

Different Mosaicism Frequencies for Proximal and Distal Duchenne Muscular Dystrophy (DMD) Mutations Indicate Difference in Etiology and Recurrence Risk

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Summary

In about 65% of the cases of Duchenne muscular dystrophy (DMD) a partial gene deletion or duplication in the dystrophin gene can be detected. These mutations are clustered at two hot spots: 30% at the hot spot in the proximal part of the gene and about 70% at a more distal hot spot. Unexpectedly we observed a higher frequency of proximal gene rearrangements among proved “germ line” mosaic cases. Of the 24 mosaic cases we are aware of, 19 (79%) have a proximal mutation, while only 5 (21%) have a distal mutation. This finding indicates that the mutations at the two hot spots in the dystrophin gene differ in origin. Independent support for the different mosaicism frequency was found by comparing the mutation spectra observed in isolated cases of DMD and familial cases of DMD. In a large two-center study of 473 patients from Brazil and the Netherlands, we detected a significant difference in the deletion distribution of isolated (proximal:distal ratio 1:3) and familial cases (ratio 1:1). We conclude from these data that proximal deletions most likely occur early in embryonic development, causing them to have a higher chance of becoming familial, while distal deletions occur later and have a higher chance of causing only isolated cases. Finally, our findings have important consequences for the calculation of recurrence-risk estimates according to the site of the deletion: a “proximal” new mutant has an increased recurrence risk of approximately 30%, and a “distal” new mutant has a decreased recurrence risk of approximately 4%.

Introduction

Duchenne muscular dystrophy (DMD) is a lethal neuromuscular disease, which affects 1 in 3,500–4,000 live male births. One in three DMD patients appears to be a new mutant (Emery 1988). In about 65% of DMD patients a partial deletion of the dystrophin gene is detected (Forrest et al. 1987; Koenig et al. 1987, 1989; Malhotra et al. 1988). The deletions are clustered at two hot spots: 30% at the hot spot in the proximal region of the gene and about 70% at the hot

spot in the distal region of the gene (Wapenaar et al. 1988; den Dunnen et al. 1989; Chamberlain et al., in press). The recent demonstration of germinal mosaicism associated with new mutants in DMD families (see table 1), in other X-linked diseases (Maddalana et al. 1987; Bröcker-Vriends et al. 1990) and in non-X-linked conditions (Byers et al. 1988), suggests that most mutations occur during mitosis (germ line or early somatic) and that mosaicism is a general phenomenon accompanying new mutations.

Assuming an identical mutation distribution spectrum for isolated, familial, and one-generation cases, we would expect no difference among these groups; however, the biased distribution that we have observed for deletions and duplications in the deletion-prone regions in the published germinal mosaicism cases and that we independently report here for spo-

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Table 1**Published Germ-Line or Somatic Mosaics for DMD Mutations**

Original Report	Contributor	Proximal pert/XJ10	Distal p20/7b8	
Bakker et al. 1987, 1989.....	Bakker (DL43)	del		
	Bakker (DL114)	del		
	Bakker (DL154)	del		
	Bakker (DL202)	dup		
	van Broeckhoven (MD6)	del		
	van Broeckhoven (MD14)	del		
Bartlett et al. 1988.....	Bartlett	del		
Bech-Hansen et al. 1987.....	Bech-Hansen	del		
Clausters et al. 1990.....	Clausters	del		
Darras et al. 1987.....	Darras		del	
Lanman et al. 1987.....	Lanman	del		
Lebo et al. 1990.....	Lebo	del		
Lindlof 1990.....	Lindlof	del		
Monaco et al. 1987.....	Monaco	del		
Wood and McGillivray 1988.....	Wood	del		
van Essen et al. 1992.....	Boileau (F9)	del		
	Corvone (DMD044)	del		
	Jeanpierre (DMD0270)	del		
	Liechti-Gallati (Be/Dol)		del	
	Poncin (DB19)	del		
	Speer (D101)		del	
	Van Essen (DMD59)	del		
	Verellen (DUL17)		del	
	Unpublished.....	Bakker (DL26)		del
	Total:			5 (.21) ^a
Reported.....		19 (.79)		
Expected.....		7 (.40)	17 (.60) ^a	

^a $P = .003$.

radic versus familial DMD, casts unexpected light on differences in etiology and recurrence risk of mutations in the two regions of the DMD gene.

Subjects and Methods

We have carried out a systematic study of the distribution of deletions in a large sample of 473 unrelated DMD patients studied in two different centers: 254 at the Centro de Miopatas in Sao Paulo (group 1) and 219 at Leiden University in Leiden (group 2). For statistical analysis we considered only the multigeneration familial and isolated cases, as selected by pedigree, prior to any DNA or creatine kinase (CK) analysis. One-generation families clearly harbor, apart from hidden familial cases, new mutations among patients' mothers (mutations that have arisen in either grandparent) and germ-line mosaic cases (arisen in

the mother). Because it is impossible, on the basis of pedigree data, to discriminate between these three possible situations, one-generation families were not included in the study.

Screening for deletions in patients from group 1 was performed by use of the PCR multiplex kit according to a method described by Chamberlain et al. (1989). Most of the results were confirmed by Southern blotting and cDNA hybridization (Passos-Bueno et al. 1990b). For group 2, all patients were analyzed by Southern blotting using cDNA probes across the gene (Bakker et al. 1989; Chamberlain et al., in press), confirming the PCR multiplex results obtained with the two multiplex kits (Beggs et al. 1990; Chamberlain et al. 1989). The reestimation of the recurrence risk, taking into account the position of the deletion within the gene, was performed according to a method described by Bakker et al. (1989).

Results

Among the 24 germinal mosaicism cases we are aware of (table 1), the proximal mutations (18 deletions and 1 duplication) are much more frequent than the distal ones: 19 (78%) are proximal, and 5 (21%) are distal; that is, the ratio is 3.8:1, which differs significantly from the expected 1:3 ratio ($\chi^2 = 8.76$; $P = .003$) observed in a large set of unrelated DMD patients ($N = 427$) with an intragenic deletion (Chamberlain et al., in press). The frequency of germinal mosaicism has a direct effect on “de novo” mutations being multiply transmitted, thus increasing their chance of eventually becoming familial. Therefore, our findings predict a difference, in the proportion of proximal versus distal deletions, between familial and isolated cases. Consistently, Passos-Bueno et al. (1990a) have observed that distal deletions were more frequent among isolated cases than among familial cases in a set of Brazilian DMD patients. We have extended this study by analyzing the site of the mutation in two large populations of unrelated DMD patients. Among the 179 isolated cases, 28% of the deletions were proximal while 72% were distal. In contrast, among the 55 familial cases, the proportions of proximal (47%) and distal (53%) deletions were very similar (table 2). The distributions of deletions in the two hot-spot regions of the gene are statistically different (fig. 1): proximal deletions occur significantly more often among familial cases, while distal deletions occur significantly more often among isolated cases ($\chi^2 = 7.14$; $P < .01$).

Discussion

The data presented here show that deletions (mutations) in the proximal part of the dystrophin gene have a higher probability of becoming a familial inherited

mutation, while distal deletions more often will be detected as a sporadic mutation. Given these data and the fact that proximal and distal deletions are apparently equally transmitted to the next generation, our observations provide the first unambiguous evidence that there is a fundamental, distributional difference, which might reflect a temporal difference in the mutational occurrence during embryogenesis. This hypothesis suggests that proximal deletions occur early in embryonic development and that distal deletions occur later (fig. 2). As a cause for the proposed temporal difference in the occurrence of two types of deletions, one could suggest that the large introns in the dystrophin gene harbor other genes that are expressed at different stages of embryonic development. This may cause differences in local chromatin structure and stability.

As described by Bakker et al. (1989) and recently confirmed by Passos-Bueno et al. (1990a) and van Essen et al. (1992), the DMD recurrence risk that one has to keep in mind is 7%–9% — or 14%–18% when the haplotype at risk is known — when a new mutation is proved. Our present results indicate that recurrence risks should be reestimated and further refined according to the site of deletion.

In 45 Leiden families in which a new mutant was proved by DNA analysis (20 proximal mutations and 25 distal mutations), seven cases of mosaicism were identified: six proximal and one distal. The frequency of mosaicism in this type of calculation reflects the recurrence risk, because in 45 families the at-risk haplotype was transmitted 44 times (7 times resulting in affected subjects and 37 times resulting in normal subjects), proximal mutations occurred 6 (approximately 30%) of 22 times, and distal mutations occurred 1 (approximately 4%) of 22 times. Therefore, if the “at-risk” haplotype is known, the recurrence risk for “de

Table 2

Number of Deletions in the Two Hot-Spot Regions of the Gene in Isolated and Familial Cases

INHERITANCE	NO. OF DELETIONS						Grand Total
	Group 1			Group 2			
	Proximal	Distal	Total	Proximal	Distal	Total	
Familial	10	8	18	16	21	37	55
Isolated	28	63	91	22	66	88	179

NOTE.— The patients were divided into the following three different groups, only on the basis of family history: (1) familial, when a typical X-linked pattern was observed; (2) isolated, when there was only one affected patient in the genealogy; and (3) one generation, when there were at least two affected in the same sibship (for this group, we did not include the deletion data; see text).

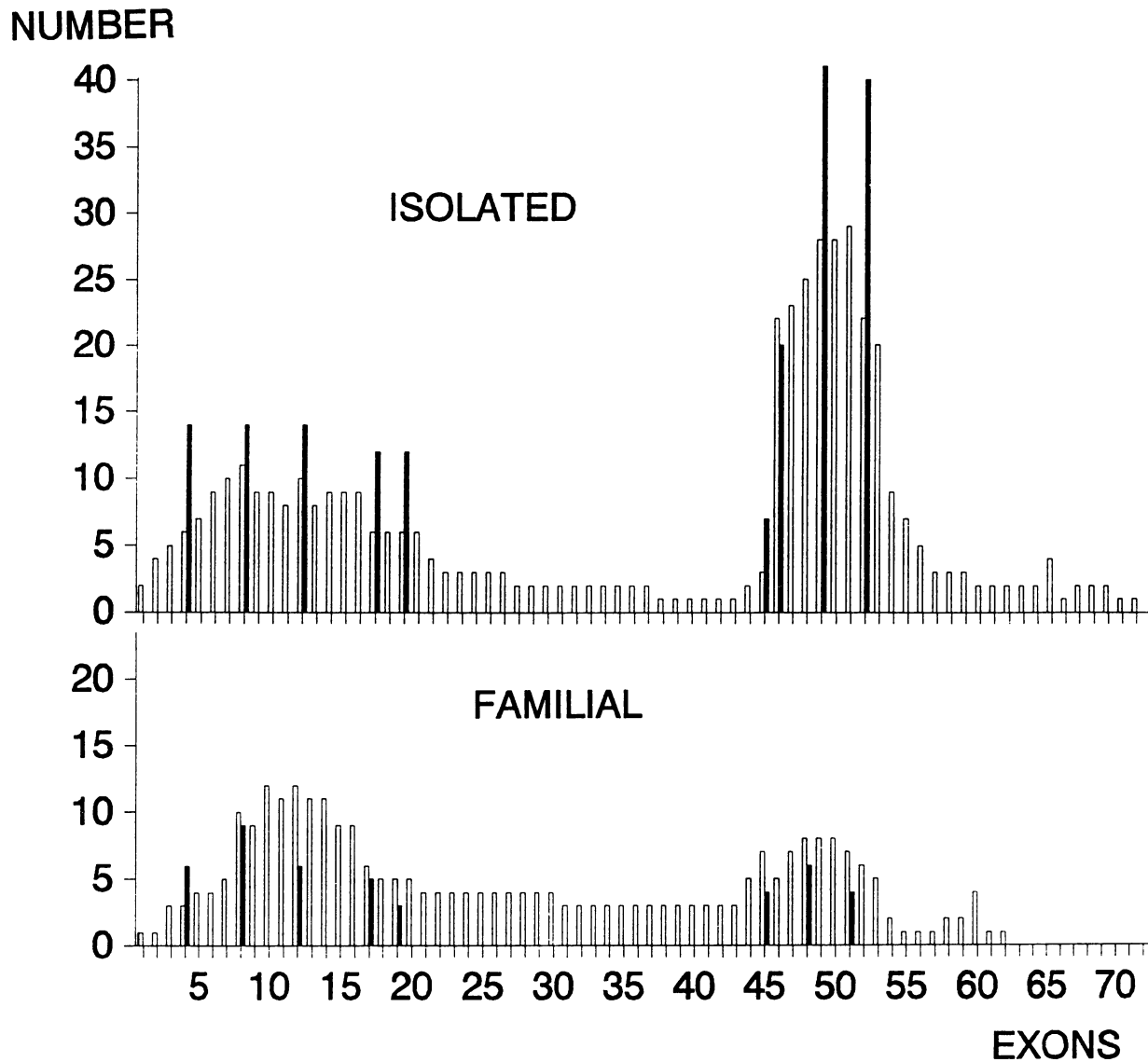


Figure 1 Distribution of deletions along the gene in isolated and familial cases. Blackened bars represent multiplex PCR results with the Chamberlain PCR set on the Brazilian patients (group 1), and unblackened bars represent Southern blot results of using all six cDNA probes testing for the presence or absence of >70 exons in the Leiden patients (group 2). Since the proportion of proximal and distal deletions did not differ statistically between the two laboratories, the two sets were analyzed together ($\chi^2 = 0.75$; $P > .05$).

novo” deletions in the proximal region could be as high as 30%, while that in the distal would be on the order of 4%.

The high recurrence risk for proximal mutations might indicate that, although they are detected as the result of a germ-line mosaicism, they probably arise very early in embryogenesis and are not necessarily restricted to the germ line. Low levels of somatic mosaicism might be detectable in mothers of new mutants,

and, if so, this might have diagnostic potential to predict the recurrence risk per case involved. Further studies on DNA samples from different somatic tissues—e.g., fibroblasts or hair roots—of proved mosaics are needed.

Finally, these findings may give insight into the nature of mutations in other genetic diseases, of which the inheritance is not very clear. As has been suggested by Edwards (1989), rare autosomal disorders recur-

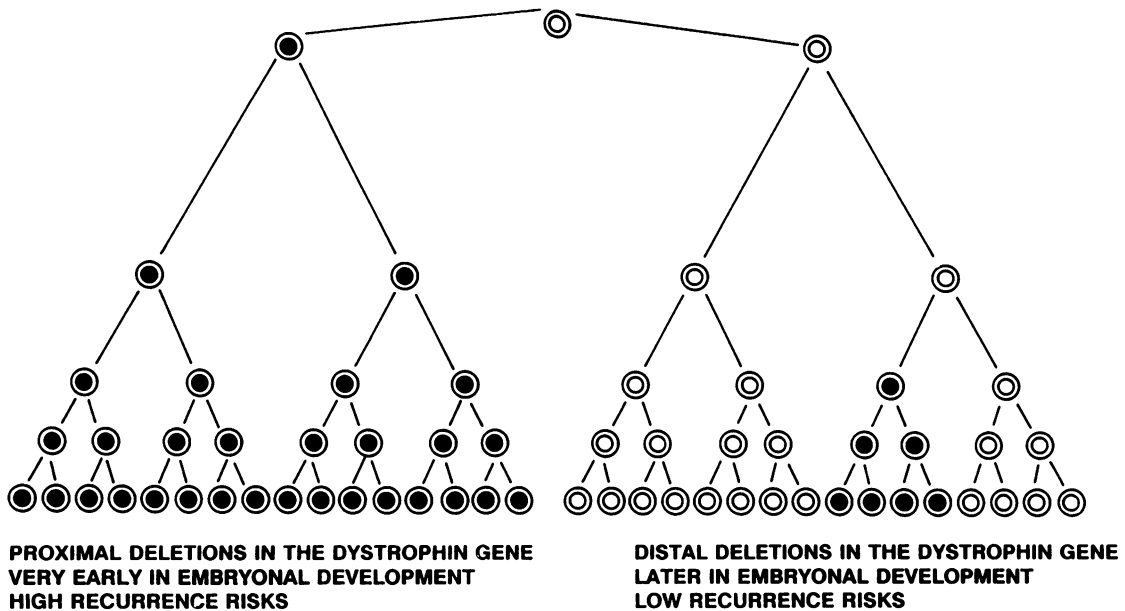


Figure 2 Schematic representation of mitotic cell divisions and an example of how very early and later (e.g., two mitotic divisions later) mutations influence the proportion of mutated cells and, consequently, the recurrence risk in the case of germ-line mosaics.

ring in the same sibship are usually classified as recessive, while in fact they are indistinguishable from autosomal dominant new mutations for which one of the parents is a germinal mosaic. It is interesting to speculate that a high mutation rate in other diseases—such as neurofibromatosis and familial adenomatous polyposis, which are frequently caused by “de novo” mutations—may be related not only to the type of mutation but also to the time of occurrence, e.g., the stage of embryonic development or gametogenesis.

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