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Ordering of Y-specific sequences by deletion mapping and analysis of X–Y interchange males and females

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Summary

We have used DNA from 23 patients with Y-chromosome aberrations and 25 patients with presumptive X–Y interchange to map 39 Yp restriction fragments and 37 Yq restriction fragments. In the majority of patients the results are consistent with a standard contiguous order of sequences along the Y chromosome. In 6 of 26 patients (23 %) with Yp aberrations and 2 of 17 (12 %) with Yq aberrations, exceptions to the consensus order have been observed. These can be accommodated by postulating the presence of inversion polymorphisms. Such variation may occur more commonly on the nonpairing part of the Y chromosome that in other chromosomes owing to the absence of homologous synapsis and recombination in male meiosis.

The Y sequence most frequently present in X–Y interchange males was that recognized by GMGY3. 18 of 19 X–Y interchange males had this sequence suggesting that it is the nearest in the series to the *TDF* locus, and indicating that the latter maps to the distal end of Yp. Several techniques, including *in situ* hybridization and DNA measurement by flow cytometry, have been used to demonstrate that in X–Y interchange males there is transfer of Y sequences to the distal end of the X chromosome; no mechanism other than X–Y interchange has been demonstrated.

Key words: Y chromosome map, Y-specific DNA sequences, X–Y interchange, deletion mapping, *TDF*.

Introduction

Karyotype–phenotype correlations in patients with structural abnormalities of the Y chromosome have been severely limited by the inadequate resolution of cytogenetic techniques (Ferguson-Smith, 1965; Davis, 1981; Goodfellow, Darling & Wolfe, 1985). Even the most sophisticated banding techniques are seldom capable of resolving the short arm of the Y into more than two bands and the long arm into more than five bands. The idiogram of the Y chromosome published by the International System for Cytogenetic Nomenclature (1981) adds further confusion, as it is clearly incorrect in several respects as indicated by Magenis *et al.* (1985) and confirmed in our laboratory (Fig. 1). It is therefore not surprising that similar Y aberrations have been interpreted differently by different cytogeneticists and that there is some confusion about the site of the locus for testis-determining factors (Davis, 1981). The most common misinterpretation has concerned a small nonfluorescent Y chromosome which was frequently mistaken as a terminal deletion of Yq; recent studies suggest that almost all these are dicentric isochromosomes of Yp, with a breakpoint in Yq (Magenis *et al.* 1985).

These difficulties could be resolved if it were possible to define Y-chromosome aberrations (and for that matter all cytogenetic aberrations) in terms of a detailed linkage map. However, the Y chromosome does not undergo recombination except in its pairing (pseudoautosomal) segment, a submicroscopic region at the distal end of the short arm (Chandley *et al.* 1984). Thus classical genetic linkage analysis in families cannot be used to order loci on the remaining 'differential' part of the Y, and physical methods must be employed. In this paper, we review the construction of a Y map by determining the order of anonymous Y-specific DNA restriction fragments, using probes randomly isolated from Y-chromosome-specific genomic libraries, in two groups of patients. The first group of patients have intrachromosomal Y

aberrations of translocations resulting in a variety of deletions and the second group are patients with X–Y interchange apparently resulting from abnormal X–Y recombination. The latter approach has been used by several groups of investigators to map Yp (Vergnaud *et al.* 1986; Affara *et al.* 1986*a*; Muller *et al.* 1986). Our findings indicate that the testis-determining region is located at the distal end of the short arm of the Y, and that there are interesting exceptions to the consensus order of Y chromosomes in both the long and short arms.

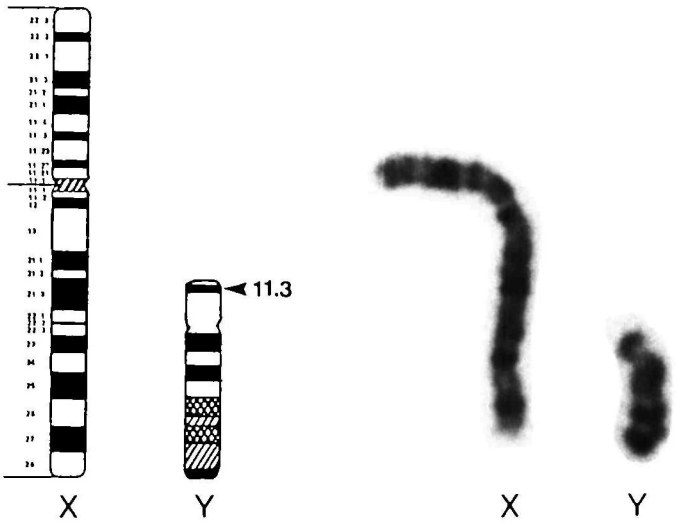


Fig. 1. The X and Y sex chromosomes and their idiograms indicating the more prominent G-bands. The Y idiogram differs from that described in the ICSN nomenclature (Fig. 2) in several important respects. (After Magenis *et al.* 1985.)

Map of the short arm of the Y

A total of 39 Y-specific restriction fragments has been mapped to the short arm of the Y by virtue of their presence in the DNA of an infertile male patient (WC) who has a monocentric isochromosome for the short arm of the Y (Fig. 2A). The probes that recognize 27 of these sequences have been isolated in Glasgow (Affara *et al.* 1986*b*), while 11 sequences are recognized by probes isolated by Weissenbach and colleagues (Vergnaud *et al.* 1986) and one by probe pDP34 of Page *et al.* (1982).

We have looked for the presence or absence of these 39 sequences in the following five patients with intrachromosomal aberrations of the Y in which the breakpoints are in Yp:

(i) DJP (Case 18 of Magenis *et al.* 1985), a patient with gonadal dysgenesis (including foci of gonadoblastoma) and short stature who had previously had a left nephrectomy for hydronephrosis secondary to ureteral stenosis. There was no evidence of masculinization. Chromosome studies revealed sex chromosome mosaicism, 45,X/46X, isodicentric (Yq); 3 of 74 cells had two idic (Yq) chromosomes.

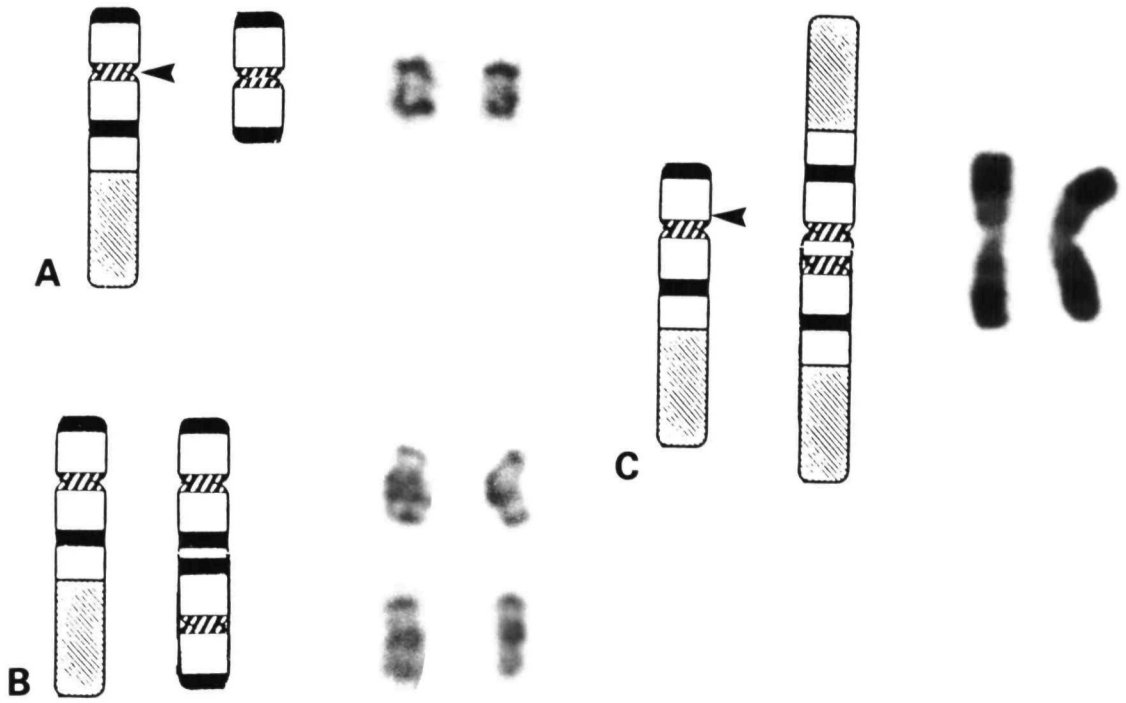


Fig. 2. Y chromosome aberrations used in deletion mapping. (A) WC. Monocentric isochromosomes for the short arm of the Y. (B) Dicentric isochromosomes for the short arm of the Y with breakpoints in Yq11.3 from two individuals (FF and WS). (C) ED. Dicentric isochromosomes for the long arm of the Y chromosome in a patient with 48,XX isodic(Yq),+isodic(Yq) and breakpoints in Yp11.2 (Trypsin Giemsa banding.)

(ii) GS, a 7-year-old male with short stature, normal intelligence and normally developed male external genitalia. Chromosome analysis revealed 45,X/46,X, isodicentric (Yq) mosaicism.

(iii) ED, a 19-year-old girl of above average stature (172 cm) and mild mental handicap (IQ = 76) who presented with late menarche (17 yrs), oligomenorrhoea and clitoral hypertrophy; there are mild facial dysmorphic features. Chromosome analysis revealed a nonmosaic 48,XX karyotype with two isodicentric (Yq) chromosomes in lymphocytes and skin fibroblasts (Fig. 2C).

(iv) TM, a 15-year-old girl of short stature with primary amenorrhoea and normal external genitalia. Chromosome analysis reveals 45,X/46,X, isodicentric (Yq).

(v) RW, a 26-year-old male of short stature (147 cm), normal intelligence and normal male external genitalia. Chromosome analysis reveals mosaicism of 45,X/46,X including a minute fragment.

A prominent feature of the chromosome analysis of DJP, GS, ED and TM is that the abnormal Y chromosomes all have bright Q bands on the distal ends of both arms.

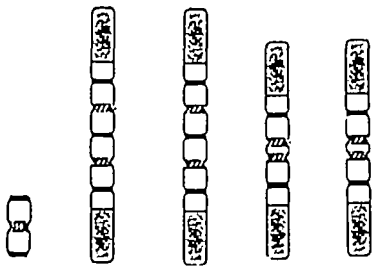
Southern analysis of the DNA of each patient has been undertaken with the series of Yp DNA probes and this is shown in Table 1. DJP and GS show no loss of Yp restriction fragments despite the fact that GS is male and DJP is female. This observation places the testis-determining factors distal to the most distal sequence, *GMGY3*, in DJP. Alternatively, the lack of male differentiation in DJP may be the result of mosaicism for 45,X cells.

ED and TM appear to have lost the most distal nine sequences from the consensus map of Yp, consistent with the localization of *TDF* to this region. However, ED has lost four more proximal sequences including *pDP34* and *GMGXY8*, and TM has also lost *GMGXY8*, *pDP34* and *p2F*(2). This may indicate that both have a paracentric inversion of Yp, or that the particular consensus sequence derived from the majority of X–Y interchange patients (Table 2) is a feature peculiar to those patients.

The results in RW are of particular interest. *In situ* hybridization using probe GMGY7 confirms that the minute chromosome fragment is derived from the Y chromosome. The most distal Y sequence (*GMGY3*) is present in the patient's DNA as is a series of ten contiguous sequences from a much more proximal region of Yp. The remaining more proximal sequences and the centromeric sequences (including *GMGY4a*) are missing, which suggests that the minute chromosome is an acentric fragment or ring with at least two interstitial deletions.

Table 2 shows the results of Southern blotting using the same Yp-specific probes in a series of 24 presumed X–Y interchange males with Klinefelter's syndrome (updated from Affara *et al.* 1986*a*). 19 (79·2%) of the 24 patients have at least one Y-

Table 1. *Pattern of Yp sequences in Yp aberrations*



Probe		WC	DJP	GS	ED	TM	RW
GMGY3		+	+	+	–	–	+
GMGXY7		+	+	+	–	–	–
47z		+	+	+	–	–	–
GMGXY6		+	+	+	–	–	–
115i		+	+	+	–	–	–
GMGXY4		+	+	+	–	–	–
13d		+	+	+	–	–	–
GMGXY9		+	+	+	–		–
GMGXY5		+	+	+	–	–	–
GMGY10	E	+	+	+	+	+	–
GMGXY2		+	+	+	+	+	–
GMGY7	A	+	+	+	+	+	–
	C	+	+	+	+	+	–
	D	+	+	+	+	+	–
50f2	A	+	+	+	+	+	–
	B	+	+	+	+	+	–
118	D	+	+	+	+	+	–
	E	+	+	+	+	+	–
GMGY46	A	+	+	+	+	.	–
GMGY22		+	+	+	+	+	–
GMGXY10		+	+	+	+	.	–
GMGY41		+	+	+	+	.	–
GMGY10	B	+	+	+	+	+	–
118e	C	+	+	+	+	+	–
118e	A	+	+	+	+	+	–
	B	+	+	+	+	+	–
GMGY23		+	+	+	+	+	+
GMGY46	B	+	+	+	+	.	+
GMGY7	B	+	+	+	+	+	+
	D′	?	?	?	?	?	+
GMGY10	E′	?	?	?	?	?	+
GMGY46	C	+	+	+	–	.	+
GMGXY8		+	+	+	–	–	+
pDP34		+	+	+	–	–	+
p2F(2)		+	+	+	–	–	+
50f2	D	+	+	+	+	+	+
GMGY10	A	+	+	+	+	+	+
	C	+	+	+	+	+	–
GMGY4(a)		+	+	+	+	+	–
CENTROMERE							
GMGY1		–	+	+	+		–
50f2	C	–	+	+	+	+	–
	E	–	+	+	+	+	–
pY3.4		–	+	+	+	+	–

specific sequence, and 18 have the most distal *GMGY3* fragment, which is thus assumed to be closest to the *TDF* locus. The cases have been arranged in Table 2 in order of decreasing number of Y-specific fragments on the assumption that in each case there is a single variable breakpoint in Yp resulting in the transfer of all Y sequences distal to the breakpoint. Only 3 of the 19 patients with Y sequences do not satisfy this hypothesis. KS, DR and HM require the occurrence of an additional rearrangement, a single paracentric inversion, to satisfy the concept of a terminal transfer of distal Yp resulting from one break (or one abnormal crossover event).

Seven of the Klinefelter patients show the transfer of 28 identical restriction fragments, suggesting that there may be a common breakpoint in each. In the five patients in whom no Y-specific sequences have been identified by our probes, it is conceivable that a smaller amount of Yp, including *TDF*, has been transferred. Alternatively, a different mechanism of sex reversal may be operating in these patients.

We have investigated the possibility that loss of Yp sequences (including *TDF*) in female patients with XY gonadal dysgenesis may result from X–Y interchange in an analogous manner to 'XX males' (Ferguson-Smith, 1966). Several female patients with partial deletions of Yp have already been described in which distal Yp sequences are missing (Magenis *et al.*

Table 2. *Pattern of Y sequences in XX males*

Probe		KS	RH	JM	TA	AG	JT	GA	WB	DR	NI	NE	OP	AP	RS	MS	MM	TK	GC	HM	AN	RT	PP	DC	5G
GMGY3		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	
GMGXY7		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	
47z		+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
GMGXY6		+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	
115i		+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
GMGXY4		+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
13d		+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
GMGXY9		+	+	+	+		+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−	−	−
GMGXY5		+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY10	E	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
GMGXY2		−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
GMGY7	A	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	C	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	D	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
50f2	A	−	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−	−	−	−	
	B	−	+	+	+	+	+	+	+	.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
118	D	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	E	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY46	A	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY22		−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGXY10		−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY41		−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY10	B	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
118c	C	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
118c	A	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	B	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY23		+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY46	B	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY7	B	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	D′	+	?	?	?	?	?	?	?	?	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY10	E′	+	?	?	?	?	?	?	?	?	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY46	C	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGXY8		+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
pDP34		+	−	−	−	−	−	−	−	.	.	−	−	−	.	.	−	−	−	−	−	−	−	−	
p2F(2)		+	−	−	−	−	−	−	−		.	−	−	−		.	−	−	−	−	−	−	−	−	.
50f2	D	−	−	−	−	−	−	−	−	.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
GMGY10	A	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY4(a)		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
CENTROMERE																									
GMGY1		−	−	−	−	−	−	−	−	−	.	−	−	−	−	−	−	−	−	−	−	−	−	−	−
50f2	C	−	−	−	−	−	−	−	−	.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
	E	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	.
pY3.4		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

1985; Disteche *et al.* 1986). The DNA from 14 such patients has been probed in our laboratory in Southern blotting experiments (Table 3) but only one patient (AM, Case 23 of Magenis *et al.* 1985) has Y restriction fragments missing from the distal end of Yp. This is the only patient in the series to have a cytogenetically detectable deletion. From our data it appears that X–Y interchange is not a frequent cause of XY gonadal dysgenesis.

The findings in patients with structural Y chromosome aberrations and in patients with X–Y interchange have been used to construct a consensus map of the short arm of the Y chromosome in which the restriction fragments identified by Yp-specific probes have been grouped in a linear series of 17 intervals (Fig. 3). The physical distances between these intervals are unknown at present, but application of the technique of pulsed-field gradient gel electrophoresis

Table 3. *Pattern of Y sequences in XY females and XO males*

		XY Females														XO Males	
Probe		JN	SM	MW	SB	DM	TG	JR	AM	RP	ST	SG	OR	JK	BK	JG	RW
GMGY3		+	+	+	+	+	+	+	−	+	.	.	.	+	+	+	+
GMGXY7		+	+	+	+	+	+	+	−	+	.	+	.	+	+	+	−
47z		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGXY6		+	+	+	+	+	+	+	−	+	.	+	+	+	+	+	−
115i		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGXY4		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
13d		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGXY9		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGXY5		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGY10	E	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGXY2		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGY7	A	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
	C	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
	D	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
50f2	A	+	+	+	+	+	+	+	−	+	.	+	+	+	+	+	−
	B	+	+	+	+	+	+	+	−	+	.	+	+	+	+	+	−
118	D	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
	E	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGY46	A	+	+	+	+	+	+	+	.	+	+	+	+	+	+	+	−
GMGY22		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGXY10		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−
GMGY41		+	+	+	+	+	+	+	.	+	+	+	+	+	+	+	−
GMGY10	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
118	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
118	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
GMGY23		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY46	B	+	+	+	+	+	+	+	.	+	+	+	+	+	+	+	+
GMGY7	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	D′	?	?	?	?	?	?	?	+	?	?	?	?	?	?	?	+
GMGY10	E′	?	?	?	?	?	?	?	+	?	?	?	?	?	?	?	+
GMGY46	C	+	+	+	+	+	+	+	.	+	+	+	+	+	+	+	+
GMGXY8		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pDP34		+	+	+	+	+	+	+	+	.	.	.	.	.	.	+	+
p2F(2)		+	+	+	+	+	+	+	+	.	.	.	.	.		+	+
50f2	D	+	+	+	+	+	+	+	+	+	.	+	+	+	+	+	+
GMGY10	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
GMGY4(a)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
CENTROMERE																	
GMGY6		.	.	.		.		.	.	.		.	.	.	.	+	−
GMGY19		.	.	.	.		.	.	.	.	.		.	.		−	−
50f2	C	+	+	+	+	+	+	+	+	+	.	+	+	+	+	−	−
	E	+	+	+	+	+	+	+	+	+	.	+	+	+	+	−	−
GMGY1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
pY3.4		+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−

(PFGE) using infrequent cutting restriction enzymes should define these distances. Application of PFGE to the DNA of individuals with normal Y chromosomes should determine if the six exceptions we have found to the consensus order among 26 patients (23%) with Yp aberrations are due to rearrangements associated with Y-chromosome abnormalities or are simply due to the occurrence of common Y polymorphisms in the normal population.

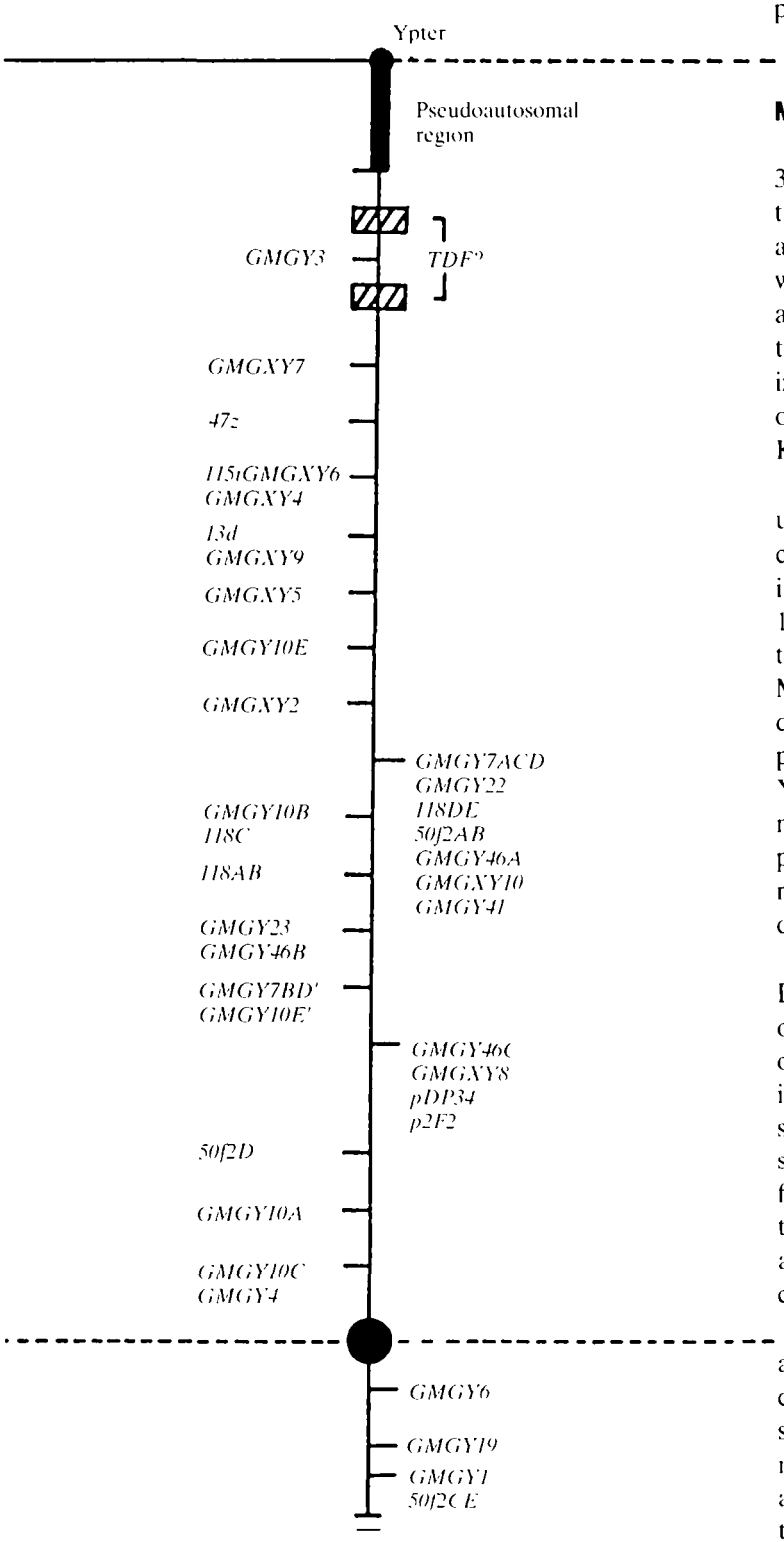


Fig. 3. Deletion map of the short arm of the Y indicating the relative order of the 37 restriction fragments which map to 17 separate intervals in Yp. The distances between the various intervals are unknown.

Map of the long arm of the Y

37 Y-specific restriction fragments have been mapped to the long arm of the Y chromosome by their absence in the DNA of WC, the infertile male patient with the monocentric isochromosome for the short arm of the Y (Fig. 2A). The 31 probes that recognize these sequences have all been isolated and characterized in Glasgow (Kwok *et al.* 1986) with the exception of pY3.4 which is described in Lau, Huang, Dozy & Kan, (1984).

The Y-chromosome aberrations that have been used to map these 37 sequences included a female carrier of an X–Y translocation with breakpoints in Xp22.3 and Yq11.2 (Ferguson-Smith *et al.* 1982), a male patient with karyotype 45X,dic t(5;Y)(p12;q11.1) and the cri-du-chat syndrome (E. Magenis, unpublished data), and 15 patients with dicentric isochromosomes for Yp, in whom the breakpoints in Yq vary in position within bands Yq11.21–23 (Fig. 2B). Almost all these patients are mosaic for 45,X cell lines, so that a wide range of phenotypes is to be expected. Thus some are infertile males and others are female patients with gonadal dysgenesis and variable degrees of masculinization.

Table 4 shows the results of Southern blotting the DNA of the two translocation patients and the series of 15 dicentric Yp isochromosomes arranged in order of increasing numbers of Y sequences present. All isochromosome cases except GC are consistent with a single breakpoint in Yq resulting in the loss of sequences distal to the breakpoint. In AM four of the five most proximal sequences are missing although the most proximal sequence is present. In both GC and AM, a simple paracentric inversion would account for the departure from the consensus order.

The findings in Table 4 have been used to construct a consensus deletion map of the long arm of the Y chromosome (Fig. 4). Only 2 of the 17 cases with structural abnormalities show deviations from this map. There are 14 intervals defined by the 17 cases, and once again determination of the distances between these intervals must await further study with the PFGE technique. The almost invariable association of 45,X mosaicism in these cases makes it difficult to establish genotype–phenotype correlations.

Variation in the order of Y-chromosome loci

The XY bivalent at meiosis is exceptional among all other bivalents, including the XX bivalent, in that recombination normally occurs in only a minute pairing (pseudoautosomal) segment. Whilst the order of loci along the nonpairing portion of the X is maintained in female meiosis by homologous synapsis and recombination, there is no such system for the nonpairing, differential segment which comprises most of the Y chromosome. Chromosome mutations which change the order of loci in the nonpairing part of the Y are likely to be tolerated provided they do not cause sterility or disturb reproductive fitness in some other way. This concept of the origin of Y-chromosome variation would seem to be supported by the relatively high frequency of harmless pericentric inversions of the Y observed in human populations. There is no reason why paracentric inversions of the type postulated in 6 of the 26 patients with Yp aberrations and 2 of the 17 cases with Yq aberrations in this study, should not also be well tolerated in a way that would be inconceivable for any other chromosome.

If such inversions are common in normal Y chromosomes, they may occasionally result in moving transcribed loci, such as the *TDF* locus, closer to the pairing segment. Accidental (illegitimate) recombination between X and Y might be expected to result in the transfer of the *TDF* locus to the X more frequently in Y chromosomes that have experienced such inversions, than in Y chromosomes in which the *TDF* locus is more proximal. A Y chromosome with a particularly distal *TDF* locus may conceivably give rise to more than one X–Y interchange male in the same family. In this regard, the family described by Page, de la Chapelle & Weissenbach (1985) in which two affected second cousins had a common great-

Table 4. *Pattern of Yq sequences in Yq deletions*

Probe		WC	GC	PZ	JG	AM	CO	FF	CC	IT	KM	RS	JC	DM	MN	FW	HM	JL	JH
CENTROMERE																			
GMGXY3		−	−	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY6		−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY8		−	−	−	.	−	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY17		−	−	−	.	−	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY35		−	−	−		−	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY9		−	−	−	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY19		−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY30		−	−	−	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY34		−	−	−	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY37		−	−	−		+	+	+	+	+	+	+	+	+	+	+	+	+	+
GMGY13		−	−	−	.	+	−	+	+	+	+	+	+	+	+	+	+	+	+
GMGY29		−	−	−	.	+	−	+	+	+	+	+	+	+	+	+	+	+	+
GMGY15		−	−	−	.	+	−	−	+	+	+	+	+	+	+	+	+	+	+
GMGY16		−	−	−	.	+	−	−	+	+	+	+	+	+	+	+	+	+	+
GMGY38		−	−	−	.	+	−	−	−	+	+	+	+	+	+	+	+	+	+
GMGY33		−	−	−	.	+	−	−	−	−	−	−	+	+	+	+	+	+	+
GMGY40	B	−	−	−	.	+	−	−	−	−	−	−	+	+	+	+	+	+	+
	C	−	−	−	.	+	−	−	−	−	−	−	+	+	+	+	+	+	+
GMGY12		−	−	−	.	+	−	−	−	−	−	−	−	+	+	+	+	+	+
GMGY36		−	−	−	.	+	−	−	−	−	−	−	−	+	+	+	+	+	+
GMGY39		−	−	−	.	+	−	−	−	−	−	−	−	+	+	+	+	+	+
GMGY14		−	−	−	.	+	−	−	−	−	−	−	−	−	+	+	+	+	+
GMGY20		−	−	−	.	+	−	−	−	−	−	−	−	−	+	+	+	+	+
GMGY26		−	−	−	.	+	−	−	−	−	−	−	−	−	+	+	+	+	+
GMGY31		−	−	−		+	−	−	−	−	−	−	−	−	+	+	+	+	+
GMGXY10	B	−	−	−	.	+	−	−	−	−	−	−	−	−	−	+	+	+	+
	D	−	−	−	.	+	−	−	−	−	−	−	−	−	−	+	+	+	+
	F	−	−	−	.	+	−	−	−	−	−	−	−	−	−	+	+	+	+
GMGY18	A	−	−	−	.	+	−	−	−	−	−	−	−	−	−	+	+	+	+
	B	−	−	−	.	+	−	−	−	−	−	−	−	−	−	+	+	+	+
GMGY21		−	−	−	.	+	−	−	−	−	−	−	−	−	−	+	+	+	+
GMGY5		−	−	−	.	+	−	−	−	−	−	−	−	−	−	−	−	+	+
GMGY1		−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+
pY3.4	B	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
GMGY2		−	−	−	.	+	−	−	−	−	−	−	−	−	−	−	−	−	−
pY3.4	A	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	C	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	D	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−

grandfather may be a case in point, as in both individuals the X–Y interchange arose as separate mutations in the same Y chromosome.

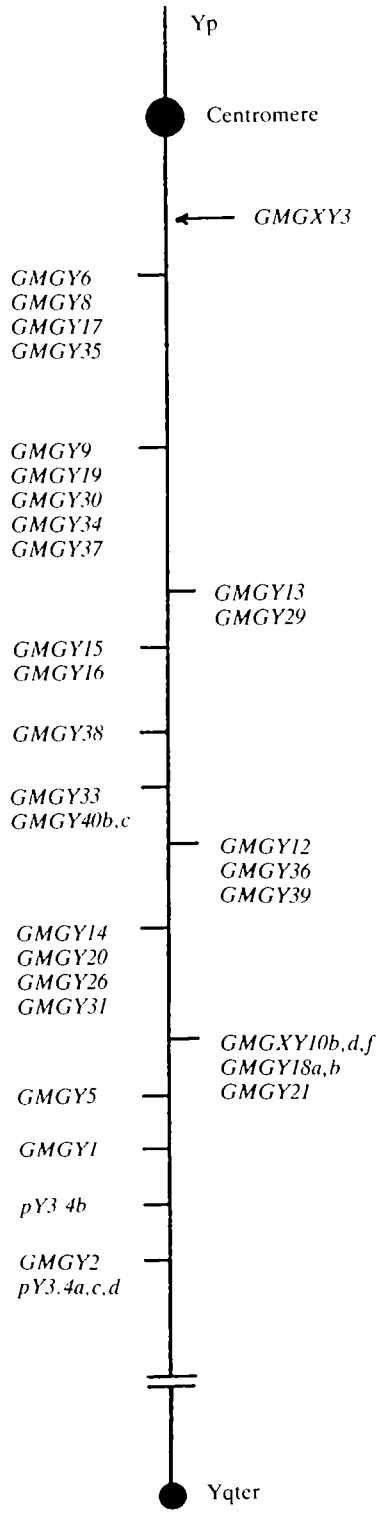


Fig. 4. Deletion map of the long arm of the Y chromosome indicating the relative order of the 38 restriction fragments which map to 14 separate intervals in Yq. The distances between the various intervals are unknown.

The destination of Y-specific sequences in X–Y interchange males

The Southern blotting analysis shown in Table 2 which reveals the presence of Y sequences in the DNA of patients with Klinefelter's syndrome and presumptive X–Y interchanges, do not indicate which chromosome carries these sequences. Earlier clues that testis-determining sequences may be transferred to the short arm of the X came from Xg blood grouping studies which revealed the coincidental loss of the paternal *Xg* allele (Ferguson-Smith, 1966), and from chromosome measurements which revealed heteromorphism of the two X's in such patients (Madan, 1976; Wachtel *et al.* 1976; Evans, Buckton, Spowart & Caruthers, 1979) due to transfer of Yp11.1-pter (Magenis *et al.* 1982). Convincing evidence of Y sequences on Xp has been provided by *in situ* hybridization (Magenis *et al.* 1984; Andersson, Page & de la Chapelle, 1986; Kalaitzidaki *et al.* abstract this symposium) but it is not clear in what proportion of patients this occurs.

We are in the process of studying the patients listed in Table 2 to determine the site of Y sequences and the possible mechanisms of transfer. So far, detailed banding studies have revealed cytogenetic evidence of transfer of the Yp11.3 band to the distal end of the short arm of the X chromosome in 10 of 15 cases. Such evidence is absent in 4 cases and uncertain in 1. DNA measurement of flow-sorted X chromosomes (Ferguson-Smith, Affara, Cooke & Boyd, 1986) has shown that one X chromosome is 3·8% larger than the other in 12 out of 20 cases: in 1 of the 8 cases with normal-sized X chromosomes (NE), cytogenetic evidence of X–Y interchange has nonetheless been found. *In situ* hybridization studies using the moderately repetitive Y-specific probes GMGY7 and GMGY10 has demonstrated the presence of the corresponding sequences at the distal end of the short arm in all 9 cases tested (Kalaitzidaki *et al.* abstract this symposium). Southern analysis of the DNA of flow-sorted chromosomes in 2 cases (RH and HM) demonstrates that the Y-specific sequences are carried by the fraction containing the X (Affara *et al.* 1986*a*). Thus in a total of 20 cases tested by one or other of these methods, we have evidence that the Y-specific sequences have been transferred to one of the X chromosomes in 14 cases (70%). In no case have we found positive evidence of a mechanism other than X–Y interchange.

Conclusion

Our studies have shown that it is possible to derive a consensus order of DNA markers along both arms of the Y chromosome by exploiting a series of structural

Y-chromosome aberrations. The map of the short arm of the Y derived by these methods is consistent with a map derived from studying patients with X–Y interchange. Exceptions to the consensus order are most readily explained by inversion polymorphisms which may be particularly common in the nonpairing part of the Y, because a strict order is not maintained by homologous pairing and recombination. The availability of Y-specific DNA markers and an extensive map of the Y chromosome have already been of great value in the interpretation of sex-chromosome aberrations referred for cytogenetic analysis, and in the elucidation of cases of presumptive X–Y interchange. The map could be further improved by studying additional patients with Y-chromosome aberrations in the short arm.

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