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Variable transfer of Y-specific sequences in XX males

N.A.Affara, M.A.Ferguson-Smith, J.Tolmie, K.Kwok, M.Mitchell, D.Jamieson, A.Cooke and L.Florentin

University Department of Medical Genetics, Duncan Guthrie Institute of Medical Genetics, Yorkhill, Glasgow G3 8SJ, UK

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ABSTRACT

A series of twelve XX males and their relatives have been examined by Southern blot analysis with fourteen different Y recombinants. The pattern of Y sequences present shows considerable variation between XX males. Furthermore, on the basis of the terminal transfer model, anomalous patterns of Y sequences are evident in certain XX males in that sequences located as proximal Yp by means of a Y deletion panel are found to be present in the absence of distal sequences. These anomalies can be resolved by proposing that the order of Yp sequences varies in the population in the form of inversion polymorphisms in the Y chromosomes of normal males. Alternatively, it is necessary to invoke multiple recombination events between the X and Y chromosomes to explain the patterns of Y sequences in these XX males. Southern analysis on DNA prepared from flow sorted X chromosomes of XX males indicates that the Y sequences in these patients are linked to X chromosomes.

INTRODUCTION

Studies on human sex chromosome aberrations reveal that male differentiation occurs only in the presence of a Y chromosome, or part of the short arm of the Y chromosome, in at least a proportion of somatic cells. The only apparent exceptions to this rule are patients with Klinefelters syndrome and a non-mosaic 46,XX karyotype (XX males) and the rare cases of XX true hermaphroditism. X-Y interchange (1) by accidental crossing-over during paternal meiosis has long been considered a possible explanation for these exceptions, and more recent studies have confirmed this by demonstrating that DNA from XX males contain Y-specific sequences (2-6). Cytogenetic analysis also reveals that in some XX males the distal G band of the short arm of the Y (Yp11.3) is transferred to the distal end of the short arm of the X (7). These studies provide evidence that the short arm of the Y chromosome normally carries a testis-determining factor locus (TDF) responsible for human male differentiation. This raises the possibility that the DNA from XX males may be a useful source for isolating and characterising those sequences on Yp responsible for male differentiation.

In this paper we exploit a series of Yp DNA probes, previously localised using a Y-deletion panel (see accompanying paper by Affara et al.), to examine the extent of transfer of Y-specific sequences in 12 XX males. It is found that there is considerable variation in the transfer of Y sequences. Moreover, the pattern of Y sequences present in XX males cannot readily be explained on the basis that the extent of the transfer is directly related to the distance of the interchange breakpoint from the centromere of the Y. Anomalous patterns of transfer exhibited by certain XX males rather indicate that the order of Y sequences in Yp may show extensive variation between Y chromosomes of different origin.

MATERIALS AND METHODS

All procedures used in this paper are described in the accompanying paper by Affara et al. The XX males in this study have had extensive chromosome analysis in order to exclude XX/XY or XX/XXY mosaicism (data not shown).

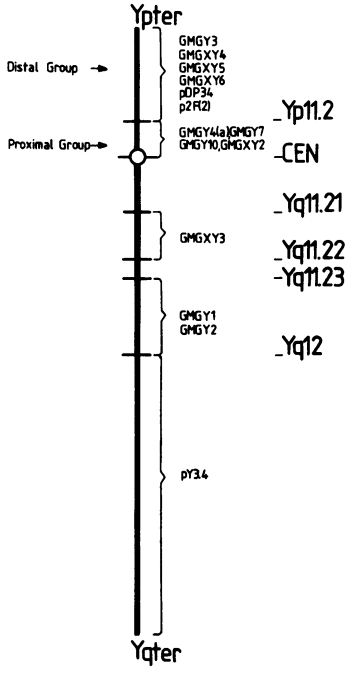

Figure 1 Chromosomal Location of Y Probes

The localisation of Y probes with respect to breakpoints defined by a Y panel.

RESULTS

Location of Yp Probes Determined by a Y Deletion Panel

Figure 1 illustrates the relative location of Y probes employed in this study, as determined using the Y deletion panel described in the accompanying paper. The Y probes fall into two groups delineated by two breakpoints in the Y deletion panel at Ycen and Yp11.2. (a) The proximal group mapping between Ycen-Yp11.2 which comprises GMGY4(a), GMGY7, GMGY10 and GMGYXY2. (b) The distal group mapping between Yp11.2-pter which contains GMGY3, GMGXY4, GMGXY5, GMGXY6, p2F(2) and pDP34.

Recombinants GMGY7 and GMGY10 detect several uniquely Y-specific fragments, whereas p2F(2) GMGXY2, GMGXY4, GMGXY5, GMGXY6 and pDP34 are X-Y homologous probes whose X-specific sequences map between Xq13-Xq24. pDP34 has been described in detail by Page et al. (8). GMGXY2 together with GMGY3 and GMGY4(a) detect autosomal sequences.

Y-specific Sequences in XX Males

In order to determine whether Y-specific sequences can be detected in this group of XX males, genomic DNA from peripheral blood lymphocytes was digested with the appropriate restriction enzyme and subjected to Southern analysis with the series of Y recombinants shown in Figure 1. The results are shown in Figure 2 where the arrows indicate the position of Y-specific fragments for each probe. Table 1 summarises the data of Figure 2, showing the pattern of Y sequences present in each XX male.

The first point to emerge is that none of the XX males have any of the sequences which map to the long arm of the Y. Several long arm sequences have been used which include GMGY1 mapping between Yq11.23-Yq12 (data shown), GMGXY3 mapping between Yq11.21-Yq11.23 (data not shown) and the major heterochromatic 3.4Kb Y repeat of Lau et al. (9) (data not shown). These findings help to rule out (but not to exclude entirely) the possibility that male determination in these XX males is due to the presence of XX/XY, XX/XXY or other forms of mosaicism.

The second point arising from the data in table 1 is the lack of any Y sequences in two of the twelve XX males (RT and AN). This may reflect the small extent of X-Y exchange involving a minute part of Yp (containing the TDF locus) within which the current probes do not map. Alternatively, it is possible that the phenotype of these XX males is not caused by an X-Y exchange event.

The remaining ten XX males reveal a marked variation in the pattern of Yp material present in their genomes. It is clear from table 1 that the transfer of a proximal group sequence is not invariably associated with the tran-

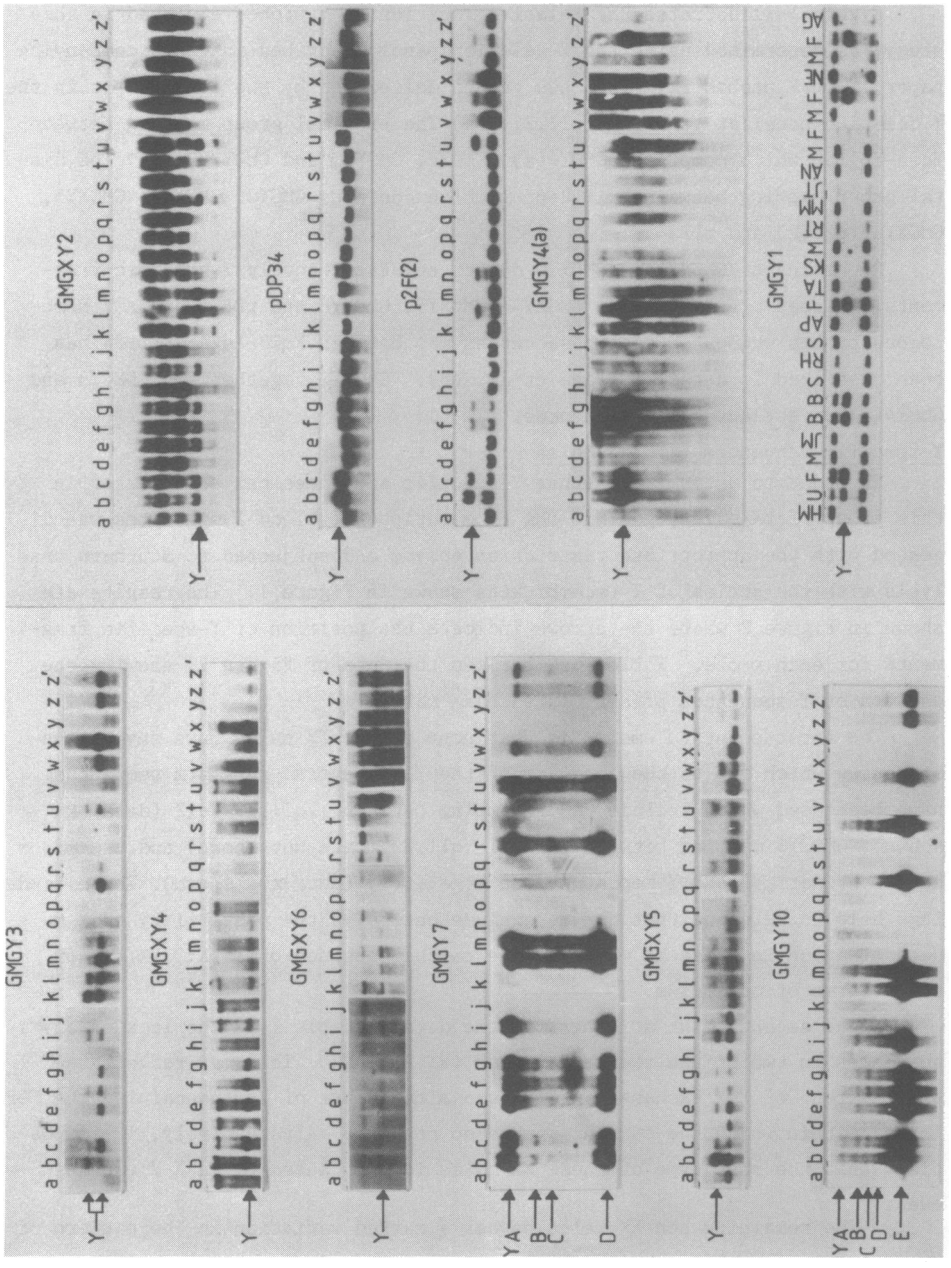
GMGY3
GMGXY4
GMGXY6
GMGY7
GMGXY5
GMGY10
GMGXY2
pDP34
p2F(2)
GMGY4(a)
GMGY1
Y
YA
B
C
D
E

TABLE 1 PATTERN OF Y SEQUENCES IN XX MALES

PROBE		KS	RH	JM	TA	AG	JT	AP	NE	MM	HM	AN	RT
GMGY3		+	+	+	+	+	+	+	+	+	-	-	-
GMGXY4		+	+	+	+	+	+	+	+	-	-	-	-
GMGXY6		+	+	+	+	+	+	+	+	-	-	-	-
GMGY7	A,C,D	-	+	+	+	+	+	-	-	-	-	-	-
	B,	+	-	-	-	-	-	-	-	-	-	-	-
	D'	+	?	?	?	?	?	-	-	-	-	-	-
GMGXY5		+	+	+	+	+	+	-	-	-	-	-	-
GMGY10	A,C	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	+	+	+	?	+	-	-	-	-	-	-
	E	+	+	+	+	+	+	-	-	-	+	-	-
GMGXY2		-	+	+	+	+	+	-	-	-	+	-	-
pDP34		+	-	-	-	-	-	-	-	-	-	-	-
p2F(2)		+	-	-	-	-	-	-	-	-	-	-	-
GMGY4(a)		-	-	-	-	-	-	-	-	-	-	-	-
CENTROMERE													
GMGXY3		-	-	-	-	-	-	-	-	-	-	-	-
GMGY1		-	-	-	-	-	-	-	-	-	-	-	-
GMGY2		-	-	-	-	-	-	-	-	-	-	-	-
pY3.4		-	-	-	-	-	-	-	-	-	-	-	-

Table 1 summarises the distribution of Y sequences in XX males. The question marks indicate uncertainty as to whether the fragment is present (see text for discussion).

sfer of all distal group sequences. On the basis of the X-Y interchange hypothesis, one might have expected a clustering of transfer breakpoints, and hence Y sequences, to be the consequence of a single recombination event. Nine of the XX males (KS, RH, JM, TA, AG, JT, AP, NE, MM) show the presence of GMGY3 sequences (the most distal marker), eight (KS, RH, JM, TA, AG, JT, AP, NE) the presence of GMGXY4 and GMGXY6 sequences and six (KS, RH, JM, TA, AG, JT) the presence of GMGXY5 sequences. It is of particular interest to note that five of the XX males (RH, JM, TA, AG, JT) clearly contain GMGY7, GMGY10 and GMGXY2 of the proximal group, but in the absence of Y sequences complementary to probes p2F(2) and pDP34 of the distal group. KS is the only XX male to contain Y sequences complementary to these latter two probes. One XX male (HM) shows the presence of sequences complementary to GMGXY2 of the proximal

Figure 2 Southern Analysis on XX Males and Relatives Using Y Probes

DNA from 12 XX males and their relatives was digested with various restriction enzymes and subjected to Southern blot analysis using the probes shown in Figure 2. The restriction enzymes used were as follows: GMGY3-Msp I; GMGXY4-Msp I; GMGXY6-Taq I; GMGY7 -EcoRl; GMGXY5-Taq I; GMGY10-EcoRl; GMGXY2-EcoRl; pDP34-Taq I; p2F(2)-EcoRl; GMGY4(a)-EcoRl; GMGY1-Msp I. The lanes for each blot are as follows: a - XX male HM; b - uncle of HM; c - father of HM; d - mother of HM; e - XX male JM; f - brother of JM; g - brother of JM; h - sister of JM; i - XX male RH; j - sister of RH; k - XX male AP; l - father of AP; m - XX male TA; n - XX male KS; o - mother of KS; p - XX male RT; q - XX male MM; r - XX male JT; s - XX male AN; t - mother of AN; u - father of AN; v - mother of NE; w - father of NE; x - XX male NE; y - mother of AG; z - father of AG; Z'- XX male AG. The arrows mark the position of Y-linked DNA fragments.

group in the absence of all other Yp sequences except GMGY10. Both GMGY7 and GMGY10 detect several Y-linked fragments all of which have been scored for transfer in this series of XX males and recorded in table 1. None of the XX males show transfer of sequences complementary to probe GMGY4(a) or to fragments A and C of GMGY10.

The strength of the signal obtained for the dominant bands of GMGY7 (GMGY7A and GMGY7D) and GMGY10 (GMGY10E) imply a degree of repetition for these sequences. Furthermore, the discrete fragment sizes for the dominant bands suggest clustered repeats with a regular occurrence of certain restriction enzyme sites (in this case EcoR1). In this respect, it is intriguing to note that a faint band can be detected with GMGY10 of the same fragment size as the dominant band in both HM and KS, but not normal females and XX males negative for GMGY10 sequences. Since this probe detects a number of more weakly hybridising fragments in normal males, this observation may reflect a further fragment on the Y chromosome normally hidden by the dominant hybridisation, but visible in HM and KS by virtue of the fact that it has been transferred in the absence of the dominant hybridising sequences. On the other hand, it may indicate a partial transfer of the clustered sequences in HM and KS hence implying that the breakpoint on the Y chromosome, in their respective fathers, has occurred within the GMGY10 cluster. A similar situation is found with the GMGY7D dominant band where KS shows weak hybridisation. If this band and that detected by GMGY10 are generated by a break in these clusters this would suggest that the GMGY7 and GMGY10 clusters are closely associated or even interspersed. If, however, the faint GMGY7D band in KS represents a further Y fragment detected by GMGY7 (GMGY7D') but normally obscured by the dominant band then close association or interspersion of these clusters need not be invoked (discussed further below).

From the pattern of Y sequences in this series of XX males, it is therefore evident that a simple X-Y exchange involving one breakpoint is inadequate to explain all the observations.

In all cases where DNA from brothers, sisters and parents was available for analysis, no anomalous distribution of Y-specific sequences was observed (see Figure 2). The expected absence of Y sequences from normal females and presence in normal males was obtained.

Origin of X Chromosomes in XX Males

It is a necessary requirement of the X-Y interchange hypothesis to demonstrate a paternal contribution to the X chromosomes present in an XX male. This can be unambiguously shown where both parents are available for analysis

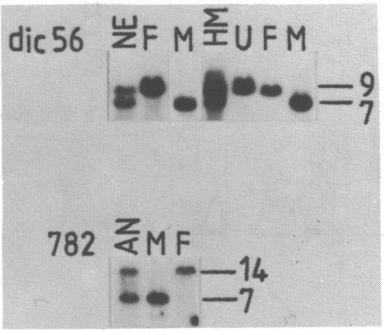

Figure 3 Origin of X Chromosomes in XX Males

Suitable XX male families were analysed using two polymorphic X probes; dic 56 and 782. DNA from mother - M, father - F and XX males NE, HM and AN were digested with Bcl I for dic 56 and EcoRl for 782 and subjected to Southern blot analysis. The bars at the right hand side indicate the size in kilobases of polymorphic DNA fragments detected by these probes.

with X-linked polymorphic probes. Figure 3 illustrates the three cases (NE, HM, AN) with parents available for study where Southern analysis has been informative using probes 782 (10) and dic56 (L. Kunkel, unpublished results). Respectively, these probes detect 7/14 Kb and 7/9 Kb X-linked polymorphisms and it can be seen that in all three cases the XX male is heterozygous for the polymorphic fragments, whereas, the parents are homozygous for different alleles. The XX male must therefore have received one paternal and one maternal X chromosome.

Chromosomal Location of Y Sequences in XX Males

The existence of Y sequences in XX males does not constitute evidence for their location on the paternal X chromosome.

In two cases (HM and RH), we have examined the chromosomal location of Y sequences by means of flow cytometric purification of human chromosomes and Southern analysis on the DNA isolated from these fractions. Figure 4 illustrates the flow karyotypes and the fractions collected to prepare DNA. In both cases, the Y-specific band of GMGXY2 (arrowed) can only be found in the X-7 fraction and not the other chromosomal fractions. In addition, RH has been analysed with probe GMGY10 where it can be seen that only sequences in the X-7 fraction react with the probe (arrowed). This strongly suggests that the Y sequences in these XX males are present on an X chromosome, although it does not formally exclude the unlikely possibility of a location on chromosome 7. Both probes fail to detect a fragment of the correct size in the 6,7,8,X fraction from a control female flow karyotype. This indicates that

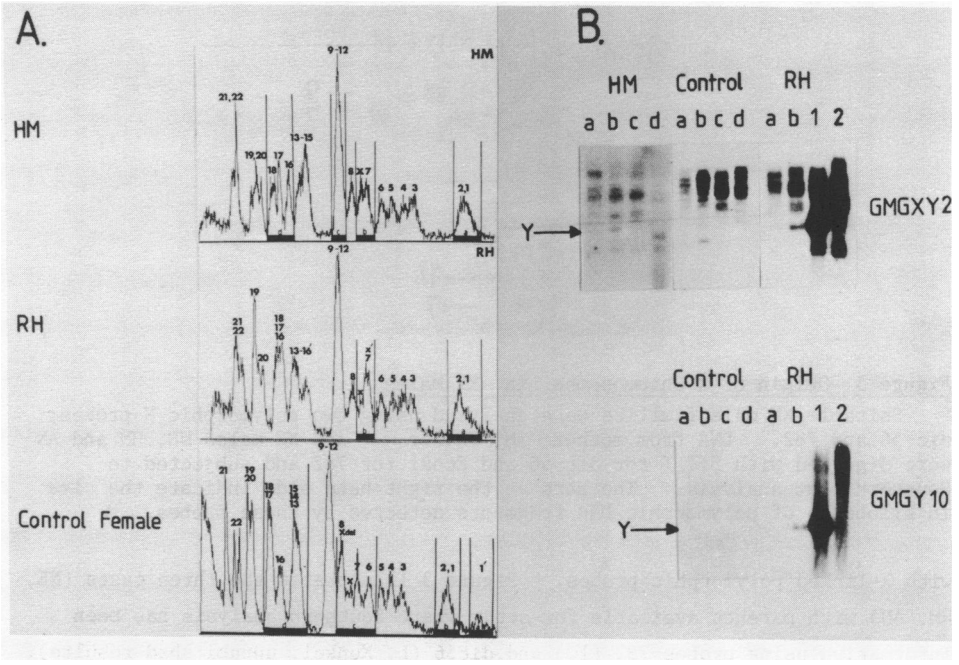

Figure 4 Chromosomal Location of Y Sequences in XX Males

DNA was prepared from the flow sorted chromosomes contained within the fractions marked by the horizontal bars in figure 4A for HM, RH and a control female (with an incidental Xq deletion). The DNA was then digested with EcoR1, blotted and probed with GMGXY2 for HM and RH and GMGY10 for RH. a,b,c and d in figure 4B correspond to the similarly marked fractions in the appropriate flow karyotype profile. 1 and 2 respectively represent DNA from the 3E7 Y-only hybrid and Horl X X-only hybrid cell lines. The arrows mark the position of Y-linked DNA fragments. The flow karyotypes are described elsewhere (12).

(a) the hybridisation to the X-7 track of RH and HM is male specific and (b) that chromosome 6,7 and 8 do not carry any autosomal sequences detected by GMGXY2 that have the same fragment size as the Y-linked sequence.

DISCUSSION

It is apparent from the data presented in this paper and the reports of other groups (2-6), that XX males contain regions of the Y chromosome which presumably carry the testis determinant(s). It is further evident from the Southern analysis of DNA from flow sorted chromosomes and the *in situ* hybridisation results reported by Magenis et al.(11), that these Y sequences may be linked to one of the X chromosomes present in XX males. This latter point is supported by flow cytometric analysis of X chromosomes in this ser-

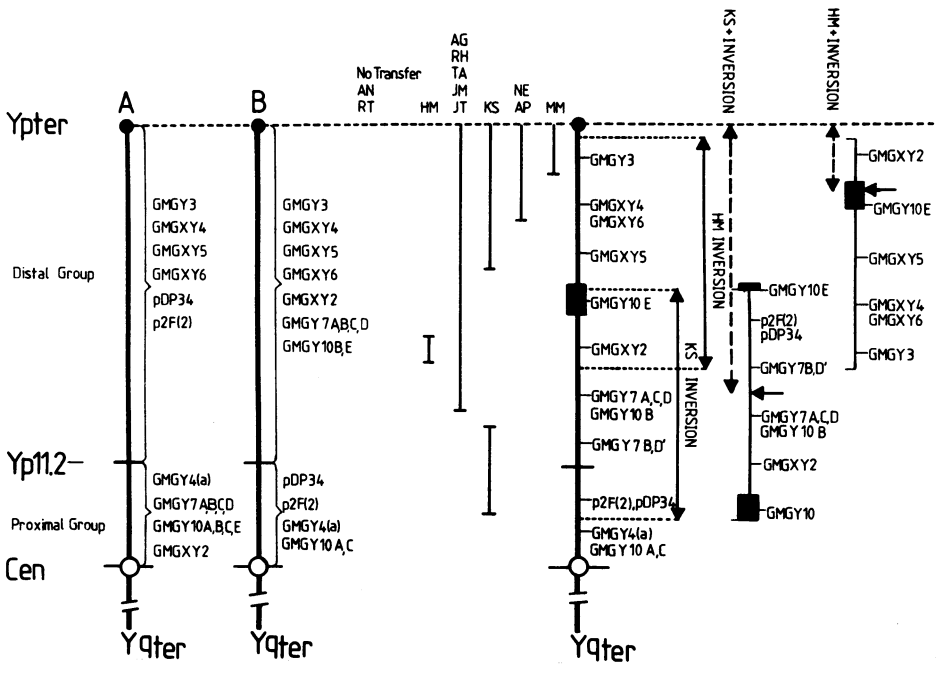

Figure 5 Model for Arrangement of Y Probes on Yp

The vertical bars indicate the sequences detectable in different XX males and form the basis of the order ascribed to Yp probes shown in the figure. A and B represent the two dispositions of Yp probes proposed to exist in the population from consideration of the order dictated by the XX males and the breakpoint on Yp contained in the key individual in the Y panel used in the accompanying paper. A shows the disposition about the break point at Yp11.2 obtained from the Y panel. B shows the disposition about the same breakpoint if there is an inversion similar to that labelled KS. GMGY4(a) and fragments GMGY10 A and C are placed in a proximal centromeric location since they are not detectable in any of the XX males (see text for discussion).

ies of XX males where it has been shown in several cases that there is an increase in the size of one X chromosome (12). These findings are most simply explained by the X-Y interchange hypothesis which invokes accidental recombination between the X and Y chromosomes in the father of the XX male (1).

The probes used in this study have been localised using the Y deletion panel described in the accompanying paper. On this basis, the pattern of transfer in certain XX males would appear to be anomalous in that sequences defined by the panel as being proximal (GMGY7, GMGY10 and GMGXY2) are sometimes transferred in the absence of sequences defined as being distal; notably pDP34 and p2F(2) for RH, JT, TA, JM, AG and all distal group sequences for HM. It is possible to explain these results by postulating the occur-

rence of three crossover events for each of RH, JM, TA, AG and JT. In the case of HM, two crossover events would be required hence transferring only sequences located in the proximal region of Yp. Solutions requiring multiple crossovers are unsatisfactory because they demand not only the concordance of two or more rare recombination events, but also for these to occur within a span of less than 20×10^6 base pairs.

If, however, one starts from the premise that there is one crossover event and that the breakpoints defining different XX males are clustered then it is possible to construct a deletion map of Yp from these XX males giving the order of Yp probes (see Vergnaud et al.(6). This is illustrated for our series of XX males in Figure 5. On this basis, GMGXY2, GMGY7 and GMGY10 are placed more distally and p2F(2) and pDP34 more proximally. More importantly, the number of discordant XX males now drops to two instead of five, namely, KS and HM. KS now transfers p2F(2) and pDP34 in the absence of the now more distally located GMGXY2, GMGY7 and GMGY10 fragments. The relative order of GMGXY2, GMGY7 and GMGY10 fragments commonly transferred in RH, JM, TA, AG and JT has been suggested on the basis of the discussion below. In addition, there is no indication of the distance between probes; the diagram merely gives their relative order.

How, then, can one reconcile the relative locations of Yp probes given by the Y deletion panel and the XX males? The contradiction is readily resolved by proposing that the order of sequences on the Y chromosome is not invariant, since the pressure of recombination in maintaining colinearity of homologous chromosomes is not operative for the non-pairing region of the Y chromosome. In so far as rearrangements do not perturb important functions, then variations in the order of sequences on the Y chromosome will be tolerated. It is suggested that inversion polymorphisms may occur in the Y chromosomes of normal males, these rearrangements being revealed when either deleted Y chromosomes or XX males are examined with a series of probes. Inversions in gene sequence order have been observed in Drosophila to be associated with a suppression of recombination between homologous chromosomes, hence leading to eventual speciation (13). It is conceivable that inversions in the human Y chromosome may serve a similar function in preventing recombination between the X and Y chromosomes.

Figure 5 details the inversions which we propose may be present in the fathers of HM and KS, and the arrows highlight the breakpoints for the single recombination event necessary to generate the pattern of Yp probes observed in these XX males. It can be seen that the locations obtained with

the Y deletion panel become compatible with those defined by the XX males if the key individual in the panel, possessing a partial deletion of Yp (Yp11.2-Ypter - see accompanying paper), also has an inversion similar to that proposed for the father of KS (see Figure 5 A and B).

It should be pointed out that the genesis of an XX male is an unusual event and may in itself be accompanied by complex rearrangements and interstitial deletions resulting in anomalous patterns of transfer. This cautionary note must be kept in mind when adducing the order of sequences on Yp from the breakpoints in XX males.

The weak band detected by GMGY10 in KS and HM raises an intriguing possibility in the light of the model presented in Figure 5. Irrespective of the inversion shown for KS, if this band represents a separate Y-linked fragment from the main GMGY10 band then it must lie distal to GMGXY2 and the GMGY10 cluster since it is transferred in the absence of both sequences. The failure of NE, AP and MM to show this weak band precludes it being located more distally than the break in NE and AP. However, it should be noted that the weakly hybridising fragment is present in HM together with GMGXY2, but in the absence of the main GMGY10 band. Given the inversion shown for HM (to minimise anomalies in the sequences transferred) the main band of GMGY10 should be evident in this XX male in view of the fact that it would now lie distal to the weak fragment. These problems are resolved if this weak band does not represent a separate Y-linked fragment, but is generated from the main band by a breakpoint within the GMGY10 cluster releasing one or a few repeat units. Figure 5 illustrates how this could be achieved to render the pattern of Yp sequences present in KS and HM consistent with the consensus order arrived at through the breakpoints in MM, NE, AP, RH, TA, JM, AG and JT. On the basis of this model, GMGXY2 is placed proximal to GMGY10 with an inversion breakpoint in KS and the X-Y recombination breakpoint in HM generating the split in the GMGY10 cluster.

Within the framework of this model one can also explain the distribution of GMGY7 fragments. GMGY7 A C D are absent from HM and KS and hence must lie proximal to both GMGXY2 and the proximal inversion breakpoint in HM. If the weak band at the same position as GMGY7D in KS is considered to represent a separate Y-linked fragment D' (but normally hidden by GMGY7D), then this avoids the necessary interspersion of the GMGY7 and GMGY10 clusters to account for the simultaneous generation of weak GMGY7 and GMGY10 bands by breakpoints within these clusters. A location of both GMGY7D' and GMGY7B proximal to GMGY7A,C,D would then explain (upon inversion in the father of KS)

the presence of only these fragments in KS. Figure 5 illustrates the necessary position of the recombination breakpoint in KS.

A distal location for the testis determinants(s) is consistent with all our XX males and the findings of others (6). If those XX males which do not show transfer of any of these Yp probes have arisen by X-Y exchange, then this would place the testis determinant(s) distal to GMGY3. However, if only those XX males showing evidence of transfer are considered, the position of the testis determinant(s) with respect to the distal probes could be Ypter-TDF-GMGY3-(GMGXY4,GMGXY6)-GMGXY5-or Ypter-GMGXY3-TDF-(GMGXY4,GMGXY6)-GMGXY5- as dictated by the breakpoints in MM, NE, AP and KS. It is, of course, impossible to examine directly the order of probes on Yp in normal males by conventional recombination analysis, except in the pseudoautosomal region (14). Therefore further examination of Y probe order and sequence rearrangements will only be possible through (a) the intermediary of more XX males and partial deletions involving the short arm and (b) detailed restriction mapping using large overlapping DNA fragments generated by restriction enzymes which cut infrequently. This latter physical approach is feasible for Yp in view of its relatively short nucleotide length.

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