

Case reports

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Y chromosome specific probes identify breakpoint in a 45,X/46,X,del(Y) (pter→q11.1:) karyotype of an infertile male

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SUMMARY An infertile male patient with a 45,X peripheral blood karyotype and a 45,X/46,X,del(Y)(pter→q11.1:) mosaic skin fibroblast karyotype is described. Steroid sulphatase (STS) activity was normal. Recombinant DNA studies using Y chromosome specific probes suggest that almost the entire long arm of the Y chromosome is deleted.

comprehensively reported.¹⁻⁴ With the advent of Y specific DNA probe analysis, a molecular map of the Y chromosome has been constructed.⁵

Yq deletions have been reported on 19 previous occasions in both mosaic and non-mosaic forms (table). We present here a 56 year old infertile male with a 45,X peripheral blood karyotype and a mosaic skin fibroblast karyotype 45,X/46,X,del(Y)(pter→q11.1:).

Case report

Genetic analysis of the Y chromosome was, until recently, hampered by a paucity of genetic markers. A classical model of the Y chromosome based on clinical and cytogenetic observations has been

The proband is a 56 year old male, the third child of normal parents (fig 1). Pregnancy and delivery were unremarkable. Since childhood he has been partially deaf. At the age of 15 years, he developed a seronegative oligoarthritis, affecting mainly the knees. On investigation for the knee complaint in

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TABLE Clinical findings in patients with 46,X,del(Y)(q).

Reference	Year	Mosaic 45,X/46,X,del(Y)(q)	Age (y)	Height (cm)	Centile	Azoospermia	Hypogonadism	Gynaecomastia	Mental retardation
(1) Lo and Kobernick ²	1965	+	28	150	<3	-	+	-	-
(2) Nakagome <i>et al</i> ²	1965	+	2.5	80.5	<3	NA	-	NA	+
(3) Surana <i>et al</i> ²	1971	-	0.9	60.5	<3	NA	+	NA	+
(4) Meisner and Inhorn ²	1972	-	36	179	75	NS	-	+	+
(5) Nielsen <i>et al</i> ²	1972	+	22	156	<3	NS	-	-	+
(6) Neu <i>et al</i> ²	1973	-	20	173	50	+	-	-	-
(7) Telfer <i>et al</i> ²	1973	-	5	NS	<3	NS	NS	NA	+
(8) Langmaid and Laurence ²	1974	-	23	152	<3	NS	-	-	-
(9) Yunis <i>et al</i> ²	1977	-	36	146	<3	+	NS	-	NS
(10) Podruch <i>et al</i> ⁶	1981	-	8.5	110	<3	NA	-	NA	+
(11) Kosztolanyi and Trixler ²	1983	-	33	160	<3	+	+	+	+
(12) Disteche <i>et al</i> ⁷	1984	-	0	54	>90	NA	-	NA	NA
(13) Fitch <i>et al</i> ⁸	1985	+	39	162.5	<3	+	+	-	-
(14) Munke <i>et al</i> ⁹ (a)	1985	-	43	165	10	+	+	NS	-
(14) Munke <i>et al</i> ⁹ (b)	1985	-	1.7	79	10	NA	-	NA	-
(15) Martin <i>et al</i> ¹⁰	1985	-	28	172	>90	+	+	-	-
(16) Chandley <i>et al</i> ¹¹	1986	+	24	159	<3	+	-	-	-
(17) Gaba <i>et al</i> ¹²	1987	-	0	NS	-	NA	+	NA	NA
(18) Beverstock <i>et al</i>	1988	+	54	154	<3	*	+	+	-

NS=not done or not stated.
NA=not applicable because of age.
*=highly probably but not confirmed.

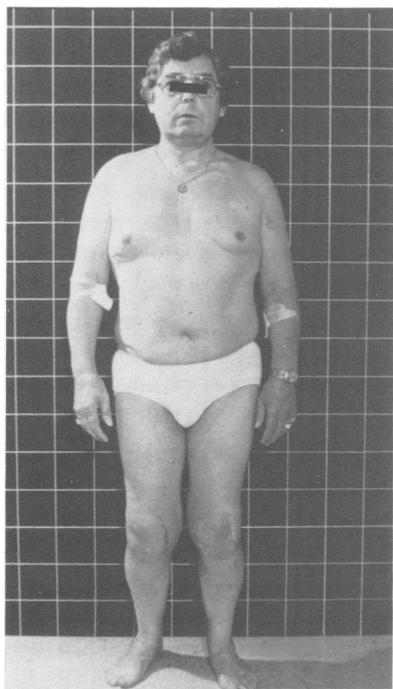


FIG 1 The proband.

1985, he was referred for further examination on account of his short stature and gynaecomastia. There have been no pregnancies despite a marriage of 25 years totally without contraceptive precautions. Infertility studies have not been undertaken. The patient's height is 154 cm (<3rd centile) and weight 64 kg. His fingertip span is 153 cm and the upper segment:lower segment ratio is 82:72. His facies are normal, head circumference 57 cm. His skin is very freckled. Hair distribution and external genitalia are of the normal male type, but the testes are small: left 2.5×1.5 cm and right 2.3×1.5 cm. The thorax is broad with wide nipples. He has cubitus valgus. Apart from his deafness, he has no neurological deficiencies. Cardiac examination was unremarkable.

Endocrinological studies showed raised FSH, LH, and prolactin with low testosterone levels. STS activity was within normal limits. Radiological studies were normal apart from secondary degenerative changes in the knees. Investigations on other family members were not undertaken since both parents were dead and according to the family history all sibs of the patient were normal with normal offspring.

CYTOGENETIC INVESTIGATIONS

Peripheral blood leucocytes and skin fibroblasts were cultured, harvested, and banded according to standard procedures. One hundred cells from the peripheral blood cultures were examined and all showed a 45,X karyotype. Analysis of skin fibroblasts, however, showed two cell lines: 45,X and 46,X,del(Y)(pter→q11.1:) (fig 2). The ratio of the two fibroblast lines was approximately 4:1. Both C banding and Cd banding were positive. DAPI/distamycin staining was negative. The deleted Y appeared to consist only of the short arms and centromere.

RECOMBINANT DNA Y PROBE STUDIES

The single or low copy human sequence probes p75/79 (*DXYS25*), p59gamma (*DXYS27*), p69/6 (*DYS20*), p47z (*DXYS5*), p50f2 (*DYS7*), and pJG1.0 were used as probes in Southern blot analysis of fibroblast DNA from our patient. Cytogenetic investigations showed that the deleted Y chromosome was only present in approximately 20% of the cultured fibroblasts. Consequently, five times more DNA from the patient (50 µg/track) was used than for normal male and female controls (10 µg/track).

All of the probes, with the exception of pJG1.0, used in this study have been described previously.⁵ Probe pJG1.0 is a 1.0 kb *EcoRI/HindIII* fragment derived from the Y specific region distal to TDF. It recognises homologous sequences in the region Yq11.2→qter in addition to the cognate sequences present on Yp. DNA digestion and Southern blot

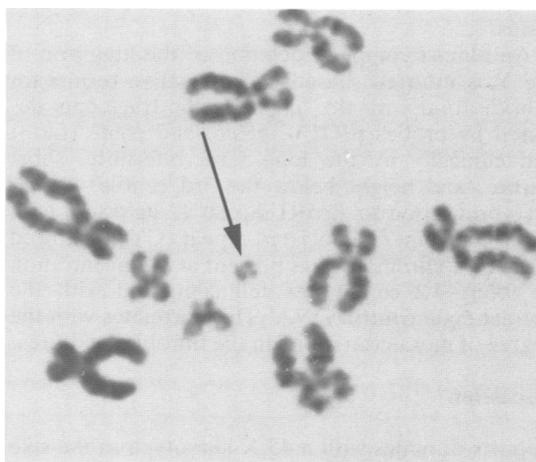


FIG 2 Partial metaphase of fibroblast culture with deleted Y chromosome (arrowed).

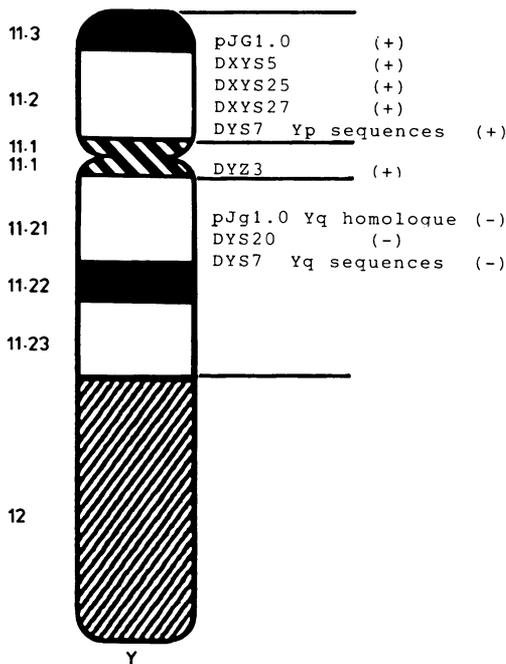


FIG 3 Ideogram of the Y chromosome showing designation and location of the Y specific probes.

analyses were essentially as described previously.¹³ The presence of a complete short arm Y in our patient was confirmed by positive results for hybridisation with the Y(p) specific fragments detected by the probes p50f2, p75/79, pJG1.0, p47z, and p59gamma (fig 3) and clinically by the presence of testes.

An almost complete deletion of the long arm of the Y is inferred, based upon negative results for hybridisation with the Y(q) specific fragments detected by probes pJG1.0, p69/6, and p50f2 (fig 3) and clinically by the long term infertility, short stature, and height below the 3rd centile.

Hybridisation to *Eco*RI digests (2 µg/track) with the Y centromere associated repeat (CY84) showed that the Y chromatin was present at a concentration of about 0.2 copies per cell compared with the normal male control DNA. This correlates with the degree of mosaicism found in the fibroblast cultures.

Discussion

Reports of males with a 45,X karyotype as the sole abnormality in all tissues are rare.² Early investigations were hampered by the unavailability of the contemporary techniques of prophase culture or

recombinant DNA technology. Published descriptions of Yq deletions indicate considerable variation in size and morphology of the deleted chromosome. For example, in six of the cases listed in the table, the Y chromosome appeared to be metacentric (patients 3, 4, 7, 9, 12, and 18). However, the length and morphology of our patient's Y chromosome corresponded most closely to those reported by Lo and Kobernick, Nakagome *et al*, Langmaid and Laurence, Nielsen *et al*, Kosztolanyi and Trixler (see Davis²), Martin *et al*,¹⁰ and Munke *et al*.⁹

A series of patients with Y(q) deletions have been expressly excluded from the table because they were investigated in the prebanding era,¹⁴ but a patient described by Chandley *et al*,¹¹ with an isodicentric Yp has been included because of the complete absence of Yq.

Seven out of the 19 patients described in the table were azoospermic. Our patient was excluded from this group of seven because he has never been investigated for his infertility. However, since he and his wife have never used any form of contraception in a marriage of 25 years, this would strongly imply that our patient too might be azoospermic.

Eight out of the 19 patients listed had hypogonadism, while seven had varying degrees of mental retardation. Six patients were mosaic 45,X/46,X,del(Y)(q) and the rest had a 46,X,del(Y)(q) karyotype in all cells. Twelve out of the 19 patients in the table were on or below the 3rd centile for growth.

All parents and sibs of these patients were found to be normal and with the exception of the unpublished observations of Klinger (Davis²), in which a tiny pericentric region of Y was present in three generations of normal fertile men, and a further report of a similarly deleted Y in 11 generations,¹⁴ all deleted Y chromosomes have originated *de novo*.

The observations that testes are present in our patient and that Southern blot analysis was positive with the probes p75/79, p47Z, p59gamma, p50f2, and pJG1.0 implies that the Y short arm is intact. Hybridisation with CY84, the centromere repeat probe, was also positive. The presence of testes and an apparently absent Yq is consistent with the belief that only the short arm/centromere region of the Y is necessary for testis formation and confirms similar observations made by Vergnaud *et al*¹⁵ and Affara *et al*.¹⁶ Candidate sequences for the human sex determining gene (TDF) from distal Yp have recently been cloned.¹⁷ The retardation of growth in our patient, his probable azoospermia (although not confirmed), and negative Southern blotting for the probes p69/6 (which maps to Yq11.2) and p50f2 long arm specific sequences would indicate that there is a

substantial, if not complete, deletion of the long arm at Yq11.1. A negative result with probe pJG1.0 also confirmed that the long arm of the Y was deleted. Munke *et al*⁹ have suggested that azoospermia in phenotypically normal males with Yq deletions is more likely to be the result of an inability of the X and Y chromosomes to pair normally during meiosis than an absence of a fertility gene located somewhere on the Y long arm.

The short stature of our patient would imply that the gene for growth promoting factors is localised somewhere on the long arm of Y, distal to q11.1, although his short stature may be entirely because of the XO cell line. Furthermore, patients with ring Y and normal height have been reported,⁹ although the actual extent of the deletion in ring Y chromosomes is difficult to estimate.

Buhler¹ has suggested that the height regulating genes may be located on both arms of the Y. Short stature has also been reported in deletions involving the proximal Yq11 region⁹ and our patient's deleted Y chromosome appears to confirm this observation and to define this locus even more accurately, that is, at or above Yq11.1. Defining the extent of a deletion in small chromosomes is difficult but the application of recombinant DNA technology has proved useful in establishing that the tiny chromosome of our patient is actually a Y, is not a ring, is substantially deleted in the long arm (apparently at q11.1), and presents as a low grade mosaic.

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References

- 1 Buhler EM. A synopsis of the human Y chromosome. *Hum Genet* 1980;55:145-75.
- 2 Davis RM. Localisation of male determining factors in man: a thorough review of structural anomalies of the Y chromosome. *J Med Genet* 1981;18:161-95.
- 3 Fryns JP, Kleczkowska A, Van den Berghe H. Clinical manifestations of Y chromosome deletions in man. In: Sandberg

- AA, ed. *The Y chromosome, Part B. Clinical aspects of Y chromosome abnormalities*. New York: Alan R Liss, 1985:151-70.
- 4 Magenis RE, Brown MG, Donlon T, Olson SB, Sheehy R, Tomor D. Structural aberrations of the Y chromosome including the non-fluorescent Y. In: Sandberg AA, ed. *The Y chromosome. Part A. Cytologic origin and consequences in the Y chromosome*. New York: Alan R Liss, 1985:537-74.
- 5 Davies KE, Mendel JL, Weissenbach J, Fellous M. Report of the committee on the genetic constitution of the X and Y chromosome. HGM9. *Cytogenet Cell Genet* 1987;46:277-315.
- 6 Podruch PE, Yen FS, Dinno ND, Weisskopf. Yq- in a child with livedo reticularis, snub nose, microcephaly, and profound mental retardation. *J Med Genet* 1981;19:377-80.
- 7 Distèche C, Luthy D, Haslam DB, Hoar D. Prenatal identification of a deleted Y chromosome by cytogenetics and a Y-specific repetitive probe. *Hum Genet* 1984;67:222-4.
- 8 Fitch N, Richer CL, Pinsky L, Kahn A. Deletion of the long arm of the Y chromosome and review of Y chromosome abnormalities. *Am J Med Genet* 1985;20:31-42.
- 9 Munke M, de Martinville B, Lieber E, Francke U. Minute chromosomes replacing the Y chromosome carry Y-specific sequences by restriction fragment analysis and in-situ hybridization. *Am J Med Genet* 1985;22:361-74.
- 10 Martin MJ, Rodriguez MT, Aller V, Abrisqueta JA, Rojo JM. Yq deletion (q11.21) in a H-Y+ azoospermic male. *Clin Genet* 1985;28:80-3.
- 11 Chandley AC, Ambros P, McBeath S, *et al*. Short arm dicentric Y chromosome with associated statural defects in a sterile man. *Hum Genet* 1986;73:350-3.
- 12 Gaba AR, Van Dyke DL, Weiss L. Dysgenetic male pseudohermaphroditism in a 45,X/46,Xdel(Y)(q11.1) mosaic infant. *Am J Med Genet* 1987;26:545-9.
- 13 Buckle VJ, Boyd Y, Fraser N, *et al*. Localisation of Y chromosome sequences in normal and 'XX' males. *J Med Genet* 1987;24:197-203.
- 14 Genest P, Laberge C, Poty J, Gagne R, Bouchard M. Transmission d'un petit "Y" durant onze generations dans une lignee familiale. *Ann Genet (Paris)* 1970;13:233-8.
- 15 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.
- 16 Affara NA, Ferguson-Smith MA, Tolmie J, *et al*. Variable transfer of Y-specific sequences in XX males. *Nucleic Acids Res* 1986;14:5375-87.
- 17 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.

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Toluene embryopathy: two new cases

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SUMMARY Toluene embryopathy is characterised by microcephaly, central nervous system

dysfunction, attentional deficits and hyperactivity, developmental delay with greater language deficits, minor craniofacial and limb anomalies, and variable growth deficiency.

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