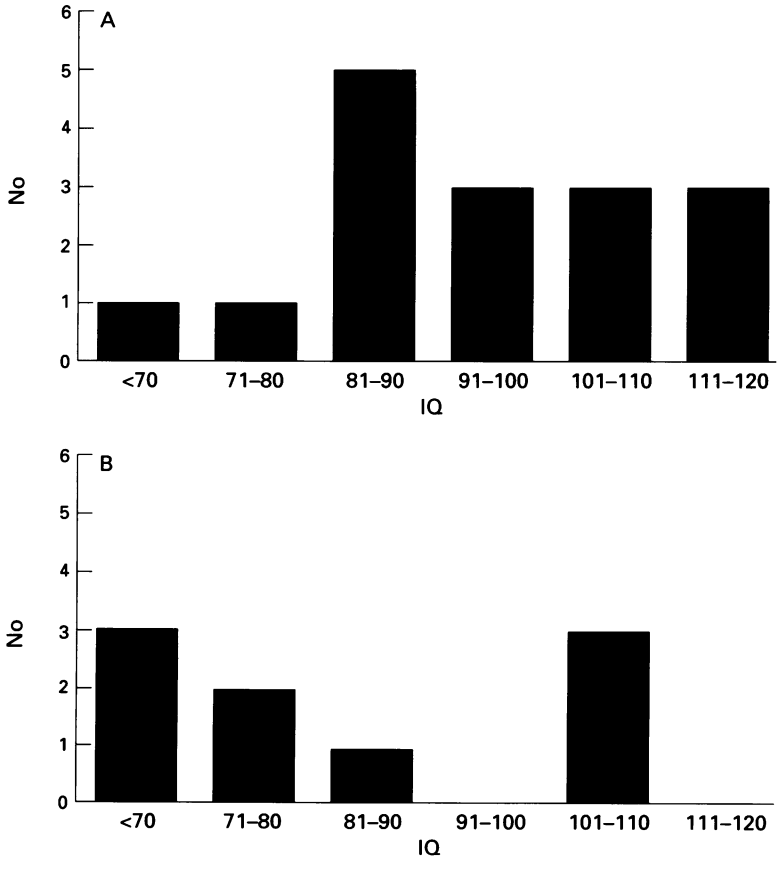

Figure 1 (A) General IQ in the 45,X patients. (B) General IQ in the ring (X) patients.

oedema, present in 12/16 (75%) of the 45,X group but in none of the ring (X) patients. Webbing of the neck, forward facing ears, and epicanthic folds, which are all thought to reflect in utero oedema, are similarly much less frequently seen in the ring (X) group.

The phenotypic differences between the two groups are reflected in the clinical presentation and age of karyotyping (table 2, fig 2). In the 45,X group, 13/16 were referred for suspicion of Turner's syndrome or short stature, and the majority (10/16) were diagnosed in the first year of life. Only one ring (X) patient (No 3) was karyotyped in the first year of life, because of congenital limb defects. The majority of ring (X) patients were not diagnosed until after the age of 11, usually because of the failure of spontaneous pubertal development.

IQ

The mean IQ of 95 (SD 15) in the 45,X group does not differ significantly from the population mean of 100. However, despite the small number, the mean IQ of 84 in the ring (X) group is significantly reduced ($p < 0.05$ Student's t test). Strikingly, the majority (5/9) of the ring (X) group had an IQ of 80 or below and four of them required special schooling, while 6/9 were felt (by themselves or their families) to be less bright than their sibs (this was true of 5/16 of the 45,X group). In the ring (X) group there is a suggestion of a bimodal distribution of IQ while a more normal distribution of IQ is seen

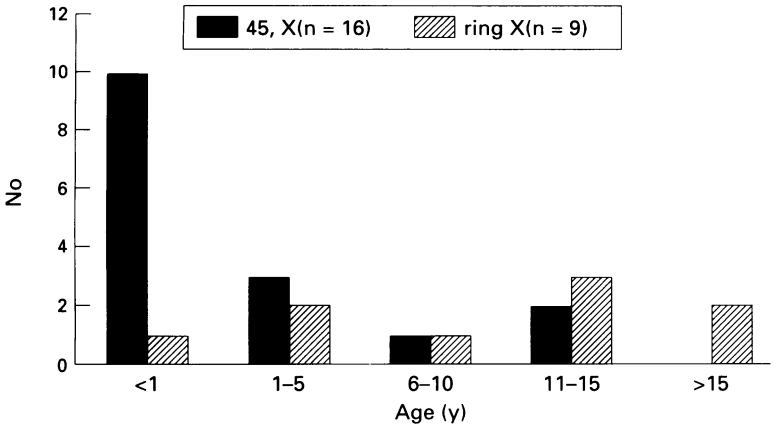

Figure 2 Age at diagnosis.

Table 2 Reason for chromosome analysis

	45,X (n=16)	*r(X) (n=9)*
Clinically ?Turner's	9	2
Short stature	4	1
Pubertal failure	1	4
Dysmorphic	1	2
Developmental delay	1	0

in the 45,X group (fig 1A and B), with only one attending special school. However, four of the 45,X group attending normal school needed special help in one or more subjects (two maths, one reading, one generally).

DYSMORPHOLOGY

Van Dyke *et al*[4] described his patients with a small ring (X) (all with mental retardation) as having growth retardation of prenatal onset, small head circumference, triangular shaped face, and occasionally seizures, simian creases, heart disease, and pigmentary dysplasia. We have previously described three other ring (X) patients with a severe phenotype[5] and facial dysmorphism reminiscent of, but distinct from, Kabuki make up syndrome. These and any other dysmorphic features were sought in both the 45,X and ring (X) groups. Fig 3 shows eight of the nine ring (X) patients. Patient 3 was included in a previous report.[5] Patients 2, 3, 4, 5, 6, and 8 all share some features with the previously described patients.[1 5–10] The characteristic features include a broad nose with anteverted nostrils, a prominent philtrum, a wide mouth with a thin upper lip, and long palpebral fissures. In many cases the hair is abundant and thick and the eye lashes are thick and dark. One of our patients (patient 3) had major limb abnormalities and syndactyly and was described previously.[5] Unlike the patients of Van Dyke *et al*,[4] the head circumference varies from less than the 3rd centile to greater than the 97th. No further cases of pigmentary dysplasia were seen.

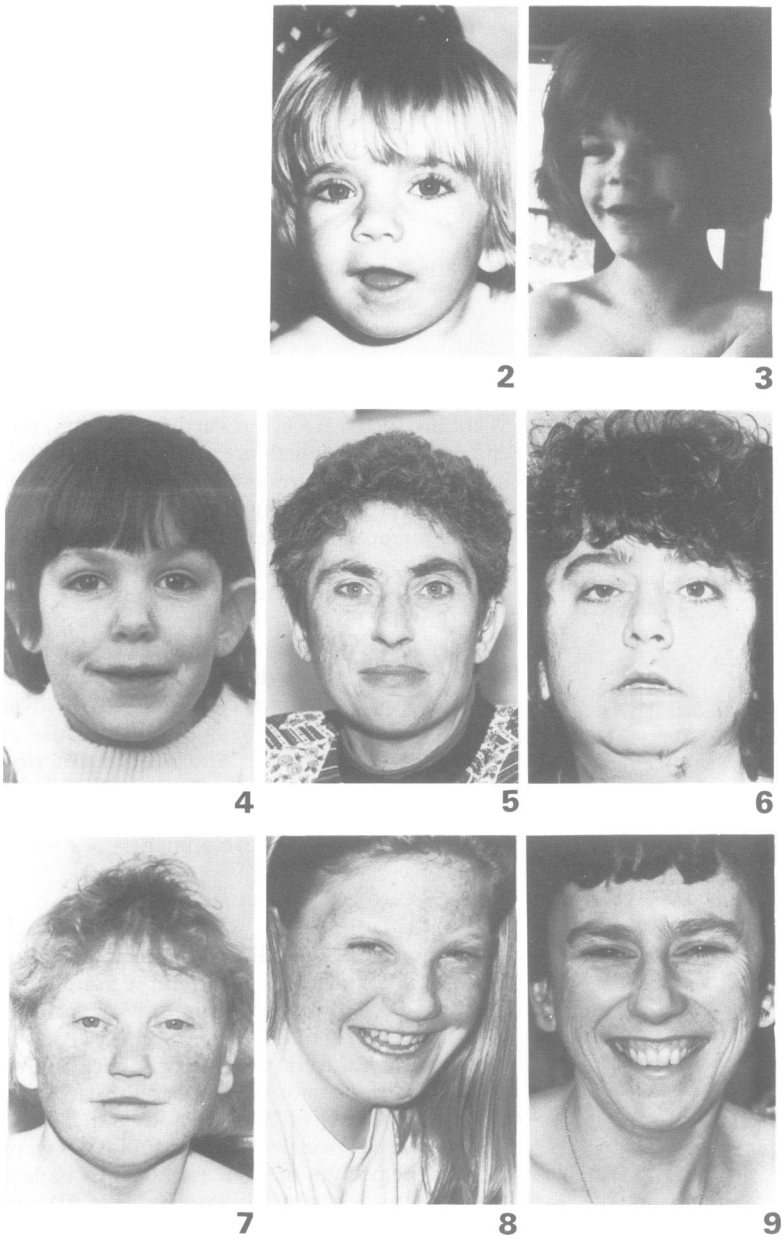

Figure 3 The ring (X) patients (No 3 reported previously.[5] Permission refused by No 1).

OTHER FEATURES

Ring (X) patients

Among these, two (Nos 2 and 7) had dry skin or eczema, one (No 4) was very obese, and one (No 3) had small, fleshy skin tags on her limbs and trunk which appeared from time to time, bled, then resolved. One patient (No 4) had broad thumbs and hirsutism and had a recurrent schizoaffective disorder, which had also been present in her mother.

45,X patients

Three patients had joint laxity, two had psoriasis, and two had spatulate fingers. In common with some of the ring (X) patients, four had very thick hair and two had long thick eyelashes.

PARENTAL ORIGIN

Fig 4 (A and B) shows the parental origin of the chromosome error related to IQ in the two groups and does not show any clear link between the two. The proportions of maternal and paternal errors in the 45,X group are approximately equal and do not reflect the preponderance (~80%) of paternal errors usually seen in these patients,[11 12] presumably because of the small sample size. The ring (X) group reflects the approximately equal maternal:paternal proportions seen in structural abnormalities of the X chromosome generally.[6]

Discussion

Of all patients with Turner's syndrome approximately half have a 45,X karyotype in peripheral blood lymphocytes, the remainder being mosaics, or having a structural X or Y chromosome abnormality. Patients with a ring (X) cell line accounted for around 23% of cases in a recent cytogenetic and molecular reappraisal of Turner's patients in Wessex.[6]

The ring (X) group lack many of the "classic" Turner's syndrome features such as webbed neck, congenital lymphoedema, anteverted ears, and epicanthic folds. These features are attributable to in utero lymphoedema and may be caused by delayed opening of the jugular lymphatic sac into the jugular vein, which normally occurs by 8 weeks' gestation.[13]

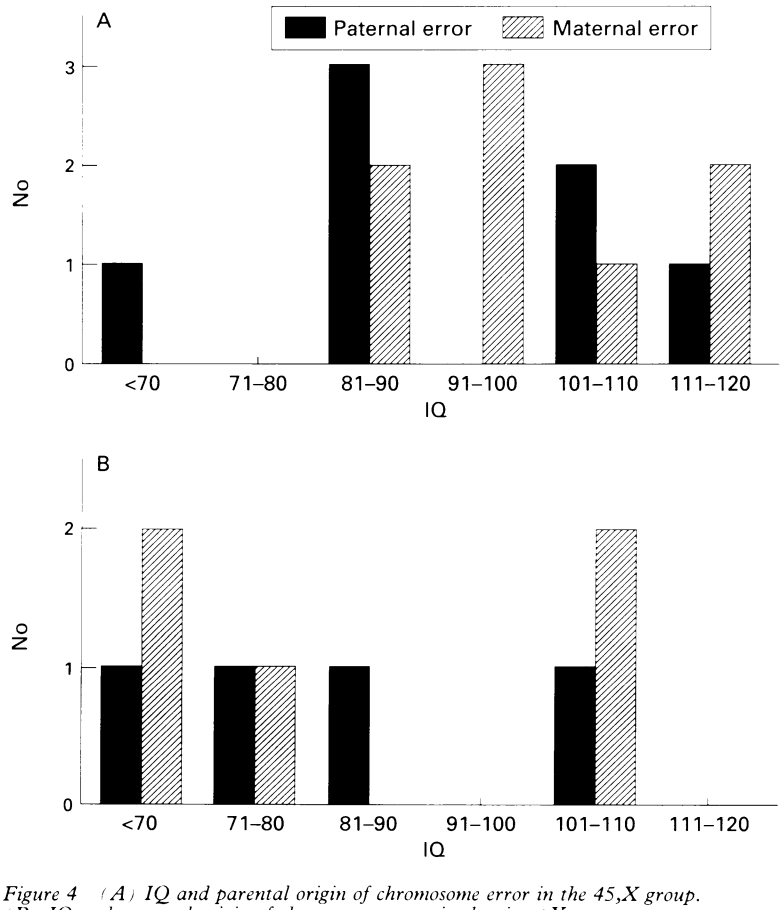

Figure 4 (A) IQ and parental origin of chromosome error in the 45,X group. (B) IQ and parental origin of chromosome error in the ring (X) group.

The absence of features related to in utero lymphoedema in ring (X) patients has been commented on previously.[14] It has recently been suggested that deletion of a gene present on both the X and Y, outside the pseudoautosomal region and escaping X inactivation, may be responsible for the somatic features of Turner's syndrome and that the S4 ribosomal protein gene located at Xq13.1 is a candidate for such a gene.[15] The position of this gene close to the centromere means that it is likely to be preserved in ring (X) chromosomes. The presence of the usual two functioning copies of such a gene or genes may explain the less "classic" phenotype of the ring (X) patients.

Turner's syndrome patients are usually of normal intelligence,[16] although some may show specific learning disabilities.[17 18] Our ring (X) patients show a reduction in general IQ of 11 points compared with the 45,X group, but with a suggestion of a bimodal distribution, and a high requirement for special education. A review of 36 patients with a ring (X) cell line in 1983 did not mention mental retardation.[19] Possibly those patients with a severe phenotype had been assumed to have an autosomal ring as the origin of small rings was difficult to assess before the advent of molecular cytogenetics. Other authors have reported mental retardation and dysmorphism associated with a 45,X/46,X(r)X karyotype.[1–5 10] Initially these features were only reported in association with small rings but, among our patients, not only IQ but also the facial dysmorphism is independent of the size of the ring. Of those patients who we feel have the "ring (X) facies" (Nos 2, 3, 4, and 6), one has a large ring,[6] one a medium ring,[4] one a small ring,[3] and one a very small ring.[2] Additionally, the presence of the "ring (X) facies" is not always associated with a low IQ, one of our dysmorphic patients (No 2) having an IQ of 106, the second highest in the group.

Several authors have recently commented on similarities between the Kabuki make up syndrome and the "ring (X) facies". Indeed, among a report of 62 cases of Kabuki make up syndrome,[10] one had a ring (X) cell line and one (female) a ring (Y) cell line. Kajii *et al*[20] also reported "Kabuki make up/Ullrich-Turner syndrome". Although the two syndromes share some features such as long palpebral fissures and growth deficiency, we feel they are distinct phenotypes and the "ring (X) syndrome" does not show arched eyebrows and lower lid eversion. However, we know of one 45,X patient with no evidence of mosaicism in blood or skin who has convincing features of Kabuki make up syndrome (Slaney, personal communication), so the Kabuki-like phenotype may not be confined to ring (X) patients.

The parental origin of the chromosome error does not appear to influence either the dysmorphism or the IQ in either group. Other authors[11 21] have also found that the phenotype is not influenced by the parental origin of the chromosome error. Lorda-Sanchez *et al*[11] found no 45,X fetuses or liveborns with a maternal error and cardiovascular abnormality, but our 45,X patient with a coarctation had a maternal error and Mathur *et al*[21] found 3/7 patients with

a maternal error had cardiac abnormalities. Structural abnormalities of the X chromosome appear equally likely to occur in either parent,[6] in contrast to X monosomy, where paternal error is found in around 80% of both liveborns and fetuses.[11 12]

Further studies are in progress to clarify the relationship between the phenotype of these patients and the presence or absence within the ring (X) of the XIST locus at Xq13,[22] deletion of which is expected to result in a failure of X inactivation and inappropriate expression of genes on the ring (X). Previous observations that patients with smaller ring (X) chromosomes were more likely to be mentally retarded would be consistent with this mechanism. The fact that we found no clear relationship between mental retardation and the size of the ring may just reflect the existence of complex intrachromosomal rearrangements in some of our patients so that proximal sequences are deleted even though the ring is large.

Our findings and those of previous authors show that Turner's syndrome patients with a ring (X) cell line are more likely to show mild to moderate mental retardation than those with a 45,X karyotype. Some patients have a characteristic facial appearance which is distinct from the Kabuki make up syndrome and there is an as yet undetermined risk of syndactyly and limb anomalies. These results have important implications for genetic counselling when a ring (X) cell line is found at prenatal diagnosis.

CASE HISTORIES OF THE RING (X) PATIENTS

Details of physical features and IQ are given in table 1.

Patient 1. Born at 36/40 gestation. Parents always concerned about her, with recurrent middle ear infections requiring grommets and hearing aids in early childhood. Karyotyping performed at 12 years because of short stature. Academically very bright, considering a career in medicine. Height just under the 3rd centile aged 16 years, following androgen therapy. Had two spontaneous menses aged 14 and 15. Two troublesome moles have been removed.

Patient 2. Born at term. Persistent vomiting and poor weight gain as a baby. Diagnosed at 14 months when Williams syndrome was considered. Recurrent middle ear infections from 1 year, requiring grommets. Height below 3rd centile aged 3 years 10 months. She has a benign cardiac murmur owing to a ventricular band. Development normal.

Patient 3. Reported previously (case 2[5]). Born at 37 weeks, polyhydramnios in pregnancy. Limb shortening, oligobrachydactyly, and syndactyly were present. Attends special school for physically and mentally handicapped. She has a bicuspid aortic valve and dilated aortic root and strikingly long palpebral fissures.

Patient 4. Born at term. Recurrent ear infections and grommets. Karyotyped around 8 or 9 years because of short stature. Had remedial teaching in primary school and attended the special needs unit in a secondary school. Very obese. Works as a care assistant with the elderly.

Patient 5. Born at term, hyperemesis in the pregnancy. Possibly slightly delayed development, walking at 16 months, and late with speech. Karyotyped aged 18 years because of failure of puberty and rapid weight gain. Developed diabetes aged 38 years, on oral hypoglycaemics. Always required special needs teaching within a normal school. Employed as a homecare worker.

Patient 6. No family available for history. In residential care for moderate/severe mental handicap. Karyotyped aged 15 years. Hypothyroid, on thyroxine. Developed a schizoaffective disorder (?with seasonal component) aged 20 years, also present in her mother. Has tried assembly work but inconsistent. IQ estimated at 70 by clinical psychologist.

Patient 7. Born at term. Middle ear infections early on, development normal. Short stature noticed around 12 years, karyotyped aged 16 years. Attended normal school throughout. Mother felt she needed remedial teaching but none was available. Attended a training centre on leaving school aged 16 and received special help there. Has held packing and weighing jobs in factories for eight and three years and has worked in a fast food restaurant.

Patient 8. Born at 42 weeks. Karyotyped because of small stature aged 4 years. Recurrent ear infections and eczema. In normal boarding school. Would like to be a children's nanny or kennel maid.

Patient 9. Born at term. Normal development, no special health problems as a child. Karyotyped aged 14 years because of short stature and pubertal failure. Normal schooling. Typist/clerk in local council.

CELL LINES

Lymphoblastoid cell lines from patients 1 and 3 (catalogue numbers DD0872 and DD0397) and a fibroblast derived cell line from patient 3 (DD0964) have been established. These are available from the European Collection of Animal Cell Cultures, PHLS, Porton Down, Salisbury, Wiltshire SP4 0JG, UK. Cell lines from all the ring (X) patients may be available in the near future.

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