Alternative splicing of dystrophin mRNA complicates carrier determination: report of a DMD family

U Lenk, S Demuth, U Kräft, R Hanke, A Speer

Abstract
Carrier determination is important for genetic counselling in DMD/BMD families. The detection of altered PCR amplified dystrophin mRNA fragments owing to deletions, insertions, or point mutations has increased the possibilities of carrier determination. However, problems may occur because of alternative splicing events. Here we present a family with a DMD patient characterised by a deletion of exons 45 to 54. At the mRNA level we detected a corresponding altered fragment which served for carrier determination. The mother and the sister of the patient showed the same altered dystrophin mRNA fragment as the patient and are therefore carriers. In the mother two additional altered dystrophin mRNA fragments were detectable, obviously resulting from alternative splicing in the normal allele. The grandmother and two other related females of the patient possess only the normal mRNA fragment. In a further female we detected an altered fragment owing to an mRNA deletion of exon 44. This fragment is created either by alternative splicing or a new mutation. Therefore, the carrier status of this female is still ambiguous indicating problems in carrier determination by the method of dystrophin mRNA analysis.
(J Med Genet 1993;30:206–9)

Reactions bridging deletions or duplications result in altered PCR products. Here we report a DMD family in which carrier determination using this method was complicated by the presence of alternative splicing events.

Material and methods

Reverse transcription and nested PCR
Total RNA was prepared from peripheral blood lymphocytes10 and about 500 ng of total lymphocyte RNA were transcribed using the primers DMD N7b and DMD 8b. Nested PCR was performed as described by Roberts et al10: 10 µl of the PCR assay was electrophoresed in a 2% agarose gel.

PRIMER SEQUENCES

Set 7
DMD N7a TCATAGCAAGAAGACAGACGC
DMD N7b CTCTGTTGATATCTCAAGGTC
DMD N7c GTGGAAGGAGTGAAGCTACAG
DMD N7d ACTTGATCAAGCAGAAAGGC
Set 8 (10)
DMD 8a CTAGAATGCCTTTCCCTTG
DMD 8b CTCAAGGAGGCCTCTCTGG
DMD 8c CTGCTCTGGCATTTACAG
DMD 8d GGGCTCTGGTAGATTTCCT

DIRECT SEQUENCING OF PCR PRODUCTS

Nested PCR products were purified from 2% agarose gels using USBioclean MP (USB) and sequenced with fmo1 DNA Sequencing System (Promega) using 32P-γ-ATP labelled primer DMD N7c.

Results

Fig 1 shows the pedigree of the DMD family requesting genetic counselling. A deletion of exon 45 to 54 inclusive was identified in patient II15 by Southern transfer/hybridisation and PCR. A junction fragment to be used for carrier determination was not detectable. Therefore we used primer sets 7 and 8 covering exons 43 to 51 and 51 to 58 respectively for dystrophin mRNA analysis.

PCR products resulting from set 7 amplifications are summarised in fig 2. For the DMD patient II15, who is deleted for exon 51, the target sequence of primers N7b,d, no PCR product is visible. The females I2, II2, II4, II12, II16, and III8 show a normal full sized fragment of 1271 bp. In the mother (II2) of the patient two additional fragments of 797 bp and 611 bp occur. By sequencing we identified

Max Delbrück Centre for Molecular Medicine, Berlin-Buch, Robert-Rösse-Strasse 10, D-11187 Berlin, Germany.
U Lenk
U Kräft
R Hanke
A Speer

Medical Academy Erfurt, Department of Medical Genetics, Arnostäder Strasse 34, D-99080 Erfurt, Germany.
S Demuth

Correspondence to Dr Lenk.
Received 11 August 1992.
Accepted 9 September 1992.
Alternative splicing of dystrophin mRNA complicates carrier determination: report of a DMD family

Figure 1 Pedigree of the DMD family requesting genetic counselling. Genomic DNA of patient III-5 was analysed by Southern transfer/hybridisation with cDNA probes cfS6a,b and by PCR covering exons 41 to 55. A DNA deletion of exons 45 to 54 was detectable. Family members I-2, II-2, II-4, III-2, III-5, III-6, and III-8 were analysed by nested amplification of reverse transcribed mRNA (RT-PCR).

Figure 2 Products of nested RT-PCR spanning exons 43 to 51 (set 7, see also diagram in fig 7) shown in an ethidium bromide stained 2% agarose gel. For the DMD patient III-5 a signal is missing owing to the deletion of exons 45 to 54. The mother (II-2) has two altered fragments of 797 bp and 611 bp in addition to the full sized 1271 bp product. Female III-6 shows a faint additional band of 1123 bp.

Figure 3 Direct sequence analysis of the 611 bp fragment of female II-2 amplified with primer set 7. The sequence data indicate splicing of exon 44 into exon 48. Therefore exons 45 to 47 are missing from the mRNA transcript.

Figure 4 Direct sequence analysis of the 1123 bp band of female III-6 amplified with primer set 7. Splicing of exon 43 into exon 45 indicates that exon 44 is missing from the mRNA transcript.
are produced from an mRNA deletion of exons 45 to 54 and 44 to 54 respectively.

A summary of all detectable RNA deletions compared to the DNA deletion is given in fig 7.

Discussion

The detection of altered PCR amplified dystrophin mRNA fragments owing to deletions, insertions, or point mutations has created new possibilities for carrier determination in DMD/BMD families. The DMD family presented in this paper underlines a problem in the application of this method for carrier determination because of alternative splicing events.

In the DMD patient of this family, we found an out frame DNA deletion of exons 45 to 54. The same deletion was also detectable at the RNA level. Alternative splicing events on the 5' or 3' end of the deletion, extending it by an exon, create two in frame RNA deletions of exons 45 to 55 and 44 to 54 respectively.

For the grandmother (I-2), aunt (II-4), and one cousin (III-8) of the patient, dystrophin mRNA analysis shows no altered fragment resulting from deletion. For the mother (II-2) and sister (III-2) of the patient we detected an mRNA deletion of exons 45 to 55 which extends the DNA deletion by one exon at the 3' end and was also present in the patient himself. Therefore both females should be treated as carriers. In addition to the disease causing deletion, the mother of the patient has two further in frame mRNA deletions of exons 45 to 47 and 45 to 48 explicable by alternative splicing in the normal allele because she also has the deleted fragment.

The carrier status of the second cousin (III-6) of the patient is still ambiguous. Here we detected an mRNA deletion of exon 44 in addition to the normal fragments. This mRNA deletion could be explained either by alternative splicing or by a new mutation. Although PCR product analyses of exon 44 at the DNA level (data not shown) indicate no strong gene dosage effect the latter possibility cannot be completely excluded.

This family illustrates a problem for the application of dystrophin mRNA analysis for DMD/BMD carrier diagnosis owing to various alternative splicing events and the possibility of new mutations.

We wish to thank Dr K E Davies for helpful discussion. The work was supported by Deutsche Forschungsgemeinschaft.

5 Hugnot JP, Riecan D, Jeanpierre M, Kaplan JC, Tolun A. A highly informative CACA repeat polymorphism in the dystrophin gene.
Alternative splicing of dystrophin mRNA complicates carrier determination: report of a DMD family

Figure 7. The diagram summarises all detectable DNA and mRNA deletions in the region of exons 43 to 58. Shaded boxes represent exons which were amplified from genomic DNA. Regions of dystrophin transcript which were amplified by RT-PCR are designated beneath the exon diagram (set 7 and set 8). Black bar represents the extent of DNA deletion identified with cDNA probes c56a,b and PCR of genomic DNA. Shaded bars designate the extent of all detectable mRNA deletions in the family members tested in our study. Effects on the translational reading frame are indicated to the right of the diagram.
Alternative splicing of dystrophin mRNA complicates carrier determination: report of a DMD family.

U Lenk, S Demuth, U Kräft, R Hanke and A Speer

doi: 10.1136/jmg.30.3.206

Updated information and services can be found at:
http://jmg.bmj.com/content/30/3/206

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/