Deletion analysis of DMD/BMD families from the German Democratic Republic and selected regions of Czechoslovakia and Hungary

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Abstract

Over the last two years we have screened 183 DMD/BMD families requesting prenatal diagnosis. Using cDNA probes cf56a,b we have detected exon deletions in 72 of them. In 62 cases the deletion was also detectable with currently available PCR primers. Deletion analysis for exons 8, 17, and 19, using either PCR or Southern blotting techniques, was performed for 65 of the 111 families which showed no deletions with cf56a,b. Eight of them were deleted for one or more of these exons.

PCR offers new possibilities for deletion analysis in families without a living patient using either Guthrie papers or histologically conserved material from the dead patient. In 20 of 25 patients, we observed concordance between the clinical picture and the molecular deletion analysis in accordance with the open reading frame hypothesis. Five patients, however, presented with DMD in spite of

our analysis showing an in frame deletion. Carrier determination in families in which DMD is caused by a deletion using linkage, dosage, or breakpoint analysis is discussed.

The introduction of DNA analysis for the diagnosis of Duchenne muscular dystrophy (DMD) and its milder allelic form Becker muscular dystrophy (BMD) has resulted in substantial progress in carrier determination and prenatal diagnosis for affected families. We describe here our methods of DNA diagnosis of DMD/BMD and discuss, in particular, the advantages and problems of the application of the polymerase chain reaction (PCR) for genomic diagnosis of this disease. A summary of the DNA analysis data collected during the last two years in the GDR and selected regions of Czechoslovakia and Hungary is given.

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Materials and methods

MOLECULAR PROBES

We used the cDNA probes cf56a and b, which correspond to exons in the central region of the gene,^{1,2} and the PCR primers described by Chamberlain *et al*³ and Speer *et al*.⁴ For carrier determination in the family shown in fig 1, a breakpoint specific PCR primer, set corresponding to the sequences surrounding the breakpoint (S England, in preparation), was applied.

MOLECULAR ANALYSIS

DNA preparation, restriction enzyme digestion, electrophoresis, transfer, and hybridisation were performed as described previously.⁵ The PCR procedure was performed according to Chamberlain *et al*.³

Results

In our experience, not only definite carriers but also women with a carrier risk of only 5% (the most common risk assessment by DNA linkage analysis)

Figure 1 Carrier determination by PCR dosage analysis. In this DMD family I.2 is a carrier according to CK estimation. II.1 is not a carrier by CK and linkage analysis (*Cf56a/PstI* restriction fragment length polymorphism flanking the deletion detected in the DMD patient II.2). 20 PCR cycles were performed for a deleted exon (E (3), a) and a non-deleted exon (D (3), b). The PCR products separated on a 2% agarose gel and stained with ethidium bromide are shown. Spectrophotometric densitometry (600 nm, Shimadzu Dual-Wavelength TCL Scanner CS-910) of the picture verified the carrier status of I.2 by half dosage for exon E (a), compared with exon D (b). II.1 shows the same dosage for both exons and is therefore not a carrier.

very often opt for prenatal diagnosis. Therefore, we usually start our DNA analysis in DMD/BMD families with a search for deletions.

The table shows the summarised data of the deletion screening in the GDR and selected regions of Czechoslovakia and Hungary from 1988 and 1989. We have screened 183 DMD/BMD families, of which 72 (39%) were deleted for exons detectable with *cf56a,b* by Southern transfer/hybridisation techniques. According to their localisation, 10 deletions would not be detectable with the PCR primers so far available

Summarised data of the deletion screening of DMD families from the GDR and selected regions of Czechoslovakia and Hungary.

	Screened	Deleted	PCR detectable
GDR			
<i>cf56a,b</i>	145	59	51
Exons 8, 17, 19*	50	5	5
CSSR			
<i>cf56a,b</i>	28	8	6
Exons 8, 17, 19*	10	3	3
Hungary			
<i>cf56a,b</i>	10	5	5
Exons 8, 17, 19*	5	0	0
Total			
<i>cf56a,b</i>	183	72	62
Exons 8, 17, 19*	65	8	8

*The screening for exons 8, 17, 19 does not include families which are deleted for the *cf56a,b* region. The table includes 36 families already published by Herrmann *et al.*⁷ and Wulff *et al.*⁸

for this part of the gene. Additional Southern or PCR analysis for the 5' part of the gene in 65 of the 111 families with no deletion in the *cf56a,b* region showed eight deletions (12%) for one or more of exons 8, 17, and 19. Up to now, 18 women from DMD/BMD families with a deletion from the GDR have requested prenatal diagnosis. This is 28% of all families in which DMD/BMD is caused by a deletion. Cytogenetic analysis diagnosed 10 females and six males. No deletion was found in three males and the diagnosis of a healthy male was confirmed after birth by serum creatine kinase (CK) estimation in two of them. The third pregnancy is still in progress. In three males we detected a deletion. All these women decided to have a termination of pregnancy. In two cases a spontaneous abortion occurred before chorionic villi sampling (CVS). PCR also allows deletion screening in families in which a living patient is missing by the use of conserved material. However, in about 56% of all DMD/BMD families the molecular basis of the disease is still unknown. Prenatal diagnosis in these families therefore relies on either sexing or linkage analysis.

Families with new mutations are confronted with the uncertainty of whether their disease is DMD or BMD; therefore we checked the open reading frame (ORF) hypothesis⁶ in them. In 25 patients we were able to compare the clinical picture with the influence of the deletion on the ORF. In 20 cases the ORF was interrupted by the deletion according to the exon/intron border type.⁹ All 20 patients showed classical DMD using this analysis. Five patients, however, had DMD and not BMD although we found in frame deletions in each case.

The detection of a deletion in a DMD/BMD family increases the possibilities for DNA carrier determination performed for women with ambiguous or normal CK data. Linkage analyses always include the risk of recombination events. About 5% have to be calculated for the whole gene. The estimation of the physical distances between deletion and polymorphism for linkage analysis allows a more correct calculation of the recombination risk and in most cases a reduction. By direct detection of a deletion or of a deletion breakpoint this risk is avoided.

Fig 1 shows a dosage analysis after PCR, in addition to published data¹⁰ based on Southern transfer/hybridisation dosage estimation. In this family the carrier status was previously determined by CK determination and linkage analysis using the *cf56a/PstI* polymorphism. In agreement with the results of the dosage analysis, I.2 also showed increased CK levels. The woman II.2 had a normal CK level and is not a DMD carrier according to the linkage and dosage analysis. Although fig 1 shows an unambiguous example of dosage analysis after PCR, technical problems cannot be disregarded, especially the reproducibility (data not shown). The detection of

Figure 2 Carrier determination by deletion bridging PCR. (a,c) cDNA probes cf56b and cf56a and the eight relevant exons (fragment in kbp after a *Pst*I digest) detected by them. (b) Localisation of the intron probes P20a and b. Localisation of *Bgl*II, *Eco*RI, and *Hind*III sites in the sequenced part of the intron. (d) Thick lines indicate the undelated parts of the gene, thin lines the deleted parts. Blocks represent exons. (e) Localisation of the oligonucleotides 1, 2, and 3 used for the PCR, in separate combination 2/3 and 1/3. (f) The control PCR product from the undelated part using oligonucleotides 2 and 3. (g) The PCR product of a person carrying this particular deletion using oligonucleotides 1 and 3. (h) The theoretical PCR product of a person without a deletion using oligonucleotides 1 and 3. Because the distance in undelated DNA is probably some hundred kbp, the PCR product is not detectable.

altered restriction fragments resulting from a deletion is another way, which is, however, based on Southern analysis. An example of a very fast and technically unambiguous method for carrier determination using PCR is shown in fig 2. Cloning and sequencing of the breakpoint in a DMD/BMD patient allows PCR bridging the deletion. Only the DNA of family members who have a deletion like the affected patient gives a PCR product (fig 2g). In normal cases, such as the grandmother, the grandfather, and the mother in this particular family, no PCR product is detectable because the distance between the two oligonucleotide primers probably covers some hundred kbp (fig 2h).

Discussion

The detection of deletions in 44% of all DMD/BMD families has created new possibilities for prenatal diagnosis. Owing to the introduction of the PCR technique, deletion analysis is becoming less expensive and time consuming. This is a particular advantage if only a small amount of material (conserved material of a dead patient, chorionic material) or degraded DNA (conserved material of a dead patient) is available, or if time is short (prenatal diagnosis). However, by PCR we can only detect about 86% of the deletions found by Southern analysis and we are unable to determine accurately the extent of a deletion, which is necessary for the differentiation between DMD and BMD. We prefer the Southern technique for deletion screening

in families with a living patient, but PCR is used for prenatal diagnosis whenever possible. Because technical problems using PCR dosage analysis are not negligible, we prefer linkage analysis for carrier determination in families in which a deletion causes DMD/BMD. However, cloning and sequencing of introns in the hot spot regions will make possible the detection of deletions in more families using either altered restriction fragments in Southern analysis or the bridging PCRs. In particular this last approach opens the way for a fast and technically simple method of carrier determination.

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