

Molecular cytogenetic characterisation of a small ring X chromosome in a Turner patient and in a male patient with congenital abnormalities: role of X inactivation

D F Callen, H J Eyre, G Dolman, M B Garry-Battersby, J R McCreanor, A Valeba, J J McGill

Abstract

The association of small accessory marker chromosomes in man with specific abnormalities has been difficult to define owing to variations in the chromosome origin and the size of the markers. In a patient with typical Turner phenotype and a 45,X/46,X,+mar karyotype the marker was shown to be a small portion of the long arm of the X chromosome which included the centromere and XIST, a candidate gene for the X inactivation centre. Therefore the lack of any additional abnormalities was attributed to inactivation of the portion of the X chromosome in the marker. In a patient with a 47,XY,+mar karyotype the mar was a small ring X chromosome which did not contain the XIST gene. For both markers the short arm breakpoints were localised between UBE1 and DXS423E. The congenital abnormalities of the male patient were attributed to the lack of X inactivation of the small ring and therefore disomic expression of normal genes possessed by the marker.

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The chromosomal origins of small accessory marker chromosomes may be identified by fluorescence in situ hybridisation (FISH) using chromosome specific pericentric repeat probes. Markers derived from the metacentric and submetacentric chromosomes can be found as small accessory ring chromosomes and examples from every chromosome except 5 and 10 have now been reported.¹⁻⁶ Patients with such marker chromosomes can have abnormal phenotypes, which, if because of the presence of the marker, must be the consequence of abnormal dosage of normal genes located in the euchromatin of the markers.

Small ring chromosomes have now been identified as derived from the X chromosome by use of in situ hybridisation procedures. Such ring chromosomes have been described in persons with Turner's syndrome with a 45,X/46,X,+mar karyotype,⁵⁻¹¹ in males with a 47,XY,+mar karyotype,³ and in patients with more than one extra marker chromosome.¹⁵ Markers which are derived from the X chromosome and contain the X inactivation centre would be

expected to be late replicating (inactive), and therefore be of little clinical significance. In contrast, markers lacking the X inactivation centre would not be inactivated and can therefore contain active genes. For such markers, it would be expected that the extra copies of the normal genes, resulting in two active copies rather than a single active copy, will be sufficient to result in an abnormal phenotype.

X inactivation may be determined cytogenetically by study of replication timing during the cell cycle. Using this approach it is difficult to assess the status of very small segments of chromosome, such as a marker. XIST is considered to be candidate gene for X inactivation since it is only expressed on the inactive X chromosome and maps to the region of the X inactivation centre.¹²⁻¹⁴ In this report two patients with small ring chromosomes were studied. These rings were identified as X derived and the presence of the inactivation centre was determined by FISH using a probe specific for the XIST gene.

Materials and methods

CASE REPORTS

Patient 1 was a male born after a normal labour and uneventful pregnancy. At the age of 2½ years he was referred because of global developmental delay. At this time, head circumference was on the 10th centile and he was dysmorphic with moderate scaphocephaly, a long philtrum, flattened nose, and a high palate with crowded dentition. Chromosome studies of 50 cells showed a 47,XY,+mar karyotype with all cells showing a small, probable ring, chromosome marker.

Patient 2 was a female born after a normal labour and uneventful pregnancy. Weight and height were within normal limits. At the age of 11 she presented with nocturnal, and occasional diurnal, enuresis. She was noted to be short (124 cm, <3rd centile) and her weight was 30 kg. Present were a low posterior hairline, cubitus valgus, and hyperconvex fingernails. Chromosome studies were performed because of a Turner syndrome phenotype and showed a 45,X/46,X,+mar karyotype. The small, apparent ring chromosome was present in two thirds of 100 metaphases scored. The patient is developing normally and is being treated with human growth hormone and oestrogen.

Department of
Cytogenetics and
Molecular Genetics,
Adelaide Children's
Hospital, South
Australia 5006
D F Callen
H J Eyre
G Dolman

Genetics Department,
Queensland Medical
Laboratory, Brisbane,
Queensland, Australia
M B Garry-Battersby

Peninsula Specialist
Centre, Kipparing,
Brisbane, Queensland,
Australia
J R McCreanor

Cytogenetics
Laboratory,
Mater Hospital,
South Brisbane,
Queensland, Australia
A Valeba

Department of
Child Health,
The Royal Children's
Hospital, Brisbane,
Queensland, Australia
J J McGill

Correspondence to:
Dr Callen.

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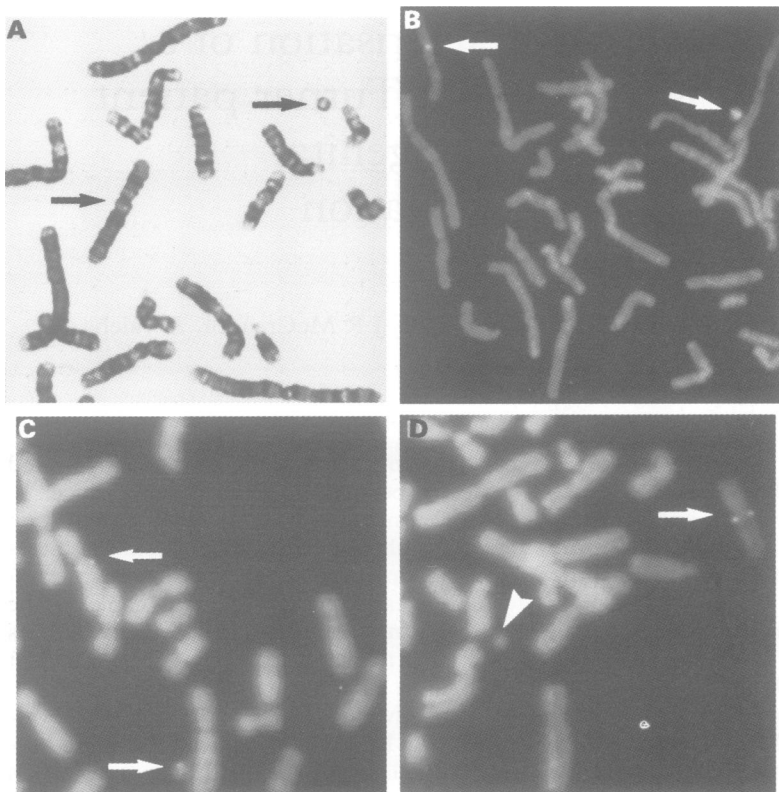


Figure 1 Cytogenetic and in situ hybridisation characterisation of marker chromosomes in patient 1. (A) G banding, (B) in situ hybridisation using X centromeric alphoid repeat probe TRX. Signal was detected by FISH. (C) FISH using the X long arm probe p8. (D) FISH using a probe to the XIST gene. Arrows indicate the normal X and the marker.

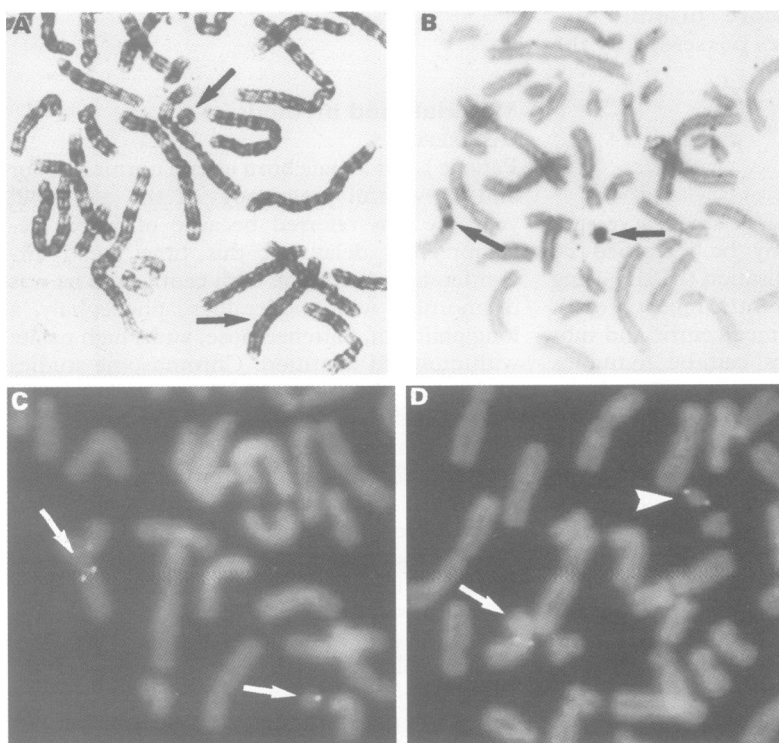


Figure 2 Cytogenetic and in situ hybridisation characterisation of marker chromosomes in patient 2. (A) G banding, (B) in situ hybridisation using the X centromeric alphoid repeat probe TRX. Signal detected by use of a silver enhancement technique. (C) FISH using the X long arm probe p8. (D) FISH using a probe to the XIST gene. Arrows indicate the normal X and the marker.

MOLECULAR CYTOGENETICS

The markers were initially recognised as r(X)s by in situ hybridisation of biotin labelled pericentric repeat probes to metaphase spreads. As previously described, signal detection was by a silver enhancement technique¹ or by fluorescence detection (FISH).¹⁵ The probe TRX is an X chromosome specific alphoid repeat probe kindly supplied by Dr K H A Choo.¹⁶ The X chromosome derived markers were investigated by FISH with two further probes: p8, DXS1 (Xq11.2-q12) and p58.1, DXS14 (Xp11.21). The location of the short arm breakpoints were also further characterised by FISH using cosmid probes containing the genes UBE1 (Xp11.23), DXS423E (Xp11.21), and ZXDA (Xp11.21).¹⁷ The presence of the gene XIST was determined by FISH using biotin labelled PCR products amplified by use of oligoprimers complementary to the published XIST gene sequence.¹²

Two primers sets were used: forward: 5'GTCCTAGTTCCTCAGTCTGTCCAGC3', reverse: 5'GTAGTTCACACTATCTAGGAGCAATG3' and forward: 5'CATTGCTCCTAGATAGTGTGAACTAC3', reverse: 5'TGACTAGAGGTCATCCACTGCTGAA3'. These products were 958 bp and 898 bp respectively and are generated from the XIST gene starting from base 2921. The human telomere probe from ONCOR was used to determine if telomere sequences were present on the markers.

Results

The ring Xs in both patients (figs 1A, 2A) were positive for the probe TRX, an alphoid repeat which, with the conditions used, is specific for the centric region of the X chromosome (figs 1B, 2B). The ring structures were confirmed by the absence of any signal on the r(X) in FISH experiments with a probe which detects all human telomeres in the same metaphase. The small ring chromosomes were further analysed by use of two additional probes, p58.1 (DXS14) and p8 (DXS1), which are derived from unique DNA adjacent to the centromere on the short and long arms of the X respectively.¹⁸ Both of the small ring chromosomes were positive for the p8 probe (figs 1C, 2C) but negative for p58.1. Localisation of the short arm breakpoint was undertaken by use of additional probes, and for both markers the breakpoints were between UBE1 and DXS423E (table).

Further characterisation by FISH using the biotinylated PCR amplified DNA of the XIST gene showed that the marker in patient 1 did

Summary of in situ hybridisation results to markers

Probe (locations)	Patient 1 (47,XY,+mar)	Patient 2 (45,X/46,X,+mar)
Telomere	-	-
DXS14 (Xp11.2)	-	-
UBE1 (Xp11.23)	-	-
DXS423E (Xp11.21)	+	+
ZXDA (Xp11.21)	+	+
TRX (X centromere)	+	+
DXS1 (Xq12)	+	+
XIST	-	+

+ indicates marker contained the probe used.

not contain XIST while the marker of patient 2 did contain the XIST gene (figs 1D, 2D). This was consistent with the larger size of the marker in the second patient. These results are summarised in the table.

Discussion

The small ring chromosomes in both patients were derived from the X chromosome and were positive for DXS1 adjacent to the centromere on the long arm. The short arm breakpoints of both rings were between UBE1 and DXS423E. These studies aim to give information which can provide prospective clinical outcomes for patients with similar marker chromosomes. This can only be achieved with both the evaluation of the clinical phenotype of the patient and the delineation of the euchromatin present in the ring. Accumulation of similar cases should allow particular phenotypic characteristics to be assigned to disomy for a defined region of the X chromosome, as has been described for chromosome 21 and Down's syndrome.¹⁹

The XIST gene is a candidate for a gene which determines X inactivation since it is only expressed from the inactive X, and maps to the region known to contain the X inactivation region.¹²⁻¹⁴ Ring chromosomes containing XIST are likely to be inactivated and the presence of the ring is therefore unlikely to contribute to the patient's phenotype. When XIST is absent, the euchromatin of the marker will be active resulting in disomic expression of normal genes. The subsequent overexpression and interactions of these genes is the genetic basis for the abnormal phenotype.

It is now established that patients with a small ring marker derived from the X chromosome in association with a 45,X karyotype show the typical Turner phenotype if the X inactivation centre is present (patient 2 of this report, patients presented in references 17 and 20). It is unlikely that the lack of phenotypic effect of the r(X) in such patients can be explained by the presence of mosaicism for the marker. A smaller r(X) lacking the XIST gene has been reported in 70% of cells in a patient with a 45,X/46,X,+r(X) karyotype.¹⁷ In addition to the expected Turner phenotype, this patient had learning difficulties. Therefore, in patient 2 of this report, the lack of additional phenotypic abnormalities is likely to be attributed to X inactivation of the r(X) rather than the presence of mosaicism. Among the X derived markers lacking the XIST gene, in addition to Turner's syndrome, there is mental retardation and multiple congenital malformations.^{17,21,22} It has been suggested that within this group the degree of clinical severity of such patients correlates with the size or the frequency, or both, of the mar(X)s.¹⁷

The male patient (patient 1) had global developmental delay and minor malformations. This phenotype may be attributed to the abnormal dosage (two rather than the single dose) of a gene or genes located in the euchromatin of the ring chromosome, since XIST was not present on this chromosome and therefore

the r(X) was not inactivated. A male patient with a similar karyotype, 47,XY,+r(X), has been reported, although the location of the euchromatin on the X chromosome was not determined.³ This patient had global developmental delay and malformations, inguinal hernias, cryptorchidism, adducted, low inserted thumbs, dysplastic ears, and a haemangioma of the upper eyelid and nasal alae.

A further male patient with 47,XY,+r(X) has been described and in this case XIST was not present or expressed.²³ The phenotype of this patient was of mild learning difficulties, joint laxity, long, thin fingers, but no other malformations. All three of these male patients were variably retarded in motor and mental development but there were no obvious similarities in congenital abnormalities. These differences are presumably because of variation in the euchromatin present in each marker chromosome. Comparisons between clinical phenotypes of these markers will require additional patients and detailed molecular characterisation of the X chromosome content of each marker.

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