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**Deletion mapping of the testis determining locus with DNA probes in 46,XX males and in 46,XY and 46,X,dic(Y) females**

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**ABSTRACT**

Eleven Y-specific DNA probes hybridizing with DNA from one or more 46,XX males were isolated from a recombinant phage DNA library constructed from flow sorted human Y chromosomes. Two probes hybridized with DNA from nine out of eleven, i.e. >80% of these 46,XX males. The relative frequency of hybridization of the probes in the 46,XX males and in a 46,X,dic(Y) female, together with in situ hybridization data, allowed mapping of the probes on Yp in relation to a putative testis determining locus. Several of those probes were also absent in a 46,XY female, further refining a model for ordering the probes on Yp. The DNA of one XX male hybridized both with probes from Yp and probes from proximal Yq (excluding the pericentral region). This suggests that complex translocations may occur into the DNA of 46,XX males that involve not only parts of Yp but also parts of Yq.

**INTRODUCTION**

The Y is the only human chromosome not shared by both sexes and is assumed to have a major function in the determination of the male sex, i.e. in testis differentiation. Various mutations in man interfering with normal primary sex differentiation (i.e., gonadal development) facilitate the search for Y chromosomal genes required for normal testicular development. These mutations include the 46,XX male syndrome, true hermaphroditism, 46,XY gonadal dysgenesis (Swyer's syndrome) and various Y chromosomal aberrations that result in female development. The 46,XX male syndrome describes grossly normal but infertile males with a 46,XX karyotype (1). Cytogenetic (2-4) as well as molecular (5-9) studies using Y-chromosomal DNA probes suggest that a small translocation of Y chromosomal material has occurred in several 46,XX males thus accounting for male development despite the 46,XX karyotype. True

hermaphroditism relates to the simultaneous presence of male and female gonadal tissue in one individual (10). Karyotypes in true hermaphrodites may be 46,XX (in which case they may be considered related to 46,XX males), 46,XY, 46,X,der(Y), or chimeric with one contributing cell line containing a normal Y or a derivative Y chromosome (10,11).

Patients with Swyer's syndrome have a 46,XY karyotype, streak gonads, and, as a result of lack of testicular development, a female phenotype (12). Recent studies have shown deletions of Y chromosomal DNA sequences in both cytogenetically normal (9) 46,XY gonadal dysgenesis individuals and in individuals with cytogenetically detectable deletions of Yp (13,14). In addition, various Y chromosomal aberrations, most of which involve the short arm of the Y, have been described that result in disturbed testicular development (15).

In the pursuit of our goal to isolate Y chromosomal DNA sequences related to testis differentiation, we have tested for the presence or absence of 18 Y chromosomal DNA sequences in 11 46,XX males, 2 46,XX true hermaphrodites, 3 46,XY gonadal dysgenesis patients and in a phenotypically female patient with a 46,X,dic(Y) karyotype. Eleven of these Y chromosomal DNA sequences were found in at least one 46,XX male, with two of the probes present in nine out of eleven XX males (>80%) tested. Furthermore, lack of several Y chromosomal DNA sequences was detected in one 46,XY gonadal dysgenesis patient. These findings together with in situ hybridization data plus data on probe hybridization with DNA from a 46,X,dic(Y) female support a model in which many of the probes may be close to a putative testis determining locus.

### MATERIALS AND METHODS

#### Patients and Fibroblast Cultures

Five of the 11 46,XX males studied in this investigation were the same as those utilized before (9). Fibroblasts from these patients were kindly provided, via Prof. U. Wolf, by: Dr. J. Flori, Strasbourg and Prof. J.L. Nivelon, Dijon (No. 102); Prof. J. Lindsten, Stockholm (Nos. 548, 775); Dr. W. Tilgen, Heidelberg (No. 460 and No. 462). In Figures 2 and 4 of a

previous study (9), the Nos. 102, 460, 548, 775, and 462 correspond to lanes 6,7,8,9,10. Additional 46,XX males were designated as #756 and #481, fibroblasts of which were the gifts of Prof. H. Hoehn, Würzburg and Dr. J.M.J. Scheres, Nijmegen. Fibroblasts from 46,XX males #GM 1886; #GM 2626; #GM 2670 and from a putative 46,X,i(Yq) (qter->cen->qter) female individual (GM 1709) and a 49,XXXXY lymphoblastoid line (GM 1202) were purchased from the Human Genetic Cell Repository, Institute for Medical Research, Camden, NJ (for further references, see 16, 17). Further cytogenetic analysis of GM 1709 revealed an asymmetric der(Y) with two probable centromeric regions plus some intervening material, thus suggesting that this chromosome is in fact dicentric, i.e. a dic(Y), (Dr. M.M. Aronson, Institute for Medical Research, Camden; personal communication and photograph). Fibroblasts from another 46,XX male carried a cytogenetically detectable translocation (X;Y) (p22.33; p11.2) as described in (4) and were kindly provided by Dr. Ellen Magenis, Portland, Oregon. Fibroblasts from two 46,XX true hermaphrodites designated as #95 and #540 respectively were kindly provided by Prof. M. Fraccaro, Pavia. In the Figures 2 and 4 of a previous study (9), DNA from patient #95 was in lane 5. Fibroblasts from 2 of the 3 46,XY gonadal dysgenesis patients tested, #651 and #740, were the same as those investigated before (lanes 13 and 14 of Figs. 2 and 4 in (9)). For further information about these patients, see (18). The third 46,XY gonadal dysgenesis patient, #620, was from Prof. E. Passarge, Essen.

In addition, DNA extracted from lymphoblastoid cells of two male patients with different deletions of Yq was studied in this investigation. One patient, an infertile 46,XYq- individual had a peripheral lymphocyte karyotype of 45,X/46,X,del(Y)(pter->q11.21:) with more than 98% of the cells carrying the deleted Y chromosome (for further reference, see 19). The second 46,XYq- individual was fertile with a peripheral lymphocyte karyotype 46,X,del(Y)(pter->q11.23:) (M. Schmid, unpublished), i.e., including the Yq11.21->Yq11.23 region not present in the first case. Probes hybridizing with DNA from the second but not the first 46,X,del(Y) patient were

thus considered to map to this interstitial segment of Yq.

Fibroblasts were grown in minimum essential medium B supplemented with 10% or 20% fetal calf serum. Lymphoblastoid cells were grown in RPMI medium supplemented with 20% fetal calf serum.

DNA Panels and Hybridization Conditions:

DNA was extracted from lymphoblastoid cell lines established from peripheral lymphocytes of some of the patients. DNA was also extracted from patients' fibroblasts and from white blood cells from normal male and female controls as described (20). Cleavage of DNA with restriction endonuclease Hind III was performed according to manufacturer's recommendations. Gel electrophoretic separation of DNA, Southern transfer, and hybridization with <sup>32</sup>P radiolabelled DNA probes was carried out as described in detail before (9).

Construction of Y-enriched Recombinant Phage Library:

The recombinant phage DNA library was constructed from flow sorted human Y chromosomes. It was enriched for sequences from the euchromatic portion of the Y chromosome. A detailed description of this library and its construction are given in (13).

Screening of Recombinant Phage Library:

Details of library screening are outlined in (13). Radiolabelled inserts from recombinant phage that hybridized with Y chromosomal DNA sequences were hybridized with various panel filters containing DNA from the patients, and from normal male and female controls.

Estimation of Copy Number:

The approximate copy number (100) of one of the Y-specific repeats detected by probe Y-190 was calculated as described before (9).

In situ Hybridization:

In situ hybridization was done according to Harper et al. (21) as modified by Donlon et al. (22).

RESULTS

The human Y library described in a previous publication (13) has now yielded 30 Y-chromosomal DNA sequences out of 53

Table 1:  
DNA probes hybridizing with either Y-specific DNA sequences or with a Y chromosomal in addition to a non-Y chromosomal DNA sequence

Probe	Probe size (kb)	Characteristics	Probe	Probe size (kb)	Characteristics
Y-202	1.5		Y-157 <sup>a</sup>	3.7	Y single copy
Y-214*	2.3	Y-specific unique	Y-198*	1.2	+ single copy, non-Y homology
Y-253*	3.6		Y-228*	2.75	
Y-294	2.1		Y-227*	2.8	
			Y-221*	2.4	
Y-97	6.7		Y-280*	2.2	
Y-156*	4.4				
Y-182*	1.85	Y-specific repeated			
Y-190*	3.5				
Y-223a*	0.75				
Y-216 <sup>a</sup>	2.4				
Y-286*	1.7				
Y-219	5.1				

Asterisks mark probes that hybridize with DNA from at least one of the 11 XX males tested in this study.

mappable phage inserts screened. Of these 30 sequences with some Y homology, 18 either hybridized solely as Y-specific single copy (4) or Y-specific repeated probes (8) or as a Y-specific single copy probe which also recognized a single X or autosomal DNA sequence (6) (Table 1). In order to identify those sequences that were closest to a putative testis determining locus, the 18 DNA probes were hybridized with DNA from 11 46,XX males, two 46,XX true hermaphrodites and three 46,XY gonadal dysgenesis patients. The karyotypes of all patients were cytogenetically normal (either 46,XY male or 46,XX female) with the exception of one of the 46,XX males, who had been shown to carry a small segment of Yp on Xp (4).

Eleven of the 18 DNA probes tested hybridized with DNA from at least one XX male (see Table 1). Figure 1 shows the hybridization pattern of one such probe (Y-190) that hybridizes with Y-specific repeated DNA sequences in 6 out of 11 46,XX males, 6 of whom are shown in Figure 1. The repeat was estimated to contain approximately 100 copies of a 3.5 kb Hind III restriction fragment in addition to less repeated Hind III restriction fragments ranging in size from 1.5 kb to 5.7 kb. In

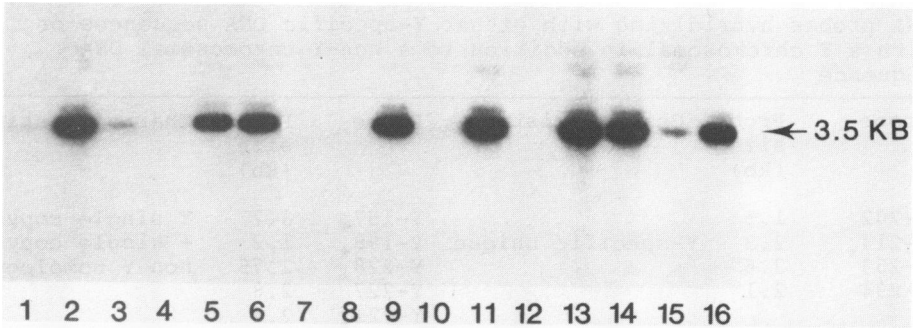


Figure 1. Autoradiograph of hybridization pattern of probe Y-190 with Hind III digested DNA from normal 46,XX females (lanes 1,12), 49,XXXXY male (lane 2), normal 46,XY male (lane 13), 46,XX males #102, #460, #462, #481, #548, #775 (lanes 4,5,6,7,9,11, respectively), 46,XY females #620, #651, #740 (lanes 14,15,16) and from two 46,XX true hermaphrodites #95 and #540 (lanes 3,8). Lane 10 is a blank.

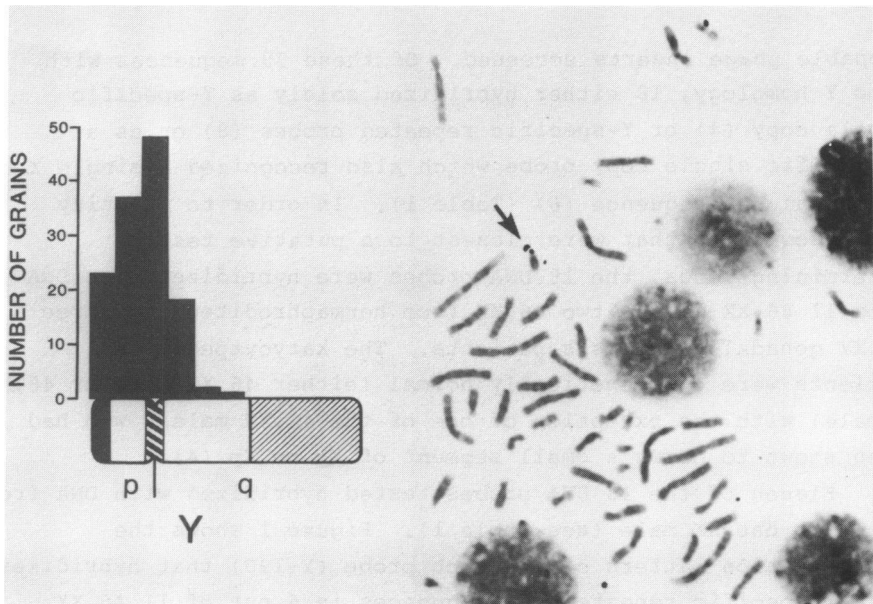


Figure 2. In situ hybridization of Y-190 to a 46,XY derived metaphase. The probe was labelled to  $2 \times 10^7$  cpm/ug, hybridized at a final concentration of 0.01 ug/ul at 42°C, and exposed for four days. >80% of all metaphases examined showed at least two grains over Yp, as shown by arrow. The sketch shows the grain distribution obtained from 50 cells analyzed.

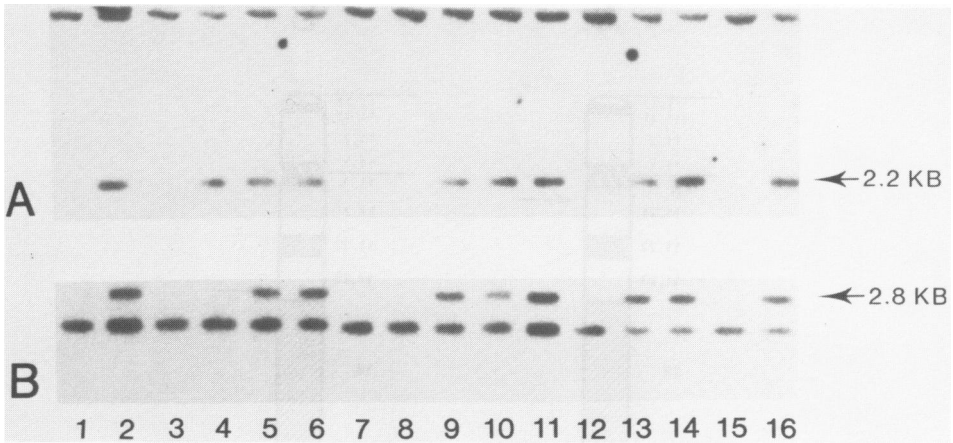


Figure 3. Autoradiograph of hybridization pattern of probes Y-280 (A) and Y-227 (B) with Hind III digested DNA from normal 46,XX females (lanes 1,12), 49,XXXXY male (lane 2), normal 46,XY male (lanes 13), 46,XX males #102, #460, #462, #481, #548, #756, #775 (lanes 4,5,6,7,9,10,11), 46,XY females #620, #651, #740 (lanes 14,15,16) and two 46,XX true hermaphrodites (lanes 3,8). Both probes also hybridized with an autosomal restriction fragment. The autosomal fragment hybridizing with probe Y-280 was polymorphic (2 alleles).

addition, a faint hybridization signal was detected in one 46,XX true hermaphrodite and in one of the three 46,XY gonadal dysgenesis patients tested (Fig. 1). No interindividual variation in the intensity of hybridization with Y-190 was detected in 8 normal males and no hybridization with Y-190 was found in any of 10 46,XX females tested (data not shown). In situ hybridization of Y-190 assigns the repeat to the proximal short arm of the Y chromosome, close to the centromere (Fig. 2). The intensity of hybridization with Y-190 was not reduced in the 46,XX males as compared to normal male controls.

Figure 3 gives an example of hybridization of two probes detecting a Y chromosomal in addition to a non-Y chromosomal DNA segment. The lanes depicted in Figure 3 contain DNA from the same individuals, plus one additional 46,XX male, as in Figure 1. Probe Y-280 of Figure 3A hybridizes with a Y-specific restriction fragment of 2.2 kb in addition to two larger autosomal polymorphic Hind III restriction fragments. Y-specific hybridization of Y-280 was found in 9 out of 11 XX males (7 of

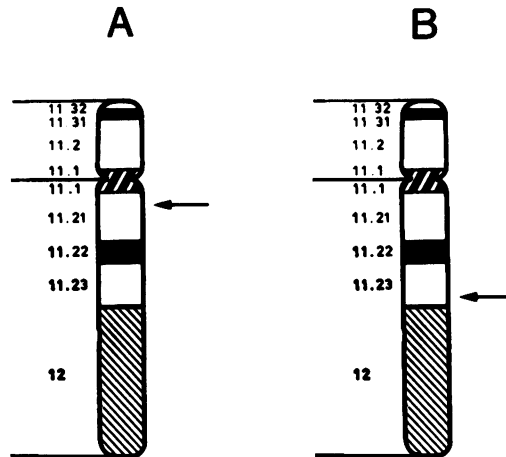


Figure 4. Diagrammatic representation of G-banded lymphocyte Y chromosomes. The breaks (arrows) are localized in Yq11.21 in the infertile patient (A) and in Yq11.23 in the fertile proband (B). The lightly cross-hatched band Yq12 is the constitutive heterochromatin.

whom are shown in Figure 3a). No Y-specific hybridization was detected in the two 46,XX hermaphrodites or in the 46,XY gonadal dysgenesis patient who exhibited reduced hybridization with probe Y-190 (Fig. 1). Probe Y-227 (Figure 3B) hybridized with a Y chromosomal Hind III restriction fragment of 2.8 kb that was detected in 8 out of 11 46,XX males, 7 of whom are shown in the figure. A smaller, non-Y specific Hind III fragment was also detected. No Y-specific hybridization was observed in the two 46,XX hermaphrodites. The Y-specific hybridization signal of probe Y-227 was also missing in the above-described 46,XY gonadal dysgenesis patient (#651).

Another probe (Y-286) hybridized with two Y-specific Hind III restriction fragments of 1.7 and 5.2 kb. The 1.7 kb Hind III fragment was assigned to the proximal long arm of the Y by comparative deletion mapping to DNA from an infertile and a fertile 46,XYq- individual that had breakpoints of Yq at Yq11.21 and Yq11.23, respectively as indicated in Figure 4. As can be seen in Figure 5, the 1.7 kb fragment was present in the fertile but absent in the infertile individual. Therefore, this



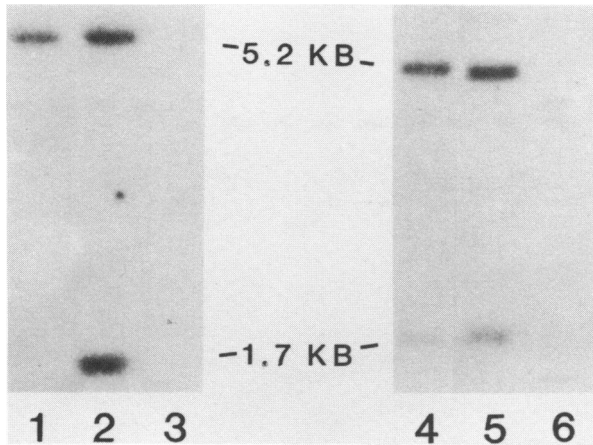


Figure 5. Autoradiograph of hybridization pattern of probe Y-286 with Hind III cleaved DNA from normal 46,XX females (lanes 3,6), normal 46,XY males (lanes 2,5) and from an infertile (lane 1) and from a fertile (lane 4) 46,XYq- proband. For assignment of the breakpoints of Yq in both patients, see Figure 4.

fragment lies within region Yq11.21→Yq11.23 of the Y long arm. The 5.2 kb Hind III restriction fragment detected by probe Y-286, however, was present in both Yq- individuals and was also found in 9 out of 11 XX males, including the t(X;Y) (p22.33; p11.2) patient, assigning this restriction fragment to Yp.

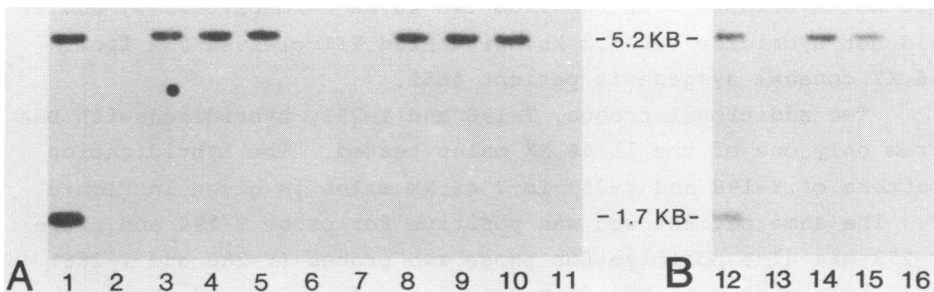


Figure 6. Autoradiograph of hybridization pattern of probe Y-286 with Hind III digested DNA from normal 46,XX females (lanes 2,13), normal 46,XY males (lanes 1,12), 46,XX males #772, #756, #548, #481, #462, #460, #102, GM2670, GM2626, GM1889 (lanes 3,4,5,6,8,9,10,14,15,16) and from two 46,XX true hermaphrodites (lanes 6,11). A and B represent the results of different experiments.

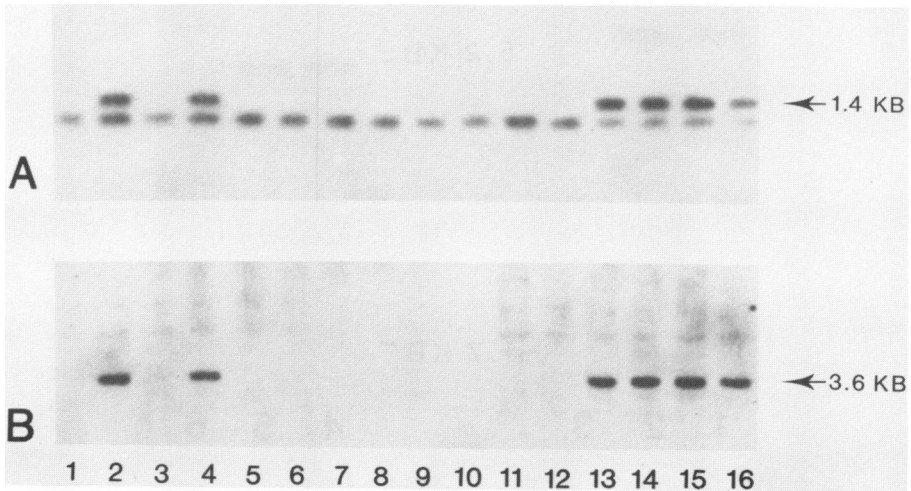


Figure 7. Autoradiograph of hybridization pattern of probes Y-198 (A) and Y-253 (B) with Hind III-cleaved DNA from normal 46,XX females (lanes 1,12), 49,XXXXY male (lane 2), normal 46,XY male (lane 13), 46,XX males #102, #460, #462, #481, #548, #756, #775 (lanes 4,5,6,7,9,10,11), 46,XY females #620, #651, #740 (lanes 14,15,16) and from two 46,XX true hermaphrodites, #95 and #540 (lanes 3,8). Both probes hybridized with DNA from one 46,XX male, #102 (lane 4) only, the same individual that was also positive with probes Y-280 and Y-286 (#4 of figure 3A and #10 of Figure 6).

Figure 6 shows the hybridization of this probe (Y-286) with Hind III cleaved DNA from eight out of ten 46,XX males. Probe Y-286 did not hybridize with DNA from two 46,XX hermaphrodites, and it did not hybridize (at 5.2 kb) with Hind III cleaved DNA from 46,XY gonadal dysgenesis patient #651.

Two additional probes, Y-198 and Y-253, hybridized with DNA from only one of the 11 46,XX males tested. The hybridization pattern of Y-198 and Y-253 in 7 46,XX males is given in Figure 7. The same patient who was positive for probe Y-198 and probe Y-253 was also positive for those two probes (Y-280 and Y-286) that hybridize with DNA from 80% of the XX males and that were absent in one 46,XY female (see Figs. 3, 6). Interestingly, deletion mapping in the two Yq- patients of Figure 4 assigned both probes Y-198 and Y-253 to the region Yq11.21->Yq11.23, rather than to Yp (Fig. 8). The hybridization of the various probes with DNA from a 46,X,dic(Y) patient was also tested. The

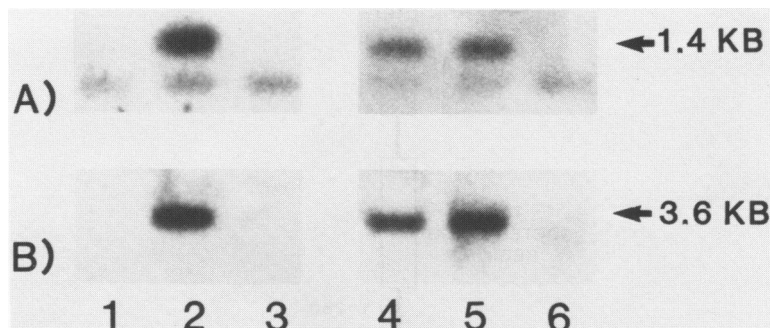


Figure 8. Autoradiograph of hybridization pattern of probes Y-198 (A) and Y-253 (B) with Hind III-cleaved DNA from normal 46,XX females (lanes 3,6), normal 46,XY males (lanes 2,5) and from an infertile (lane 1) and from a fertile (lane 4) 46,XYq- male. For assignment of the breakpoints of Yq in both patients, see Figure 4.

results obtained in this patient with Y-homologous probes are summarized in Table 2.

#### DISCUSSION

Eleven out of eighteen DNA probes from a recombinant phage DNA library enriched for the human Y chromosome were shown to hybridize with DNA from at least one 46,XX male (Tables 1,2).

Table 2:

Hybridization of 11 Y-chromosome-specific DNA probes with DNA from eleven 46,XX males and from a 46,X,dic(Y) female

Probe	46,XX males											
	460	462	548	756	775	t(X;Y) (p22.33; p11.2)	GM 2626	GM 2670	102	GM 1889	481	GM 1709 46,X,dic(Y)
Y-156	+	+	+	+	+	+	-	-	-	-	-	+
Y-182	+	+	+	+	+	+	-	-	-	-	-	+
Y-190	+	+	+	+	+	+	-	-	-	-	-	+
Y-223a	+	+	+	+	+	+	-	-	-	-	-	+
Y-219	+	+	+	+	+	+	-	-	-	-	-	+
Y-227	+	+	+	+	+	+	+	+	-	-	-	+
Y-228	+	+	+	+	+	+	+	+	-	-	-	-
Y-286	+	+	+	+	+	+	+	+	+	-	-	-
Y-280	+	+	+	+	+	+	+	+	+	-	-	-
Y-198	-	-	-	-	-	-	-	-	+	-	-	+
Y-253	-	-	-	-	-	-	-	-	+	-	-	+

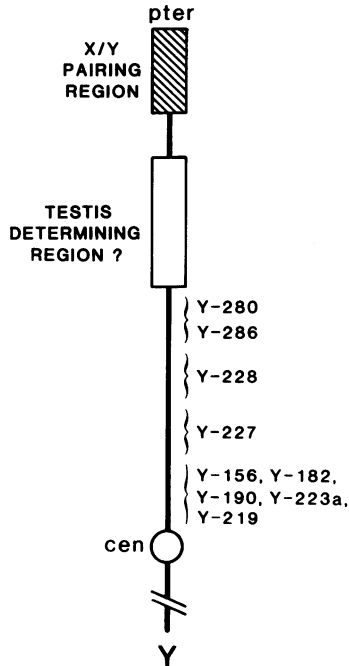


Figure 9. Schematic diagram of the relative arrangement of Y-chromosomal DNA sequences hybridizing with various probes in relation to a putative testis determining locus on Yp. The guiding assumption behind this diagram is that probes detecting DNA in a greater proportion of 46,XX males are closer to the testis determining locus. Note that probes Y-280 and Y-286 could thus be proximal or distal from this locus, but in either case, closer to this locus than are any of the other probes shown. For further details, see text. Probes Y-198 and Y-253, found in one 46,XX male, are not included in this map; they localize to Yq11.21-Yq11.23. Distances given in the map are intended to reflect probe order without regard to the actual magnitude of physical or genetic distances of the probes from the testis determining locus.

All probes tested were either Y-specific or hybridized with one Y specific in addition to one X or autosomal DNA sequence. Probes that detected one Y chromosomal and multiple X or autosomal homologous restriction fragments (8,13) were not used in this study since they are suboptimal for chromosome walking experiments and thus for the eventual cloning of testis determining genes. Nine probes listed in Table 2 hybridized with DNA sequences of the Y short arm, and 2 probes hybridized

with DNA sequences of the proximal long arm of the Y chromosome. Major evidence for Yp mapping of the first 9 probes was the observation that they hybridized with DNA from an XX male that carried a translocation from Yp (Yp 11.2->Ypter) on Xp (4). In situ hybridization, carried out with three of these probes (Y-190, Figure 2; Y-156 and Y-182; ref. 9), confirmed their assignment to Yp. Our observations are therefore in agreement with both cytogenetic (23) and molecular findings (8,9) that assign a putative testis determining locus to the short arm of the Y.

Several probes of Table 2 hybridized with Y chromosomal DNA sequences of a 46,X,dic(Y) phenotypic female individual, reinforcing the revised cytogenetic impression that the patient carried a dic(Y) rather than an i(Yq), including some Y short arm material (most likely fusion point Yp11.2). The finding in the 46,X,dic(Y) patient extended fine mapping of the probes in relation to the testis determining locus. Four regions in Yp are defined by our studies of these probes (Table 2 and Figure 9). Probes Y-156, Y-182, Y-190, Y-223a, and Y-219 hybridized with the same set of six out of eleven 46,XX males (including the 46,X,t(X;Y) male containing Yp11.2->Ypter) and with DNA from the 46,X,dic(Y) female and are thus likely to be closest to the centromere, most likely within Yp11.2, and furthest away from the testis determining locus. Probe Y-227 also hybridized with DNA from the 46,X,dic(Y) female and the above 46,XX males but with DNA from two additional 46,XX males as well, and may therefore detect sequences closer to a testis determining locus than do the former group. Probe Y-228 detects Y chromosomal sequences in the same group of 46,XX males as does probe Y-227, but not in the 46,X,dic(Y) female, and thus appears to be closer to a testis determining locus than is probe Y-227. Finally, probes Y-280 and Y-286 detect Y-specific DNA sequences in nine of eleven 46,XX males (>80%) but not in the 46,X,dic(Y) female. These probes may thus be closest of those studied to a testis determining locus. As yet, it cannot be decided, however, whether the last two probes are proximal or distal from this locus.

A tentative map of Yp based on these observations and the

assumption of contiguous translocations from Yp to the DNA of 46,XX males is given in Figure 9. This arrangement is more consistent with Model "A" than Model "B" of (8) for the distribution along the Y of DNA probes found in 46,XX males. Model "A" of (8) is based on the assumption that each 46,XX male carries a terminal contiguous portion of the short arm of the Y chromosome. Probes that hybridize with the DNA of more 46,XX males are located more distal on Yp. Model "B" of (8) assumes that each 46,XX male carries an internal contiguous portion of the short arm of the Y chromosome, with the reverse positioning of probes as a function of hybridization with the DNA of 46,XX males. In contrast to either of these models, however, we also observe two probes (Y-198 and Y-253), which hybridize with the DNA of one 46,XX male, that derive from Yq. This suggests a lack of complete contiguity of all Y probes observed in 46,XX males. This finding raises the possibility of non-contiguous translocations of Y material in XX males. It is possible, however (see below), that the position of probes Y-198 and Y-253 is not relevant to maps of testis determining segments on Yp.

The probes of this investigation found to hybridize with DNA of most XX males (Y-280, Y-286) do not seem to be related to probes found to hybridize with DNA from approximately 60% of 46,XX males studied by others, e.g. probes 47a and 47c (8). While 47a and 47c show X and Y homology, our probes either exhibit Y and autosome homology (Y-280) or are Y-specific (Y-286).

The testis determining locus is clearly excluded from the paracentromeric region of Yp, given the minimum of 7 proximal Yp probes not present in all 46,XX males (Fig. 9). Furthermore, the telomeric region of Yp almost certainly does not carry the testis determining locus, since this region, that exhibits DNA replication homology with Xp (24), has been shown to undergo obligatory crossing over with Xp during male meiosis (25-28). As a result, any testis determining locus on Yp must lie somewhere between these extremes, probably closer to the latter, in the short arm of the Y. Given a total DNA content of the Y of  $50 \times 10^6$  base pairs and provided that the Y short arm makes up 1/3 to 1/4 of its total length, i.e.  $10-15 \times 10^6$  bp, our

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probes may be assumed to lie within less than  $5-10 \times 10^6$  base pairs of the testis locus.

The Yq sequences recognized in one 46,XX male (#102) by probes 198 and 253 are not likely to be related to testis determination. These sequences are not present in any of the other 46,XX males, and they are not deleted in any of the 46,XY gonadal dysgenesis patients examined (including the two described in reference 14). Also, none of the proximal Yq or centromeric sequences recognized by other probes are present in patient #102. However, probes Y-280 and Y-286, located more distal on Yp, detect sequences present in this patient. It follows then, that the sequences from Yp and Yq occurring in the fibroblasts of this individual 46,XX male must have originated from a complex process of Y DNA sequence transfer involving at least two translocation events.

The lack or reduced copy number of some Y-specific sequences in one of the 46,XY gonadal dysgenesis patients tested confirms and extends earlier data in this (9) and in other 46,X,der(Y) gonadal dysgenesis patients (13,14). Two other patients tested (#620 and #740) showed hybridization with all Y probes utilized. This observation may reflect point mutations or at most very small deletions in the testis determining locus of these two patients. The results overall support the notion that the mutations underlying 46,XY gonadal dysgenesis are heterogeneous at the molecular level.

Of the two 46,XX true hermaphrodites tested, one typed Y-DNA positive with one probe. This probe (Y-190), gave only a weak signal with DNA from the 46,XX hermaphrodite patient #95, even though this probe hybridizes with approximately 100 copy intensity in normal males and in many of the patients described in this report (Figure 1). The basis for this finding remains unknown. Perhaps it reflects low level chimaerism (10,11) in patient #95.

In conclusion, using multiple Y-specific DNA probes and examining several patients with disorders of primary sex differentiation, evidence was obtained for the existence of the putative testis determining locus somewhere proximal to the telomeric X-Y pairing region of the Y short arm. Two of the

probes tested hybridized with Y-specific DNA sequences in 9 of the 11 46,XX males available for testing. This, together with the lack of hybridization of these probes with the DNA of a 46,XY female, indicate that they may be very close to that portion of the Y containing the testis determining locus. These two and the seven other Yp probes found to hybridize with 46,XX male DNA should assist ultimate molecular definition of this locus.

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