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Long-range restriction map of the terminal part of the short arm of the human X chromosome

(sex reversals/X-recessive chondrodysplasia punctata/Kallmann syndrome)

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ABSTRACT **The terminal part of the short arm of the human X chromosome has been mapped by pulsed-field gel electrophoresis (PFGE). The map, representing the distal two-thirds of Xp22.3 spans a total of 10,000 kilobases (kb) from Xpter to the *DXS143* locus. A comparison with linkage data indicates that 1 centimorgan (cM) in this region corresponds to about 600 kb. CpG islands were essentially concentrated in the 1500 kb immediately proximal to the pseudoautosomal boundary. Several loci, including the gene encoding steroid sulfatase (*STS*) and the loci for the X-linked recessive form of chondrodysplasia punctata (*CDPX*) and for Kallmann syndrome (*KAL*) have been placed relative to the Xp telomere. *CDPX* is located between 2650 and 5550 kb from Xpter, and *STS* is located between 7250 and 7830 kb from Xpter. *KAL* maps to an interval of 350 kb between 8600 and 8950 kb from the telomere. The X-chromosomal breakpoints of a high proportion of XX males resulting from X–Y interchange cluster to a 920-kb region proximal and close to the pseudoautosomal boundary.**

The existence of the pseudoautosomal region, a region of strictly homologous sequences shared and exchanged between the human X and Y chromosomes, has several consequences that are reflected by the unique properties of the terminal part of the X chromosome short arm.

First, genes from the pseudoautosomal region, located at the tip of each of the short arms of the sex chromosomes, are present in a double dose in both sexes and, therefore, should not be subject to X-inactivation (1), which affects one X chromosome in each cell of female mammals. This has been shown for *MIC2*, the only pseudoautosomal gene identified at present (2). Two chromosome X-specific genes (*XG* encoding the Xg blood group and *STS* encoding steroid sulfatase), mapping to Xp22.3, also escape, at least partially, this inactivation (3–5). By analogy, it has been suggested that such X-specific genes from Xp22.3, which are at least partially insensitive to X-inactivation, are remnants of an ancestral pseudoautosomal region that once extended more proximally on Xp22.3. This is further supported by the pseudoautosomal location of murine *STS* (6) and, in man, by the existence of numerous sequence homologies between Xp22.3 and different parts of the Y chromosome (7–12).

Second, the high frequency of translocations involving Xp22.3 and the Y chromosome is possibly related to these numerous sequence homologies. These translocations involve either the short arm of the Y chromosome (X–Y interchange sex reversals) or the long arm (Xp22.3;Yq11 translocations).

In addition to these unique biological features, the loci for five genetic diseases have been mapped to Xp22.3. While loci for short stature (SS), X-linked recessive chondrodysplasia punctata (CDPX; McKusick no. 21510), and mental retardation (MR) are all localized distal to the X-linked ichtyosis (XLI) locus (13–16) (*STS* locus), Kallmann syndrome (KAL) locus maps proximal to *STS* (17).

Finally, a genetic map expansion has been observed in telomeric regions of human chromosomes (18–20). While in male meiosis the genetic map expansion in the pseudoautosomal region is dramatic (21–24), in female meiosis no such expansion has been observed (21, 24). However, map expansions in more subtelomeric parts of Xp22.3 cannot be excluded, and the occurrence of a recombination hot spot within locus *DXS278* from Xp22.3 has been proposed (10).

An accurate physical map of Xp22.3 remains a prerequisite to an understanding of the questions outlined above. This paper reports the establishment of a long-range pulsed-field gel electrophoresis (PFGE) restriction map extending 10 megabases (Mb) from Xpter to *DXS143*.

MATERIALS AND METHODS

DNA Probes. Twelve probes detecting chromosome X-specific loci were used. The loci detected by probes IP415 (*DXF28S1*), J15 (*DXS284*), IP147 (*DXS431*), IP402 (*DXS432*), 38j (*DXS283*), IPJ32 (*DXF28S2*), and J502 (*DXS285*) all map distal to the locus (DXS31) detected by probe M1A (12, 16) (see Fig. 1). The other probes used were obtained from the following investigators: M1A (*DXS31*) (8) (J. L. Mandel, Strasbourg), CRI-S232 (*DXS278*) (10) (Collaborative Research), Stb14 (25) (*STS*) (A. Ballabio), GMGX9 (*DXS237*) (26) (N. Affara and M. Fergusson Smith), and dic56 (*DXS143*) (27) (L. Kunkel).

DNA Sources and Analysis. Peripheral lymphocytes from three 45,X Turner, five 46,XY, and one 48,XXXX individuals were studied. Cells from two patients were analyzed: peripheral lymphocytes from a 46,Y,(del)(X)(pter → p22.3) patient (LIL155) with SS, MR, XLI, CDPX, but without KAL (J.L., unpublished data) and fibroblasts from a 46,Y,der(X),t(X;Y)(Xp22.3;Yq11.2) patient (445) with SS, MR, CDPX, and KAL (28). In both patients, the terminal deletion included the *DXS237* locus. In patient LIL155, a strong hybridization signal was still observed with probe CRI-S232, whereas in patient 445, very faint bands were detected.

Digestions, PFGE conditions, and DNA transfer have been reported (24). Electrophoresis of DNA fragments larger than 1.3 Mb was performed in a Beckman vertical apparatus. Because of space restriction, some data used to establish the map are not reported and are available on request.

RESULTS

The present mapping studies have been carried out with a total of 12 DNA probes previously ordered to six intervals by deletion mapping as summarized in Fig. 1 (12, 16). Interval 6

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Abbreviations: PFGE, pulsed-field gel electrophoresis; SS, short stature; CDPX, X-linked chondrodysplasia punctata; MR, mental retardation; KAL, Kallmann syndrome; XLI, X-linked ichtyosis; STS, steroid sulfatase; PAB, pseudoautosomal boundary.

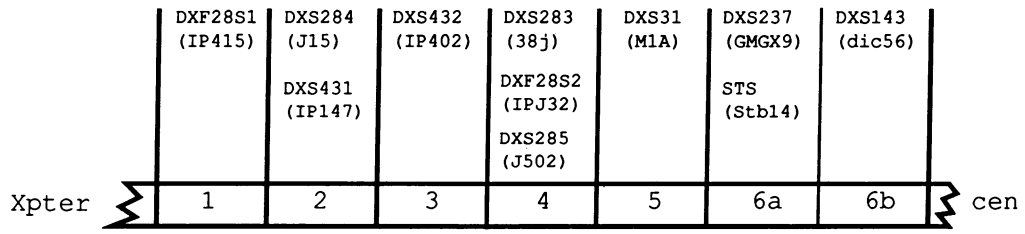

FIG. 1. Deletion map of region Xp22.3. Intervals 1–6 are defined by the sex chromosome rearrangements observed in a somatic cell hybrid (JUCH1) (interval 1) (12), X–Y interchange sex reversals (XX males and XY females) (intervals 2–4) (12), patient PORa (16) (interval 5), and Xp22.3;Yq11 translocations (interval 6) (29). Interval 6 was further subdivided into 6a and 6b as deduced from genetic linkage data (10). The loci are assigned to one interval; their probes are indicated in parentheses. The short arm telomere and the centromere are indicated respectively by "Xpter" and "cen."

can be further subdivided into two parts by deletion mapping, with gene *STS* in the distal part and locus *DXS143* in the proximal one (29). Based on linkage data reported by others (10, 30), it has been proposed that locus *DXS278* detected by probe CRI-S232 maps between *STS* and *DXS143*.

For convenience a restriction fragment detected by a probe is designated in brackets by its size in kilobases (kb), followed by the relevant locus or probe name and ended by the abbreviation(s) of the restriction enzyme(s) used to generate this fragment—e.g., [630 kb IP415 *Not* I/*Nru* I] designates a restriction fragment of 630 kb obtained after double digestion with *Not* I and *Nru* I that hybridizes to probe IP415. Fragments are sometimes designated by only two of these characteristics—e.g., [*MIC2 Not* I] or [4800 kb *Mlu* I]. Since the map presented in this report extends our previous map of the pseudoautosomal region, we indicate positions of restriction sites as previously (24), starting from the pseudoautosomal telomere—e.g., ptel7400 means 7400 kb from the Xp telomere. The description below intends essentially to establish the contiguity of a number of key fragments. Positions of other restriction sites are shown in Fig. 2. The region spanning the pseudoautosomal boundary (PAB) of chromosome X (*PABX*) to *DXS143* (probe dic56) has been subdivided into two parts: the more distal one includes deletion intervals 1–5, and a proximal block is defined by interval 6.

Linking Intervals 1–5 to Xpter. Three large chromosome X-specific fragments detected by 19B (locus *MIC2*) hybridize also to several other probes mapping more proximally. The largest fragment (4800 kb) is obtained with *Mlu* I digestion and can be observed with all of the probes from intervals 1–5. A *Not* I fragment of 1200 kb is detected by probes from intervals 1–3, whereas a *Nru* I fragment of 1200 kb is only observed with IP415, the probe defining interval 1. The distal restriction sites of these three fragments have been localized previously on the physical map of the pseudoautosomal region (24). Two of these sites were shown to correspond to the most proximal restriction sites of the pseudoautosomal region for *Not* I (ptel 2350) and *Nru* I (ptel 1530). In contrast, the [4800 kb *Mlu* I] fragment is also detected by probe 601 (locus *DXYS17*), indicating that the most proximal pseudoautosomal *Mlu* I site remains apparently uncleaved in the DNA samples presently analyzed. The distal end of the large *Mlu* I fragment was localized to ptel 1450. Thus, the proximal restriction sites of the large *Mlu* I, *Not* I, and *Nru* I fragments map respectively to coordinates ptel 6250 (*Mlu* I), ptel 3550 (*Not* I), and ptel 2730 (*Nru* I). Another *Nru* I fragment of 2800 kb hybridizes to all of the probes from intervals 2–5. It can be shown that this *Nru* I fragment is contiguous to the [1200 kb *MIC2–DXF28S1 Nru* I] fragment as follows. [500 kb *DXF28S1–DXS284 Bss*HII] and [430 kb *DXF28S1–DXS284 Sst* II] fragments were cleaved in only two fragments by *Nru* I digestion. In addition, a partial *Nru* I digestion product of 4000 kb is detected by all of the probes from intervals 1–5. This maps the proximal end of the [2800 kb *Nru* I] fragment to ptel 5530. The distal end of the [3650 kb *DXF28S2–DXS283–DXS285–DXS31 Not* I] was positioned by *Mlu* I/*Not* I and *Nru* I/*Not* I double digestions, which indicate that the [3650 kb *Not* I] fragment is contiguous to fragment [1200 kb *MIC2–DXF28S1–DXS284–DXS431–DXS432 Not* I]. This localizes the proximal end of [3650 kb *DXF28S2–DXS283–DXS285–DXS31 Not* I] to ptel 7200.

Mapping Interval 6. All fragments detected by GMGX9 (*DXS237*) that are mentioned below were also observed with Stb14 (*STS*) (25). Since the former probe gave stronger hybridization signals, we have used GMGX9 to map *STS* on a long-range restriction map. Probes GMGX9, CRI-S232, and dic56 hybridize to a *Mlu* I fragment of 3500 kb. This fragment has been found in all individuals tested. A [3350 kb *Nru* I] fragment is detected with CRI-S232 and GMGX9 but not with dic56. Since *Mlu* I/*Nru* I double digests yield a unique [2700 kb GMGX9–CRI-S232 *Mlu* I/*Nru* I] fragment, it follows that fragments [3350 kb GMGX9–CRI-S232 *Nru* I] and [3500 kb GMGX9–CRI-S232 *Mlu* I] overlap. Both probes GMGX9 and CRI-S232 also detect a 1700-kb fragment in *Bss*HII and *Not* I digests and in *Not* I/*Bss*HII double digests. However CRI-S232 detects additional [975 kb *Bss*HII] and [3650 kb

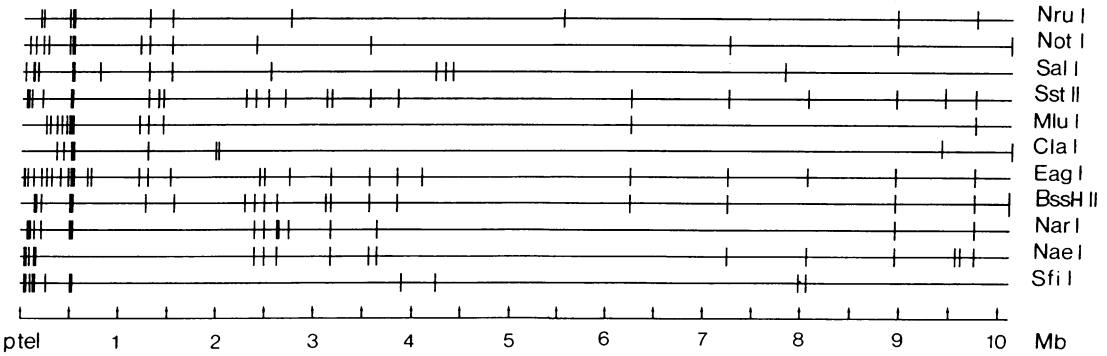

FIG. 2. Long-range restriction map of the terminal part of the human X chromosome short arm. Coordinates indicate distances in kilobases from the X short-arm telomere.

Not I] fragments, whereas dic56 hybridizes to a *Not* I fragment of 1100 kb. The [3650 kb CRI-S232 *Not* I] fragment cannot represent a partial digestion product composed of fragment [1700 kb GMGX9–CRI-S232 *Not* I], since the former is not revealed with GMGX9. Only two of the three *Not* I fragments detected by GMGX9, CRI-S232, or dic56—namely, fragments [3650 kb CRI-S232 *Not* I] and [1100 kb *DXS143 Not* I] can be recut by *Mlu* I. Since *DXS143* (probe dic56) has been mapped proximal to *STS* (29) and to *DXS278* (detected by probe CRI-S232) (10), the following order can be proposed: Xpter–[CRI-S232 *Not* I/*Mlu* I]–[GMGX9–CRI-S232 *Not* I]–[dic56 *Not* I/*Mlu* I]. The total size of these three fragments amounts to that of the [3500 kb CRI-S232–GMGX9–dic56 *Mlu* I] fragment, indicating that the three fragments are contiguous.

Linking Interval 6 to Xpter. Since CRI-S232 labels two *Bss*HII and *Not* I fragments, which do not overlap, we will consider the fragments detected by this probe separately and designate the more distal as *S232A* and the more proximal one as *S232B*. *S232A* is distal to *STS*. *Nru* I/*Mlu* I double digests yields a single [2700 kb GMGX9–CRI-S232] fragment. Therefore, the proximal ends of [3350 kb GMGX9–CRI-S232 *Nru* I], [1700 kb GMGX9 (*DXS237*)–*CRI-S232B Not* I], and [1700 kb GMGX9 (*DXS237*)–*CRI-S232B Bss*HII] must map in the same CpG island. Coincidence of the *Nru* I and *Not* I sites is further supported by the identical size of [800 kb dic56 *Not* I/*Mlu* I], [800 kb dic56 *Nru* I] and [800 kb dic56 *Nru* I/*Mlu* I]. Since [GMGX9 *Sal* I] and [GMGX9 *Sfi* I] can be recut by *Not* I, *Bss*HII, and *Sst* II but not by *Nru* I, this localizes these fragments as overlapping the distal end of the [GMGX9–CRI-S232 *Not* I] fragment. Locus *CRI-S232A* is included in fragments [3650 kb *Not* I], [975 kb *Mlu* I/*Not* I], and [975 kb *Bss*HII] distal to *STS*, whereas locus *CRI-S232B* is present in fragments [1700 kb *STS Not* I] and [1700 kb *STS Bss*HII]. One 45,X case and a 46,XY individual display a polymorphism characterized by the absence or nondetection of locus *CRI-S232A* in fragments [3700 kb *Not* I], [975 kb *Mlu* I/*Not* I], and [975 kb *Bss*HII], whereas the [1700 kb *Not* I] and [1700 kb *Bss*HII] fragments containing locus *CRI-S232B* can be observed in both of these individuals (Fig. 3). Comparison of the hybridization patterns of CRI-S232 in individuals with both loci and individuals displaying only locus *CRI-S232B* indicates that fragments [975 kb *Xma* III] and [975 kb *Sst* II] contain the distal locus *CRI-S232A* (Fig. 3). The majority of the hybridization signal corresponding to the locus *CRI-S232B* is associated with the [875 kb *Nae* I] and [875 kb *Sst* II] fragments, thus indicating that this locus is essentially proximal to *STS*. The proximal end of the distal block of intervals 1–5 shows the coincidence of restriction sites *Bss*HII, *Mlu* I, *Sst* II, and *Xma* III at ptel 6250. Conversely, restriction sites of the same four enzymes coincide in the distal part of interval 6, suggesting that these latter sites could also represent the proximal end of interval 5. This hypothesis implies that locus *CRI-S232A* from interval 6 and *DXS285* and *DXS31* from intervals 4 and 5 share the same *Not* I fragment of 3650 kb. This was tested with partial *Not* I digestions. Fragment [1700 kb GMGX9 (*DXS237*)–*CRI-S232B Not* I] remains unaffected with milder digestions, whereas fragment [3650 kb *CRI S232A Not* I] disappears progressively and is replaced by larger fragments of about 5 Mb; all of these large fragments are also detected with M1A (*DXS31*) (Fig. 4). This conclusion is further supported by the analysis of the DNA of an individual (PORa) carrying an interstitial deletion with a proximal breakpoint between intervals 4 and 5 (16).

Mapping the *KAL* Locus. The chromosome X breakpoints present in patients LIL155 and 445 frame the locus encoding KAL. In patient LIL155, a fragment of 350 kb hybridizing to probe CRI-S232 was observed in *Not* I, *Bss*HII, *Sst* II, and *Nru* I digests as in double digests using combinations of these enzymes. Probes CRI-S232 and dic56 reveal a [1150 kb *Mlu* I] fragment. This confirms the coincidence of sites *Sst* II, *Bss*HII, *Not* I, and *Nru* I at position ptel 8950 and maps the breakpoint of LIL155 at 350 kb distal to this CpG island. In patient 445, probe dic56 reveals, as in normal individuals, [800 kb *Bss*HII], [800 kb *Xma* III], and [1100 kb *Not* I] fragments.

DISCUSSION

The map presented in this paper extends the map of the pseudoautosomal region previously reported (24). Altogether a region spanning 10 Mb from the telomere of Xp to *DXS143* has been physically mapped. This allows us to order 11 of 12 chromosome X-specific loci (Fig. 5).

The comparison of physical and genetic linkage maps is restricted by the few linkage data available in this region. The only locus mapped with a high lod (logarithm of odds) score is the *DXS278* locus. However, as shown, it corresponds to at least two loci detected by probe CRI-S232. Assuming that linkage data reported for *DXS278* corresponds to the most distal locus (*CRI-S232A*), the genetic distance of 12.4 centimorgans (cM) to *DXYS14* (10), which maps at less than 50 kb from the telomere (36, 37), would correspond to a physical distance of 7200 kb—i.e., 580 kb for 1 cM. The increase in recombination frequency observed in the subtelomeric regions of a number of autosomes (18–20) is thus rather slight in the case of Xp22.3. Knowlton *et al.* (10) observed recombination between the different DNA bands detected by CRI-S232 with a frequency of 1.4% and proposed that locus *DXS278* was a recombinational hot spot. The present analysis, which took into consideration only the fragments giving a very strong hybridization signal with CRI-S232, shows the existence of at least two distinct loci detected by this probe. The minimal physical distance of 1475 kb observed between the two extreme loci could well account for the recombination frequency of 1.4% detected between different bands (10).

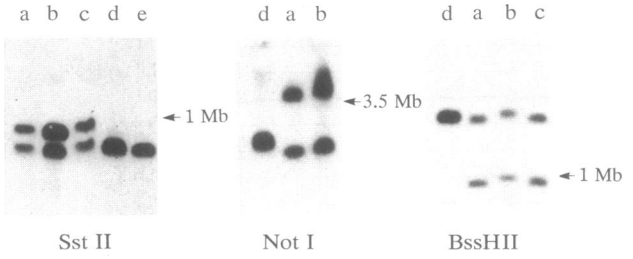

FIG. 3. PFGE analysis of peripheral lymphocytes from different individuals. Products of restriction digestions by *Sst* II, *Not* I, and *Bss*HII are probed by CRI-S232. Lanes: a and d, DNAs from two 45,X individuals; b, DNA from a 48,XXXX individual; c and e, DNA from two 46,XY individuals.

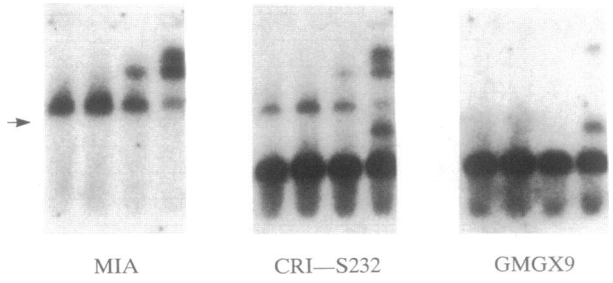

FIG. 4. PFGE analysis of 45,X peripheral lymphocytes. Partial *Not* I digests with decreasing amounts of enzyme from left to right, probed by M1A, CRI-S232, and GMGX9. The arrow indicates the localization of the smallest *Schizosaccharomyces pombe* chromosome (3.5 Mb).

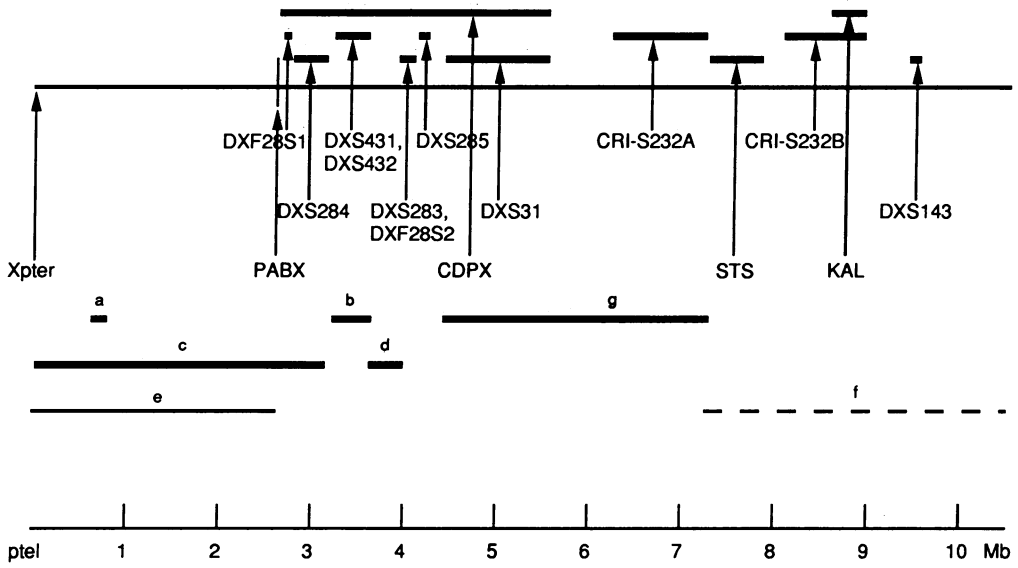

FIG. 5. (*Upper*) Mapping of several X chromosome loci on the long-range restriction map. Localization intervals of the loci (designated by arrows) are represented by horizontal solid bars. Probe CRI-S232 detects at least two loci flanking *STS*. (*Lower*) Mapping of the X chromosome breakpoints observed in XX males and XY females. Solid bars a, b, c, d, and g correspond to cases studied in the laboratory [a, one XX male CON101 (31); b, five XX males (12); c, four XX males (12); d, 10 XX males (12); and g, two XY females (32)]. Solid and dashed lines e and f correspond to cases from the literature [e, two or three XX males (33, 34); f, one XX male (35)]. Coordinates indicate distances in Mb from the telomere of Xp (Xptel).

Since both loci are characterized but not identified by restriction fragment length polymorphisms, accurate genetic distances with other close loci cannot be established. Moreover, since the most distal locus is not detectable in a number of X chromosomes (two of the eight tested), the genetic distance of 1.4% between both loci detected by CRI-S232 is underestimated.

The occurrence of chromosomal breakpoints in Xp22.3 in cases of sex reversal resulting from an abnormal X–Y interchange has been reported (31–33, 38). Among 24 cases of Y(+)XX males analyzed, 23 showed an X-chromosomal breakpoint distal to the *DXS283* and *DXF28S2* loci (12, 31, 33, 34) and a single one proximal to *STS* (35). The physical map presented here indicates that these 23 breakpoints are localized in a region spanning 4 Mb from the telomere. Fifteen breakpoints are clustered in an interval of 920 kb (Fig. 5). It should be pointed out that this interval shares homologies with sequences located on Yp (12). Therefore, this raises the possibility that these homologies may promote abnormal homologous X and Y recombination. In the two cases of XY females that we studied, the breakpoint on the X chromosome is proximal to *DXS31* and distal to *STS*—i.e., within an interval of 3.4 Mb from ptel 4400 to ptel 7800 (see also Fig. 5).

A total of 10 CpG islands have been observed proximal to *PABX* at ptels 2650, 2760, 3180, 3570, 3850, 6250, 7250, 8070, 8950, and 9750. CpG islands of genes undergoing X-inactivation are hypermethylated (see ref. 39) on the inactive X chromosome. However, restriction digestions of DNA from 45,X or 48,XXXX individuals with enzymes sensitive to methylation showed identical restriction patterns for the sites indicated above. Similarly, it was observed previously, that the pseudoautosomal CpG islands remain undermethylated on the inactive X chromosome(s) (24, 40). This suggests that genes associated to these CpG islands escape X-inactivation or that their activity is not related to the methylation status of these CpG islands. A higher density of CpG islands can be noticed in the vicinity of *PABX*.

It is also noteworthy that probes J15, IP402, IP147, IPJ32, 38j, and J502, which were all selected for the presence of a STIR (subtelomeric interspersed repeat) element (12), are clustered between *PABX* and ptel 4100. This observation is in line with the subtelomeric location of STIR elements on autosomes (20).

On this physical map, we are able to map the *CDPX* locus within an interval of 3 Mb between *PABX* and ptel 5550.

KAL has been previously mapped proximal to *DXS237* and distal to *DXS143* (41). The study of the chromosome X breakpoints in patient LIL155 and 445 refines this localization to an interval of 350 kb between ptel 8600 and ptel 8950. A single CpG island only (at ptel 8950) maps in this interval.

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