

Minute Y chromosome derived marker in a child with gonadoblastoma: cytogenetic and DNA studies

V Petrovic, S Nasioulas, C W Chow, L Voullaire, M Schmidt, H Dahl

Abstract

A 12 year old girl referred for chromosome analysis because of short stature was found to have karyotype mos 45,X/46,X,+mar. The marker chromosome was observed in 58% of her blood lymphocytes. It was a small, pale staining, spherical fragment with GTL banding and showed faint differentiation along its length with CBG banding. DNA analysis using Y specific probes showed the absence of the testicular determining region and the presence of some short arm and centromeric Y chromosomal material. In situ hybridisation confirmed that the Y chromosomal material was associated with the marker chromosome. At laparotomy the patient was found to have streak gonads. Gonadectomy was subsequently performed and histological examination showed dysgenetic gonads with a dysgerminoma arising from a gonadoblastoma in the left gonad. This case shows that even very small Y derived marker chromosomes with pericentric material can predispose the phenotypic female to gonadal neoplasia.

An increased risk for gonadoblastoma in phenotypic females with dysgenetic gonads and the presence of Y chromosome material is well known.¹ The Y chromosome material can be present as a low level Y chromosome mosaicism or as a cytogenetically indefinable marker, in which case its identification using DNA methods is important in determining the patient's risk for developing gonadoblastoma.

Page² has postulated a gene (GBY) on the Y chromosome with an undefined physiological function in normal males, but which predisposes dysgenetic gonads to develop malignancy when present in females. On the basis of two reported cases³⁻⁵ of females with dysgenetic gonads, deleted Y chromosomes, and gonadoblastoma, Page² has argued that the GBY gene is located either near the centromere or on the long arm of the Y chromosome and is distinct from the testis determining factor. Identification of the minimal regions of the Y chromosome that are present in patients with gonadoblastoma will clarify those females who are at risk and may lead to an understanding of the oncogenic mechanism.

The presence of a very small Y derived marker chromosome in a girl who was subsequently found to have gonadal neoplasia has

allowed us, using DNA techniques, to refine the region of the Y chromosome that may contain the hypothetical GBY gene.

Case report

The patient was a 12 year old girl who was referred for chromosome analysis because of short stature. She was born at term with a birth weight of 2183 g to a 26 year old mother and a 28 year old father. At birth she was noted to have swollen ankles and feet. At 3 years of age she had bilateral inguinal hernias repaired and at 9 years she had an eosinophilic granuloma removed from her right clavicle. At 12.9 years her weight was 34.5 kg (<10th centile) and her height 130.5 cm (<3rd centile) with normal body proportion and a bone age in keeping with her chronological age. There were other features of Turner's syndrome including increased carrying angle, lack of pubertal development, breasts, and pubic hair, and mild lymphoedema of the left ankle. Webbing of the neck and ear deformities were absent. Ultrasound showed a markedly atrophic uterus, normal cervix and vagina, and the ovaries were not clearly visualised. There was a mild degree of pectus excavatum which was familial. At 13 years of age, after cytogenetic studies, a laparotomy was carried out which showed bilateral streak gonads and gonadectomy was performed.

HISTOPATHOLOGY

The right gonad was a streak 23 × 3 × 1.5 mm with ovarian stroma but no germ cells. The left gonad was 16 mm in length with a 5 mm nodule on one side. Section of this nodule showed infiltration by clusters and trabeculae of a dysgerminoma associated with many lymphoid follicles, focal calcification, and an occasional nest of gonadoblastoma was present (fig 1).

Materials and methods

CYTOGENETICS

Metaphase cells were obtained from PHA stimulated blood lymphocytes using standard techniques. Chromosomes were examined using various staining methods including GTL banding, CBG banding, Ag-NOR banding, and GBG banding. Three separate cell cultures were established from the gonadal tissue and chromosomes from the dividing fibroblasts were examined.

Murdoch Institute,
Royal Children's
Hospital, Flemington
Road, Parkville,
Melbourne, Victoria
3052, Australia.
V Petrovic
S Nasioulas
L Voullaire
M Schmidt
H Dahl

Department of
Anatomical
Pathology, Royal
Children's Hospital,
Melbourne, Australia.
C W Chow

Correspondence to
Ms Petrovic.

Received 10 June 1991.
Revised version accepted
3 January 1992.

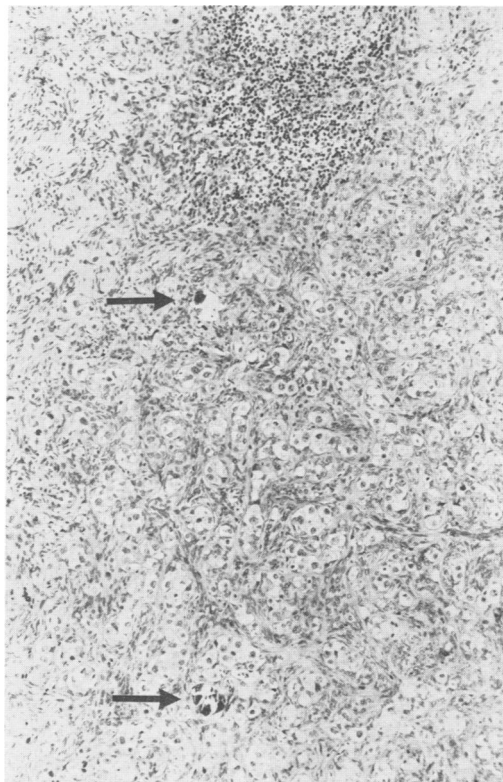


Figure 1 Section through left gonad of the patient showing infiltration by dysgerminoma with lymphoid follicle and focal calcification (arrowed) (H&E).

IN SITU HYBRIDISATION

Fluorescent in situ hybridisation to the patient's chromosomes using the biotin labelled Y centromeric probe pY84⁶ was carried out according to published methods.⁷ Chromosomes were photographed using Ektachrome ASA 400 slide film and printed directly onto black and white paper. The slides were subsequently stained with Giemsa and rephotographed.

DNA STUDIES

DNA was extracted from white blood cells from the proband and from a normal male and a normal female as described by Miller *et al.*⁸ Genomic DNA, digested with restriction endonucleases *EcoRI*, *TaqI*, *HindIII*, or *HaeIII*, was electrophoresed on a 0.7% agarose gel, blotted onto a Gene Screen Plus membrane (New England Du Pont), hybridised, and washed at high stringency (0.1 SSC, 0.1% SDS, 70°C) following manufacturers' recommendations. To determine gene dosage, equal amounts of DNA were loaded onto the gels.

The following probes which were used have been assigned to a deletion interval map of the human Y chromosome.⁹ A schematic representation of this map is shown in fig 2.

Probe pDP1007 detects Y chromosome specific sequences which map to deletion interval 1 at Yp11 and cross hybridises to the short arm of the X chromosome. On *TaqI* digests it detects a 2.8 kb Y specific fragment and an X linked fragment of 2.3 kb. This probe recognises the ZFY locus.¹⁰

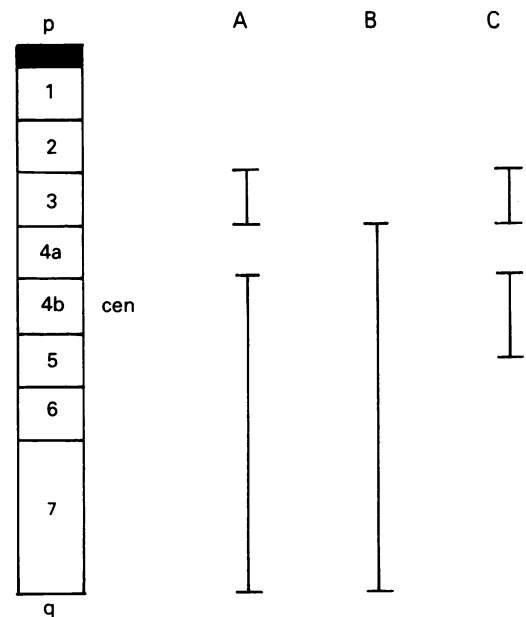


Figure 2 Interval map of the human Y chromosome. On the right the bars indicate the regions remaining in the deleted Y chromosomes in patients described by (A) Distche *et al.*,³ (B) Magenis *et al.*,^{4,5} and (C) the present case.

Probe pDP61 is a subclone derived from plasmid p115.¹¹ It detects homologous X and Y chromosome sequences (*DXYS8*) of 1.8 and 2.8 kb. On *TaqI* digests, it detects a Y linked fragment of 2.1 or 2.6 kb (Y linked RFLP) mapping to deletion interval 2.³

Probe pDP105(A/B) defines a family of Y specific repetitive sequences. On *TaqI* digests it detects Y linked fragments of 8.0, 5.2, and 2.5 kb. The 2.5 kb fragment A maps to deletion interval 3 (Yp), while the 5.2 kb fragment B maps to deletion interval 6 (Yq).^{3,12}

Probe pDP34 detects highly homologous sequences on the X and Y chromosome. On *TaqI* digests it detects a Y linked fragment of 15 kb specific to deletion interval 4A. It also detects an X linked allele at 11 kb and 12 kb fragments.¹³

Probe pDP97 is a subclone derived from cosmid Y97. It detects an alphoid repeated sequence at the centromere of the Y chromosome. On *EcoRI* digests it detects a Y linked fragment of 5.5 kb specific for deletion interval 4b.¹¹

Probe pDP527 on *TaqI* digests detects a 7 kb Y specific fragment which maps to deletion interval 5 or 6 on Yq, and two X specific fragments of 4.2 and 3.3 kb (D C Page, personal communication).

Probe 116/21 is a subclone derived from an independent cosmid 116. On *HindIII* digests it detects 5.5 and 9.0 kb Y specific sequences mapping to the boundary of intervals 6 and 7.⁶

Probe pY431-*HinfA* detects highly repeated Y specific sequences which map to deletion interval 7 (the heterochromatic region of Yq). Using *HaeIII* digests an intense 2.1 kb Y linked fragment is detected (probe made by K Smith, D C Page, personal communication).

The probes were labelled with ³²P using the random oligonucleotide method.¹⁴

Presence or absence of the SRY gene was determined by the polymerase chain reaction.¹⁵ The SRY specific primers were 5'-AGTGTG-AAACGGGAGAAAAC-3' (nucleotides 544-563¹⁶) and 5'-TACAACCTGTTGTCCAG-TTG-3' (nucleotides 882-901¹⁶).

Results

CYTOGENETIC STUDIES

Fifty G banded metaphase cells obtained from peripheral blood lymphocyte culture showed a mosaic karyotype, mos 45,X/46,X,+mar. The marker was present in 58% of cells (29/50 of cells counted). With GTL banding the marker appeared to be a small pale staining spherical

fragment (fig 3A).

With CBG banding there was faint differentiation along the marker (fig 3B). It stained negatively with Ag-NOR banding and showed late replication with GBG banding (data not shown). Parental chromosomes were normal.

Chromosome analysis of fibroblast cells from gonadal tissue showed the same mosaic karyotype seen in the blood lymphocytes but the proportions of the two cell lines varied slightly. The marker chromosome was present in 32% of cells (16/50 of cells counted) from the fibroblast culture of the right gonad, in 82% of fibroblast cells (41/50 of cells counted) from the left gonad, and in 62% of fibroblast cells (31/46 of cells counted) from the nodule in the left gonad.

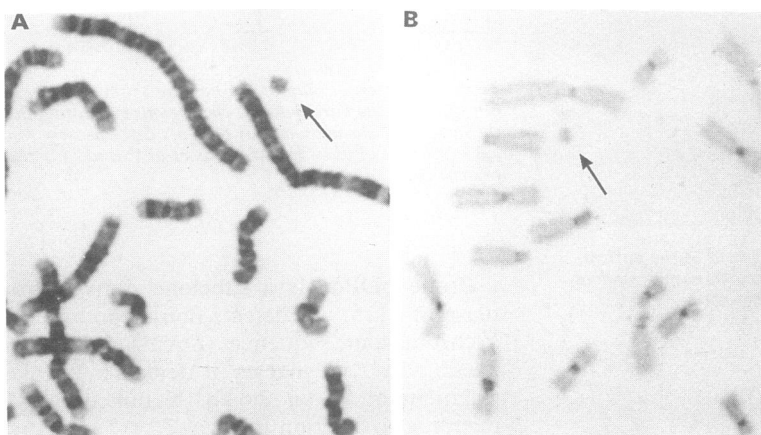


Figure 3 Marker chromosome seen in the patient after (A) GTL banding, (B) CBG banding.

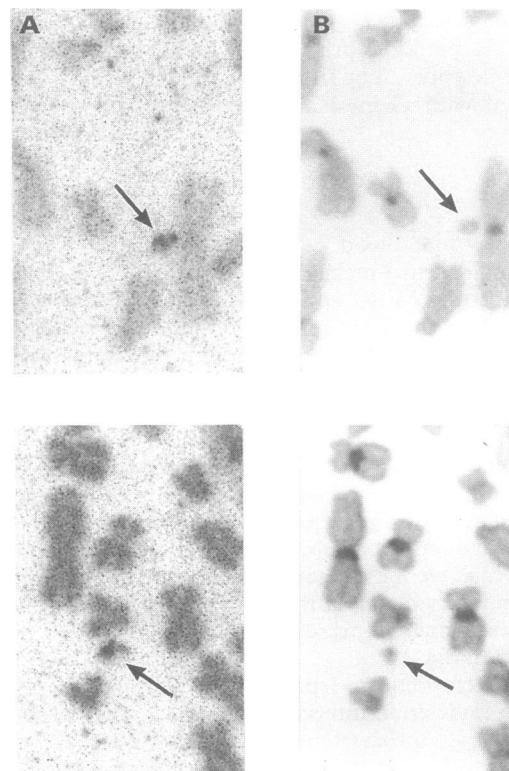


Figure 4 In situ hybridisation using the biotin labelled probe pY84 (A) showing signal on marker chromosome and (B) showing the same cell without signal.

IN SITU HYBRIDISATION

The Y centromeric probe pY84 was found to hybridise to the marker seen in the patient's karyotype (fig 4).

DNA ANALYSIS

DNA from the patient was examined for the presence of Y chromosome material using the Y specific probes listed in Materials and methods. These probes were chosen to cover the seven intervals of the Y chromosome.⁹ The results of Southern blot analysis using the eight probes described are shown in fig 5. Probes pDP105A (2.5 kb fragment) and pDP97 hybridised to the patient's DNA. Probes pDP1007, pDP61, pDP105B, pDP34, pDP527, 116/21, and pY431 gave no Y specific signals in the patient. DNA from the normal male control showed hybridisation with all the probes used. These results are summarised in fig 6. The SRY gene was not detected in the patient by use of specific primers in the PCR reaction (fig 7). Thus, as summarised in fig 6, the marker chromosome in the patient is deleted for intervals 1, 2, and 4a of the short arm, and, of the long arm, intervals 6 and 7 and possibly some of 5 has been deleted. The patient's marker chromosome, then, carries some Y short arm and centromeric material and possibly some long arm material.

Discussion

A very small marker chromosome was seen in a girl with features of Turner's syndrome who was found to have gonadoblastoma and dysgerminoma. DNA studies showed that the patient's karyotype contained Y short arm and centromeric material and possibly some proximal long arm material. The SRY gene was not detected. In situ hybridisation confirmed that the marker chromosome carried the Y centromeric material. We therefore interpret the marker chromosome as a Y derived ring chromosome where the breakpoints have occurred at p11.2 and q11.2.

On the deletion interval map of the human Y chromosome (fig 6) our patient is deleted for

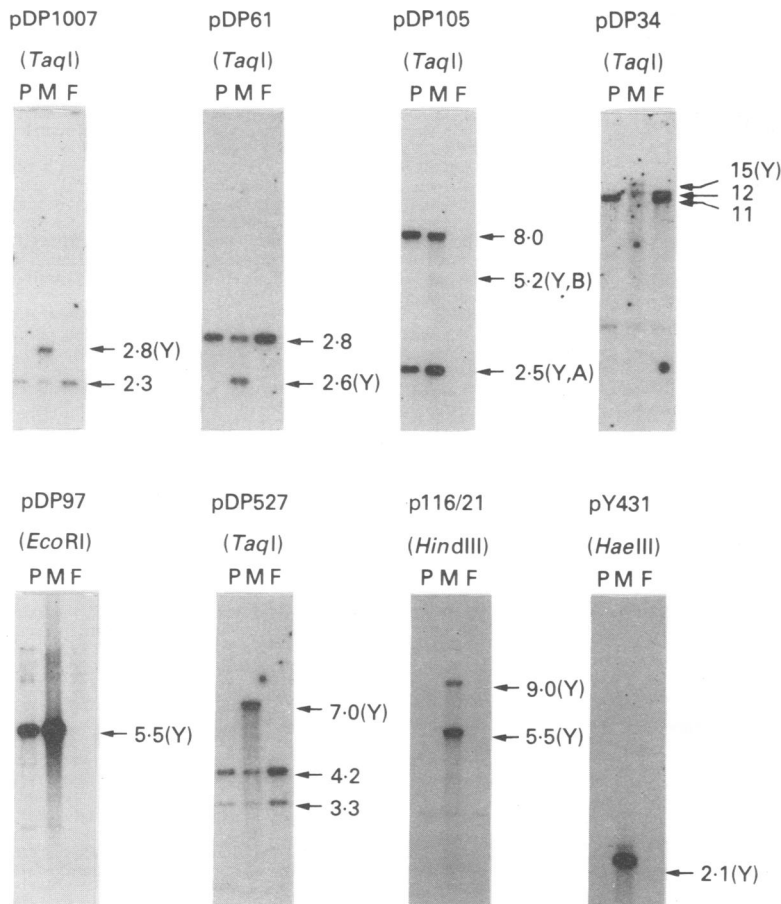


Figure 5 Detection of Y chromosome DNA in the patient by Southern blot analysis. DNA from the patient (P), a normal control male (M), and a normal control female (F) was digested with restriction endonucleases *TaqI*, *EcoRI*, *HaeIII*, or *HindIII* as indicated. The filters were hybridised to the eight DNA probes as shown. The sizes of the detected fragments, the Y specific fragments (Y), and two intervals identifying Y specific fragments detected by probe pDP105 (A and B) are also indicated.

(patient 2) described by Disteché *et al*³ (fig 2). This has been interpreted by Page¹⁷ to be a result of the rearrangement occurring in a Y chromosome that has an inversion of intervals 3 and 4a. Our report of another case supports the hypothesis that this inversion is a population dimorphism.¹⁷

It is known that the testis determining gene (TDF) is located towards the terminal end of the Y chromosome short arm in interval 1.⁸ The gene SRY, which has recently been isolated and proposed as TDF,¹⁶ is located within this region. The loss of interval 1 and the absence of SRY in the proband's marker Y chromosome implies the absence of TDF in the patient and is consistent with the lack of testicular development. The presence of gonadoblastoma in our patient without testis development and in the absence of the testis determining region of the Y chromosome gives support to the view that the development of this tumour is not dependent on either the presence of testicular tissue or the TDF gene.

If the GBY gene postulated by Page² exists, then it must have been present on the marker chromosome in our patient. The marker chromosome seen in our patient is smaller than those previously investigated with DNA techniques in patients with similar clinical features^{3,5} (summarised in fig 2). In particular, interval 7, which is the heterochromatic region of the Y chromosome, and interval 6 are absent in our patient. The DNA studies on previously published cases together with those on the present case suggest that the postulated GBY gene must map close to the centromere. Thus, we conclude that currently a Y centromeric probe is the most suitable probe for screening for malignancy risk in females with dysgenetic gonads and small marker chromosomes of uncertain origin. However, a centromeric probe could fail to indicate the presence of the GBY gene in the event of this gene being translocated onto another chromosome.

intervals 1, 2, and 4a of the short arm, and, of the long arm, intervals 6 and 7 and possibly some of interval 5 has been deleted. The deletion of interval 4a, with the presence of interval 3 and 4b has also occurred in an XY female

Y chromosome deletion intervals	Probe	Locus	Hybridisation		
			Normal P	Normal M	Normal F
1	pDP1007	ZFY, ZFX	-	+	-
2	pDP61	DXYS8	-	+	-
3	pDP105/A	DYZ4/A	+	+	-
4a	pDP34	DXYS1	-	+	-
4b	pDP97	DYZ3	+	+	-
5	pDP527	STSP	-	+	-
6	pDP105/B	DYZ4/B	-	+	-
7	pY431-Hinf-A	DYZ2	-	+	-

Figure 6 DNA probes used to test for the presence (+) or absence (-) of Y specific restriction fragments (see fig 2) in the patient, normal male, and normal female controls. The loci have been assigned to a deletion interval map of the Y chromosome as shown.

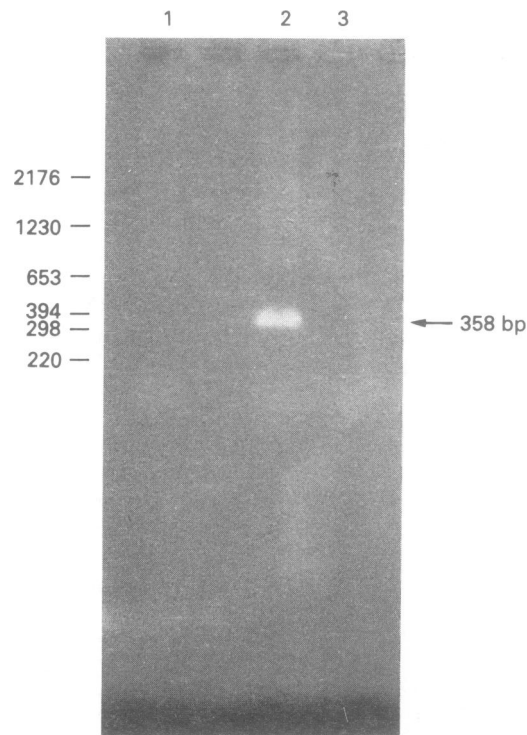


Figure 7 *SRY* gene analysis. Using the *SRY* gene specific primers in the polymerase chain reaction, the presence of the *SRY* gene is indicated by a 358 base pair amplified band. Lane 1 is the amplification product from the patient, lane 2 from a control male, and lane 3 from a control female.

A lymphoblast cell line (MICY880044) established from this patient is available from the Tissue Culture Laboratory, Murdoch Institute. We thank Dr D C Page and Dr N Fraser for the Y specific probes.

- 1 Verp MS, Simpson JL. Abnormal sexual differentiation and neoplasia. *Cancer Genet Cytogenet* 1987;25:191-218.
- 2 Page DC. Hypothesis: a Y-chromosomal gene causes gonadoblastoma in dysgenetic gonads. *Development* 1987;101(suppl):151-5.
- 3 Distèche CM, Casanova M, Saal H, *et al.* Small deletions of the short arm of the Y chromosome in 46 XY females. *Proc Natl Acad Sci USA* 1986;83:7841-4.
- 4 Magenis RE, Tochen ML, Holohan KP, Carey T, Allen L, Brown MG. Turner syndrome resulting from partial deletion of Y chromosome short arm: localization of male determinants. *J Pediatr* 1984;105:916-19.
- 5 Magenis RE, Casanova M, Fellous M, Olson S, Sheey R. Further cytologic evidence for Xp-Yp translocation in XX males using in situ hybridization with Y-derived probe. *Hum Genet* 1987;75:228-33.
- 6 Wolfe J, Darling SM, Erickson RP, *et al.* Isolation and characterization of an alphoid centromeric repeat family from the human Y chromosome. *J Mol Biol* 1985;182:477-85.
- 7 Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA* 1986;83:2934-8.
- 8 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- 9 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.
- 10 Page DC, Mosher R, Simpson EM, *et al.* The sex-determining region of the human Y chromosome encodes a finger protein. *Cell* 1987;51:1091-104.
- 11 Bishop C, Guellaen G, Geldwerth D, Fellous M, Weissenbach J. Extensive sequence homologies between Y and other human chromosomes. *J Mol Biol* 1984;173:403-17.
- 12 Bernstein R, Rosendorff J, Ramsay M, Pinto MR, Page DC. A unique dicentric X;Y translocation with Xq and Yp breakpoints: cytogenetic and molecular studies. *Am J Hum Genet* 1987;41:145-56.
- 13 Wolfe J, Erickson RP, Rigby PW, Goodfellow PN. Regional localization of 3 Y-derived sequences on the human X and Y chromosomes. *Ann Hum Genet* 1984;48:253-9.
- 14 Feinberg AP, Vogelstein B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 1986;132:6-13.
- 15 Saiki RK, Gelfand DH, Stoffel S, *et al.* Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988;239:487-91.
- 16 Sinclair AH, Berta P, Palmer MS, *et al.* A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* 1990;346:240-4.
- 17 Page DC. Sex reversal: deletion mapping the male-determining function of the human Y chromosome. In: *Molecular biology of Homo sapiens*. Cold Spring Harbor Symposium on Quantitative Biology. Vol 51. New York: Cold Spring Harbor Laboratory Press, 1986:229-35.