A *TaqI* map of the dystrophin gene useful for deletion and carrier status analysis

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Abstract

We describe a partial *TaqI* map of the dystrophin gene, obtained mainly by analysis of 87 overlapping DMD/BMD deletions with small fragments of the dystrophin cDNA probes; exon 6 of the dystrophin gene was identified on the *TaqI* map using the polymerase chain reaction. The cDNA probes detect five polymorphisms with *TaqI*, more than with *HindIII* (one), *BglII* (four), or *PstI* (three). The five polymorphisms are analysed concomitant with screening for deletions on the *TaqI* map, and in the one-third of DMD/BMD cases with no detected deletion the polymorphism information may be used for counselling. Correlation of the *TaqI* map with the *HindIII* map in the region of probes 5b-7 and 8 has allowed the establishment of reading frame. In this region of the dystrophin gene, all of 41 DMD deletions resulted in a shift of reading frame and all of 10 BMD patients maintained reading frame, in agreement with the 'reading frame hypothesis'.

Duchenne and Becker muscular dystrophies (DMD/BMD) both arise from mutation of the dystrophin gene on the short arm of the X chromosome. In most cases, deletions of the dystrophin gene are detected, allowing direct DNA based prenatal and carrier diagnosis.¹⁻³ However, in approximately one-third of cases, no mutation is detected and counselling is offered by linkage analysis of polymorphic markers. The entire dystrophin cDNA has been cloned and the *HindIII* exon containing fragments have been ordered into a partial map.^{4,5} The normal exon containing restriction fragments and polymorphisms detected by hybridisation with the cDNA probes have been described for *HindIII* and *BglII*, and polymorphisms were reported for two additional restriction enzymes, *PstI* and *TaqI*.⁶ In order to facilitate concomitant screening for deletions, duplications, and polymorphisms, we characterised the normal cDNA hybridisation pattern for *TaqI* and arranged the exon containing fragments into a partial *TaqI* map.

Materials and methods

CLINICAL EVALUATION

A total of 128 families (98 DMD and 30 BMD) was examined by neurologists of the MDA clinics at Duke University Medical Center, Durham, NC, USA, and the Australian

Neuromuscular Research Institute, Queen Elizabeth II Medical Centre, Perth, WA, Australia. One additional sample of patient DNA was provided by Dr M J Denton, the Prince of Wales Hospital, Sydney, NSW, Australia. The probands were diagnosed using standard clinical diagnostic criteria for DMD/BMD in conjunction with raised creatine kinase activity and typical dystrophic changes on muscle biopsy. Diagnosis was also confirmed in 14 families by dystrophin analysis (Genica, Inc, Worcester, MA) or immunohistochemistry using antidystrophin antibodies.⁷

RESTRICTION DIGESTION, SOUTHERN TRANSFER, AND HYBRIDISATION

DNA isolated from whole blood or from lymphoblast cell lines was digested with restriction enzymes *TaqI*, *HindIII*, *BglII*, or *PstI* (BRL, Gaithersburg, MD, USA; NEB, Beverly, MA, USA; Toyobo, Kitaku, Osaka, Japan). The resulting DNA fragments were separated by agarose gel electrophoresis, Southern transferred onto GeneScreen Plus membrane (NEN DuPont, Boston, MA), hybridised to an oligolabelled probe, and autoradiographed. These methods have been described in detail previously.^{1,8,9}

cDNA PROBES

The six probes comprising the 14 kb dystrophin cDNA were kindly provided by Dr L M Kunkel⁴ or were obtained from ATCC. To order the *TaqI* exon containing fragments, probes 1-2a, 5b-7, and 9-14 were each divided into smaller probe fragments (fig 1) for

Figure 1 Dystrophin cDNA probe fragments. Restriction enzymes: B = *BamHI*, C = *HincII*, E = *EcoRI*, G = *BglII*, H = *HindIII*, P = *PstI*, X = *XbaI*.

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Figure 2 Normal hybridisation pattern of the cDNA probes for *TaqI*. (A) cDNA probes 1-2a to 5b-7, (B) cDNA probes 8 to 11-14. Constant bands are indicated by horizontal bars; the five polymorphisms are indicated by arrows (sizes in kb). DNA in the left hand lane for probe 2b-3 and the right hand lane for probe 8 is from a female; all other lanes contain DNA from males.

hybridisation in some experiments. Probe 5b-7 was used to prepare two different sets of

probe fragments, so that the order of *TaqI* exon containing fragments at the 5' end of this

Table 1 *TaqI* polymorphisms detected with dystrophin cDNA probes.

Probe	Allele size (kb)		Frequency	No
	Frequent allele	Rare allele		
2b-3	3.4	3.6	0.54/0.46	112
5b-7-a	3.3	1.7	0.52/0.48	105
8	6.7	5.9	0.94/0.06	125
9-14-b	1.7	2.2	0.93/0.07	106
9-14-c	1.2	1.4	0.73/0.27	63

region could be resolved. Digestion of the *EcoRI* insert of probe 5b-7 with *XbaI* and *HincII* yielded probe fragments 5b-7-a, 5b-7-b, and 5b-7-c, which were used for routine deletion screening; digestion of the 5b-7 insert with *PstI* and *BglII* yielded alternative probe fragments 5b-7-P and 5b-7-G (fig 1), plus two small fragments (0.2 and 0.5 kb) not used for hybridisation.

PCR AMPLIFICATION

Oligonucleotide primers for exon 6 were designed from the published sequence of the gene¹⁰: forward 5'-GTC AAA AAT GTA ATG AAA AAT ATC ATG-3'; reverse 5'-TAT GAC TAT GGA TGA GAG CAT TCA AAG-3'. Amplification was performed using *Taq* DNA polymerase (Biotech International, Bentley, Western Australia) with 5 ng target DNA in a final volume of 25 µl under the supplier's reaction conditions. The cycle profile was: 94°C for three minutes, 55°C for one minute, 75°C for two minutes (one cycle); 94°C for 30 seconds, 55°C for one minute, 75°C for two minutes (39 cycles).

Results

The normal hybridisation pattern of the cDNA probes on *TaqI* digested DNA shows a total of 68 exon containing *TaqI* fragments, representing approximately 250 kb of genomic DNA (fig 2). The cDNA probe 1-2a detected 10 *TaqI* fragments, with four of these detected by 1-2a-a and seven by 1-2a-b. Both parts of 1-2a detected the 1.8 kb fragment containing the cDNA *HincII* site of exon 4.¹⁰ Probe 2b-3 detected a two allele RFLP and eight constant fragments. One of the constant fragments (2.4 kb) is also detected by probe 1-2a owing to overlap of the two probes in exon 11.^{6,10} Probe 4-5a detects eight fragments. Probe 5b-7-a detects six constant fragments and a two allele

RFLP. Probes 5b-7-b and 5b-7-c detect three and four fragments, respectively. Probe 8 detects a two allele RFLP and four constant fragments, one of which (3.9 kb) is also detected by 5b-7-c. A common fragment is also detected by these two probes on *HindIII* digests.⁶ Probe 9 detects five constant fragments; probes 10 and 11-14-a both detect a two allele RFLP, as well as five and eight constant fragments, respectively. Probe 11-14-b detects four constant fragments; one of these (4.4 kb) is also the only fragment detected by 11-14-c.

The cDNA probes 2b-3, 5b-7-a, 8, 10, and 11-14-a each identified a *TaqI* RFLP, making a total of five cDNA RFLPs which are detected with this enzyme, spanning the length of the dystrophin gene^{6,11,12} (table 1). When intact cDNA 5b-7 is hybridised to *TaqI* blots, the lower 1.7 kb allele comigrates with a constant band, so that densitometric scanning of the autoradiograph is necessary for RFLP analysis.⁶ However, the smaller cDNA fragment 5b-7-a detects the RFLP but not the comigrating constant fragment, so that RFLP analysis can be performed directly (fig 2). The 1.7 kb constant band is detected by probe segment 5b-7-c.

A total of 128 patients (98 DMD, 30 BMD) was screened with the cDNA probes, and deletions were observed in 72% (71/98) of DMD cases and 53% (16/30) of BMD cases, for an overall deletion frequency of 68%. In two of the DMD cases, all exon containing bands were detected, plus an additional junction fragment, seen both on *TaqI* and *BglII* digests; these cases were presumed to arise from duplication events. *TaqI* and *HindIII* digests of individual DNA samples were screened sequentially with the entire cDNA in most cases, allowing the extent of any deletion to be determined. Deletions in different families were compared, and regions of overlap were used to determine the order of *TaqI* exon containing fragments (fig 3). The *TaqI* fragment corresponding to exon 6 was identified by hybridising radiolabelled reaction product from a PCR reaction to a *TaqI* digest previously hybridised with cDNA 1-2a. This was done since no patients were deleted solely for either the 7.7 or 1.4 kb bands, now identified as corresponding to exons 6 and 7, respectively. In the region detected by probe fragments 5b-7-a and 5b-7-b (fig 1), only one deletion was informative for fragment order. Hybridisation of alternative probe fragment 5b-7-P (fig 1) to *TaqI* digests detected the 4.6, 2.5, 0.9, 0.8, and 3.6 kb bands also seen by probe fragment 5b-7-a. The alternative probe fragment 5b-7-G detected the 3.6, 7.0, and 3.3/1.7 kb bands of probe fragment 5b-7-a, plus the 9.4 and 2.2 kb bands of probe fragment 5b-7-b. Combining information derived from the informative deletion and from hybridisation with the two sets of probe fragments, the order of *TaqI* fragments at the 5' end of 5b-7 could be determined (fig 3). In the region detected by probes 5b-7-c and 8, which is the region of most frequent deletion, each exon containing *TaqI* restriction fragment was correlated with the

Table 2 Deletion frequencies detected with cDNA probes on *TaqI* digested DNA.

Probe	Deletions/families tested	%
1-2a	18/133	14
2b-3	19/136	14
4-5a	10/127	8
5b-7-a	5/115*	4
5b-7-b	4/135	3
5b-7-c	42/133	32
8	57/140*	41
9	7/117	6
10	1/107	1
11-14-a	0/63	0
11-14-b	0/52	0
11-14-c	0/56	0

* One probable duplication also detected (see Results).

Figure 3 A *TaqI* map of exon containing fragments of the Duchenne muscular dystrophy gene. The cDNA probes are shown in the left hand column. Exon containing fragments hybridising to each probe are shown in the box, in the most likely order (sizes in kb). Brackets indicate bands for which the order has not yet been determined. The positions of genomic probes XJ1.1, pERT87-15, pERT87-JBir, and P20, and the exon containing fragments which detect *BanI* and *PstI* polymorphisms, are indicated on the *TaqI* map. Vertical bars show the extent of *TaqI* deletions detected in 83 DMD/BMD families. Solid bars denote definite deletions; dashed bars denote the possible maximum extent of deletion for faintly hybridising bands difficult to score accurately. Family numbers are given above each deletion. * denotes BMD families. J = junction fragment.

corresponding fragment of the *HindIII* map.^{4,5,13} The fragment order for *TaqI* was consistent with that for *HindIII* in all cases tested. This allowed the exon border type of deletions in this region to be established.¹⁴ In all 41 DMD and 10 BMD cases tested, DMD patients had deletions which are predicted to cause a frameshift in the translational reading frame, while BMD patients had deletions which are predicted to maintain the reading frame (fig 4). Additional screening with intragenic genomic probes was performed, and XJ1.1, pERT87-15, pERT87-JBir, and P20 were located on the *TaqI* map. The *TaqI* RFLP detected by genomic probe pERT87-15 was found to be identical to that detected by cDNA 2b-3 (fig 5).

Discussion

Deletion screening on *TaqI* digested DNA allows for concurrent analysis of five RFLPs, so in those families with no detected deletion

or duplication counselling by conventional linkage analysis may be facilitated. The *TaqI* and *HindIII* maps were correlated over nine exons detected by cDNA probes 5b-7 and 8. Although absolute confirmation of order would require sequencing of the *TaqI* exon containing fragments to ensure that no small fragments had been omitted, in all 51 cases tested in this region the deduced order of exon containing fragments for *TaqI* and the *HindIII* order⁴ were consistent.

Similar deletions were observed in DMD and BMD patients, although in no case were the same exon containing fragments deleted in a DMD and a BMD family (figs 3 and 4). The 'reading frame hypothesis'¹⁴ predicted that frameshift deletions result in production of a truncated, unstable dystrophin protein, leading to severe phenotypes, while deletions that maintain the reading frame result in production of an internally deleted dystrophin protein that is partially functional, leading to mild phenotypes. Testing in a number of different

Figure 4 Correlation of *TaqI* and *HindIII* maps in the region of frequent deletion. The *TaqI* and corresponding *HindIII* exon containing fragments (ECF) are indicated for probes 5b-7-c and 8 (sizes in kb). The positions of genomic probes JBir and P20, and of the *BanI* and *PstI* cDNA RFLPs for probe 8 are indicated. P20 bands 1 and 2 are the 0.8 kb and 6.7 kb *TaqI* fragments detected with this probe, respectively.¹⁵ The border type number indicates the relative position of the intron/exon border with respect to the translational reading frame. A border of '3' denotes an exon/intron border occurring between intact codons of the mRNA (after codon position 3). Borders of '1' and '2' denote a border occurring after the first or second nucleotide of the codon, respectively.¹³ The predicted effect that a deletion would have upon frameshift of dystrophin mRNA is indicated. The extent of deletions in 10 BMD cases (open bars) and 10 DMD cases (solid bars) is indicated. An asterisk indicates a *TaqI* junction fragment detected with P20. To the right of the deletion is the family number; to the left is the total number of families observed with that deletion. (a) from reference 16; (b) one *TaqI* fragment contains two *HindIII* fragments; (c) two *HindIII* fragments contain a single exon¹⁷; (d,e) from reference 13.

patient populations has shown that in the majority of cases the predictions of disease severity based on the expected effects of deletion on dystrophin translation are correct; however, a few exceptions have been observed, particularly at the 5' end of the gene.¹³⁻²⁰ In the frequent deletion region of probes 5b-7 and 8 that we tested, all BMD patients had deletions that preserved the translational reading frame downstream to the deletion; all DMD patients had deletions that resulted in frameshift (fig 4). In the present study, deletion of the 4.1 kb *HindIII* exon containing fragment in family 91 produced a DMD phenotype. Baumbach *et al*¹⁹ reported that this same exon deletion could result in either a DMD or an 'intermediate' DMD/BMD phenotype, so that the predicted effects of exon deletions on transcriptional reading frame do not always allow accurate prognosis of disease severity.

Four genomic probes have been localised on the *TaqI* map, and permit more precise delineation of deletions. Genomic probe pERT87-15 was found to detect the same polymorphic exon containing *TaqI* fragment that is detected by the cDNA probe 2b-3. Fig 5 shows the use of cDNA 2b-3 in a DMD family for screening of deletions and polymorphisms concurrently.

Division of the cDNA to yield 15 probe segments ordered the *TaqI* exon containing fragments into 15 groups. For routine deletion

screening, however, it was not necessary to divide probe 1-2a, as all the bands were well separated; the alternative probe fragments 5b-7-P and 5b-7-G were also not needed for routine screening. Fig 3 shows the distribution of deletions detected, with a central high frequency deletion region and a secondary region of frequent deletion towards the 5' end. The overall frequency of deletions observed was 68%. No deletions were observed to extend 3' to cDNA 10 in our data set. Probes 1-2a, 2b-3, 5b-7-c, and 8 together detected 83 of 87 (95%) of all deletions in our data set, suggesting that these probes should be used in initial screening for deletions.

In conclusion, a partial *TaqI* map of exon containing fragments of the dystrophin gene is described, allowing concurrent detection of deletions/duplications and analysis of five polymorphisms. Genomic probes have been localised on the *TaqI* map, improving resolution of deletion detection. Oligonucleotide primers are described for the amplification of exon 6, allowing its identification on the *TaqI* map, so that fragments 6 and 7 could be ordered. The *TaqI* map has been correlated with the *HindIII* map in the high deletion frequency region of probes 5b-7-c and 8; all 51 deletion families screened in this region conformed with the 'reading frame hypothesis'. Although multiplex polymerase chain reaction (PCR) will probably be increasingly used for initial deletion screening, owing to the rapidity

Figure 5 Concurrent screening for deletions and polymorphisms on the TaqI map. (A) cDNA probe 2b-3 hybridised to TaqI digests of DMD family 423 DNA. Arrows indicate the polymorphic fragments at 3.6 and 3.4 kb. In this family, deletion involves the fragments at 8.8, 3.5, and 2.4 kb. (B) After stripwashing and autoradiography to verify removal of probe, the same blot was hybridised with the genomic probe pERT87-15. The position and inheritance of the alleles was identical to those of the 2b-3 RFLP, indicating that genomic probe pERT87-15 hybridises to the same exon containing fragment which shows a polymorphism in 2b-3, thus locating this genomic probe on the TaqI map.

of the process,¹⁷ primers for all exons have not yet been identified and characterised. Southern hybridisation allows conventional linkage analysis to be used in those families with no detected deletion, and will remain an important analytical tool until better techniques are developed for identification of putative micro-deletions and point mutations in the more than 2 Mb of the dystrophin gene.

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