

Abstract

Analysis of dystrophin mRNA show nonsense, splice and cryptic splice site mutations cause Becker muscular dystrophy

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We have applied the protein truncation test to screen muscle RNA from 9 BMD patients with no gross rearrangements of the dystrophin gene. Four cryptic splice site mutations were identified. An A to G change 2 kb into intron 25 of BMD1 creates a cryptic acceptor site, resulting in two mutant cDNAs with 95bp and 207bp of intronic sequence inserted between exons 25 and 26. The effect on RNA splicing is similar to the donor site mutation reported by Ikezawa et al., despite the mutations being 206bp apart. This same acceptor site mutation has also been reported by Tuffery-Giraud et al. but only results in the 95bp insertion.

In BMD2 an A to T change 636 bp 3' of exon 32 creates a cryptic acceptor site adding 43bp of intronic sequence between exons 32 and 33. An A to T change 9kb 5' of exon 45 creates a cryptic donor site in BMD3 and results in an extra 71bp incorporated between exons 44 and 45 in the cDNA. In BMD4 an A insertion more than 55kb 5' of exon 45 produces a cryptic donor site causing an extra 74bp insertion in the cDNA. In all cases despite premature stop codons, alternative splicing produces full-length protein, accounting for the milder phenotypes.

Nonsense mutations in exons 25 and 49 (BMD5 & 6) and donor site mutations in exons 4 and 64 (BMD7 & 8) were found to cause alternative splicing of inframe exons in the mRNA. The 1bp deletion at the donor site of exon 64 also resulted in an additional methionine residue in BMD8's dystrophin. Although BMD9 has an out-of-frame 5bp deletion in exon 71, it is thought that failure of nonsense mediated decay means that some mutant mRNA is not completely degraded but is translated into near full-length dystrophin that can rescue the predicted severe phenotype.

Four of these mutations would not have been detectable with DNA-based mutation screening strategies. In order to offer comprehensive mutation screening in genes with large introns such as dystrophin, an RNA-based approach is likely to be required for the foreseeable future.