Analysis of deletions in DNA from patients with Becker and Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic disorder for which the biochemical defect is as yet unknown. Recently, two cloned segments of human X-chromosome DNA have been described which detect structural alterations within or near the genetic locus responsible for the disorder[1,2]. Both of these cloned segments were described as tightly linked to the locus and were capable of detecting deletions in the DNA of boys affected with DMD. In an attempt to determine more precisely the occurrence of these deletions within a large population of DMD patients and the accuracy of one of the segments, DXS164 (pERT87), in determining the inheritance of the DMD X chromosome, the subclones 1, 8 and 15 were made available to many investigators throughout the world. Here we describe the combined results of more than 20 research laboratories with respect to the occurrence of deletions at the DXS164 locus in DNA samples isolated from patients with DMD and Becker muscular dystrophy (BMD). The results indicate that the DXS164 locus apparently recombines with DMD 5% of the time, but is probably located between independent sites of mutation which yield DMD. The breakpoints of some deletions are delineated within the DXS164 locus, and it is evident that the deletions at the DMD locus are frequent and extremely large.

The previously described deletions of the DXS164 region and the XJ-1.1 junction clone[1,2] were assumed to be large, for neither set of cloned segments exhibits any common sequence overlap and yet both cloned segments are absent from most of the same deletion DNA samples[2]. To increase the possibility that a particular deletion would exhibit a break within cloned DNA, the DXS164 region was expanded further by chromosome walking[3] in human genomic libraries constructed in the phage vector EMBL-3 (ref. 4). Following five bidirectional walks, a 137-kilobase (kb) contiguous stretch of DNA was obtained from the DXS164 region. All DNA segments obtained from the 137 kb of DNA were subcloned in plasmid vectors and segments of unique sequence were identified for the entire length. The two previously described subclones (pERT87-1 and -8; ref. 1) and a new unique-sequence segment (pERT87-15) which also detects restriction fragment length polymorphisms (RFLPs; ref. 5) were sent to other investigators interested in DMD.

The three DXS164 subclones, which are spaced over a 50-kb section, were tested for deletion by hybridization against the DNA isolated from males exhibiting the DMD and BMD phenotype. The results of this analysis for 1,346 males are presented in Table 1. As the results were obtained in many different laboratories, they are presented separately for each laboratory. Of all DMD and BMD males tested, 6.5% show deletions at the DXS164 locus, a slightly lower percentage than that found previously[1]. A difference was observed in the incidence of deletions between DMD males with a clear family history of the disease (8.3%) and those with no family history (5.8%). Such a difference might be a reflection of the incidence of deletion mutation in maternal and paternal meiosis. Any true sporadic case of DMD where the mother is not a carrier of the disease represents a mutation which must occur only in female meiosis with no paternal contribution. Familial cases would be assumed to have a contribution of both male and female meiotic deletion events. Thus, if the frequency of deletion mutations at the DMD locus were nearly equal in males and females, one might assume that a higher incidence of deletions, as presented in Table 1, would be observed in familial cases. Prediction of affected individuals in families segregating a deletion which is assumed to be the primary genetic cause of the disease (of more than 150 normal boys, no deletions were observed) should be highly accurate.

As indicated in Table 1, two boys with BMD were found to have a deletion of the DXS164 region. Linkage analysis has shown that the locus for BMD is localized near, or is a potential allele of, DMD[6-9]. The finding of deletions in the DNAs of BMD males which overlap with deletions found in DMD males suggests that if they are two separate loci they are indeed close to one another. Alternatively, if BMD and DMD are caused by different alleles at the same locus, then the milder BMD phenotype might be expected to be the result of a low-level expression of the DMD/BMD gene product. If this were the case, the BMD deletions might not involve DNA sequences that are absolutely necessary for the expression of the DMD/BMD gene product.

The three DNA probes from the DXS164 region that were distributed to other investigators each recognize the informative RFLP alleles given in Table 2. The separate RFLP alleles of pERT87-1 and -8 exhibit a degree of disequilibrium and are only informative in ~60% of women tested, despite there being many different enzyme-defined loci. The three separate loci of pERT87-15 defined by different enzymes, although in some degree of disequilibrium with each other, are closer to equilibrium with the pERT87-1 and -8 loci. When the three enzyme-defined loci of the pERT87-15 probe were used in combination with the *Bst*XI locus of pERT87-8 and the *Xmn*I locus of pERT87-1, 25 of 28 (89%) unrelated women at risk for DMD or BMD were observed to be informative for linkage between one or more of the RFLP-detecting loci and the disease locus.

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Table 1 Structural alterations detected at the DXS164 locus in DMD males

Contributing laboratory	Clinical description			Deletions		
	DMD-F	DMD-S	BMD	DMD-F	DMD-S	BMD
R. Bartlett, Durham*	11	9	2	3	1	0
M. Bobrow, London	75	50	24	7	2	1
T. Caskey, Houston	22	18	0	5	3	0
K. Davies, Oxford†	42	68	0	0	2	0
A. de la Chapelle, Helsinki	10	20	2	0	1	1
M. Denton, Sydney*	12	15	0	6	0	0
R. Doherty, Rochester	32	61	8	4	4	0
A. Emery, Edinburgh†	0	30	0	0	0	0
M. Ferguson-Smith, Glasgow	37	44	17	3	3	0
K. Fischbeck, Philadelphia*	25	27	4	1	1	0
U. Francke, New Haven	11	1	1	1	0	0
C. Greenberg, Manitoba	3	0	1	1	0	0
P. Harper, Cardiff	50	43	35	4	1	0
V. Ionasescu, Iowa City	16	21	5	2	1	0
C. Junien, Paris	51	2	0	1	1	0
C. Boehm, Baltimore	12	5	0	1	0	0
J. C. Kaplan, Paris	52	29	7	3	2	0
L. Kunkel, Boston*	14	29	0	1	3	0
J.-L. Mandel, Strasbourg	12	13	0	0	0	0
P. Pearson, Leiden	43	24	8	5	3	0
A. Read, Manchester†	22	32	0	2	2	0
G. Romeo, Genova	7	6	10	1	1	0
A. Speer, E. Berlin†	51	0	18	1	0	0
U. Tantravahi, Boston	8	1	0	2	0	0
R. Worton, Toronto*	32	3	3	0	1	0
Total	650	551	145	54	32	2
Percentage				8.3%	5.8%	1.4%
Overall total			1,346		88 (6.5%)	

The DNAs isolated from 1,346 DMD and BMD males from separate kindreds were cleaved with various restriction enzymes. Diagnosis of DMD and/or BMD was established by characteristic history, physical examination, increased CPK levels and, in many cases, a positive muscle biopsy[19-21]. Following separation of digested DNA by electrophoresis, the DNA samples were hybridized[22] with one or more of the DXS164 subclones pERT87-1, pERT87-8 and pERT87-15. Not all samples were hybridized with all the subclones, but most were hybridized with pERT87-8. The investigator's laboratory in which the DNA isolations and, in most cases, the hybridizations were accomplished are indicated. The patients were divided into three categories: DMD-F, DMD males from a family in which there was more than one affected individual or, in a few cases, where the mother had an elevated CPK, but no clear family history of DMD; DMD-S, DMD males where there was no family history of the disease and the mother had a normal CPK value; BMD, males who exhibited the less severe X-linked myopathy. The number of boys studied within each of these three categories is shown for each laboratory. Deletions were defined as complete absence of hybridization for a DXS164 subclone and the number of individuals with absent fragments is given in each category. Those DMD males who exhibited deletions were representative of the clinical spectrum for DMD and included males who were mentally retarded as well as those not mentally retarded. Many DMD boys who were diagnosed as mentally retarded did not exhibit a deletion of DXS164 subclones. Those BMD males who exhibited deletion of DXS164 subclones were both on the more severe side of the BMD clinical spectrum, but both were wheelchair-bound later than a typical DMD patient: one was from a familial case of BMD and the other was a sporadic occurrence of BMD. Both are functioning well in their mid-twenties and one male is still able to drive a car.

* Most of these DNA samples were obtained from the individual listed and were sent to L.M.K. in Boston, where they were tested for deletion.

† Most of these DNA samples were sent to K.E.D. at Oxford, where they were tested for deletion. K.E.D. received other DNA samples from Dr Schwarts (Denmark) and Drs Ch. Coutelle, Spiegler, Herrmann and Szibor (FRG).

Segregation analysis of these RFLP-detecting loci in multiple families at risk for BMD and DMD has shown that recombinants are possible between DXS164 and the disease locus; one such recombinant is shown in Fig. 1. The family consists of two affected sons and a daughter whose level of creatine phosphokinase (CPK) and muscular hypertrophy indicate a carrier status. The boy indicated by an arrow in the figure has clearly received from his mother a 2.2-kb hybridizing *Bst*XI fragment for pERT87-8, whereas his affected brother and carrier sister have each received a 4.4-kb fragment. Thus, the X chromosome of the boy carrying the 2.2-kb fragment must be the result of a recombination event between DXS164 locus and DMD.

To determine more accurately the meiotic exchange points in this family and to minimize the possibility of sample error, the same family was also tested for segregation with other Xp cloned loci. The *Pst*I RFLP detected by the cloned probe 754 (DXS84; ref. 10) also exhibited recombination in the same individual, whereas the other loci, DXS41 (99-6) and DXS43 (D2)[11,12], appear to be non-recombinant. This is an unexpected result based on previous notions of gene order for these loci on the X chromosome[10-15]. Physical mapping of these marker loci using panels of hybrid cells[16,17] containing translocation chromosomes from two independent X; autosome translocations found in females with DMD localize DXS164 (ref. 14), DXS41 and DXS43 (refs 11, 12) on the pter side and DXS84 (refs 13, 17) on the centromere side of the translocation breakpoints. If the translocation exchange points in these two patients are in the DMD gene[17,18], the order of these loci would be as presented in Fig. 1 ('old order'). The recombination data of Fig. 1 are inconsistent with this order in this particular family. There must be a recombination event between DMD and DXS84(754), one between DMD and DXS164(pERT87-8), and another between DXS164(pERT87-8) and DXS41(99-6) for this latter marker to appear non-recombinant. The presumed order would require three meiotic exchanges within a region of previously closely linked markers[9,15]. An alternative, and perhaps more likely explanation is that the DMD mutational site in this family maps between DXS41 and DXS164 and a single recombination (indicated at the bottom of Fig. 1) between DXS164 and the DMD mutation site would explain the results.

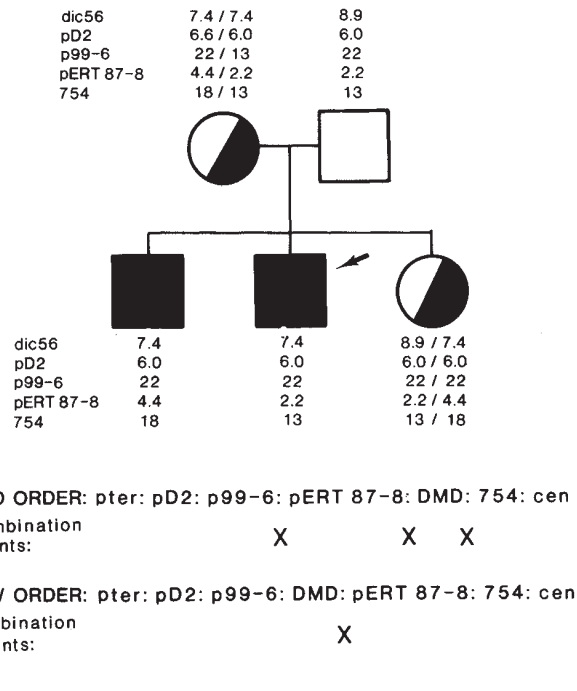

Fig. 1 Segregation of five Xp cloned loci in a DMD family. ○, Females; □, males; ■, DMD-affected individual; ◑, DMD carrier. DNA was isolated[11] from five members of a DMD kindred. Diagnosis of DMD was established as described in Table 1 legend, and the mother was judged to be a carrier of the disease as she exhibited an increased concentration of CPK[19,20] and had given birth to two affected individuals. The mother, though, had no knowledge of any other case of DMD in her family. The daughter was judged to be a carrier as she had extremely elevated CPK (relative to other female carriers) and exhibited hypertrophy of the leg muscles, although no weakness. The DNA samples were cleaved with the various restriction enzymes appropriate for each of the cloned DNA probes. Gel electrophoresis, transfer and hybridization were as described previously[11]. The different loci were tested by hybridization with the following cloned DNA fragments (given from the most distal): DXS143, dic56; DXS43, pD2; DXS41, p99-6; DXS164, pERT87-8; and DXS84, 754 (the most proximal). The various cloned segments had been physically mapped previously[10-14]. The numbers above and below each symbol indicate the size of hybridizing restriction fragments (alleles). Segregation of the locus DXS143 (ref. 23) is included to show that the samples from the non-recombinant boy and the father were not inverted. Two possible orders for the loci are indicated at the bottom of the figure: the first places the DMD locus at the point where the translocation breaks occur in two females with DMD; the second order (new order) represents an interpretation of the results obtained in the family study.

In the cases where DXS164 has been observed to recombine with DMD (eight known instances) and where DXS84 was also informative (five instances), both markers were recombinant. These results are consistent with the fact that the mutations giving rise to DMD in some families lie distal to DXS84, two translocation breaks in Xp21 (refs 16, 17) and the DXS164 locus. Thus, DXS164 may lie between these two translocation breaks and additional sites of mutation, all of which yield the phenotype of DMD and/or BMD. Alternative explanations for the linkage results could include a different X-linked locus with a phenotype very similar to DMD or new mutations which would appear as recombinants. Potentially consistent with the latter possibility is the observation that only one of the eight DXS164 recombinants observed occurs in the third generation or thereafter in a multi-generation DMD family.

For whatever reason, the exclusive use of DXS164 cloned probes as linked markers would lead to some mistakes when predicting the inheritance of the DMD X chromosome. This result has major implications for the prediction of the DMD genotype. Although the exact error rate is difficult to calculate (because only recombinants for other closely linked markers were tested for the segregation of DXS164 clones), best estimates can be made from the observation that approximately one-half of DXS84 recombinants (estimated at 10% recombination; ref. 9) are also DXS164 recombinants. This leads to the assumption that DXS164 will be in error in predicting the DMD X chromosome 5% of the time. It is strongly advised that the DXS164 locus be used in combination with other Xp21 flanking markers to construct an Xp21 haplotype. Given that the DXS164 locus seems to be localized between mutations found in different families which yield the DMD phenotype, DXS164 should not be considered as a flanking marker to DMD with other Xp21 loci. In a diagnostic situation, it would be unknown in which direction the particular mutation actually occurred relative to DXS164. Combined multi-locus information should improve the accuracy of genotype prediction in DMD families, assuming, of course, that the observed prediction errors for the DXS164 locus are caused by recombinants and not by new mutations or a different, as yet uncharacterized, locus.

The deletions observed in the DNAs of boys with DMD (and also BMD) provide the means to start to define important segments of Xp21 which might be involved in the normal functioning of the DMD locus. Therefore, 53 of the 88 DNA samples which constituted deletions for the three DXS164 subclones distributed were tested for the absence or presence of additional subclones from the entire 137 kb of DNA from the DXS164 locus. A representative hybridization analysis is shown in Fig. 2 for five DXS164 subclones hybridized to nine different deletion DNA samples. The subclones are located within the DXS164 locus on either side of pERT87-1 and -8, and the five subclones combined can detect size differences between *Pst*I-hybridizing fragments over 45 kb of the DXS164 locus. Among the nine deletion DNA samples, five showed no DXS164-hybridizing *Pst*I fragment. The remaining four DNA samples exhibited some fragments but not others; these samples thus have breakpoints within the DXS164 locus, and one (Fig. 2, lane 3) of the breakpoints is preliminarily determined as an altered *Pst*I hybridizing fragment. By using additional subclones from the DXS164 locus, each breakpoint can be mapped more precisely to a specific subregion. Once localized to a specific region, the appropriate restriction enzyme can be chosen to best demonstrate the actual breakpoint (within 100–1,000 base pairs (bp)).

Table 2 RFLP-detecting subclones of DXS164

Subclone	Insert size (kb)	Enzyme	Allele size (kb)		Allele frequency	
			p	*q*	*p*	*q*
pERT87-1	1.3	*Bst*NI	3.1	2.5/0.6	0.63	0.37
		*Xmn*I	8.7	7.5	0.66	0.34
pERT87-8	1.3	*Bst*XI	4.4	2.2	0.6	0.4
		*Taq*I	2.7/1.1	3.8	0.71	0.29
pERT87-15	1.5	*Bam*HI	7.1/2.3	9.4	0.62	0.38
		*Taq*I	3.1	3.3	0.67	0.33
		*Xmn*I	1.6/1.2	2.8	0.68	0.32

The three DXS164 subclones are listed together with the sizes of their cloned human DNA inserts. The enzyme site variation detected by each probe is represented by the sizes of the hybridizing fragments and the respective frequencies of the common allele (p) and the rare allele (q). The allele frequencies were calculated in each case from the results obtained with over 75 X chromosomes.

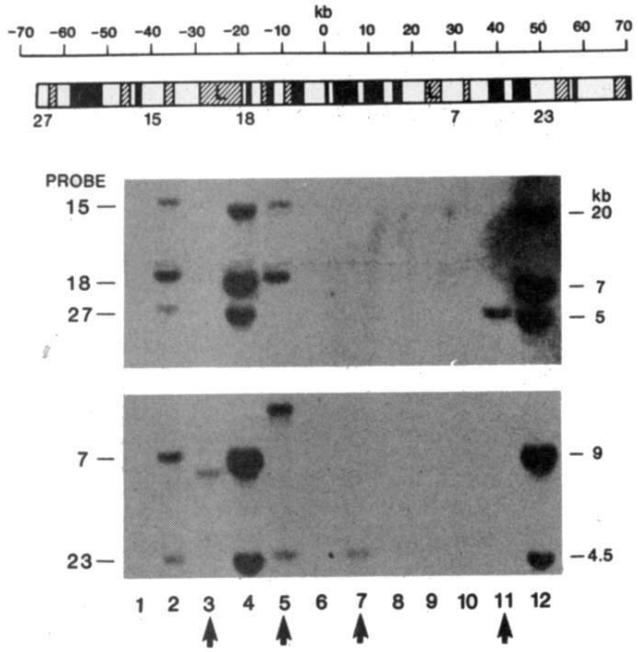

Fig. 2 Demonstration of four deletion breakpoints within the DXS164 locus. The DXS164 locus was expanded by chromosome 'walking'[3] as described prevously[1]. A schematic drawing of 137 kb of contiguous human DNA is presented at the top. Solid bars represent regions of repeated DNA sequences; open boxes represent regions of unique sequence. Cross-hatched boxes represent moderately repeated sequences, and two copies of LINE sequences[24] are indicated by the letter L. The numbers beneath the schematic diagram give the designations for five of the unique-sequence subclones used to determine the extent of deletions in DNA samples from nine DMD males. After digestion with *Pst*I, each DNA sample was tested for the presence or absence of various human *Pst*I fragments by hybridization[22] with the various subclones. In the two different autoradiographs presented, the hybridizing *Pst*I fragment for each subclone is indicated to the left of the figure; these are evident in lanes 2, 4 and 12, where the DNA samples isolated from individuals who do not bear deletions of DXS164 subclones are presented. The sizes of the respective *Pst*I hybridizing fragments for normal individuals are given to the right of the figure. The DXS164 subclones pERT87-18, -15 and -27 were radiolabelled separately[11] and hybridized simultaneously to a nitrocellulose filter containing the 12 immobilized *Pst*I-cleaved DNA samples. The DXS164 subclones pERT87-7 and -23 were also radiolabelled separately and hybridized together to a duplicate nitrocellulose membrane with the same immobilized *Pst*I-cleaved DNA samples. The different hybridization intensities of the *Pst*I fragments were due to differences in specific activity and hybridization characteristics of the individual probes. Lines 4 and 12 contained 5 μg of *Pst*I-cleaved DNA, whereas the others each contained ~1 μg. Lanes 1, 6 and 8–10 show no hybridization for any of the five DXS164 subclones. Lanes 3, 5, 7 and 11 (arrowed) exhibit at least one DXS164-hybridizing *Pst*I fragment. The deletion DNA sample in lane 3 exhibits an absence of the subclones (left-hand side), pERT87-27, -15 and -18 and has a single altered *Pst*I fragment for one of the subclones on the right-hand side (either pERT87-7 or -23). The altered fragment is presumably detected by the 23 subclone, and the hybridization results indicate that the deletion breakpoint occurs near pERT87-23 and the deletion extends in a leftward direction. The DNA sample in lane 7 exhibits similar hybridization results to the sample in lane 3, but the pERT87-23 hybridizing *Pst*I fragment is normal in size. This DNA sample is presumed to have a different deletion breakpoint between pERT87-7 and pERT87-23 compared with that in lane 3 and the deletion also extends to the left. The DNA sample in lane 5 exhibits a normal-sized *Pst*I hybridizing fragment for pERT87-23, a rare allele of a *Pst*I RFLP detected by pERT87-7 (~10% of both normal and DMD males exhibit this larger *Pst*I fragment) and normal-sized hybridizing *Pst*I fragments for pERT87-18 and -15. The pERT87-27 subclone exhibited no hybridization. This DMD male has not been previously shown to have a deletion of pERT87-8 or -1 and has a deletion which starts between pERT87-15 and pERT87-27 and extends also to the left. The DNA sample in lane 11 carries a deletion which breaks near that presented in lane 5, but the deletion extends in the opposite direction. pERT87-27 hybridizes to a normal-sized *Pst*I fragment whereas the remaining subclones are absent.

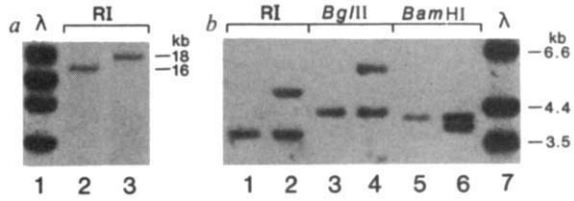

Fig. 3 Demonstration of deletion breakpoints for two independent deletions. Restriction enzyme cleavage, radiolabelling and hybridization were as described in Fig. 2 legend and ref. 1. In *a*, a deletion sample previously shown to carry pERT87-1 and a normal-sized hybridizing *Pst*I fragment, showed no hybridization for pERT87-8 or other subclones to the left of this one. Subclone pERT87-14, between pERT87-1 and -8, was used to detect by hybridization an altered *Eco*RI fragment in the deletion DNA sample (lane 2; compare with a normal-sized *Eco*RI fragment in lane 3). The normal *Eco*RI fragment stretches across pERT87-8 and, based on other restriction enzyme sites in the region, the breakpoint of this deletion has been determined to fall between the right side of pERT87-8 and a *Pst*I site 1.5 kb farther to the right. *b*, Demonstration of a deletion breakpoint detected with the pERT87-1 subclone. The deletion sample originally shown to be a deletion for pERT87-15 and -8 exhibits a break just to the left of pERT87-1. Lanes 1, 3 and 5, *Eco*RI, *Bgl*II and *Bam*HI digests, respectively, of a normal male; lanes 2, 4 and 6, *Eco*RI, *Bgl*II and *Bam*HI digests, respectively, of DNA isolated from a female heterozygous for the deletion. Both *Eco*RI and *Bgl*II reveal a larger altered fragment than *Bam*HI. The deletion breakpoint lies ~500–1,000 bp to the left of pERT87-1.

Figure 3 shows two such determinations for two independent deletions. In one case (Fig. 3*a*, lane 2), an altered hybridizing *Eco*RI fragment is detected with the pERT87-14 subclone (located between pERT87-8 and pERT87-1). Comparison of the size of the altered *Eco*RI fragment with that seen in normal individuals (Fig. 3*a*, lane 3) and the knowledge that pERT87-8 is absent indicate that this deletion breakpoint maps between the pERT87-8 segment and an unaltered *Pst*I site 1.5 kb towards pERT87-14. In the other example, different-sized hybridizing fragments for *Eco*RI (5.0 kb), *Bgl*II (5.8 kb) and *Bam*HI (3.9 kb) were observed in the DNA sample isolated from a female heterozygous for a deleted X chromosome. A sample isolated from an unaffected male (Fig. 3*b*, lanes 1, 3, 5) exhibited the normal hybridizing fragments for the three enzymes.

Most deletions were found to be larger than 137 kb, for no subclone from within the entire cloned region was found to be present. Among the 57 deletions tested, 24 showed the presence of some subclones but not others (the data are summarized in Fig. 4); these were deletions which had breakpoints within previously cloned DNA of the DXS164 locus. Fourteen deletions break on the left side of the cloned DNA and extend in the rightward direction off the DXS164 map towards the centromere (the direction of the DXS164 map relative to the centromere was established by analysing some deletion samples with the XJ-1.1 cloned segment; see Fig. 4 legend). Nine deletions break on the right of the map and extend in a leftward direction away from the centromere. Although some deletions are presented as similar, most have been shown to have independent breakpoints within the DXS164 locus (see Fig. 4 legend) and are schematically presented together when the breakpoints are near each other. It is apparent from Fig. 4 that there is a common region which is absent from almost all deletions except the six shown in the lower part of the figure. This region of common deletion overlap may be slightly biased due to the fact that the first probe made available to other investigators was pERT87-8, which resides near the overlap region. Further studies on additional DMD boys using all the DXS164 subclones should confirm whether this common region of overlap contains DNA sequences important for the expression of the DMD phenotype, or whether it represents an ascertainment bias.

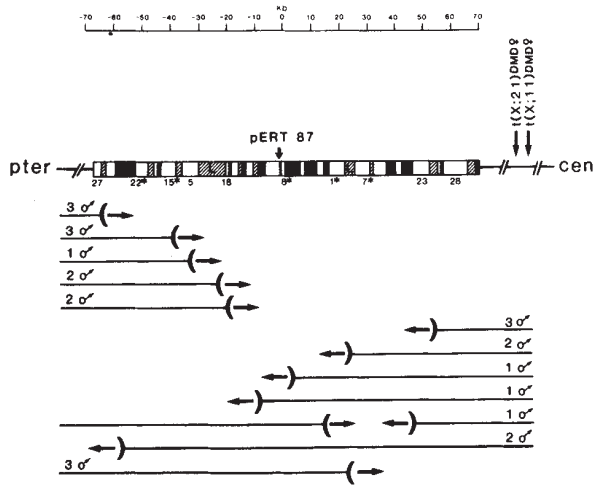

Fig. 4 A schematic representation of deletion breakpoints in the DXS164 locus. Fifty-seven DNA samples (53 samples shown to be deletions of one of the distributed subclones and 4 additional DNA samples shown to be deletions of either XJ-1.1 (ref. 2) or other DXS164 subclones) were tested for the absence or presence of various DXS164 subclones as presented in Figs 2, 3. Twenty-four of the 57 DNA samples showed the presence of at least one DXS164-hybridizing *Pst*I fragment, whereas the remaining 33 DNA samples exhibited no DXS164-hybridizing *Pst*I fragment—the entire 137 kb was missing. By using a combination of subclones including those exemplified in Fig. 2, the approximate positions of the 24 different breaks were determined and are indicated by a bracket with an arrow indicating the direction of the missing DNA. Certain breakpoints which were similar are combined, and the number of males combined is indicated at the side of the figure. Nine deletion breakpoints were determined to the extent where an aberrant hybridizing restriction fragment was identified; these are localized within a few kilobases within the DXS164 locus. Nine deletions were determined to be different from the first nine, as revealed by the pattern of their hybridization with DXS164 subclones, but an altered restriction fragment has yet to be identified and these are thus only localized to within 1–5 kb. Six deletion breakpoints (the most recently acquired) were only mapped to 10–30-kb regions of the DXS164 locus and have not been definitely shown to differ from the other 18. Some of the deletions presented have also been tested for hybridization with the previously described clone XJ-1.1 (ref. 2): of those tested that break within DXS164 and extend to the right, all show no hybridization with XJ-1.1. For those tested that break in DXS164 and extend to the left, XJ-1.1 is present. The hybridization results with XJ-1.1 allow localization of the DXS164 subclones relative to the centromere as well as the (X; 21) translocation breakpoint, as indicated in the figure. Four of the six deletion DNA samples depicted at the bottom of the figure were not determined to be deletions by the original subclones pERT87-1, -8 or -15; two were found to be deletions for the XJ-1.1 cloned segment and were subsequently mapped with additional DXS164 clones, and the remaining two deletions were detected only with the leftmost DXS164 subclone, pERT87-27.

Screening of boys with additional pERT87 subclones and with the XJ-1.1 clone detected four deletions which would not have been detected with the originally distributed clones. These deletions were found among 269 boys who had been shown previously not to carry deletions of the pERT87-1, -8 or -15 subclones. These four deletions are not included in the data presented in Table 1 but are shown in Fig. 4; it is assumed that the true proportion of DMD and BMD boys who have deletions of portions of their X chromosomes would be greater than that presented in Table 1, where only three probes were used. Analysis of DMD or BMD DNA samples with the most distal pERT87-27 subclone, the three central subclones pERT87-1, -8 and -15, and the XJ-1.1 subclone should detect most of the deletions that are currently detectable.

The breakpoints of six deletions are different from most others; these are shown at the bottom of Fig. 4. One deletion is completely encompassed within cloned DNA and removes only 45 kb of DNA. Three deletions break within this 45-kb region and extend off the map towards the centromere; two of these were originally detected by the more centromeric cloned segment XJ-1.1 and subsequently mapped within the DXS164 locus. Two other deletions (detected with subclones of DXS164 other than pERT87-1, -8 and -15) break at the extreme left end of the cloned DNA and extend off the map for an undetermined distance. The central location of DXS164 subclones 1, 8 and 15 to DMD and/or BMD mutational sites, inferred from family studies and translocation breakpoint mapping, is substantiated by these additional deletion DNA samples. The profile of deletion breaks indicates that there must be a large segment of DNA which, when disrupted, can yield the phenotype of DMD and/or BMD. The fact that large deletions yield a DMD phenotype similar to that of other DMD boys who have not been demonstrated to bear deletions, indicates that the product of the locus can be either completely absent or aberrant and still yield a similar clinical picture.

The pattern of deletions implies that the gene(s) responsible for DMD must lie on a very large segment of X-chromosomal DNA. This is substantiated by the fact that recombinants are observed between cloned segments in the region and some disease mutation sites. For the future of diagnosis, the deletions represent a ready means of obtaining additional cloned segments from the other side of those deletions that break within DNA that has already been cloned. These junctional fragments from deletion patients are presently being cloned and the development of new RFLP-detecting probes to mark the entire region in which mutation can yield DMD and BMD is imminent. The deletion breakpoints also demarcate the regions of the DXS164 locus that might contain information important for the expression of the gene responsible for DMD and/or BMD. Analysis of these breakpoints at the level of the nucleotide sequence may also elucidate the mechanism underlying the deletions.

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