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An extremely polymorphic locus on the short arm of the human X chromosome with homology to the long arm of the Y chromosome

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ABSTRACT

A genomic DNA clone named CRI-S232 reveals an array of highly polymorphic restriction fragments on the X chromosome as well as a set of non-polymorphic fragments on the Y chromosome. Every individual has multiple bands, highly variable in length, in every restriction enzyme digest tested. One set of bands is found in all males, and co-segregates with the Y chromosome in families. These sequences have been regionally localized by deletion mapping to the long arm of the Y chromosome. Segregation analysis in families shows that all of the remaining fragments co-segregate as a single locus on the X chromosome, each haplotype consisting of three or more polymorphic fragments. This locus (designated DXS278) is linked to several markers on Xp, the closest being dic56 (DXS143) at a distance of 2 cM. Although it is outside the pseudoautosomal region, the S232 X chromosome locus shows linkage to pseudoautosomal markers in female meiosis.

In determining the X chromosome S232 haplotypes of 138 offspring among 19 families, we observed three non-parental haplotypes. Two were recombinant haplotypes, consistent with a cross-over among the S232-hybridizing fragments in maternal meiosis. The third was a mutant haplotype arising on a paternal X chromosome. The locus identified by CRI-S232 may therefore be a recombination and mutation hotspot.

INTRODUCTION

Although quite disparate in size, genetic functions and DNA sequence content, the human X and Y chromosomes have been found to contain many loci with sequence homology (1-8). These homologies are ascribed either to the common evolutionary origin of the X and Y chromosomes, or to more recent convergent evolution brought about by transposition of DNA sequences between the X and Y. In the case of sequences on the distal short arms of these two chromosomes which pair (9,10), there is presumably a functional requirement for homology to promote meiotic pairing and segregation. In the telomeric pseudoautosomal regions, the X and Y chromosomes are completely homologous and undergo regular meiotic crossing-over and exchange (11-15).

We have discovered a human genomic clone that will be useful in elucidating both the evolutionary relationship between the X and Y chromosomes and the functional relationships involved in meiotic pairing. As an exceptionally polymorphic X chromosome locus in the X-Y pairing region, it will be an informative genetic marker for

normal and aberrant crossing-over between the sex chromosomes. Because the cloned DNA detects homologous sequences on the Y chromosome outside the pairing region, it also sheds light on the evolution of the human sex chromosomes.

MATERIALS AND METHODS

Isolation of clone CRI-S232.

CRI-S232 is a human genomic DNA cloned in bacteriophage Charon 21A, isolated in a random screening of phage from a human chromosome 7-specific library (LA07NS01) obtained from the Los Alamos National Laboratory (16). This particular clone was one of 42 "single-copy" clones tested, i.e., a phage with a DNA insert that gave no detectable signal in plaque hybridizations with ^{32}P-labeled human DNA or hamster DNA (17). (The flow-sorted chromosomes used to construct the LA07NS01 library had been prepared from a hamster-human hybrid line, MR3.31-6TG6.) Each randomly selected phage clone was screened in Southern transfer hybridizations for restriction fragment length polymorphisms (18,19). CRI-S232 was selected for further study because of the unusual polymorphism.

Human DNA.

Human genomic DNA was prepared as described previously (18) from whole blood or from transformed lymphoblast lines. Blood samples from unrelated Caucasians were obtained from the American Red Cross. DNA samples from three-generation CEPH families were provided by CEPH (Centre d'Etude du Polymorphisme Humain, Paris) or were prepared from lymphoblast lines obtained from the Camden Cell Repository or from R. White.

Hybridization.

Standard procedures for restriction enzyme digestion, agarose gel electrophoresis, gel transfer and probe hybridization were followed (18). Bacteriophage clone DNA was prepared according to Helms et al. (20), and labeled with ^{32}P by nick translation (21). Hybridization was carried out in 10% dextran sulfate, 50% formamide, 5X SSC, 40 mM sodium phosphate, pH 7, 5X Denhardt's solution, and 50 ug/ml denatured, sonicated salmon DNA for 16-20 hr at 42°C.

Genetic linkage analysis.

Pairwise linkage relationships were analyzed using the LIPED program (22). Multipoint linkage analysis employed the program CRI-MAP (19).

RESULTS

CRI-S232 detects an array of highly polymorphic DNA sequences.

The DNA clone designated CRI-S232 was isolated from a recombinant bacteriophage library (LA07NS01) prepared by the Los Alamos National Laboratory from flow-sorted

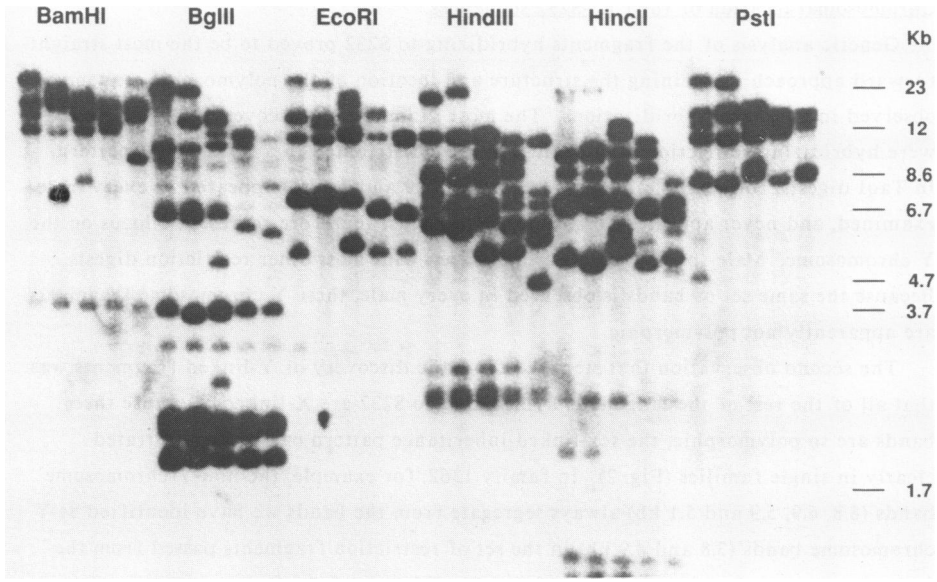

Figure 1. Hybridization pattern of probe CRI-S232 in DNA restriction digests of unrelated individuals. DNA from five unrelated individuals was digested with each of the restriction enzymes indicated, separated on a 0.8% agarose gel, transferred and hybridized with S232. Molecular weights of markers are shown at right.

chromosomes enriched for human chromosome 7 (16). The clone CRI-S232 was one of a set of phage selected for human single-copy sequence inserts (19), and contains a single human EcoRI fragment of 7.0 kb. Hybridization to restriction digests of human DNA with this probe displays a complex and polymorphic pattern of restriction fragments in every enzyme digest, and the presence of almost every band is variable in comparisons of unrelated individuals (Fig. 1).

The hybridization of S232 to so many restriction fragments in each individual digest suggests that the probe contains a low-copy repeat sequence. For instance, in some individuals the probe hybridizes to as many as six distinct EcoRI fragments over 6 kb each, even though the probe is a genomic EcoRI fragment only 7.0 kb in length. Furthermore, the observation that the array of bands detected with S232 is polymorphic with every restriction enzyme tested suggests that the variation is the result of DNA rearrangements such as insertion and deletion of DNA sequences. The hypervariability of the fragments detected resembles that of the "mini-satellite" or variable number tandem repeat (VNTR) sequences (23-25). However, we have not yet confirmed that the S232 clone or the complementary genomic restriction fragments contains direct, tandem repeats.

Chromosomal location of the CRI-S232 sequences.

Genetic analysis of the fragments hybridizing to S232 proved to be the most straightforward approach to defining the structure and location of the polymorphic sequences observed in Southern hybridizations. The most illuminating discovery was that there were hybridizing restriction fragments that were distributed in a sex-specific pattern. In TaqI digests, for example, at least two bands (4.9 and 3.8 kb) appeared in every male examined, and never appeared in females. They must therefore represent a locus on the Y chromosome. Male-specific bands are also seen with most other restriction digests. Because the same set of bands is observed in every male, these Y chromosome fragments are apparently not polymorphic.

The second observation that stemmed from the discovery of Y-linked fragments was that all of the rest of the fragments hybridizing to S232 are X-linked. Because these bands are so polymorphic, the sex-linked inheritance pattern can be demonstrated clearly in single families (Fig. 2). In family 1362, for example, the non-Y-chromosome bands (8.8, 6.9, 5.9 and 5.1 kb) always segregate from the bands we have identified as Y chromosome bands (3.8 and 4.9 kb) in the set of restriction fragments passed from the father to each of the offspring. All of the non-Y bands of the father co-segregate as a group or haplotype to each of his daughters. Thus, these bands show an X-linked inheritance pattern. Similarly, the bands from the mother segregate to each of the children in one of two allelic sets: haplotype D (13, 8.0, 7.5 and 6.1 kb) or haplotype C (13.5, 10, 9.5 and 8.6 kb). Extension of this analysis to 19 large sibship CEPH families showed the same sex-linked inheritance pattern of all polymorphic restriction fragments hybridizing to S232. Since all of the S232 fragments were polymorphic in some restriction digests (such as EcoRI) and all co-segregated with the X or Y chromosome, there is no autosomal sequence detected by the probe, and it must therefore have been an X chromosome contaminant in the chromosome 7-specific clone library. (It is unlikely to have come from the Y chromosome because of the origin of the chromosome 7 library, the weaker hybridization of the probe to the Y-specific bands, and the absence of a corresponding 7 kb EcoRI fragment among the Y chromosome fragments observed in genomic DNA hybridizations).

The set of TaqI fragments constituting each X chromosome haplotype in family 1362 shown in Fig. 2 were found to co-segregate, even in the maternally derived X chromosomes which had the opportunity to undergo recombination. The X chromosome TaqI fragments hybridizing to S232 therefore originate from a single locus or are closely linked to each other. The segregation patterns of polymorphic restriction fragments generated with different restriction enzymes (TaqI, EcoRI and BglII) are identical in a given family, confirming that all of the hybridizing RFLPs map to the same locus on the X chromosome. The Human Gene Mapping Workshop designation of the locus is DXS278.

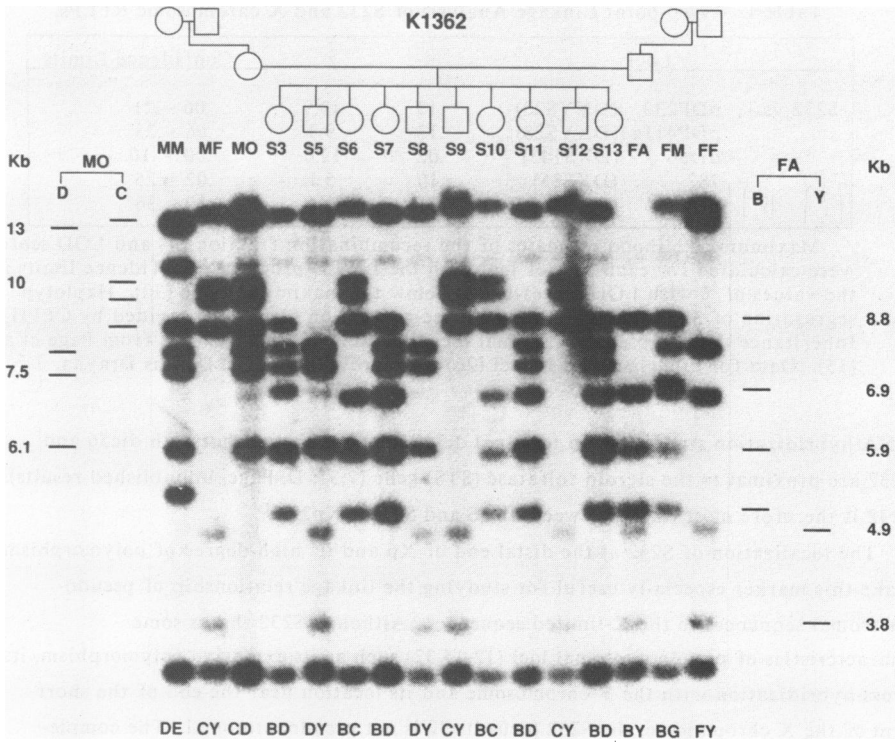

Figure 2. Segregation of S232 TaqI fragments in family 1362. The bands seen in each individual's DNA are grouped in inferred X and Y chromosome haplotypes (designated B - G, Y). Bands comprising each haplotype are shown schematically beside each autoradiogram. The letters under each lane indicate the two haplotypes inherited by the individual. The 3 kb TaqI band at the bottom of the autoradiogram and several faint smaller fragments are constant in all individuals and not listed in the haplotype descriptions. Molecular weights of bands are shown (in kb).

Mapping the S232 locus on the X chromosome.

The extraordinary degree of polymorphism of the S232 locus make it a fully informative marker for linkage analysis. We have collected data on segregation of the S232 haplotypes in 19 of the CEPH families. We measured recombination distances to several X-chromosome loci for which segregation data in the CEPH families were available (15,26). Linkage to S232 with LOD scores greater than 3 was observed for five markers (15,27-29), all on Xp: pD2 (DXS43), 782 (DXS85), dic56 (DXS143), pDP411a (DXYS28) and pDP230 (DXYS20). The closest of these markers is dic56 (DXS143, located at Xp22.3), at a recombination fraction ($\hat{\theta}$) of 0.02, with a maximum LOD score of 12.0 (Table 1). Multipoint linkage analysis places the S232 locus 1.6 cM distal to dic56 (Fig. 3). This order for S232 and dic56 is favored over the alternative by a factor of 98.

Table 1. Two-point Linkage Analysis of S232 and X chromosome RFLPs.

Loci			$\hat{\theta}$	$\hat{z}$	Confidence Limits
S232 vs.	pDP230	(DXYS20)	.13	13.9	.06 - .21
	pDP411a	(DXYS28)	.12	9.3	.06 - .23
	dic56	(DXS143)	.02	12.0	.001- .10
	782	(DXS85)	.10	5.1	.02 - .25
	pD2	(DXS43)	.21	3.4	.10 - .36

Maximum likelihood estimates of the recombination fraction ($\hat{\theta}$) and LOD score ($\hat{z}$) were calculated for each pair of loci with the LIPED program. Confidence limits are the values of θ with LOD scores 1 unit below the maximum value (30). Haplotype segregation of S232 was scored in 19 three-generation families (provided by CEPH). Inheritance data of pseudoautosomal loci (DXYS20 and DXYS28) is from Page et al. (15). Data for other mapped X loci (26) were provided by Dr. Dennis Drayna.

DNA hybridization studies of Xp terminal deletions have shown that both dic56 and S232 are proximal to the steroid sulfatase (STS) gene (7,31; D. Page, unpublished results). S232 is therefore most likely between dic56 and STS at Xp22.3.

The localization of S232 at the distal end of Xp and its high degree of polymorphism make this marker especially useful for studying the linkage relationship of pseudoautosomal sequences to the X-limited sequences. Although S232 shares some characteristics of pseudoautosomal loci (12-15,32) such as its extensive polymorphism, its cross-hybridization with the Y chromosome and its location near the end of the short arm of the X chromosome, the S232 locus itself is not pseudoautosomal. The complementary bands on the X and the Y chromosomes are limited to the respective chromosomes and have not been observed to exchange in the CEPH families. As discussed below, the Y chromosome locus identified by S232 is not even in the pairing region.

The recombination map distance between S232 and two pseudoautosomal markers (15) in female meiosis is 12.4 cM (Fig. 3). The observed recombination fractions (Table 1) were 0.12 with the more proximal locus, pDP411a (DXYS28), and 0.13 with the more distal, pDP230 (DXYS20), a marker very close to the Xp telomere. In male meioses in the same families, these two pseudoautosomal loci recombined with S232 (and all other X-specific loci) at rates of 38% and 49%, respectively. Of course, the recombination rate with S232 in males indicates only the high rate of crossing-over within the pseudoautosomal region and the distance of the DXYS20 and DXYS28 markers from the boundary of the strictly sex-linked portions of the sex chromosomes.

Regional localization of S232 sequences on the Y chromosome.

As shown in Figs. 2, 5 and 6, S232 hybridizes to a set of restriction fragments that are common to all males and co-segregate with the Y chromosome. More precise mapping of DNA sequences on the Y chromosome can be achieved by hybridizing to DNA from individuals carrying various deletions of the Y chromosome. By hybridizing S232 to a

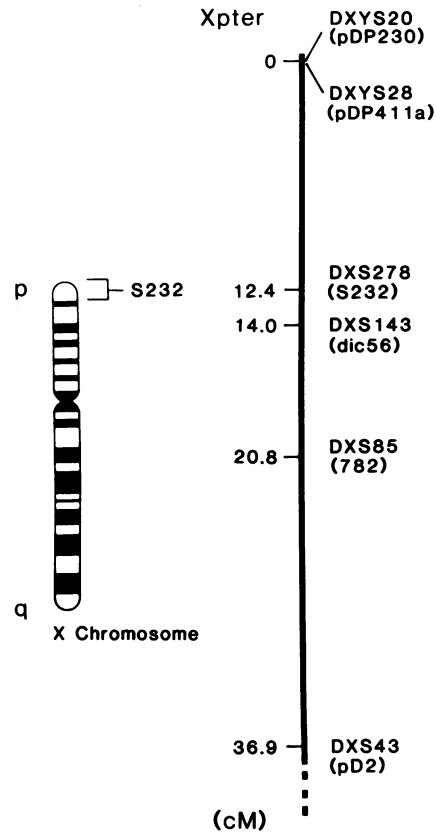

Figure 3. Multipoint linkage map of S232 and other X chromosome markers. The most likely order and map intervals were determined with the program CRI-MAP (18) from RFLP data described in Table 1. Map distances are centimorgans (cM) from the most telomeric marker, DXYS20. The map order shown is over 100 times more likely than any other, with two exceptions. First, an order with S232 and dic56 reversed is 98-fold less likely. Second, the order of DXYS20 and DXYS28 could not be determined from recombination in female meiosis. However, DXYS20 has been shown to be more distal by linkage analysis in male meiosis (15).

set of DNAs with Y chromosome deletions, the Y-specific sequences were mapped to interval 6 in the map of Vergnaud et al. (33), corresponding to a location in the middle of the long arm of the Y chromosome (Fig. 4). Interestingly, three other cloned sequences that have been mapped to this region of the Y chromosome (D. Page, unpublished results), M1A (34) and p71-7a (35) and STS (7) are also homologous to sequences on the distal short arm of the X (Xp22.3). The hybridization of S232 and these three clones to the Y chromosome deletion panel are discordant in one individual, however,

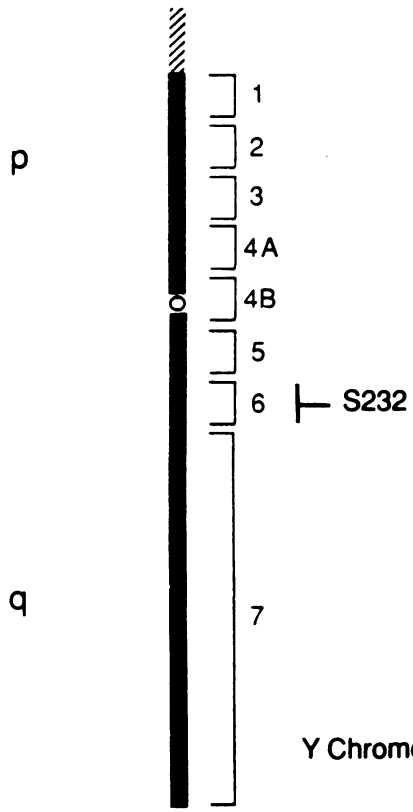

Figure 4. Regional mapping of CRI-S232 sequences on the Y chromosome. The presence of the S232 Y-specific bands in a panel of individuals with various Y chromosome deletions (32) was determined by Southern transfer hybridization. In the Y chromosome deletion map (32,38), the sequences homologous to S232 map to interval 6.

defining two subregions within interval 6, one including S232 and the other with M1A, 71-7a, and STS. The region of homology between this portion of Yq and the distal short arm of the X may therefore be extensive, but we do not yet know the distance between these three loci on either the X or Y chromosomes. M1A has been shown to be distal to 71-7a, STS and dic56 on the X chromosome (36).

Recombination within the X chromosome S232 locus.

In inheritance studies of the S232 polymorphism in 19 large multi-generational families, no recombination of the parental fragment haplotype was observed in 136 of 138 informative chromosomes in female meioses. Thus, S232 defines a haplotype which usually segregates as a single locus. In two of the 138 maternal meioses we have scored, however, neither of the complete parental haplotypes is maintained in the transmitted X chromosome. In these two cases the novel S232 haplotypes consisted of restriction fragments derived from each of the maternal haplotypes, results consistent with recombination within the S232 locus.

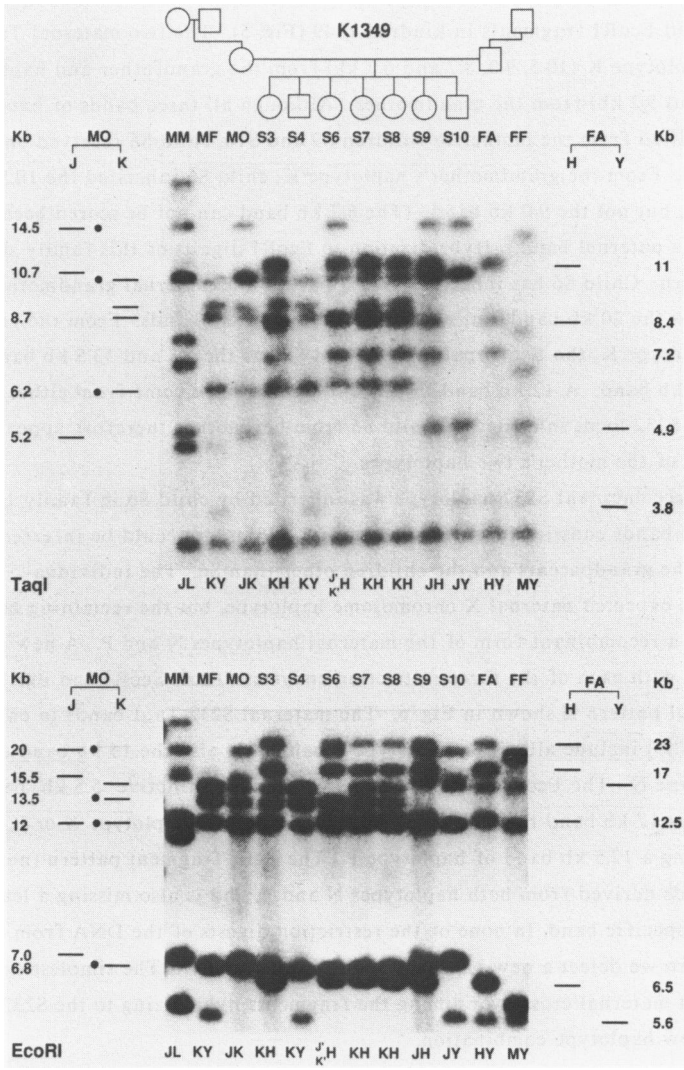

Figure 5. Inheritance of recombinant haplotype in family 1349. Hybridization pattern of S232 in TaqI (top panel) and EcoRI digests (bottom panel) of family 1349 members. The maternal X chromosome fragments are shown in the diagram at left, grouped by grandmaternal haplotype (J) and grandpaternal haplotype (K). Similarly, the diagram at right indicates the father's X chromosome haplotype (H) and Y chromosome bands (Y). Letters below each lane are the two haplotypes of each family member. J'K' is the recombinant haplotype inherited by individual S6. Bands in this haplotype are indicated by dots adjacent to bands in the diagram of J and K bands at left.

An example of such an apparent recombination event is shown in the segregation of S232 TaqI and EcoRI fragments in kindred 1349 (Fig. 5). The two maternal TaqI haplotypes are haplotype K (10.5, 9.0, 8.7 and 6.2 kb) from the grandfather and haplotype J (14.5, 10.7 and 5.2 kb) from the grandmother. Although all three bands of haplotype J were transmitted from the mother to children S9 and S10, child S6 received only the 14.5 kb band. From the grandmother's haplotype K, child S6 inherited the 10.5 and 6.2 kb bands, but not the 9.0 kb band. (The 8.7 kb band can not be scored because it is obscured by a paternal band.) Hybridization to EcoRI digests of this family shows a similar pattern. Child S6 has inherited only a part of the maternal grandmother's haplotype J -- the 20 kb band, but not the 15.5 kb or 7.0 kb bands. From the grandfather's haplotype K, the EcoRI pattern of child S6 has the 6.8 and 13.5 kb bands but lacks the 15 kb band. A 12 kb band in this child could have come from either grandparent. The S232 locus inherited by child S6 from her mother therefore appears to be a recombinant of the mother's two haplotypes.

Another recombinant S232 haplotype was inherited by child S6 in family 1331 (Fig. 6). The bands constituting the four parental haplotypes could be inferred from the patterns of the grandparents and the children other than S6. The individual S6 has inherited the expected paternal X chromosome haplotype, but the remaining bands appear to be a recombinant form of the maternal haplotypes N and P. A new pattern is recognizable with each of the three restriction enzymes (TaqI, EcoRI and BglII) tested. Only the TaqI pattern is shown in Fig. 6. The maternal S232 TaqI bands in child S6 (haplotype N'P') include all of the haplotype P bands but also the 15 kb band derived from haplotype N. The EcoRI pattern (not shown) has a distinctive 5.5 kb band of haplotype P, a 12 kb band that could have come from either haplotype N or P, but is clearly missing a 12.5 kb band of haplotype P. The BglII fragment pattern (not shown) includes bands derived from both haplotypes N and P, and is also missing a least one haplotype P-specific band. In none of the restriction digests of the DNA from this individual can we detect a new band not found in the mother. The simplest explanation is therefore a maternal cross-over among the fragments hybridizing to the S232 probe to generate a new haplotype combination.

The cross-over events within the S232 locus itself that were detected in two meioses in families 1331 and 1349 allow us to order the restriction fragments in the recombinant haplotypes with respect to the flanking X chromosome genetic markers. In family 1349, for example, the recombinant X chromosome in child S6 carries telomeric pseudoautosomal alleles and the 20 kb S232 EcoRI fragment from the grandmother, and centromere-proximal marker alleles with the 6.8 kb EcoRI fragment from the grandfather (Fig. 5). Thus, when the S232 restriction fragments of the X chromosomes in this family and in family 1331 are incorporated into a physical map of the S232 locus, it will be possible to orient that map with respect to the X chromosome genetic map.

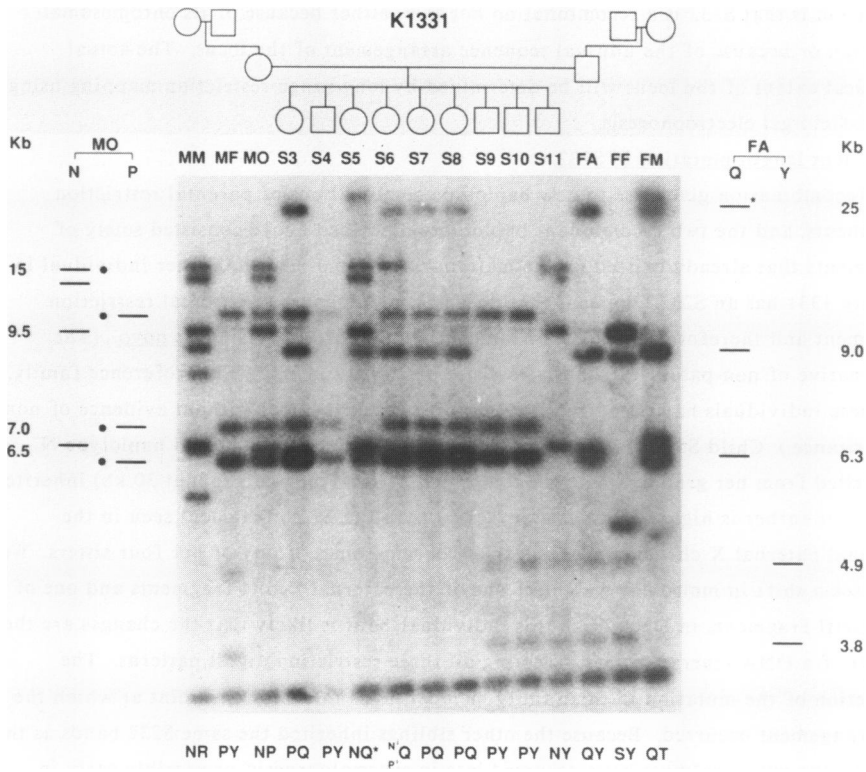

Figure 6. Recombinant haplotype and fragment length mutation in family 1331. Hybridization patterns of S232 in TaqI digests of family 1331 members. Fragments comprising each parental haplotype are shown in diagrams at the left and right of the autoradiogram as in Figs. 2 and 5. The letters below each lane indicate the two haplotypes of each individual. N'P' is the recombinant haplotype inherited by individual S6, and the bands in this haplotype are those in N and P marked with a dot in the diagram at left. Q* is the paternal X chromosome haplotype inherited by individual S5, but differs from haplotype Q in the highest molecular weight fragment (marked with an * in the diagram at right).

The 1.4% rate of recombination (2/138 meioses) observed within the S232 region is a lower limit on the cross-over frequency within the interval defined by the most widely separated S232-hybridizing sequences. Because each individual hybridization pattern consists of combined bands of two complex haplotypes and gel resolution is limited, some bands occasionally overlap, so that some haplotype changes could go undetected. In any case, the high rate of recombination within the S232 locus suggests that fragments hybridizing to this probe are separated at a considerable distance. On average, one centimorgan in the human genetic map represents about 1000 kb of DNA, leading to an estimated span of 1.5 million base pairs for the S232 locus. An alternative inter-

pretation is that S232 is a recombination hot spot, either because of its chromosomal location or because of the unusual sequence arrangement of the locus. The actual physical extent of the locus will be determined by long-range restriction mapping using pulse-field gel electrophoresis.

Fragment length mutation in S232.

Recombination gives rise to new haplotype combinations of parental restriction fragments, and the two recombinant haplotypes described above consisted solely of fragments that already existed on the maternal X chromosomes. Another individual in family 1331 has an S232 fragment that does not correspond to a parental restriction fragment and therefore represents a fragment length variant arising de novo. (The alternative of non-paternity is not a realistic possibility in this CEPH reference family, as these individuals have been typed at hundreds of RFLP loci without evidence of non-inheritance.) Child S5 in this family (Fig. 6) has the complete maternal haplotype N inherited from her grandmother, but the largest of the fragments (about 30 kb) inherited from her father is higher in molecular weight than the 25 kb fragment seen in the original paternal X chromosome or in the X chromosomes of any of her four sisters. We also see a shift in molecular weight of one of the paternal EcoRI fragments and one of the BglII fragments in DNA from this individual, so it is likely that the changes are the result of a DNA rearrangement affecting all three restriction digest patterns. The detection of the mutation in this family member helps to define the point at which the rearrangement occurred. Because the other siblings inherited the same S232 bands as the father, the mutation must have occurred late in spermatogenesis, or possibly early in embryogenesis of individual S5. In either case, because the new allele originated from a male, the mutation occurred without meiotic pairing between X chromosome homologues. This result is significant, because it shows that a fragment length mutation, whether by unequal homologous recombination or not, can occur without the aid of meiotic pairing and exchange.

DISCUSSION

The X chromosome locus defined by the probe S232 includes a set of restriction fragments that are highly variable in length. Variable fragments within the locus segregate in meiosis as a group, and the haplotype combinations are so diverse that we have not yet observed any individual homozygous for an S232 haplotype. Except for the occasional recombinant haplotype, the S232 locus has been fully informative as a genetic marker. In the 19 CEPH families studied, we were able to determine unambiguously the grandparental origin of both S232 alleles of every child, including two individuals that carried recombinant haplotypes.

The S232 probe identifies two loci, one on the long arm of the Y chromosome that

appears to be non-polymorphic, and the highly polymorphic locus on the short arm of the X chromosome. On the X chromosome, the nearest previously mapped marker appears to be the locus DXS143 defined by the BclI polymorphism detected with the probe dic56. S232 is also close to the pseudoautosomal region of X-Y meiotic exchange, and as a fully informative marker, provides the maximum data on genetic distance between the pseudoautosomal loci and the X-specific sequences. Two loci, DXYS28 and DXYS20, have been mapped at a distance of 12.4 cM from S232 in female meiosis.

A detailed map of the X chromosome adjacent to the pseudoautosomal segment will be important in localizing translocations between the X and Y chromosomes. Illegitimate exchanges between the X and Y outside of the normal cross-over region have been shown to be responsible for many genetic disorders in sex determination, particularly in the cases of XX males and XY females (37-42). Many of these individuals appear to have inherited aberrant sex chromosomes as a result of the testis-determining factor (TDF) gene having been transferred from the Y chromosome to the X chromosome during or prior to paternal meiosis. It has been hypothesized that similar unequal exchanges may be involved in deletions of the STS gene from the X chromosome (7). With S232 and other mapped loci from the X-Y pairing region, it is possible to localize the breakpoints of exchanges between the X and Y chromosomes.

The sequence organization of the S232 locus on the X chromosome is complex, and difficult to characterize because of the high degree of variability of most of the restriction fragments detected, the motif of reiterated sequences, and the size of the locus, which must extend far beyond the 7.0 kb clone from the genomic library. Our working model is that there are several homologous, hypervariable segments, interspersed with stretches of non-homologous DNA. We are investigating the structure of the extended locus by pulse-field gel electrophoresis, and are isolating additional genomic clones hybridizing to the S232 probe.

The number of different restriction fragment lengths that distinguish the X chromosome S232 alleles among virtually any set of unrelated individuals suggests that new fragment length variants appear at a high frequency in the population. Not surprisingly, therefore, one such mutational event was detected in our family inheritance studies of the S232 locus. One child was found to have a higher molecular weight band (in three different restriction digests) than one of the expected bands from the X chromosome of her father. Having arisen from the male germ-line, the DNA rearrangement responsible for the mutant allele occurred in the absence of meiotic pairing and recombination of X chromosome homologues. Meiotic recombination also contributes to the variety of S232 haplotypes, however. We observed two instances of crossing over within the locus during female meiosis that generated non-parental haplotypes.

S232 and at least three other clones (M1A, p71-7a and STS) are representatives of

what may be a block of DNA sequences at Xp22.3 with homologous sequences on Yq. Determination of the map order and separation of these loci on both the X and the Y chromosomes would help to define the continuity and extent of the shared sequences. Given the polymorphic variability of the X chromosome sequences and the invariance of the Y chromosome fragments, it will also be instructive to compare the DNA sequence organization of the X and Y loci hybridizing to S232. That analysis might reveal distinctive elements of the S232 X chromosome sequence that confer its extraordinary variability.

ACKNOWLEDGEMENTS

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