Possibilities and limitation of prenatal diagnosis and carrier determination for Duchenne and Becker muscular dystrophy using cDNA probes

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SUMMARY Two cDNA probes, cf23a and cf56a, identify deletions of selected exons in about 50% of our DMD/BMD patients. We have estimated the most likely order of the 11 exons detectable with both probes with respect to the different extensions of the deletions. In one of our BMD pedigrees, the observed deletion could be traced in the affected males through three generations. This result shows that with the use of cDNA probes detecting deletions, the only risk of error in genomic prenatal diagnosis is the general high frequency of new mutations for DMD/BMD. This is important progress in diagnosis compared to the 2 to 5% risk of misdiagnosis because of crossing over events using conventional linkage analysis with bridging or intragenic probes. The first prenatal diagnosis of an unaffected fetus of a woman who is a DMD carrier according to ultrasound examination is described. In one of our DMD males, the cDNA probe cf56a detects a deletion breakpoint. His sister also shows the altered band and is therefore a DMD carrier, while his mother has a totally normal band pattern. The interpretation of this observation could be either germline mosaicism or two identical new mutations. The identification of deletion breakpoints is a new diagnostic strategy, especially for carrier determination, which excludes misdiagnosis owing to crossing over events and the problems of dosage estimation. It is, however, limited by the low frequency of breakpoints detectable with cDNA probes. Therefore, the generation of new intron probes in this region is an important goal.

Duchenne muscular dystrophy (DMD) and its clinically milder allelic form, Becker muscular dystrophy (BMD), are the most common X linked recessive genetic disorders affecting about one in 3000 males. DNA diagnosis has resulted in dramatic progress in carrier detection and prenatal analysis for affected families.¹⁻³ However, since up to now it has been predominantly based on linkage analyses, its reliability was limited by the possibility of crossing over events. This has caused difficulties in decision making for pregnant women, particularly in prenatal diagnosis.

Material and methods

MOLECULAR PROBES

The cDNA probes used were Cf23a^{4 5} and Cf56a.⁵

MOLECULAR ANALYSIS

DNA preparation, restriction enzyme digests, and electrophoresis were performed as described previously.¹ As transfer matrix we used either Hybond N TM¹ or CCA paper.⁶ The labelling of the DNA probes was carried out by nick translation or hexanucleotide primed labelling. Creatine kinase was determined with the N-acetyl-cystein (NAC) activated creatine kinase (monotest Boehringer 126.322). Normal values have been determined for

different age groups.⁷ Ultrasound carrier determinations were performed according to van Rohden and Steinbicker.⁸

Results

We searched for deletions in affected males from 13

BMD families with the cDNA probes cf23a and cf56a. In four of these families a deletion was detectable with cf23a. Twenty-one DMD males out of a total of 45 showed a deletion with the cDNA probes cf23a and cf56a (13/cf23a, 14/cf56a, 6/cf23a and cf56a). Different hybridisation patterns of these deletion patients are shown in fig 1. Fig 2 summarises

FIG 1 (a) *Pst*I digest of 14 DMD/BMD males showing different deletion patterns observed by hybridisation to cf56a (three different blots). Normal band patterns visible in lanes 2, 5, 6, 9, 10, and 12 (* deleted band, p polymorphic band, << altered size band). (b) *Pst*I digest of 10 DMD/BMD males showing different deletion patterns observed by hybridisation to cf23a (four different blots). Normal band patterns visible in lanes 2, 5, and 7 (symbols as in fig 1a).

marises schematically 18 of the 25 described deletions and the most likely order of the exons. Five deletions extend to the whole region and two are different from the given exon order.

Fig 3 shows the pedigree of a BMD family, in which BMD males of the third, fourth, and fifth generation are alive. This allows us to follow the exon deletions found in this family over three generations. The observed deletion of the 3-55 kbp exon detectable with cf23a in a *Pst*I digest and the 4-95 kbp and 4-7 kbp exon detectable with cf56a in a

*Pst*I digest (data not shown) were inherited by every BMD male but not by the unaffected ones. This shows the conserved inheritance over many generations of such exon deletions.

The results of our first prenatal diagnosis in a DMD family using a deletion of all five exons detectable in a *Pst*I digest with cf56a is shown in fig 4. The cytogenetically identified male fetus (lane 2) of a DMD carrier identified by ultrasound examination (III.7) shows, in contrast to one of the affected boys of the family (lane 3), the same hybridisation

FIG 2 Compilation of 18 deletion cases. The figures to the left give the numbers of identical exon deletions. The top numbers show the most likely order of the detectable bands (exons) after *Pst*I digestion and hybridisation to cf56a and cf23a respectively, as deduced from the deletions of our patients. The total number of breakpoints within the same intron region is given at the bottom.

FIG 3 Pedigree of a BMD family.

FIG 4 Pedigree of two DMD families and *Pst*I digests of family members who are relevant for prenatal diagnosis hybridised to *cf*56a. (● DMD carrier according to ultrasound examination, ▲ fetus with DMD, △ fetus without DMD, n normal male control.)

pattern as the normal male control (lane 4). We therefore diagnosed an unaffected boy. The pregnancy is still in progress.

Lanes 5 and 6 in fig 4 show DNA analysis with the probe *cf*56a of a woman and the aborted material of her male fetus. This woman (III.4) was identified as a DMD carrier by DNA analysis with the probe 754. Prenatal diagnosis based on a deletion with cDNA probes was not possible because no DMD male is alive in this family. The woman, who was not prepared to accept an error of 2 to 10% in the prenatal diagnosis, requested termination of pregnancy after the cytogenetic determination of a male fetus. The DNA hybridisation pattern of the fetal material in a *Pst*I digest with *cf*56a shows a deletion of three exons. Therefore, we concluded that the fetus was affected. This result can be used as a reference for the DNA analysis of further male pregnancies of this woman.

In one case (lane 13, fig 1a) one deletion breakpoint was detectable with *cf*56a by the appearance of an altered 2.9 kbp band instead of the

FIG 5 Pedigree of a DMD family and *Pst*I digest of family members who are relevant for carrier determination using an altered band. Hybridisation is to *cf*56a. (Symbols as in figs 1a and 3.)

normal 3.25 kbp one. We used this observation for carrier determination in the affected family (fig 5). The sister (II.2) of the DMD male (II.1) has also inherited the altered band and is therefore a DMD carrier, while the mother (I.1) shows a normal band pattern with the two polymorphic alleles, 10.8 kbp and 3.25 kbp. This could be interpreted as germline mosaicism or the occurrence of two identical new mutations.

Discussion

The discovery of deletions detectable with DMD gene probes in about 10% of DMD males^{9 10} resulted in new possibilities for prenatal diagnosis, excluding the risk of misdiagnosis owing to crossing over events. However, this new diagnostic strategy in prenatal diagnosis of DMD/BMD has so far been limited by the relatively small number of deletions detectable with these probes. The isolation of cDNA clones from the DMD/BMD gene region^{4 5 11-13} has changed the situation dramatically, because deletions have been detected in about 50% of all DMD/BMD cases by application of such exon probes. This makes this strategy applicable on a much broader scale, including the investigation of fetal tissue after termination of male pregnancies in families in which no living DMD/BMD male is available for reference.

Obviously the main field for deletion analysis is in prenatal diagnosis, while carrier determination suffers from the problems of dosage analysis with a risk of misdiagnosis of about 5%, comparable to that of linkage analysis. This limitation could be overcome by identifying deletion breakpoints, detectable by a size change of hybridisation bands, but in general such breakpoints are so far away from the exons that cDNA probes can detect them only in a few cases. Therefore, the isolation of specific intron probes is necessary for a broader application of breakpoint detection in carrier determination of DMD/BMD.

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