the various hypotheses that have arisen around specimens described as *A. afarensis*. Further material may show the same large range of variation as these later specimens or it may provide more explicit evidence of an early radiation in the Hominidae between 5 Myr and 3.7 Myr.

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Chromosome Y-specific DNA in related human XX males

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Human 'XX males' are sterile males whose chromosomes seem to be those of a normal female. About 1 in 20,000 males has a 46, XX karyotype, and most cases are sporadic, that is, they are without familial clustering[1]. It has long been argued that maleness in XX males may result from the undetected presence of a small, testis-determining fragment of the Y chromosome[2], and there is strong evidence for this in sporadically occurring XX males[3–5]. Indeed, the genomes of three of four sporadic XX males tested were found to contain certain Y-specific DNA sequences[6]. A pedigree in which three XX males occur has been interpreted as being consistent with autosomal recessive inheritance of maleness[7,8], and it has been argued that the basis of XX maleness in this family is fundamentally different from that in the sporadic cases[9]. However, we report here that these related XX males, like the sporadic cases, contain portions of the Y chromosome. The portion of the Y chromosome present in one of the three XX males differs from that present in the other two.

In one of the few families reported to contain two or more XX males, the pattern of inheritance suggests a dominant male-determining locus segregating according to an autosomal mode[10]. Affected siblings[11] and affected identical twins[12] have also been described. The family reported here has been described previously[7,8]. Briefly, two XX males are second cousins, and they are distantly related to a third XX male via a common male ancestor (Fig. 1*a*). The mothers of two of these XX males are related in several ways. It has been argued that an autosomal[8] or 'pseudoautosomal'[9] recessive male-determining gene is segregating in this family.

In the present study, DNAs from three related XX males and their relatives were tested for the presence of certain Y-specific sequences using probes derived from the human Y chromosome. Some of these Y-specific DNA sequences were previously found to be present in, and others absent from, the genomes of sporadic XX males[6]. We have reported previously the absence in these three related XX males of the Y-specific 15-kilobase (kb) *Taq*I fragment at the X–Y homologous locus *DXYS1* (ref. 13). DNAs prepared from cultured fibroblasts or from peripheral blood leukocytes were digested with the restriction endonuclease *Taq*I and gel transfers[14] of the resulting fragments were hybridized with radiolabelled DNA probes detecting Y-specific *Taq*I fragments. Table 1 summarizes the results of these tests and Fig. 1*b, c* shows autoradiograms of some of the original data. All three XX males carry certain Y-specific sequences. The two XX males who are second cousins, X-1 and X-2, seem to carry similar if not identical portions of the Y chromosome; that is, their genomes contain the two Y-specific *Taq*I fragments detected by probe 47c, but they are negative for all other Y-specific sequences tested. (In this respect, they resemble the sporadic XX male '3' previously reported by Guellaen *et al.*[6].) X-3, the distantly related XX male, is positive not only for the two Y-specific *Taq*I fragments detected by 47c, but also for some of the Y-specific *Taq*I fragments detected by probes 50f2, 52d and 118. (The patterns observed were identical with those seen in sporadic XX male '4' reported by Guellaen *et al.*[6].) None of the three XX males is positive for all of the Y-specific sequences. We conclude that each carries only a part of the Y chromosome.

By this test, there is no evidence of abnormality at the DNA sequence level in any of the relatives. The fathers of the XX males and a brother, X-4, are positive for all of the Y-specific sequences tested, while the mothers and a sister, X-5, are negative for all of the Y-specific sequences. Moreover, the intensities

of the Y-specific bands (relative to autosomal or X-specific bands) in the fathers are similar to those seen in normal unrelated males (data not shown). Thus, as the fathers all have a normal male karyotype, it is likely that the Y-specific DNA sequences they display are present in normal copy number and only on the Y chromosome. As in *Sxr* mice[15,16], 'sex reversal' in these humans may be the result of an X; Y translocation. However, unlike *Sxr*, duplication of the testis-determining portion of the Y chromosome in carrier males is apparently not involved.

We conclude that these three related XX males are males because, like the three sporadic XX males reported by Guellaen *et al.*[6], they carry a testis-determining fragment of the Y chromosome. Note that the patterns of Y-specific fragments shown by these related XX males are among the patterns shown by sporadic XX males. In the case of each of the three XX males, a new mutation must have occurred in the father, during or before the meiotic divisions. That mutation apparently involved breaking the Y chromosome and perhaps translocating one of the resulting pieces to the X chromosome. We detected no Y-specific sequences in any of the three mothers. (Guellaen *et al.*[6] previously reported the same finding in the mother of one sporadic XX male.) These results seem incompatible with a maternal contribution to XX maleness in this family; such a contribution had been suggested by weak positivity for the H-Y antigen in the mothers[8]. If there is an inherited defect predisposing to XX maleness in this family, it is operating in the fathers, in whom Y chromosomes are broken.

The two closely related XX males (X-1 and X-2) carry a similar if not identical portion of the Y chromosome, and they are related through males only. Thus, it is possible that the Y chromosome common to their fathers is susceptible to the sort of gross mutation described above, perhaps at a particular site on the Y chromosome. Indeed, a Y-linked mutation predisposes to reciprocal X; Y translocation in the mouse[17]. If a Y-linked mutation is operating in the fathers of the two closely related XX males, the occurrence of XX maleness in a distant relative (X-3), related through a lineage that includes females as well as males, may be merely coincidental. This is consistent with the finding that X-3 carries a different, albeit overlapping, fragment of the Y chromosome.

Using X-linked restriction fragment length polymorphisms, we have shown previously that most, if not all, XX males, including the three studied here, inherit one X chromosome from their father and one from their mother[13]. However, like many XX males, X-3 does not express his father's allele for the dominant, X-linked marker *Xg*. (*Xg* segregation is uninformative in the other two XX males.) As reported above, X-3 has inherited part of his father's Y chromosome. This is reminiscent

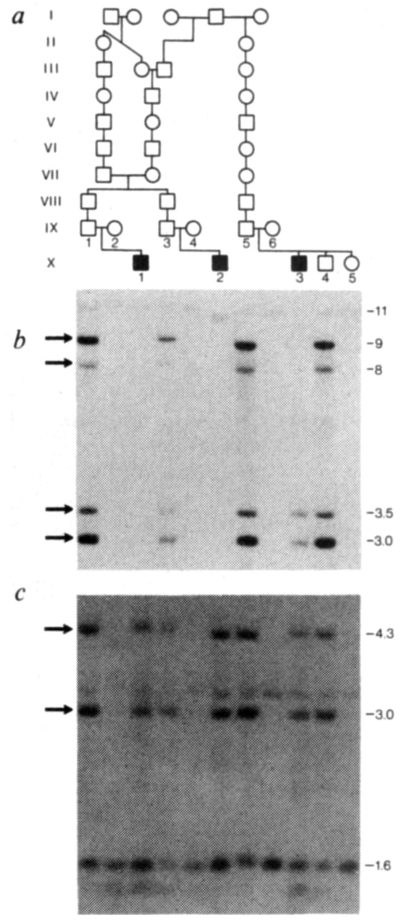

Fig. 1 *a*, Simplified paternal pedigree of XX males X-1, X-2 and X-3 (black squares) (from ref. 8). *b*, *c*, Hybridization of *Taq*I-digested DNAs from the XX males and their relatives with 50f2 (*b*) and 47c (*c*). Each lane corresponds to the individual immediately above that lane in the pedigree shown in *a*. DNA samples were digested with *Taq*I, electrophoresed on agarose gels, transferred[14] to Pall Biodyne (1.2-μm pores) nylon paper and hybridized with radiolabelled probes largely as described previously[13]. However, following transfer, the air-dried papers (DNA-coated side up) were exposed to germicidal (short-wave) ultraviolet light at an intensity of 400 μW cm^{-2} for 1 min; they were not baked. The arrows on the left indicate the Y-specific *Taq*I fragments detected. The sizes of restriction fragments (in kilobase pairs) are shown on the right.

Table 1 Y-specific DNA sequences in XX males and their relatives

	Probes							
Individual	47c	50f2	52d	118	27	49f	12f3	pDP34*
Normal male	2	4	3	8	1	3	1	1
Normal female	0	0	0	0	0	0	0	0
IX-1 (GM2672) father	2	4	3	ND	ND	ND	ND	1
IX-2 (GM2671) mother	0	0	0	ND	ND	ND	ND	0
X-1 (GM2626) XX male	2	0	0	0	0	0	0	0
IX-3 (GM2624) father	2	4	3	ND	ND	ND	ND	1
IX-4 (GM2625) mother	0	0	0	ND	ND	ND	ND	0
X-2 (GM2670) XX male	2	0	0	0	0	0	0	0
IX-5 (LGL118) father	2	4	3	ND	ND	ND	ND	1
IX-6 (LGL117) mother	0	0	0	ND	ND	ND	ND	0
X-3 (LGL115) XX male	2	2	2	4	0	0	0	0
X-4 (LGL116) brother	2	4	3	ND	ND	ND	ND	1
X-5 (LGL119) sister	0	0	0	ND	ND	ND	ND	0

Genomic DNAs from normal unrelated males and females and from the indicated members of the pedigree in Fig. 1*a* were digested with *Taq*I, blotted and hybridized with probes detecting Y-specific sequences. Methods were as described for Fig. 1*b*, *c*. The number of male-specific *Taq*I fragments detected by each probe are shown. ND, not done.

* The data for probe pDP34 (*DXYS1*) are from ref. 13.

of our finding of a sporadic XX male who expresses his father's allele for 12E7, a Y-linked marker, but fails to express his father's *Xg* allele[5]. Both cases can be explained by an interchange of a portion of the Y chromosome with a portion of the paternal X chromosome[2]. However, such an interchange cannot easily account for XX males X-3's apparent failure to express his father's allele for the dominant, X-linked marker *Xm* (ref. 1), which maps to the long arm of the X chromosome[18]; *Xg* maps to the short arm. It is possible that the Xm typing of this family was in error. Unfortunately, as Xm antiserum is no longer available, the typing cannot be repeated.

Taken together, our findings and those of Guellaen *et al.*[6] suggest that, though variable in size, the segment of the Y chromosome present is similar in at least some familial and sporadic XX males. It is likely that that segment of the Y chromosome carries the genetic information which is male-determining.

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X-linkage of steroid sulphatase in the mouse is evidence for a functional Y-linked allele

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In the human there is an X-linked gene affecting steroid sulphatase (STS) activity which, when deficient, is associated with X-linked congenital ichthyosis[1,2]. The gene (*STS*) is located on the distal tip of the short arm[3–6] and is only partially inactivated when it is on the inactive X-chromosome[7–10]. In the mouse, the genetics of *STS* are not clear; the results of one study using XX:X0 oocyte comparisons indicated X-linkage[11], but three other studies using STS variants have produced segregation data compatible with autosomal linkage of murine *STS*[12–14]. Here we present the results of STS assays of crosses of deficient C3H/An male mice[12] to normal X0 animals which demonstrate X-linkage of *STS* in the mouse and indirectly indicate the existence of a functional *STS* allele on the Y-chromosome which undergoes obligatory recombination during meiosis with the X-linked allele.

Table 1 Results of crosses of STS-deficient C3H/An with normal mice

Cross		No. of offspring			
		Females		Males	
Female	Male	STS^+	STS^-	STS^+	STS^-
*C3H/An (STS^-)	BALB/c (STS^+)	13	0	17	0
*BALB/c (STS^+)	C3H/An (STS^-)	6	0	8	0
*C3H/An (STS^-)	Feral mouse (STS^+)	3	0	5	0
*C3H/An (STS^-)	C57B (STS^+)	Not tested		7	0
*(C3H/An × C57B) F_1 (STS^+)	C3H/An (STS^-)	10	13	13	15
*(C3H/An × C57B) F_1 (STS^+)	C57B (STS^+)	16	0	17	0
C3H/An (STS^-)	(C3H/An × BALB/c) F_1 (STS^+)	2	6	2	4
C3H/An (STS^-)	(BALB/c × C3H/An) F_1 (STS^+)	1	6	2	1

STS assays were carried out on sonicated spleen extracts using ^{3}H-dehydroepiandrosterone sulphate as substrate, according to Shapiro *et al.*[32]. Pure-breeding STS^+ mice have STS activity of >250 pmol per h per mg protein and pure-breeding STS^- mice (C3H/An) have <7 pmol per h per mg protein. The STS activity of STS^+ hybrid mice varied from 70 to 160 pmol per h per mg protein.
* Unpublished data from Balazs *et al.*[12].

When C3H/An STS-deficient mice are crossed with STS normal animals in various combinations, the results seem compatible with simple autosomal inheritance of STS deficiency (Table 1). However, one litter from a cross of an STS-deficient female with a supposed pure-breeding STS normal male yielded one STS-deficient female. If the exceptional female was X0, it would suggest that STS deficiency follows an unusual form of sex-linked inheritance. Unfortunately, materials were not available for cytological analysis of the exceptional animal nor could the male parent be tested for possible heterozygosity.

We therefore instituted crosses of C3H/An STS-deficient males with STS normal X0 mice marked with the tabby (*Ta*) gene. The X-linked tabby marker permits separation of mice carrying *Ta*, in either the hemizygous or homozygous condition, from heterozygotes and wild-type mice by its characteristic coat markings. From these crosses, three classes of offspring are expected with regard to tabby and sex: *Ta*/+♀♀, +/0♀♀ and *Ta*/Y♂♂. Table 2 gives the results of these crosses with respect to tabby, sex and STS level. If the STS variant we are studying is autosomal, there should be no differences in STS levels between the three classes of offspring. On the other hand, if the STS variant lies on the X-chromosome, then the X0 progeny should be STS-deficient, in contrast to the normal or intermediate values for the *Ta*/+ and *Ta*/Y animals. In fact, the X0 animals show STS values not significantly different from their deficient male parent. The absence of STS-deficient animals among 15 *Ta*/Y and *Ta*/+ siblings argues against the possibility that the X0 parents are heterozygous for STS deficiency at an autosomal locus. These results demonstrate X-linkage of the C3H/An STS deficiency.

The studies reported here (Table 1) and elsewhere[12] using the C3H/An STS-deficient mouse strain in various crosses yield results compatible with simple autosomal inheritance of the deficient *STS* gene in the C3H/An strain. However, when deficient C3H/An males are crossed to STS normal X0 mice, the segregation pattern observed in the offspring demonstrates X-linkage of the *STS* deficient gene (Table 2). These apparently contradictory results are best explained by postulating a functional Y-linked *STS* gene that undergoes obligatory recombination with its X-linked allele. As both the C3H/An and BALB/c Y chromosomes exhibit recombination with the X-linked *STS*