Deletion patterns of Duchenne and Becker muscular dystrophies in Greece

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Abstract

We present molecular data from 90 Greek boys with Duchenne or Becker muscular dystrophy using cDNA analysis or multiplex PCR or both. Deletions were detected in 63.3% of patients and were mainly clustered in two areas of the gene, one in the 3' and one in the 5' end of the gene (exons 3-19 and 44-53). Almost 17% of deletion breakpoints lay in intron 44 while 29% of deletions have a breakpoint in intron 50. Thus the distribution of deletions in our DMD/BMD patients differs from that previously reported. Furthermore a 1:4.35 proximal:distal ratio was observed in familial cases and a 1:2.45 ratio in isolated ones.

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Southern blot analysis using cDNA probes and multiplex PCR reactions have shown that 65-70% of DMD or BMD patients have deletions¹⁻⁴ or duplications⁵ of one or more exons of the gene. In the remaining 35%, the disease is assumed to result from point mutations.⁶ The percentage of detectable deletions in DMD/BMD patients is similar for all the populations studied so far, with the exception of an Israeli study, where the reported incidence is very low (39%).⁷

Deletions in the dystrophin gene are preferentially clustered in two areas, one in the 5' end, between exons 3 to 7, and one in the central to 3' end with the majority of breakpoints in intron 44.¹⁸ Thus with the use of multiplex PCR for the most commonly deleted exons, it is possible to detect 98% of the deletions detected with cDNA probes.^{8,9}

In the present study, we estimated the frequency and extent of deletions in 90 Greek DMD/BMD boys using cDNA analysis and multiplex PCR analysis and compared the map of intragenic deletions and breakpoint "hot spots" detected in the Greek population with those of other populations. It is obvious that the experience obtained from this study greatly facilitates diagnosis and prenatal testing for the muscular dystrophies in Greece.

Materials and methods

This study performed for the first time in Greece included 90 boys, 74 with Duchenne and 16 with Becker muscular dystrophy. The diagnosis and classification of the patients was based on the classic clinical criteria, age of

onset, CK values, electromyography (EMG), and muscle biopsy.

The first 55 patients were studied both with cDNA¹⁴ probes and multiplex PCR for the simultaneous amplification of 18 exons of the gene⁸ and the results have been previously reported.¹⁰ The remaining patients were studied with multiplex PCR and only when a deletion was not detected were they studied with the use of sets of primers for Ca1A, Cf56a, J66, and MP1P (Mountford *et al*, unpublished data)¹¹ and subsequently with cDNA probes.

Microsatellite repeats STR44 and STR50 were amplified¹² in patients with a deletion end point in the respective intron.

Results

Deletions were detected in 63.3% of the patients, were heterogeneous in size, and were mainly clustered around exons 44-53 and 3-19 (figure). Specifically, in 48.8% of the patients (75.8% of detectable deletions) deletions were detected primarily at the 3' end of the gene and between exons 44-53. Ten patients (17.2%) had one deletion end point between exons 44 and 45. Their DNA was further screened with microsatellite repeat STR44 which lies in intron 44. It was found that STR44 was preserved in all nine deletions starting in intron 44 but was removed in the one deletion terminating in intron 44. Seventeen deletions (29.3%) had at least one end point between exons 50 and 51. Screening with microsatellite repeat STR50 showed that of the 14 patients with a deletion ending at exon 50, STR50 was present in 13 of them (95% of these deletions stop before this marker). Furthermore STR50 was absent from three patients with a deletion of exon 51. Almost 12% of deletions involved exons 48 to 50. Only one deletion extended as far as exon 60 (figure).

In 15.5% of the patients (24.2% of detectable mutations) mutations were detected at the 5' end of the gene and were mainly clustered around exons 3-19. Seven of the deletions (12% of deletions) had an end point possibly in intron 2, while no patient had a deletion of the promoter or the first two exons of the gene. Only one deletion extended from exons 24 to 34, and was detected first with Ca1a and secondly with the use of cDNAs 4-5a and 5b-7.

A discontinuous deletion was identified with cDNA analysis, in which exons 19-21 and 26-29 were missing, while exons 22-25 were detected as a 20 kb *HindIII* restriction fragment. Further investigation of this patient's DNA (digestion with several restriction en-

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Position and extent of deletions detected in Greek DMD/BMD patients using cDNA analysis or multiplex PCR or both for 18 exons of the dystrophin gene. Thin lines: deletions detected using cDNA; bold line: duplication detected with cDNA; triangles: deletions detected using PCR; JF: junction fragment.

zymes and comparison with normal male controls) excluded the possibility that this could be a junction fragment of exactly the same size as the 20 kb fragment.

A duplication was detected in our sample shown with cDNA 1-2a for exons 7-9. The duplication was identified by comparing the intensities of different bands in the same track and in the tracks of the two adjacent samples. No systematic dosage analysis was undertaken in order to detect the actual percentage in our patients.

In one patient a deletion of exon 47 was

detected with both techniques but multiplex PCR detected in addition to this a deletion of exon 48 and further investigation is considered. Reduction of the annealing temperature did not succeed in amplifying exon 48. In another patient cDNA analysis showed a deletion of exons 48-50, plus an additional junction fragment. PCR did not show a deletion of exon 48, suggesting a deletion of exons 49-50, with exon 48 being present in the junction fragment.

Of the 16 boys with Becker muscular dystrophy, deletions were detected in eight cases. Three patients had deletions of exons 45-47,

No of Duchenne familial and isolated cases with a detectable deletion in the 3' and 5' end of the dystrophin gene

	No of 5' end deletions (%)	No of 3' end deletions (%)
No of familial cases = 11	2 (18.8%)	9 (81.8%)
Ratio: 1 proximal:4.35 distal		
No of isolated cases = 38	11 (28.94%)	27 (71.06%)
Ratio: 1 proximal:2.45 distal		

and the other five had deletions of exons 38–43, 45–53, 47–48, 50–52, and 50–53. All BMD patients seemed to have a deletion in the 3' end of the gene.

In our sample there were 65 isolated and 25 familial cases and the incidence of Duchenne or Becker patients was the same in both groups. Deletions were detected in 66.2% of DMD patients and 50% of BMD patients. More deletions were detected among sporadic cases (67.6%) than familial (56%) ones. Specifically there is a 17% increase in deletions detected among isolated cases of DMD rather than among familial cases, while in Becker families there is a 15% increase of detectable deletions among familial cases. Moreover, we have observed a 1:4.35 proximal:distal ratio in Duchenne familial cases as opposed to a 1:2.45 ratio in isolated cases (table).

Discussion

The percentage of deletions (63.3%) detected in our DMD/BMD patients is in agreement with those reported for other populations^{1 2 4 13 14} with the exception of the Israeli study.⁷ The distribution of the observed deletions agrees with the reported ones. We have shown that 17.2% of detectable deletions had a breakpoint in intron 44 supporting previous reports of a breakpoint "hot spot" in this intron.^{15 16} STR44 segregated preferentially with exon 44, so the breakpoint maps distal to this marker.¹⁷ Although our results support that intron 44 is a breakpoint "hot spot", the percentage of deletions detected in this area (17.2%) is lower than the 32% published.^{11 16} On the other hand a higher percentage (29.3%) of our patients had at least an end point in intron 50 and STR50 segregated preferentially with exon 51. Thus in our population the major deletion breakpoint "hot spot" is located proximal to this marker in intron 50 rather than in intron 44. In contrast to other reports,^{1 18} deletions of the promoter or the first two exons of the gene were not observed in our DMD/BMD patients. Almost 12% of detectable deletions had a breakpoint in intron 2 which could be regarded as a minor deletion "hot spot" of the 5' end of the gene, in agreement with previous reports.¹⁸ The observed differences in the distribution of deletions in our sample could be explained by the hypothesis that intronic sequences could accumulate differences in different populations¹⁸ and that the so called "local DNA environment" might play a role in the creation of deletions and mutations.^{19 20}

The discontinuous deletion detected may be the result of a complex rearrangement within the gene in that particular region. Very few

discontinuous deletions have been reported so far.^{21 22}

A small increase was noted in the percentage of detectable deletions in isolated rather than familial cases but not to the extent reported by others.²² However, while this is true for the Duchenne cases the opposite was observed for the Becker cases and this is in contradiction to the above report. Moreover, our data do not support the hypothesis that proximal and distal deletions occur equally in familial cases²³ and we observed that in isolated cases the ratio is much higher than the reported one.

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