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Accidental X-Y Recombination and the Aetiology of XX Males and True Hermaphrodites [and Discussion]

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Accidental X–Y recombination and the aetiology of XX males and true hermaphrodites

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Accidental recombination between the differential segments of the X and Y chromosomes in man occasionally allows transfer of Y-linked sequences to the X chromosome leading to testis differentiation in so-called XX males. Loss of the same sequences by X–Y interchange allows female differentiation in a small proportion of individuals with XY gonadal dysgenesis. A candidate gene responsible for primary sex determination has recently been cloned from within this part of the Y chromosome by Page and his colleagues. The observation that a homologue of this gene is present on the short arm of the X chromosome and is subject to X-inactivation, raises the intriguing possibility that sex determination in man is a quantitative trait. Males have two active doses of the gonad determining gene, and females have one dose. This hypothesis has been tested in a series of XX males, XY females and XX true hermaphrodites by using a genomic probe, CMPXY1, obtained by probing a Y-specific DNA library with synthetic oligonucleotides based on the predicted amino-acid sequence of the sex-determining protein. The findings in most cases are consistent with the hypothesis of homologous gonad-determining genes, *GDX* and *GDY*, carried by the X and Y chromosomes respectively. It is postulated that in sporadic or familial XX true hermaphrodites one of the *GDX* loci escapes X-inactivation because of mutation or chromosomal rearrangement, resulting in mosaicism for testis and ovary-determining cell lines in somatic cells. Y-negative XX males belong to the same clinical spectrum as XX true hermaphrodites, and gonadal dysgenesis in some XY females may be due to sporadic or familial mutations of *GDX*.

Introduction

From the early days of chromosome cytology the X and Y chromosomes of several species, including man, were described as having paired homologous segments and non-homologous differential segments (Darlington 1976). The male testis determining factors (*TDF*) are contained in the differential part of the Y chromosome. The paired segments are kept homologous by synapsis, chiasma formation and crossing over, and these processes ensure the proper segregation during first meiosis of the X and Y chromosomes into different gametes and thus an approximately equal sex ratio. In man, failure of synapsis and chiasma formation within the pairing segment leads to non-disjunction (or non-conjunction) of the sex chromosomes and the production of sterile individuals with the 45, X and 47, XXY syndromes of Turner & Klinefelter respectively. The maintenance of an approximately normal sex ratio and normal sex determination and differentiation also depends on the absence of crossing over within the differential segments which might allow transfer of *TDF* from Y to X. Such accidental crossing over outside the X–Y pairing segment was postulated in an earlier paper (Ferguson-Smith, 1966) as the most likely cause of Klinefelter's syndrome in sterile males with an apparently normal female karyotype and of gonadal dysgenesis in females with an

apparently normal male karyotype. The rare occurrence of this form of X–Y interchange could be regarded as the price paid by the species for the comparatively low frequency of 47, XXY and 45, X individuals.

Evidence from the XG blood group

The evidence that X–Y interchange might be involved in the aetiology of XX males came first from studies of the sex-linked dominant XG blood group which was later found to map to the tip of the short arm of the X (Ferguson-Smith *et al.* 1982). Several of the earlier cases were observed who had failed to inherit the XG^a allele from their father. Data are now available on 102 XX males, 71.6% of whom are Xg(a+), a frequency significantly different from the 88.4% expected in a sample of normal females, and rather more than the 65.9% expected in normal males (R. Sanger & P. Tippett, unpublished observations). This is consistent with loss of the *XG* locus from the paternal X in many XX males. The reciprocal situation, namely the transmission of the father's XG^a allele to an XY female on an interchanged Y, has not been demonstrated and it is not known what proportion of XY females are Xg(a+).

Evidence from chromosome analysis and flow cytometry

With the improvement of cytogenetic techniques, several authors reported that one X chromosome was slightly larger than the other in some XX males (Madan 1976; Wachtel *et al.* 1976; Evans *et al.* 1979) and, later, that this was due to transfer of Yp11.2→pter from the Y (Magenis *et al.* 1982). It has been possible to measure the DNA content of X chromosomes by flow cytometry in a series of XX males and this reveals an increase of 3.8% (equivalent to approximately 6 million base pairs) in one of the two Xs in 12 of 20 cases (Ferguson-Smith 1988); in the other 8 cases the two X chromosomes are indistinguishable in total DNA content. As far as XY females are concerned, a much smaller proportion of cases have been found to have loss of Y chromosome material. In one case the Y chromosome is about 10% smaller than the father's Y chromosome indicating a loss of about 6 megabases from the Y short arm (Affara *et al.* 1987).

Evidence from molecular genetics studies

Confirmation of transfer of Y material from the Y to the X came with the demonstration that the Y-linked locus *MIC2*, associated with high expression of the 12E7 cell-surface antigen on red cells (see Goodfellow *et al.*, this symposium), was present in an Xg(a−) XX male who had failed to inherit the paternal Xg(a+) allele (de la Chapelle *et al.* 1984). The *MIC2* locus on the Y had apparently been interchanged with the *XG* locus. This report was followed by several studies in which the presence of Y-specific DNA sequences was demonstrated in the DNA of an increasing proportion of XX males by using DNA probes isolated from Y-chromosome-specific libraries in Southern blotting experiments (Guellaen *et al.* 1984; Page *et al.* 1985; Muller *et al.* 1986*a*; Affara *et al.* 1986*a*). However, these studies could not determine whether or not the Y sequences had been transferred to Xp in proximity to the pairing segment and this was not clarified until Y-specific probes were annealed direct to metaphase chromosomes by using the technique of *in situ* hybridization (Magenis *et al.* 1984; Andersson *et al.* 1986; Buckle *et al.* 1987; Kalaitzidakis *et al.* 1987). In our own series of XX males, all nine cases known to have Y-specific sequences so far tested have these sequences located at the proximal tip of one of the

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two X chromosomes (Kalaitzidakis *et al.* 1987). Similarly, loss of Y-specific sequences has been demonstrated in several XY females (Disteche *et al.* 1986; Muller *et al.* 1986*b*; Affara *et al.* 1987).

Several XX males are described in which no Y-specific sequences have been demonstrated and there are, similarly, many XY females without any apparent loss of Y sequences. It is therefore likely that mechanisms other than X–Y interchange are responsible for the sex reversal in these exceptions. There seem to be some interesting clinical differences between those XX males with and without evidence of X–Y interchange (see below).

Cloning the sex determining factors on Yp

The primary signal for male (testis) differentiation has long been regarded as a Y-linked dominant trait determined by a gene (*TDF*) or genes located on the short arm of the Y chromosome. Thus testicular differentiation occurs only in individuals who have a specific segment of the short arm of the Y chromosome containing *TDF* in at least some of their somatic cells. Y-negative XX males and XX true hermaphrodites remain the only exceptions to this rule. Attempts by several groups to isolate and clone *TDF* have therefore focused on the Y-specific sequences carried by XX males and deleted in some XY females. It is almost certain that this has now been accomplished. Page *et al.* (1987*a*) have reported details of a likely candidate for *TDF*, some sequences of which were cloned from an X–Y interchange male and found to be missing in a female patient who had a reciprocal translocation between the short arm of the Y and chromosome 22 associated with the loss of a 160 kilobase segment from Yp. The gene codes for a DNA binding protein, characterized by a series of zinc fingers, which presumably acts in the regulation of transcription of a key substance in the pathway to testis differentiation. The DNA sequence is highly conserved in mammals and an important finding is that there is a homologous sequence carried on the short arm of the X. The X-linked sequence is located in Xp21–22.3 and is therefore likely to be subject to X-inactivation. If both X and Y genes code for the same sex-determining protein, normal male somatic cells have a double dose and normal female somatic cells a single active dose of the *TDF* product. Similarly, females with abnormal sex chromosome constitutions including X0, XXX or XXXX have one active dose, males with XXY, XXXY and XXXXY have two active doses and males with XYY, XXYY have three active doses (suggesting that more than one dose is male determining). XX males have two doses because *TDY* is transferred to part of the X that normally escapes X-inactivation. The implication is that man now falls into line with many other invertebrate species including *Drosophila*, where the primary sex-determining system depends on gene dosage (see McLaren, this symposium). In fact, all mammalian species so far tested have X and Y homologues of *TDF*. As the sex difference depends on X-inactivation in female somatic cells, the suggestion of Chandra (1985) that X-inactivation has evolved in mammals primarily as a sex-determining device seems highly plausible. In chickens, where there is no mechanism for dosage compensation analogous to X inactivation, it seems likely that the Z chromosome but not the W chromosome carries *TDF* so that the ZZ male has two doses and the ZW female only one.

If it is confirmed that the testis-determining signal is not a Y-linked dominant trait, but as suggested above, a quantitative trait determined by X and Y homologous gonad-determining genes, it would seem preferable to drop the *TDF* symbol and in future follow German (1988) and refer to the X and Y loci as *GDX* and *GDY* respectively.

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The contribution of X–Y interchange males and females to mapping the X and Y chromosomes

The results quoted above strongly support the concept of accidental recombination of *GDY* as an important factor in the aetiology of XX males and XY females. A substantial number of patients have now been studied with a variety of X- and Y-specific probes, and data are accumulating on the extent of X–Y interchange and on the chromosomal sites of interchange in both X and Y. Most data come from the study of XX males, and considerable variation has been found in the amount of Y material transferred to the X from case to case. On the assumption that the interchange involves a single terminal transfer of Y short arm to the X, preliminary maps of the differential part of the Y chromosome have been constructed (Vergnaud *et al.* 1986; Affara *et al.* 1986*a*; Muller *et al.* 1986*b*). Affara *et al.* 1986*b* (updated in Ferguson-Smith *et al.* 1987) have used 58 DNA probes that recognize 76 Y-specific DNA fragments to construct deletion maps of the Y chromosome in two groups of patients, namely those with structural Y chromosome aberrations defined by classical cytogenetic analysis, and those with X–Y interchange. The results show that both maps are approximately consistent and that all Y sequences involved in 18 patients with X–Y interchange map to the short arm of the Y. There are a few exceptions to the consensus order that can be explained by inversion polymorphisms. These polymorphisms may be comparatively common in the differential part of the Y chromosome, as there is, of course, no mechanism of synapsis and chiasma formation to maintain any particular order of loci on the non-pairing part of the Y. Chromosome mutations that change the order of loci are likely to be tolerated provided they do not disturb reproductive fitness.

Different approaches have led to the isolation of DNA sequences from the homologous pairing (or pseudoautosomal) segment of the sex chromosomes (Cooke *et al.* 1985; Simmler *et al.* 1985; Rouyer *et al.* 1986; Goodfellow *et al.* 1986). Those sequences that identify X- and Y-specific restriction fragment length polymorphisms have been used to study recombination between the X and Y (partial sex linkage). The results are consistent with a single obligate cross-over in male meiosis (that is probably essential for segregation of the X and Y) and show that recombination is much higher in male meiosis than in female meiosis. Most work has been accomplished using probes at four pseudoautosomal loci: *DXYS14* (p29C1), *DXYS15* (p113), *DXYS17* (p601, 602) and *MIC2* (pSG1, p19B). Linkage analysis reveals a gradient of partial sex linkage, the most proximal locus (*MIC2*) recombining with sex infrequently (2.5%) whereas the most distal locus DXYS14 shows virtually no sex linkage (Rouyer *et al.* 1986). No examples of double recombination have been found within the pairing segment. A comparison of recombination between males and females demonstrates that, between the most proximal and most distal loci, there is 45% recombination in male meiosis compared with 2.0% recombination in female meiosis. The high frequency of recombination within the pairing segments is also associated with hypervariability of several of the sequences (e.g. *DXYS14* and *DXYS15*) used as probes. This is the result of variation in copy number of small repeats or minisatellites, and makes the probes particularly informative in family studies.

These pseudoautosomal probes can therefore be used to study X–Y interchange, first to determine the X or Y origin of the pairing segment contributed by the father, and secondly to help map the breakpoints of the interchange. Thus Petit *et al.* (1987) have studied six Y-positive XX males and have shown that in each case the complete pseudoautosomal region of

the father's Y chromosome had been transferred to the paternal X. In addition, the authors demonstrated loss of Xp sequences tightly linked to the pairing segment in the paternal X. The three Y-negative XX males studied showed inheritance of proximal pseudoautosomal loci from the paternal X, indicating that terminal X–Y interchange had not occurred.

In another XX male (Page *et al.* 1987*b*) not only the entire pseudoautosomal region from the paternal Y chromosome but the proximal part of the pseudoautosomal region from the paternal X was present in the interchange showing that the X breakpoint involved in the interchange had occurred within the pseudoautosomal region. The molecular basis of X–Y interchanges can thus be studied with existing mapping data, and now reveals considerable heterogeneity. The interesting paper of Rouyer *et al.* (1987) shows that in one XX male the abnormal homologous recombination occurred between two Alu sequences, one in the pseudoautosomal region of the paternal X, the other located in the differential segment of Yp. Cloning of the breakpoints in other examples of XX males should indicate if this type of homology is commonly found to be associated with X–Y interchange.

Our own studies in a series of 23 XX males provide information about the variation in the location of X and Y breakpoints in X–Y interchange.

(*a*) *Xg studies*

Five patients are informative for *XG*. Four are Xg(a−) negative and have failed to inherit the father's XG^a allele; in each case they show high expression of 12E7 indicating that the *MIC2* locus has been transferred to the X from the paternal Y. The X breakpoint is thus proximal to the *XG* locus on the paternal X in these cases. One Y-negative XX male, is Xg(a+) positive and has inherited his father's XG^a allele, indicating that if X–Y interchange has occurred the breakpoint is distal to the *XG* locus.

(*b*) *STS studies*

Eleven patients have had steroid sulphatase activity (STS) assayed in hair roots. The locus for this enzyme maps to the end of Xp within measurable distance of *XG* and escapes inactivation. Seven XX males have levels of STS consistent with the double dose expected in normal female controls, and three have considerably elevated levels approximately twice the levels found in normal female controls. No explanation for the increased activity in these cases has been found, other than a suggestion that there may have been some disturbance at a regulatory locus. None of the patients have an STS activity in the male range, as would be expected if the STS locus had been included in the interchange. One such case with reduced STS activity has been noted previously (Wieaker *et al.* 1983).

(*c*) *Variation in transfer of Y-specific sequences*

We have tested our series of 23 XX males with 27 DNA probes that recognize 30 Y short arm sequences. All except one of the 17 Y+XX males have retained GMGY3 (table 1), which is therefore regarded as being that most closely linked of our probes to the *GDY* locus (Affara *et al.* 1987). The studies of Page *et al.* (1987*a*) show that GMGY3 is distal to *GDY* but proximal to *MIC2Y*. GMGY3 is the only sequence found to be transferred in two cases. Eight cases appear to have transferred 28 Y sequences (approximately 70% of the Y short arm sequences) and in these cases the breakpoint is in exactly the same interval, suggesting a possible recombination 'hot-spot'. In four cases, six to eight Y sequences have been transferred,

Table 1. Pattern of Y sequences in XX males

(The table summarizes the results of Southern blot analysis on genomic DNA on an extensive group of XX males by using a series of DNA probes that detect Y linked fragments. The order of sequences given on the table reflects the order derived with this series of patients and a series of patients with deletions of Yp (Affara *et al.* 1986*b*). CMPXY1 recognizes X and Y fragments of the sex-determining locus.)

probe....		KS	RH	JM	TA	AG	JT	GA	WB	DR	NI	NE	OP	AP	RS	MM	TK	GC	HM	MS	AN	RT	PP	DC
GMGY3		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
CMPXY1		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	.	−	−	−	−
47z		+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
GMGXY7		+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
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		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

suggesting a further recombination 'hot-spot'. It is noteworthy that all cases with a large X chromosome by flow cytometry have transferred at least six Y-specific sequences.

More recently, we have tested the same XX males with a DNA probe (CMPXY1), which we believe maps within the locus reported by Page *et al.* (1987*a*) to be a likely candidate for *GDY* and which recognizes both X- and Y-specific sequences. CMPXY1 was obtained by

probing a Y-specific DNA library with a series of synthetic oligonucleotides constructed from the predicted amino-acid sequence of the DNA binding protein described by Page *et al.* (1987*a*). Sixteen of the seventeen XX males who have previously been found to be Y-positive also have the Y sequence recognized by CMPXY1, which proves to be absent in the six previously Y-negative XX males (figure 1). The exception has normal-sized X chromosomes and ambiguity of the external genitalia, and otherwise seems similar to Y-negative XX males.

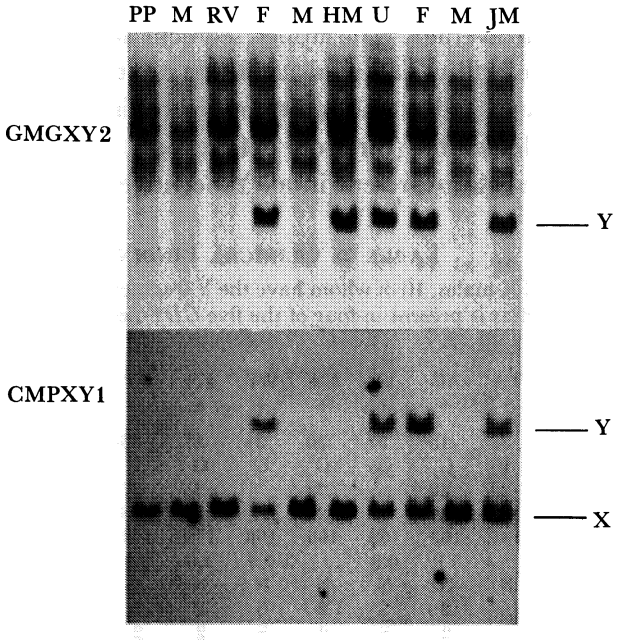

FIGURE 1. Eight micrograms of genomic DNA from patients and their relatives were digested with the restriction enzyme *Eco*RI and subjected to Southern blot analysis in the usual manner. DNA blots were successively probed with GMGXY2 and CMPXY1. From left to right: PP, Y-negative XX male; M, mother of PP; RV, XX true hermaphrodite; F, father of RV; M, mother of RV; HM, Y-positive XX male; U, uncle of HM; F, father of HM; M, mother of HM; JM, Y-positive XX male. Note that although HM has the Y-specific fragment of GMGXY2 he lacks the Y fragment of CMPXY1. JM has both Y-specific fragments, and PP and RV have neither.

(*d*) *DNA analysis using X-specific probes*

The DNA from our series of Y-positive XX males was probed with several probes that map to the distal end of the X chromosome, either within or outside the pairing segment, to look for evidence of deletion or duplication of X-linked sequences. The probes used include 782(*DSX85*), dic56 (*DXS143*), GMGX9 (*DXS237*); GMGXY19, GMGXXY3 and p19B (*MIC2*). There is no evidence for loss of any of these sequences in the 17 patients tested. Dosage studies in the 17 patients tested with p19B, however, strongly suggest that there are three alleles at the *MIC2* locus in five cases, indicating that the breakpoint in the paternal X is distal to *MIC2* as in the case described by Page *et al.* (1987*b*). It is therefore clear that several XX males have retained both the paternal *MIC2* locus and X-linked loci proximal to it, including *XG*. With regard to the cluster of probes tightly linked to the *STS* locus (GMGX9, GMGXXY3 and GMGXY19), all patients tested show no evidence of either duplication or deletion, indicating that *STS* is not commonly deleted in XX males, and that the increased *STS* activity noted above in some patients cannot be ascribed to triple dose of the *STS* allele.

Genotype-phenotype correlations in XX males and XY females

The majority of XX males have the same type of prepubertal testicular atrophy and endocrinological features described in XXY Klinefelter's syndrome (Ferguson-Smith 1965*a*). Gynaecomastia is a more frequent feature, present in 11 of 14 post-pubertal patients in our series (table 2). All patients have azoospermia and most show clinical and biochemical evidence of androgen insufficiency with reduced body hair and lack of temporal recession. Unlike XXY Klinefelter's syndrome, patients have average intelligence and are smaller in stature instead of taller than average. The upper segment:lower segment ratio tends to be normal instead of reduced. Infertility and gynaecomastia are the usual indications for referral. Genital anomalies such as hypospadias and undescended testis occur only among those of our patients who have either no detectable (three out of four cases) or few Y-specific sequences (one out of two cases). Gynaecomastia is present in all Y-negative cases.

Table 2. Clinical Findings

(Clinical features in a series of 15 XX males, 10 of whom have the Y fragment of CMPXY1 (*GDY*) in their genomic DNA samples. Note that hypospadias is present in four of the five *GDY*-negative XX males, but not in any of the *GDY*-positive XX males.)

	RH	JM	TA	AG	JT	GA	WB	KS	AP	NE	HM	GC	AN	RT	PP
CMPXY 1	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
age at examination	45	30	32	15	27	30	34	27	23	10	21	17	20	39	20
reason for referral	I	I	I	G	G	G	G	G	I	SSE	GC	HG	H	I	HG
maternal age at birth	30	39	29	26	·	22	·	35	27	35	32	·	25	22	·
paternal age at birth	29	39	37	25	·	23	·	36	28	41	29	·	32	24	·
height	163	165	161	163	166	166	166	170	172	127	172	·	160	172	·
US:LS ratio	0.9	·	·	1.0	0.9	·	0.9	1.0	0.9	1.3	1.0	·	1.1	0.9	
external genitalia	N	N	N	N	N	N	N	N	N	N	Per.H	Pen.H	GH	SP	Per.H
testes right/cm	2	2	·	2	S	2	S	2	2	N	2.5	3.5	S	2.5	2
testes left/cm	2	2	·	2	S	2	S	2	2	N	UND	2	S	2.5	2
gynaecomastia	0	0	+	++	+(R)	+	+	+	0	−	++	++	+	++	++
pubic & axillary hair	↓	+	·	↓	↓	+	+	↓	+	−	+	+	↓	+	+
chest hair	+	0	·	0	·		0	+	0	−	0	+	0	+	+
temporal recession	+	0	·	0	·	0	0	+	0	−	0	0	0	+	+
shaving frequency (per week)	1	3	1	2	1	1	0	3	7	−	2	·	0	7	7
plasma testosterone	·	↓	↓	·	↓	·	↓	N	↓	·	·	·	↓	·	↓
intelligence	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Key to abbreviations used: I, infertility; G, gynaecomastia; SSE, short stat., epilepsy; GC, gynaecomastia, cryptorchidism; HG, hypospadias, gynaecomastia; H, hypogonadism; Per.H, perineal hypospadias; Pen.H, penile hypospadias; GH, glandular hypospadias; SP, small penis; S, small; N, normal; +, present; 0, absent; −, not applicable; ·, no information; ↓, less than normal.

One can speculate that the phenotypic differences between XXY and Y-positive XX males are directly related to the amount of Y material present. Karyotype–phenotype correlations in patients with the Klinefelter and Turner syndromes (Ferguson-Smith 1965*b*) show that determinants for skeletal growth are carried by the short arms of both the X and Y chromosomes. Patients with 47, XXY and XYY are taller and 45, X and 46, XXp- are smaller than average. However, patients with a 47, XXX karyotype are of average stature (Johnston *et al.* 1961) and it is clear that loss of Xp or the addition of a Y have a more profound effect on stature than gain of an X. On the other hand, the gain of either an X or Y seems to have a more harmful effect on intellectual function than loss of an X. It is possible that the normal intelligence of a Y+XX male is in fact due to the presence of a normal complement of

X/Y homologous segments and his smaller than average male stature is due to absence of skeletal growth determinants from the proximal part of the short arm of the Y.

The phenotype of the few XY females who have been shown to have both X and Y pairing segments but who lack Yp sequences, have in addition to gonadal dysgenesis (including bilateral gonadoblastoma in some cases) some features of Turner's syndrome, such as webbed neck, peripheral lymphoedema, short IVth metacarpals, hypoplasia of the nails, etc. However, they are unlike patients with Turner's syndrome in that they are not short in stature. In other words, those determinants on the Y which normally prevent Turner's stigmata in normal males have been lost along with *GDY*, although the determinants for stature are retained, presumably because they are located proximal to the breakpoint of the X–Y interchange in the Y. It is therefore concluded that the main Y-linked determinants for stature are excluded from these abnormal X–Y interchanges.

The aetiology of true hermaphroditism

Testis differentiation in the absence of Y chromosome material occurs also in XX true hermaphrodites. These are individuals who have ambiguity of the external and internal genitalia associated with either bilateral ovotestes, unilateral testis and ovary, ovary and ovotestis, or testis and ovotestis. There is variable regression of the Müllerian ducts depending on the extent of testicular differentiation. An identical phenotype in man can result from XX/XY chimerism or in the mouse from experimental chimerism induced by injecting embryonic XY cells into an XX blastocyst. In these chimeras the somatic cells are of two types: those with an XY complement are testis determining and those with an XX complement are ovary determining. X–Y interchange with random inactivation of the interchanged X could well account for the gonadal findings (Ferguson-Smith 1966) but so far Y-specific sequences have not been found in XX true hermaphrodites. Wiberg & Scherer (1987) have investigated 7 cases with Y-specific probes and we have tested 12 others with 14 Yp probes including CMPXY1 (*GDY*). Other hypotheses must be sought for the presence of both ovarian and testicular tissues in the same individual. One that would satisfy the current gene-dosage theory of sex determination involves the possibility of escape from X-inactivation at the *GDX* locus in one of the two X chromosomes. This release of a *GDX* allele from the normal constraints of random X-inactivation might be achieved by mutation involving a regulatory sequence close to *GDX* or by an inversion or transposition that moves *GDX* into the distal end of Xp (which normally escapes inactivation). Random X-inactivation during early embryogenesis would lead to somatic-cell mosaicism in which some cells would have two doses of *GDX* whereas others have only one dose. The former would be testis determining and the latter ovary determining. The hypothesis is testable by a combination of molecular genetic techniques including pulsed-field gradient electrophoresis, restriction mapping and DNA sequencing, which might reveal changes in the locus of one *GDX* allele or mutations in adjacent sequences.

It seems likely that Y-negative XX males have a similar aetiology to XX true hermaphrodites if only because spontaneous and experimental XX/XY chimeras sometimes have bilateral testes and a male phenotype. It may also be significant that Y-negative XX males may have abnormalities of the external genitalia, such as hypospadias (page 140). In our exceptional Y + XX male (HM) with hypospadias and unilateral cryptorchidism but without *GDY* (table 1),

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the transfer of other Y sequences from Yp to the X may have been the event that led to the escape from X-inactivation of the *GDX* locus on one X chromosome. The *GDX* activation hypothesis could also account for the occurrence of familial cases in which siblings are affected with XX true hermaphroditism, or XX Klinefelter's syndrome, or both in the same sibship. In these cases the activated *GDX* allele might be transmitted by unaffected fertile XX mothers or fertile XY fathers as an X-linked dominant trait with incomplete penetrance. All known pedigrees with multiple affected relatives (see de la Chapelle 1987) can be interpreted in this way without invoking autosomal inheritance, and this would seem to be a powerful argument in favour of the gene-dosage hypothesis of sex determination.

The gene-dosage hypothesis may also help to explain the origin of some cases of gonadal dysgenesis in XY females. If *GDY* and *GDX* are truly homologous and have an identical gene product, loss or mutation of *GDX* should have the same consequence as loss or mutation of *GDY*. We have so far tested nine patients with XY gonadal dysgenesis by using CMPXY1 and only one (case AM in Affara *et al.* (1987)) shows loss of *GDY*. None shows loss of *GDX* but a 'null' mutation in either *GDX* or *GDY* has not yet been excluded. Once again, familial cases showing X-linked recessive inheritance could conceivably be due to a familial mutation of *GDX*.

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Discussion

P. N. Goodfellow (*Imperial Cancer Research Fund, London, U.K.*). Could Professor Ferguson-Smith's Yp^+, CMPXY1 (−) XX males represent intragenic breaks, so explaining maleness and the specific phenotype he identifies?

M. A. Ferguson-Smith, F.R.S. Yes. If that part of *GDY* without the CMPXY1 homology was transferred to the X and was functional, it would be possible to generate a Y-positive, CMPXY1-negative XX male. However, because of the phenotypic similarity of our patient to XX true hermaphroditism, other possibilities seem more likely.

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B. M. Cattanach (*MRC Radiobiology Unit, Didcot, U.K.*). The situation regarding STS expression in the mouse may provide a model for Professor Ferguson-Smith's hypothesis. Thus recent data from Harwell clearly establish that the locus in the pseudo-autosomal segment is subject to the X-inactivation process in females and, because there are functional X and Y loci in this species that are equally expressed, there is a 1:2 ratio of STS expression in females and males.

M. A. Ferguson-Smith, F.R.S. This new observation on STS expression in the mouse is of great interest. A 1:2 ratio of sex-determining protein expression in females and males is exactly what I propose. The STS locus is also unusual in man, although not comparable to the mouse. The 1.7:1 ratio of STS expression is due to its partial escape from X-inactivation because it maps sufficiently close to the pairing segment, and also because the STS allele is non-functional on the Y.

D. C. Deeming (*Department of Cell and Structural Biology, University of Manchester, U.K.*). Is Professor Ferguson-Smith suggesting that sex determination in *Drosophila*, nematodes, reptiles and humans is essentially the same?

M. A. Ferguson-Smith, F.R.S. Yes, in that in all these species primary sex determination seems to depend on quantitative effects, rather than the effect of a Y-linked dominant gene as was previously thought for mouse and man.

However, in *Drosophila* and *Caenorhabditis*, females and hermaphrodites have two doses of the sex-determining factors whereas males have only one. In mammals and birds, females seem to have only one dose and males have two.

D. C. Deeming. Intersexes in turtles could also be explained by a dosage–mosaic model.

M. Hulten. (*Regional Cytogenetics Laboratory, East Birmingham Hospital, U.K.*). This is a question not only for Professor Ferguson-Smith but also for others who have done molecular investigations on parents and sibs of XX males. I wondered if there is now enough data to tell whether or not the XpYp meiotic recombination in fathers of XX males is generally occurring in aberrant positions. One of the reasons I am asking this question is that many years ago, I had the opportunity to investigate meiosis in a father of an XX male. In air-dried preparations about 30% of cells at diakinesis/MI showed a clear XY chiasma. A chiasma is not normally seen in this position as the X and Y appear associated end to end. I have taken the occurrence of a visible chiasma in this father of an XX male to indicate a proximalization of the XY recombination. I was wondering if he is exceptional or if other fathers might also show a general tendency to proximalization of their XpYp meiotic recombination?

M. A. Ferguson-Smith, F.R.S. The information is mounting (see table 1) that the Y breakpoint in X–Y interchange may be non-random, but it appears at present that the X breakpoint may be at any position either within the pairing segment or even proximal to the *STS* locus, if the results of STS activity in XX males can be interpreted as evidence of gene dosage. It is tempting to speculate that the observed variation in the order of gene loci in the differential segment of the Y may sometimes lead to proximalization of recombination sites, thus providing an alternative explanation of the high frequency of cells in diakinesis showing an XY chiasma mentioned by Dr Hulten.

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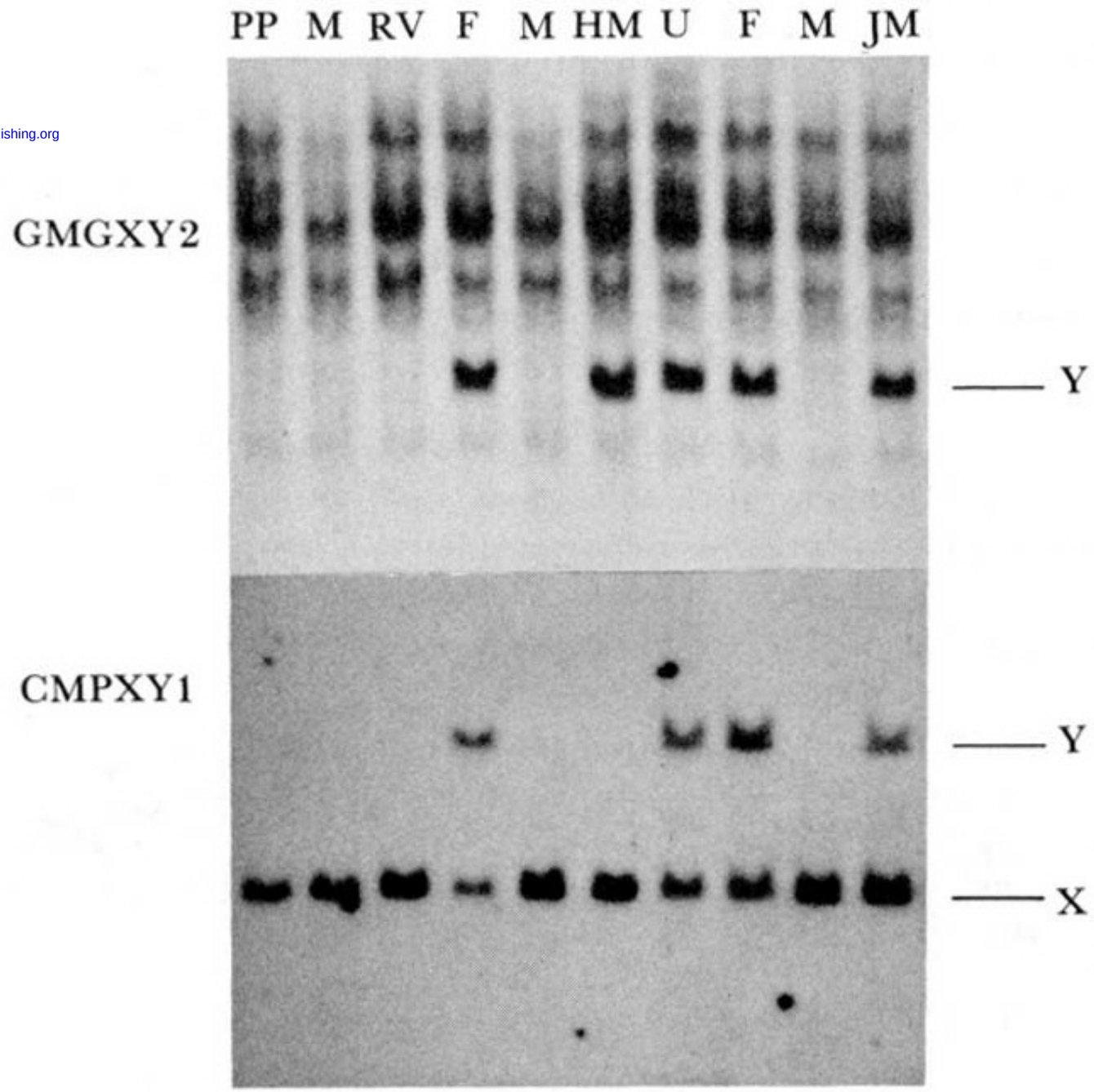

FIGURE 1. Eight micrograms of genomic DNA from patients and their relatives were digested with the restriction enzyme *Eco*RI and subjected to Southern blot analysis in the usual manner. DNA blots were successively probed with GMGXY2 and CMPXY1. From left to right: PP, Y-negative XX male; M, mother of PP; RV, XX true hermaphrodite; F, father of RV; M, mother of RV; HM, Y-positive XX male; U, uncle of HM; F, father of HM; M, mother of HM; JM, Y-positive XX male. Note that although HM has the Y-specific fragment of GMGXY2 he lacks the Y fragment of CMPXY1. JM has both Y-specific fragments, and PP and RV have neither.