A molecular and FISH analysis of structurally abnormal Y chromosomes in patients with Turner syndrome

D O Robinson, P Dalton, P A Jacobs, K Mosse, M M Power, D H Skuse, J A Crolla

Abstract

Fourteen patients with Turner syndrome and a structurally abnormal Y chromosome were analysed by PCR amplification and fluorescence in situ hybridisation for the presence of sequences specific to defined regions of the Y chromosome. Thirteen patients had a mosaic karyotype including a 45,X cell line and one case was non-mosaic in cultured lymphocytes. Ten patients had a pseudodicentric Yp chromosome, two an isodicentric Yq, one a pseudodicentric Yq, and one a derived Y chromosome. Two of the patients with a psu dic(Yp) chromosome had complex karyotypes with more than two cell lines, one of which exhibited five morphologically distinct mar(Y) chromosomes, presumably derived from a progenitor psu dic(Yp). Nine of the ten psu dic(Yp) chromosomes were positive for all Yp and Yq probes used except DYZ1 which maps to Yq12, suggesting a common breakpoint near the Yq euchromatin/heterochromatin boundary. In the three patients with a dicentric Yq chromosome two different breakpoints were observed; in two it was between PABY and the subtelomeric repeat sequence and in one it was between DYZ5 and AMGY in proximal Yp. Our results suggest that the great majority of structurally abnormal Y chromosomes found in Turner syndrome mosaics contain two copies of virtually all of the functional Y chromosome euchromatin.

(*J Med Genet* 1999;**36**:279–284)

Keywords: Turner syndrome; Y chromosomes; marker chromosomes; fluorescence in situ hybridisation

Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, Wiltshire, SP2 8BJ, UK
D O Robinson
P Dalton
P A Jacobs
M M Power
J A Crolla

Institute for Hereditary Diseases, Orlovskaya St 66, Minsk 220053, Belarus
K Mosse

The Behavioural Sciences Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
D H Skuse

Correspondence to:
Dr Robinson.

Received 10 June 1998
Revised version accepted for publication 20 August 1998

Turner syndrome affects 1 in approximately 3000 liveborn females[1] and is thought to be caused by the absence of genes normally present on the second sex chromosome. Approximately half of all patients have a 45,X karyotype and the remainder are demonstrable mosaics for a cell line containing a second sex chromosome. This is usually a normal or structurally abnormal X, but in about 6% of cases[2–5] a second cell line containing a structurally abnormal Y chromosome is present. The presence of Y chromosome sequences is significant in that such patients have an increased risk of developing gonadoblastoma.[6]

Y chromosome aneuploidy has been reviewed by Hsu,[7] who described 350 cases with abnormal Y chromosomes characterised using conventional cytogenetic techniques. As these were analysed by karyotyping no detailed molecular information on the Y sequences present could be given. A number of reports have described the use of PCR to identify the presence or absence of specific Y sequences in patients with Turner syndrome,[8–11] but such analysis does not give information about the structure of the Y chromosome present. Fluorescence in situ hybridisation (FISH) is a more useful technique for determining the detailed structure and content of abnormal chromosomes. However, reports describing the FISH analysis of abnormal Y chromosomes are mostly limited to the identification of centromeric sequences,[12–16] although Muller *et al*,[17] Diekmann *et al*,[18] Bukvic *et al*,[19] and Liou *et al*[20] have made some use of Yp and Yq FISH probes. In this paper we present our findings from an extensive FISH and PCR analysis of 14 Turner syndrome patients with abnormal Y chromosomes.

Materials and methods

SUBJECTS

Fourteen cases ascertained for Turner syndrome and known from conventional karyotype analysis to carry an abnormal Y chromosome were analysed. Twelve of these were identified during a systematic survey of 211 patients with Turner syndrome previously described by Jacobs *et al.*[5] A further two patients, 97/1158 and 95/4053, were identified during the routine analysis of such cases at the Wessex Regional Genetics Laboratory and could be included in the present study because cell lines were available. The karyotypes are shown in table 1.

CONVENTIONAL CYTOGENETIC AND FISH METHODS

Conventional cytogenetic analysis following GTL banding and BrdU late labelling studies were carried out on all patients on metaphases derived from either PHA stimulated peripheral blood lymphocytes, skin fibroblasts, or lymphoblastoid cell lines recalled from the European Cell and Culture Collection, Porton Down, Salisbury, Wilts SP4 OJ4, UK.

The 14 cases were studied using a standard in situ hybridisation technique based on the method of Pinkel *et al.*[21] The YACs, cosmids, and plasmid probes (fig 1) were nick translated using either biotin or digoxigenin (Boehringer-Mannheim), and then applied either singly or

Table 1 Karyotypes of cases studied

Case No	*Karyotype*
95/2899	45,X[76]/46,X,idic(Y)(qter→p11::p11→qter)[24]
96/6937	45,X[90]/46,X,psu dic(Y)(qter→p11.3::p11.3→qter)[10]
96/7949	45,X[43]/46,X,idic(Y)(qter→p11.3::p11.3→qter)[57]
96/8698	45,X[92]/46,X,der(Y)(qter→p11::q11.2→qter)[8]
95/2580	45,X[9]/46,X,psu dic(Y)(pter→q11::q11→pter)[91]
95/2553	45,X[21]/46,X,psu dic(Y)(pter→q11::q11→pter)[79]
95/2900	45,X[77]/46,X,psu dic(Y)(pter→q11.2::q11.2→pter)[23]
96/5408	46,X,psu dic(Y)(pter→q11.2::q11.2→pter)[100]
96/7171	45,X[82]/46,X,psu dic(Y)(pter→q11::q11→pter)[18]
95/4323	45,X[73]/46,X,psu dic(Y)(pter→q11::q11→pter)[27]
96/8253	45,X[63]/46,X,psu dic(Y)(pter→q11::q11→pter)[37]
95/4053	45,X[27]/46,X,psu dic(Y)(pter→q11::q11→pter)[8]
97/1158	45,X[26]/46,X,psu dic(Y)(pter→q11::q11→pter) [26]/47,X,psu dic(Y)(pter→q11::q11→pter), + psu dic(Y) (pter→11::q11→pter)[3]
97/2598	45,X[9]/46,X,psu dic(Y)(16)/46,X,r(Y)[20]/46,X,mar(Y) [13]/47,X,r(Y),+mar(Y)[42]

more usually in a pairwise combination consisting of the Y84 (DYZ3) alphoid centromere probe and one of the YACs or cosmids differentially labelled. The site(s) of hybridisation were examined after stringent washing (1.0 × SSC in 50% formamide, two five minute washes at 42°C) and visualised using one layer of avidin conjugated (FITC or Texas Red) for biotin labelled probes or antidigoxigenin (FITC or TRITC conjugated) for digoxigenin labelled probes. The chromosomal DNA was counterstained with 0.05 mg/ml DAPI suspended in an antifade solution (Vectashield, Vector Labs, UK).

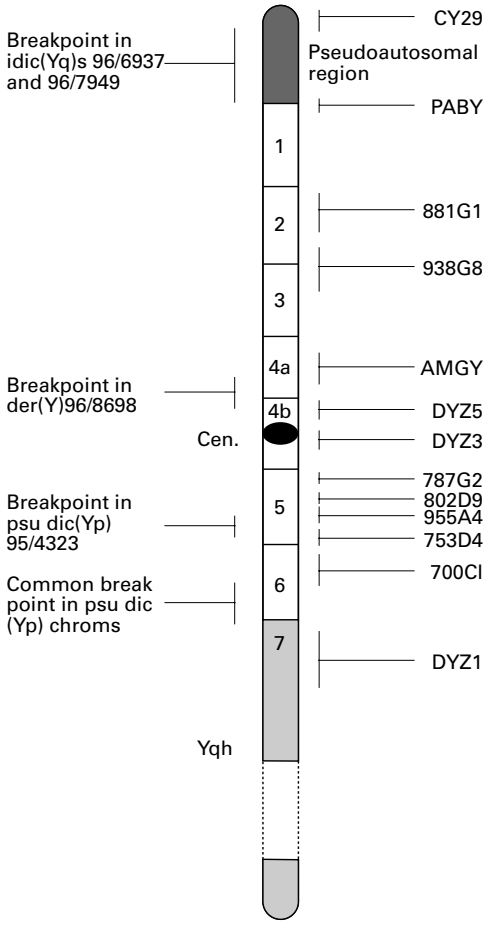

Figure 1 Y chromosome map locations of probes used and breakpoints in the abnormal Y chromosomes studied. Map locations are as described by Vergnaud et al,[22] Jones et al,[23] and Vollrath et al.[28] The area of the Y chromosome designated "Common breakpoint in psu dic (Yp) chroms" indicates the breakpoints in cases 95/2580, 95/2900, 96/5408, 96/7171, 96/8253, 95/4053, 97/1158, and 97/2598.

Slides were examined using a Carl Zeiss Axioskop epifluorescent microscope fitted with Chroma Technology's Pinkel fluorescent No 83 filter series. Images were captured using a cooled CCD camera and the digitised data were analysed and visualised using "Smartcapture" software (VYSIS, UK). A minimum of five metaphases containing the structurally abnormal Y chromosome were examined after hybridisation with each probe, but in case 97/2598, more cells were scored in order to detail the extent of mosaicism (see below). In cases where a negative FISH result was found on the structurally abnormal Y, analysis was repeated using the patient's cells in parallel with metaphases from a normal male and confirmed negative in the abnormal Y when the probe showed >95% hybridisation efficiency (usually 100%) to the control's normal Y chromosome. The relative positions of the probes were inferred from the maps in Vergnaud *et al*,[22] Jones *et al*,[23] and Kirsch *et al*.[24]

IDENTIFICATION OF Y CHROMOSOME SEQUENCES BY PCR AMPLIFICATION

DNA was extracted from peripheral blood using a salt precipitation technique.[25] DNA samples were analysed for the presence of Y chromosome sequences using the Y specific PCR primers AMXY in Yp11.1,[26] DYZ3 at the Y centromere, DYZ1 at Yq12, SRY at Yp11.32, and PABY at the Yp pseudoautosomal boundary (fig 1).[27 28] With the exception of the AMXY primers which amplify sequences of different sizes from the X and Y chromosomes, all PCR amplifications were carried out as duplex reactions containing a second set of X chromosome specific primers amplifying a larger product than the Y chromosome amplicon as a control.

The AMXY reaction conditions were as described by Nakahori *et al*[26] with primer AMXYNR end labelled with α^{32}PdATP. The reaction products were separated by electrophoresis through 6% polyacrylamide and visualised by autoradiography. DYZ3, DYZ1, and PABY were coamplified with the PABX X chromosome specific primers using the conditions described by Coto *et al*.[11] The amplification products were separated by electrophoresis through 1.6% agarose and visualised by ethidium bromide staining. The SRY primers were coamplified with the dystrophin exon 48 primers described by Beggs *et al*[29] using the same protocol except that the magnesium chloride concentration was 2.1 mmol/l and the annealing temperature was raised to 62°C. The amplification products were visualised by electrophoresis through 8% polyacrylamide and ethidium bromide staining.

Results

Tables 1, 2, and 3 show the results. Thirteen of the 14 patients had a mosaic karyotype with a 45,X cell line and one patient, 96/5408, with a

Table 2 Summary of PCR and FISH results

	Case	*Subtel*	*PABY*	*SRY*	*SRY*	*881G1*	*938G8*	*AMGY*	*DYZ5*	*DYZ3*	*DYZ3*	*787G2*	*802D9*	*955A4*	*753D4*	*700C1*	*DYZ1*
i(Yq)	95/2899	PP	p	PP	p	PP	PP	p	NT	PP	p	PP	PP	PP	PP	PP	p
i(Yq)	96/6937	N	p	PP	p	PP	PP	p	NT	PP	p	PP	PP	PP	PP	PP	p
i(Yq)	96/7949	N	p	PP	p	PP	PP	p	NT	PP	p	PP	PP	PP	PP	PP	p
i(Yq)	96/8698	NT	n	NT	n	NT	NT	n	P	P	p	PP	PP	NT	PP	PP	p
i(Yp)	95/2580	PP	p	PP	p	PP	PP	p	NT	PP	p	PP	PP	PP	PP	PP	n
i(Yp)	95/2553	NT	p	PP	p	NT	NT	p	NT	NT	p	NT	NT	NT	NT	NT	n
i(Yp)	95/2900	PP	p	PP	p	PP	PP	p	NT	PP	p	PP	PP	PP	PP	PP	n
i(Yp)	96/5408	PP	p	PP	p	PP	PP	p	NT	PP	p	PP	PP	PP	PP	PP	n
i(Yp)	96/7171	PP	p	PP	p	PP	PP	p	NT	PP	p	PP	PP	PP	PP	PP	n
i(Yp)	95/4323	PP	p	PP	p	PP	PP	p	PP	PP	p	PP	PP	PP	N	N	n
i(Yp)	96/8253	PP	p	PP	p	PP	PP	p	NT	PP	p	PP	PP	PP	PP	PP	p
i(Yp)	95/4053	PP	p	PP	p	PP	PP	p	PP	PP	NT	PP	PP	PP	PP	PP	n
i(Yp)	97/1158	PP	p	PP	p	PP	PP	p	NT	PP	NT	PP	PP	PP	PP	PP	n
i(Yp)	97/2598	PP	p	PP	p	PP	PP	p	NT	PP	p	NT	PP	NT	PP	PP	n

PP = two FISH signals; P = single FISH signal; N = negative with FISH; p = positive with PCR; n = negative with PCR; NT = not tested.

Table 3 Additional FISH results in case 97/2598

	Case	*Subtel*	*PABY*	*SRY*	*SRY*	*881G1*	*938G8*	*AMGY*	*DYZ5*	*DYZ3*	*DYZ3*	*787G2*	*802D9*	*955A4*	*753D4*	*700C1*	*DYZ1*
i(Yp)	97/2598	PP	p	PP	p	PP	PP	p	NT	PP	p	NT	PP	NT	PP	PP	n
$r(Y_1)$	97/2598	N	NA	P	NA	P	P	NA	NT	P	NA	P	P	NT	P	P	NA
$r(Y_2)$	97/2598	N	NA	N	NA	P	P	NA	NT	P	NA	N	N	NT	N	N	NA
$r(Y_3)$	97/2598	N	NA	NT	NA	NT	NT	NA	NT	PP	NA	NT	NT	NT	NT	NT	NA
mar(Y)	97/2598	PP	NA	P	NA	P	P	NA	NT	P	NA	P	P	NT	N	N	NA

PP = two FISH signals; P = single FISH signal; N = negative with FISH; p = positive with PCR; n = negative with PCR; NT = not tested; NA = PCR result not applicable (see text).

psu dic(Yp) chromosome was non-mosaic in blood lymphocytes (table 1). All patients had the Y centromeric sequence DYZ3. The testis determining gene SRY was present in all cases except 96/8698 with a derived Y chromosome (tables 2 and 3).

DICENTRIC (YQ) CHROMOSOMES

Case 95/2899 was positive for all probes used and clearly showed two foci of hybridisation with all FISH probes including the subtelomeric repeat CY29, indicating that the breakpoint had occurred distal to the subtelomeric repeat array (fig 2A). Subsequent hybridisation with the ONCOR all telomere probe did not show the presence of the $(TGAGGG)_n$ telomeric repeat unit, indicating a breakpoint close to the distal end of Yp. Cases 96/6937 and 96/7949 were both negative for the subtelomeric repeat but positive for the remainder of probes used including PABY, showing that a breakpoint had occurred between PABY and the subtelomeric probe CY29 (fig 2B). Karyotype analysis showed 95/2899 and 96/7949 to be dicentric and 96/6937 to be pseudodicentric.

DERIVED Y CHROMOSOME

Case 96/8698 gave a single signal for both the centromere probe DYZ3 and for DYZ5 in proximal Yp. However, the Yq specific FISH probes 787G2, 802D9, 753D4, and 700C1 showed a signal on each arm of the derived chromosome. A combination of FISH and PCR data identified a breakpoint in Yp between AMXY (AMGY) and DYZ5.

PSEUDODICENTRIC (YP) CHROMOSOMES

Nine of the 10 psu dic(Yp) chromosomes were studied with the whole panel of FISH probes but there was insufficient material available for detailed FISH analysis of the remaining case, 95/2533. Eight of the nine cases were positive with all FISH probes used and showed two distinct signals from the abnormal Y (fig 2C). PCR analysis showed DYZ1 to be absent, indicating a breakpoint between 700C1 (Yq11.2, interval 6A-B) and DYZ1 (Yq12, interval 7). In the remaining case, 95/4323, a breakpoint was identified between 955A4 (Yq11.22, interval 5J) (fig 2D[2]) and 753D4 (Yq11.22, interval 5Q).

Case 97/2598, one of the seven cases with a breakpoint between 700C1 and DYZ1, was exceptional in that FISH analysis identified five morphologically distinct marker chromosomes, compared with three defined by conventional cytogenetic analysis.[5] The results from these five mar(Y)s were as follows and are summarised in table 3.

(1) A psu dic(Yp) as described above (fig 2E).

(2) A small ring shaped marker, r(Y1), which was negative for the Yp subtelomeric probe and positive with a single focus of signal with all probes used, including SRY and the Y centromere probe, DYZ3, indicating that the r(Y) was monocentric (fig 2F).

(3) A small monocentric (DYZ3+) ring shaped marker, r(Y2), negative for the Yp subtelomere and SRY but positive with the two Yp YACs, 881G1 and 938G8 (fig 2G). This r(Y2) was negative for all the Yq YACs used.

(4) A small dicentric (DYZ3++) ring shaped marker, r(Y3), negative for the Yp subtelomere repeat probe (fig 2H).

(5) A small monocentric (DYZ3+) and metacentric shaped marker Y chromosome with copies of the Yp subtelomere repeat probe at the ends of both arms (fig 2H). FISH with cosmids and YACs showed that the mar(Y) was positive with SRY (single signal) and also the two Yp YACS, 881G1 and 938G8. Results with the Yq YACs showed a breakpoint between 802D9 and 753D4.

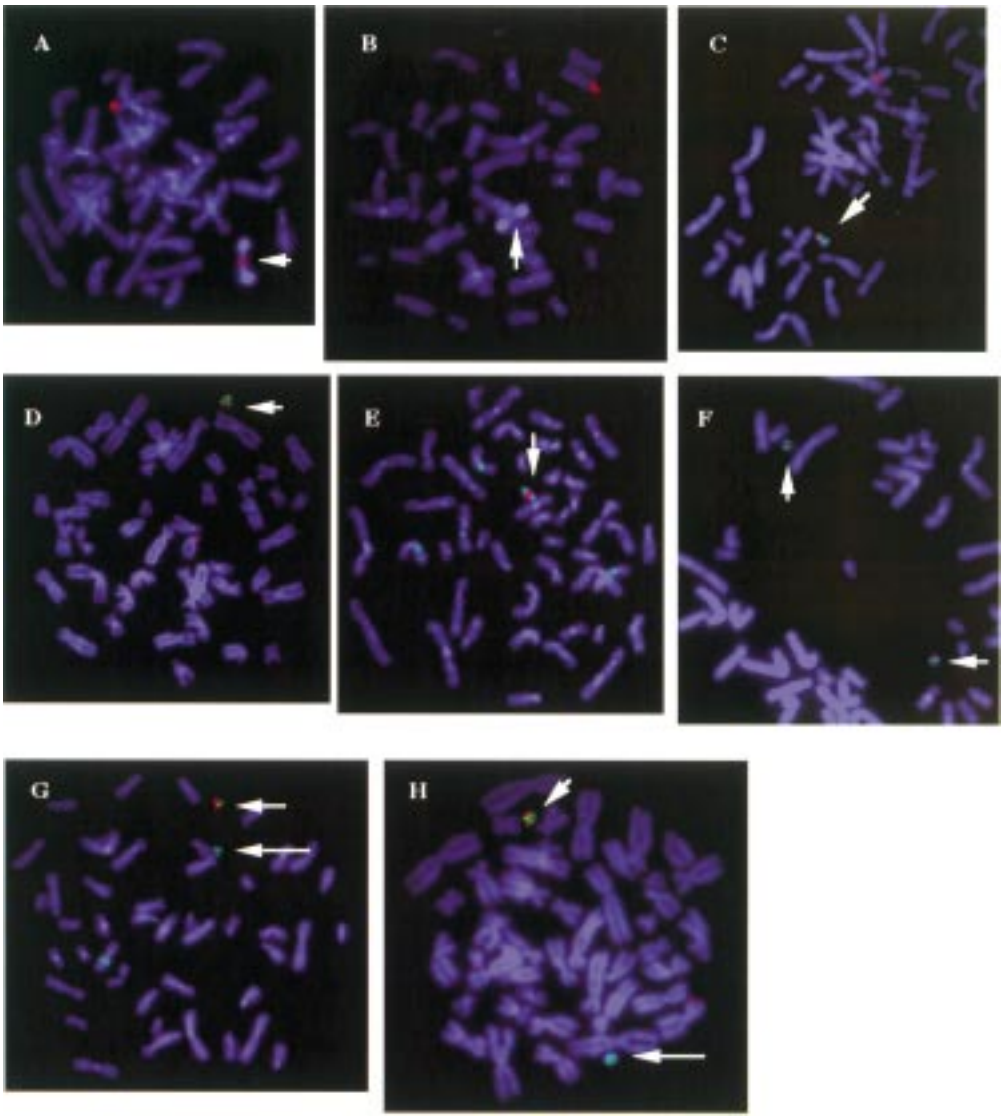

Figure 2 (A) Results following FISH using the Xp and Yp subtelomeric repeat cosmid CY29 in case 95/2899. Both the distal tip of the normal Xp and and the interstitial region of the iso(Yq) (arrowhead) are positive with the probe (red signal). (B) Results following FISH using the Xp and Yp subtelomeric repeat cosmid CY29 in case 96/6937. In contrast to (A), the iso(Yq) (arrowhead) does not show signal in the interstitial region, although the normal Xp is positive and therefore acts as an internal positive control. The same result was obtained in case 96/7949 (data not shown). (C) Dual colour FISH using DYZ3 (Y centromere, green signal) and 881G1 (red signal) in case 95/4053. The psu dic(Yp) (arrowhead) shows two distally located copies of red 881G1 signal and two more medially located Y centromeric signals. (881G1 is chimeric with cross hybridisation to Xq21.) (D) Dual colour FISH using DYZ3 (Y centromere, green signal) and 995A4 (red signal) in case 95/4323. The psu dic(Yp) (arrowhead) is positive for both probes. (E) Dual colour FISH result for the psu dic(Yp) in case 95/2598. The psu dic(Yp) (arrowhead) shows two copies of signal with probe 938G8 (green) and two coincident signals with 700C1 (red). (938G8 is chimeric with cross hybridisation to the chromosome 10 centromeres.) (F) Dual colour FISH using DYZ3 (green signal) and a cosmid from the SRY region (red signal) in patient 95/2598. Two copies of the small r(Y1) show a single focus of hybridisation with both probes (arrowheads). (G) Dual colour FISH using 881G1 (green signal) and 753D4 (red signal) in patient 95/2598. Two r(Y) chromosomes are present in this cell. Both rings are positive with the Yp probe 881G1, but only the upper ring, r(Y1), is positive with the Yq probe (small arrow), with the smaller r(Y2)s having only 881G1 present (large arrow). (H) Dual colour FISH using DYZ3 (green signal) and the Xp and Yp subtelomeric repeat cosmid CY29 (red signal) in patient 95/2598. The dicentric r(Y3) (large arrow) can be seen at the bottom of the metaphase which is also negative for the CY29 probe in contrast to the mar(Y) (arrowhead) at the top of the cell showing two copies of CY29 and a monocentric DYZ3 signal.

Discussion

Although extensive physical mapping of the normal human Y chromosome using a variety of methodologies has been published,[23 24] few reports of detailed molecular investigations of the structurally abnormal Y chromosomes seen in a proportion of Turner syndome mosaics have been published to date.[17–20 25] In the present study we have used a combined PCR and FISH approach to determine the structure of 12 abnormal Y chromosomes found during a systematic survey of patients with Turner syndrome[5] and two additional previously unpublished cases also referred because of Turner syndrome.

Thirteen of the 14 patients carried a dicentric Y isochromosome, 10 of which were psu dic(Yp), two idic(Yq), and one psu dic(Yq). These fall into two principal morphological groups corresponding to the subtypes identified by standard karyotype analysis described by Hsu *et al*[7]: firstly, morphologically monocentric psu dic(Yp) chromosomes comprising two copies of the Y short arm and cen-

tromere with a break/join within the Yq euchromatin proximal to the heterochromatic segment, and secondly dicentric iso(Yq) chromosomes comprising two copies of almost all of the Y chromosome with a break/join at the distal tip of the short arm. All 13 of the Y isodicentric chromosomes carry the testis determining gene SRY and the putative gonadoblastoma gene thought to lie in the Y pericentromeric region.[30 31] The remaining case was a monocentric derived Y chromosome with Yq sequences on both arms and only the proximal region of Yp present excluding SRY.

Of the 14 patients analysed 13 also had a 45,X cell line in blood lymphocytes, whereas one psu dic(Yp) case (96/5408) was non-mosaic (table 1). All cases analysed in this study were ascertained because they had Turner syndrome, but only a proportion of subjects mosaic for Y chromosome material exhibit features of Turner syndrome, the spectrum of phenotypes ranging from female to male depending upon the presence or absence of the testis determining gene SRY and, perhaps more importantly, the degree of mosaicism and the tissue distribution of SRY containing cells. All the cases presented here, apart from case 96/8698, have cells containing SRY but exhibit a female phenotype. It is thought that the development of a male phenotype is initiated early in development by action of the SRY gene product in the cells of the developing gonadal ridge.[32] It appears that in the patients in the present study, all of whom exhibit a female habitus, no or insufficient SRY transcript to specify a male phenotype was present in this tissue at the appropriate time during development. Patient 96/5408, however, has a non-mosaic karyotype in blood lymphocytes with a psu dic(Yp) chromosome containing SRY, but still has a female phenotype. We conclude that she must be a cryptic mosaic with an unidentified 45,X or other cell line lacking SRY. It is also possible that she carries a point mutation in SRY inhibiting its action.

Some indication as to the origin of Y isochromosomes can be deduced from the fact that a normal 46,XY cell line was absent from all 13 cases. This may be the result of ascertainment bias because of selection for Turner syndrome which is more likely to occur in the absence of a normal cell line. However, in a review of Y chromosome aneuploidy by Hsu *et al*,[7] no normal 46,XY cell line was found in 99 of the 102 isodicentric Y chromosomes described. This suggests that the abnormal Y chromosome was either (1) present in the sperm before fertilisation and resulted from an error during gametogenesis before the spermatid stage because two chromatids are required to generate these rearrangements, or (2) arose from an error in the first zygotic division. Errors occurring after the first zygotic division would result in mosaicism including a normal cell line. It can be argued that if the error occurred at a very early stage in development such mosaicism would be in many cases undetectable, the normal cell line being absent from the blood lymphocytes analysed in this study. However, the absence of a detectable 46,XY cell line in all 13 isochromosome cases described here and in 99 of the 102 described by Hsu *et al*[7] strongly suggests that such errors are more likely to occur during gametogenesis before the spermatid stage, or during the first division after fertilisation, rather than during subsequent cell divisions.

Thirteen of the 14 abnormal Y chromosomes in the current study are either dicentric or pseudodicentric and in all of these the breakpoints on both arms have the same cytogenetic location (table 1), FISH analysis being consistent with this. This suggests a mechanism involving a single break in the Y chromosome followed by fusion of the broken ends of sister chromatids and loss of the unstable acentric fragment. Similarly, James *et al*[33] presented evidence that X isochromosomes are usually generated by sister chromatid breakage and reunion. The derived Y case 96/8698, however, gives evidence of a mechanism other than a single breakage. Sequences distal to DYZ5 in Yp are absent and have been replaced by Yq sequences indicating breakpoints in both Yp and Yq.

There appears to be a common breakpoint or region prone to breakage in distal Yq11. Eight of the psu dic(Yp) chromosomes showed a duplicated FISH signal at all loci tested (table 2) but were negative for the DYZ1 sequence in the heterochromatic region of Yq. There was also no heterochromatin visible upon karyotype analysis. These eight therefore appear to contain all or virtually all of the Y euchromatin and may share a common distal Yq breakpoint in the interval between the most distal FISH marker used (700C1) and the border betwen Yq11 and Yq12. Kirsch *et al*[24] describe repetitive sequence blocks in Yq11 where sister chromatid breakage and inappropriate fusion of broken ends could occur to form isodicentric chromosomes.

The three dicentric Yq chromosomes were shown to have two different Yp breakpoint regions, either between the Yp subtelomere repeat array and PABY (96/6937 and 96/7949) or in the Yp telomeric region (95/2899). In all three cases, all positive FISH markers were shown to be present in two copies and the PCR results were positive at all loci tested (table 2). Similarly, Tuck-Muller *et al*[14] and Bergendi *et al*[15] showed the presence of virtually all Y euchromatin, including SRY in a patient with features of Turner syndrome and an isodicentric(Yq) chromosome.

Three of the psu dic(Yp) cases were exceptional. In case 95/4323 the Yq breakpoint was more proximal than in the other cases, lying between 955A4 and 753D4 (table 3, fig 2D). Case 95/2598 was previously found to have a psu dic(Yp) in 16% of cells together with r(Y) and mar(Y) chromosomes.[5] Additional FISH studies showed a total of four morphologically distinct abnormal Y derivatives (fig 2 E-H), all presumably descendants of a progenitor psu dic(Yp) chromosome or possibly from an initial unstable dicentric ring. The heterogeneous euchromatic content of the rings and mar(Y)s observed suggests a com-

plex aetiology with multiple breakpoints in the progenitor abnormal Y presumably resulting from mitotic instability. Although instability of ring chromosomes is a very well described phenomenon, dynamic mosaicism of the type described here resulting in morphologically distinct derivatives of the same chromosomal origin is an extremely rare phenomenon. As far as we are aware, it has only been observed in three patients with markers derived from chromosomes 22,[35] 15 (Crolla *et al*, in preparation), and the Y.[17] Patient 97/1158 also had a complex karyotype which included 46,X psu dic(Yp) in some cells, 45,X in some cells, and in a minority of cells two copies of a psu dic(Yp), presumably the result of mitotic nondisjunction.

In conclusion, it appears that the most common abnormal Y chromosome present in Turner syndrome patients is an isodicentric Y present as part of a mosaic karyotype including a 45,X cell line. It is probable that isodicentric Y chromosomes are usually generated during gametogenesis before spermatid formation, or during the first division after fertilisation, and that almost all are present as part of a mosaic karyotype. Eight of 10 isodicentricYp chromosomes examined had a breakpoint between the most distal probe used in Yq11, 700C1, and the Yq11/Yq12 boundary suggesting a breakage prone region or common breakpoint. It appears that the majority of structurally abnormal Y chromosomes found in Turner syndrome patients contain two copies of virtually all the Y euchromatin, but although all 13 patients with a Y isochromosome carried the testis determining factor gene SRY, the mosaic nature of their karyotypes rendered this insufficient to induce a male phenotype.

We are very grateful to the patients who made this study possible. We also thank Sarah Beal for technical assistance, Morag Collinson for advice, and the Wellcome Trust for support and the provision of image enhancement equipment The study was also supported by the Child Growth Foundation.The panel of Y specific YAC probes was obtained from the YAC Screening Centre, Milan; the Xp/Xp subtelomeric repeat, CY29, was a gift from Dr Lyndal Kearney (Institute of Molecular Medicine, Oxford); the pHuR14 (SRY) cosmid and Y84 (DYZ3) were provided by Dr Peter Goodfellow (University of Cambridge).

1 De la Chapelle A. Sex chromosome abnormalities. In: Emery AEH, Rimoin DL, eds. *Principles and practice of medical genetics*. London: Churchill Livingstone, 1990:273-99.
2 Hassold T, Benham F, Leppert M. Cytogenetic and molecular analysis of sex chromosome monosomy. *Am J Hum Genet* 1988;**42**:534-41.
3 Robinson A. Demography and prevalence of Turner syndrome. In: Rosenfeld RG, Grumbach MM, eds. *Turner syndrome*. New York: Dekker, 1990:93-100.
4 Lippe B. Turner syndrome. *Endocrinol Metab Clin North Am* 1991;**20**:121-52.
5 Jacobs P, Dalton P, James R, *et al*. Turner syndrome: a cytogenetic and molecular study. *Ann Hum Genet* 1997;**61**:471-83.
6 Page DC. Hypothesis: a Y-chromosomal gene causes gonadoblastoma in dysgenic gonads. *Development Suppl* 1987; **101**:151-5.
7 Hsu LYF. Phenotype/genotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. *Am J Med Genet* 1994;**53:** 108-40.
8 Medlej R, Lobacarro JM, Berta P, *et al*. Screening for Y derived sex determining gene SRY in 40 patients with Turner syndrome. *J Clin Endocrinol Metab* 1991;**75**:1289-92.
9 Kocova M, Siegel SF, Wenger SL. Detection of Y chromosome sequences in Turner syndrome by Southern blot analysis of amplified DNA. *Lancet* 1993;**342**:140-3.
10 Chu CE, Connor JM, Donaldson MDC, Kelnar CJH, Smail PJ, Greene SA. Detection of Y mosaicism in patients with Turner's syndrome. *J Med Genet* 1995;**32**:578-80.
11 Coto E, Toral JF, Menendez MJ, *et al*. PCR-based study of the presence of Y-chromosome sequences in patients with Ullrich-Turner syndrome. *Am J Med Genet* 1995;**57**:393-6.
12 Bernstein R, Steinhaus KA, Cain MJ. Prenatal application of fluorescent in situ hybridisation (FISH) for identification of a mosaic Y-chromosome marker, idic(Yp). *Prenat Diagn* 1992;**12**:709-16.
13 Kuznetzova T, Baranov A, Scwed N, *et al*. Cytogenetic and molecular findings in patients with Turner's syndrome stigmata. *J Med Genet* 1995;**32**:962-7.
14 Tuck-Muller CM, Chen H, Martinez JE, *et al*. Isodicentric Y chromosome: cytogenetic, molecular and clinical studies and review of the literature. *Hum Genet* 1995;**96**:119-29.
15 Bergendi E, Plochl E, Vlasak I, Rittinger O, Muss W. A Turner-like phenotype in a girl with an isodicentric fluorescent Y chromosome mosaicism. *Klin Padiatr* 1997;**209**: 133-6.
16 Patsalis PC, Hadjimarcou MI, Velissariou V, *et al*. Supernumerary marker chromosomes (SMCs) in Turner syndrome are mostly derived from the Y chromosome. *Clin Genet* 1997;**51**:184-90.
17 Muller U, Donlon TA, Kunkel SM, Lalande M, Latt SA. Y-190, a DNA probe for the sensitive detection of Y-derived marker chromosomes and mosaicism. *Hum Genet* 1987;**75:**109-13.
18 Diekmann L, Palm K, Pfeiffer RA, *et al*. Multiple minute marker chromosomes derived from Y identified by FISH in an intersexual infant. *Hum Genet* 1992;**90**:181-3.
19 Bukvic N, Susca F, Gentile M, Tangari E, Iannirubertо A, Guanti G. An unusual dicentric Y chromosome with a functional centromere with no detectable alpha-satellite. *Hum Genet* 1996;**97**:453-6.
20 Liou JD, Ma YY, Gibson LH, *et al*. Cytogenetic and molecular studies of a familial paracentric inversion of Y chromosome present in a patient with ambiguous genitalia. *Am J Med Genet* 1997;**70**:134-7.
21 Pinkel D, Landegent L, Collins C, *et al*. Fluorescence in situ hybridisation with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. *Proc Natl Acad Sci USA* 1988;**85**:9138-42.
22 Vergnaud G, Page DC, Simmler MC, *et al*. A deletion map of the human Y chromosome based on DNA hybridization. *Am J Hum Genet* 1986;**38**:109-24.
23 Jones MH, Khwaja OSA, Briggs H, *et al*. A set of ninety seven overlapping yeast artificial chromosome clones spanning the human Y chromosome euchromatin. *Genomics* 1994;**24**:266-75.
24 Kirsch S, Keil R, Edelmann A, *et al*. Molecular analysis of the genomic structure of the human Y chromosome in the euchromatic part of its long arm (Yq11). *Cytogenet Cell Genet* 1996;**75**:197-206.
25 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;**16**:1215.
26 Nakahori Y, Hamano K, Iwaya M, Nakagome Y. Sex identification by polymerase chain reaction using X-Y homologous primers. *Am J Med Genet* 1991;**39**:472-3.
27 Weissenbach J, Goodfellow PN. Report of the committee on the genetic constitution of the Y chromosome. *Cytogenet Cell Genet* 1991;**58**:967-85.
28 Vollrath D, Foote S, Hilton A, *et al*. The human Y chromosome: a 43 interval map based on naturally occurring deletions. *Science* 1992;**258**:52-9.
29 Beggs AH, Koenig M, Boyce FM, Kunkel LM. Detection of 98% of DMD/BMD gene deletions by polymerase chain reaction. *Hum Genet* 1990;**86**:45-8.
30 Salo P, Kaariainen H, Petrovic V, *et al*. Molecular mapping of the putative gonadoblastoma locus on the Y chromosome. *Genes Chrom Cancer* 1995;**14**:210-14.
31 Tsuchiya K, Reijo R, Page D, Disteche C. Gonadoblastoma: molecular definition of the susceptibility region of the Y chromosome. *Am J Hum Genet* 1995;**57**:1400-7.
32 Lovell-Badge R, Hacker A. The molecular genetics of SRY and its role in mammalian sex determination. *Philos Trans R Soc B* 1995;**350**:205-14.
33 James RS, Sharp AJ, Cockwell AE, Coppin AE, Jacobs PA. Evidence for a cryptic 46,XX cell line in a 45,X/46,X,psu idic(Xq) patient with normal reproduction. *J Med Genet* 1997;**34**:1030-2.
34 Tyler-Smith C, Oakey RJ, Larin Z, *et al*. Location of DNA sequences required for human centromere function through analysis of rearranged Y chromosomes. *Nat Genet* 1993;**5**:368-75.
35 Urioste M, Visedo G, Sanchis A, *et al*. Dynamic mosaicism involving an unstable supernumerary der(22) chromosome in cat eye syndrome. *Am J Med Genet* 1994;**49**:77-82.