

ORIGINAL ARTICLE

Deletions, not duplications or small mutations, are the predominant new mutations in the dystrophin gene

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Examination of the carrier state was performed in 744 unrelated mothers of the Duchenne muscular dystrophy/Becker muscular dystrophy (DMD/BMD) probands with identified mutations in the dystrophin gene. Owing to that it was possible to assess frequency and type of new mutations in the gene. Contrary to the Japanese observations of Lee *et al.* published in this journal, we did not find significant differences in the carrier frequency between mothers of DMD and BMD patients. However, we found that new mutations in patients with deletions were significantly more frequent than in those with duplications and small mutations: of 564 unrelated patients with deletions, 236 (41.8%) carried new mutations, the respective values for duplications and small mutations were 21 of 95 patients (22.1%) and 18 of 85 patients (21.2%)—the differences highly significant ($P < 0.0001$).

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INTRODUCTION

Duchenne muscular dystrophy (DMD; OMIM 310200) caused by mutations in the dystrophin gene is characterized by progressive and irreversible atrophy of the muscles resulting in death of the affected persons usually in the end of their second or the beginning of the third decade of life. Prevalence of DMD is 1:3500 among male live births. An allelic variant of this disease, Becker muscular dystrophy (BMD; OMIM 300376), is milder and five times less frequent.¹ Sixty percent of mutations causing DMD/BMD are deletions of different sizes that comprise at least one exon of the dystrophin gene; 10% are the duplications of similar size while 30% of cases are small mutations such as substitutions, deletions, duplications or insertions of one or several nucleotides.^{2,3}

Detected mutations are distributed within the gene in the characteristic, well-known manner for DMD/BMD, that is, deletions group in two hot spots within exons 44–55 and 3–17, duplications occur mostly at 5′-end of the gene and small mutations are distributed throughout the whole dystrophin gene. In-frame deletions, with few exceptions, cause a mild form of the disease (BMD). Similar effects have in-frame duplications but in these cases exceptions of the severe form of the disease are more frequent. Out-of-frame deletions and duplications cause a severe form of the disease (DMD). Small mutations that create a stop codon, or disturb splicing, usually cause a severe form of the disease.^{4,5}

A majority of the patients affected with DMD/BMD never produce offspring and owing to that the mutated allele is eliminated from the population. According to a formula proposed in 1935 by Haldane, one-third of mutated genes are eliminated in each generation, and two-thirds remain in the female carriers' genome. The resulting

stable frequency of the disease results from the balance between new mutations and the selection of the mutated genes.⁶

Results obtained by Lee *et al.*⁷ indicating a different carrier frequency between mothers of patients with DMD and mothers of patients with BMD led us to verify their results in much larger group of 744 unrelated families. In addition, we explored these authors' data concerning different frequencies of new mutations in cases of deletions, duplications and small mutations.⁷

MATERIALS AND METHODS

DMD was diagnosed in 609 families and BMD in 135 families. Parents of the children and adult persons whose DNA was analyzed signed the informed consent. DNA was isolated from peripheral white blood cells derived from members of 744 families tested in the Department of Genetics in 1990–2015. DNA was isolated by a standard phenol–chloroform extraction method and using a MagNa Pure Compact appliance (Roche Instrument Center AG, Rotkreuz, Switzerland).⁸ Diagnostic criteria for DMD and BMD were same as those of Lee *et al.*,⁷ that is, based on clinical picture: later onset of the disease in BMD and the age of ambulation loss, 10–13, in DMD, and considerably longer survival in BMD. Clinical diagnosis was supported by the dystrophin gene analysis and in a few cases by dystrophin testing in a biopsied muscle.

Molecular examination of DMD/BMD patients was initially performed using a PCR-multiplex method and then using multiplex ligation-dependent probe amplification (MLPA; SALSA MLPA KIT P034/035 DMD/Becker, MRC-Holland, Amsterdam, The Netherlands).^{9,10} Since 2010 a direct sequence analysis has been applied.¹¹

The carrier status of mothers of the affected sons was initially examined by PCR of the microsatellites' sequences located in the dystrophin gene (2, 7, 44, 45, 48, 49, 50 and 63 intron; www.dmd.nl). Exclusion of the deletion mother carrier status was based on finding two different microsatellite sequences—corresponding to the site of the deletion in the affected

Table 1 Mutations detected in 744 families with DMD and BMD; familial and isolated cases; unrelated cases only (probands).

	Deletions	Duplications	Small mutations	Overall
DMD	440 (72.2%)	90 (14.8%)	79 (13.0%)	609 (100%)
BMD	124 (91.9%)	5 (3.7%)	6 (4.4%)	135 (100%)
Overall	564 (75.8%)	95 (12.8%)	85 (11.4%)	744 (100%)

Abbreviations: BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy.

son.¹² Duplications and deletions have been tested using the multiplex ligation-dependent probe amplification: an increase or reduction of a peak by ~50% in relation to the control sample was interpreted as the detection of the carrier state's duplication or deletion. The carrier state of small mutations was set by means of direct sequence analysis of the dystrophin gene fragment, including mutations detected in the probands.

The number of detected duplications and small mutations in patients with DMD/BMD is lower than real, as only the PCR-multiplex method was used in years 1990–2008.

Statistical analysis was performed using the Fisher's exact test or the χ^2 -test as appropriate.

RESULTS

The number and type of detected mutations in the dystrophin gene present among 744 unrelated DMD/BMD patients are shown in Table 1. Most frequently the disease was caused by deletions (75.8%), and significantly more deletions were found in BMD (91.9% vs 72.3% in DMD; $P < 0.00001$) while more duplications and small mutations were detected in DMD (Table 1). Small mutations are more common in DMD because they usually produce stop codon or disturb open reading frame. In effect a proportion of the deletions (often in frame) in milder form of the disease, BMD, is higher.

Carrier status was found in 469 out of a group of 744 mothers of the probands (63.0%). The remaining 275 mothers (37.0%) was found to be non-carriers (based on analysis of DNA extracted from white blood cells), although among them there were 9 families with more than one affected individual: evidence of germinal mosaicism in 3.3% of the 'non-carrier mothers'. Among 526 mothers of single (isolated) cases of the disease 260 mothers (49.4%) were the mutation carriers.

The numbers of *de novo* mutations detected in the dystrophin gene in each form of the disease are shown in Table 2. The carrier's frequency in mothers of sons with BMD (92/135—68.1%) was a little higher than in the mothers of sons with DMD (377/609—61.9%), but the difference was not statistically significant ($P > 0.05$) (Figure 1). Thus, our data do not seem to confirm the observations of Lee *et al.*⁷

The majority the disease cases due to new mutations were caused by deletions—236/564 (41.8%), and less often by duplications—21/95 (22.1%) and small mutations—18/85 (21.2%). Therefore, among DMD/BMD patients, the frequency of *de novo* deletions differed significantly from the frequency of *de novo* duplications ($P = 0.0005$) and small mutations ($P = 0.0006$). The frequency of new mutations of other types, duplications and small mutations, was nearly identical (Table 2).

DISCUSSION

The main goal of this work was to compare our analysis to the results of Lee *et al.*⁷ published in this journal. The analyzed material is shown in Table 1 in which overall group of the 744 unrelated patients is divided into DMD and BMD cases, and the types of mutations found in both forms of the disease are indicated.

Table 2 Frequency of new mutations in DMD and BMD according to the types of the mutations: deletions; duplications; and small mutations.

	New deletions	New duplications	New small mutations	Overall
DMD	194/440 (44.1%)	20/90 (22.2%)	18/79 (22.8%)	232/609 (38.1%)
BMD	42/124 (33.9%)	1/5 (20.0%)	0/6 (0.0%)	43/135 (31.9%)
Overall	236/564 (41.8%)	21/95 (22.1%)	18/85 (21.2%)	275 ^a /744 (37.0%)

Abbreviations: BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy.

^aIncluded nine germinal mosaics (five deletions in DMD, two small mutations in DMD, one duplication in DMD and one deletion in BMD).

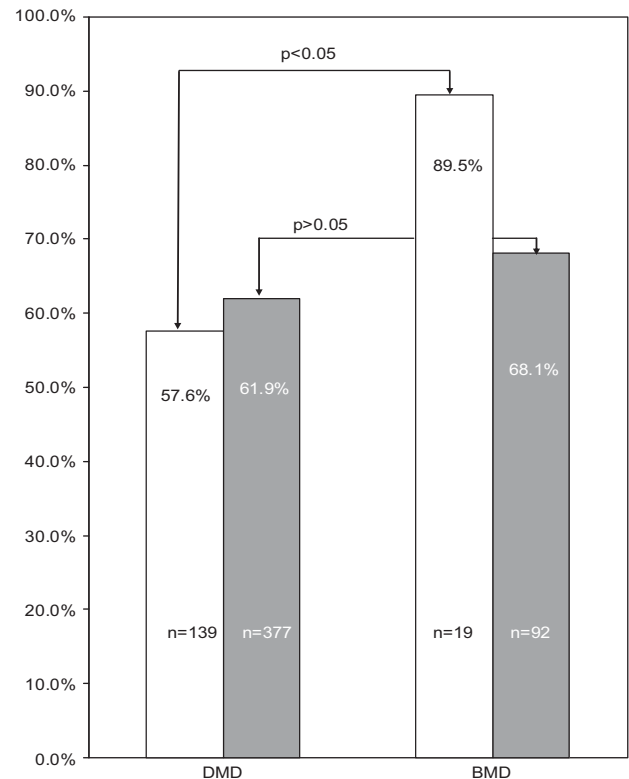


Figure 1 The carrier frequency of mothers of Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) patients. Comparison of presented data (shaded bars) and the Japanese data (Lee *et al.*⁷). In the Japanese material the carrier frequency was significantly lower in mothers of DMD patients compared to that in mothers of BMD patients ($P < 0.05$). In the Polish data the respective difference was not significant ($P > 0.05$). In both instances Fisher's exact test was used (Table 2).

The results presented above do not confirm the Japanese observations of a statistically significant difference between the frequency of the mutations' carriership in the mothers of patients affected with DMD and BMD.⁷ In our material of 744 cases the frequency of the respective carrier status of mothers of the probands was 61.9% (377/609) and 68.1% (92/135; Figure 1). A little higher frequency of the maternal carriers in BMD may be due to the mutation transfer from some patients to all their daughters, especially from those with the mildest form of BMD. According to the Haldane theory new mutations should be more frequent in the severe form of the disease, that is, DMD with zero fitness. In fact we noticed like Lee *et al.*⁷ some difference but not significant

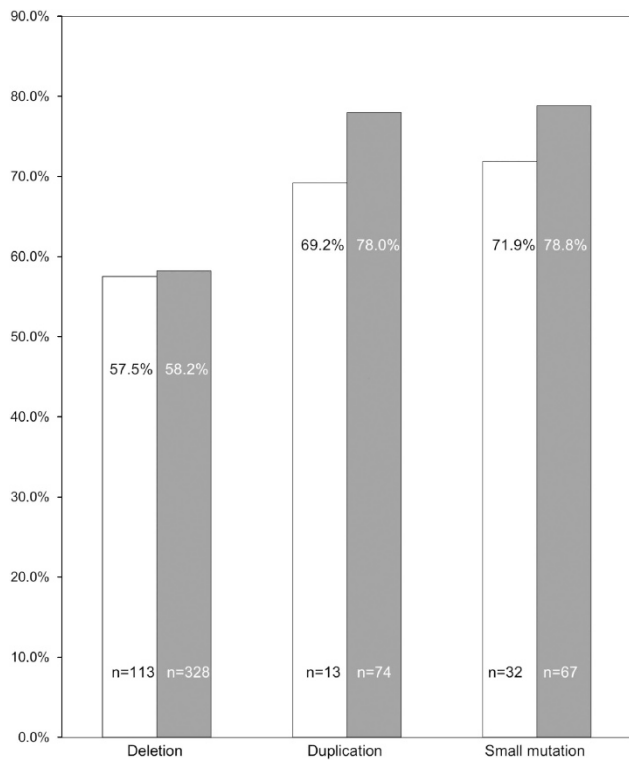


Figure 2 The carrier frequency of mothers of Duchenne/Becker muscular dystrophy patients according to a type of the mutation. Comparison of the Polish data (shaded bars; Zimowski *et al.*) and the Japanese data (Lee *et al.*⁷). Both in the Japanese and Polish data the carrier frequency in the mothers of patients with deletions was lower than in those with duplications and small mutations. In the Polish data carrier frequency of mothers of patients with deletions was significantly lower than that in the mothers with other types of mutations.

statistically. Lower frequency of small mutations in this study might have some effect to the lower carrier frequency of BMD (Figure 1) as the frequency of new deletion mutations (33.9%) is higher than that of other mutations (Table 2). Lower than one might expect carrier frequency of BMD may also result from some bias—due to the fact that milder cases of BMD with preserved capacity of the affected males to reproduce tend to be unnoticed and avoid being referred to the health service units.

Thus, we were able to confirm the suggestion of the Japanese authors that among the new mutations, deletions are significantly most frequent. Among deletions, the new mutations constitute as much as 41.8% (236/564), that is, the highest percentage among the various types of mutation. Duplications and small mutations appear *de novo* in every fifth patient's mother: 22.1% (21/95) and 21.2% (18/85), respectively (Table 2; Figure 2). The observations of Lee *et al.*⁷ were similar, however, perhaps due to the small number of the examined women, their results were not statistically significant.

Table 3 represents a compilation of molecular studies from other countries that illustrate the frequency of new mutations. The results demonstrating the frequency of DMD/BMD mutation carriers in different populations are rather divergent (Table 3). For example, results from India and Mexico on several small groups of mothers of isolated cases of the disease or familial cases do not seem to be very reliable due to small sizes of the studied groups.^{13–17}

Table 3 Frequency of new mutations in the dystrophin gene in different countries (literature).

Country of origin	Tested mothers		New mutations		Isolated/familial cases
	No.	No.	%		
India ¹³	29 ^a	18	62.1		Isol.
India ¹⁴	17 ^a	10	58.8		Isol.
Netherlands ¹⁸	189	91	48.1		Isol.
Australia ¹⁹	201	109	54.2		Isol.
India ¹⁷	109	66	60.6		Isol. and Fam.
India ¹⁵	31 ^a	22	71.0		Isol. and Fam.
Mexico ¹⁶	37 ^a	23	62.2		Isol. and Fam.
Japan ⁷	158	61	38.6		Isol. and Fam.
France ²⁰	1472	361	24.5		Isol. and Fam.
Own data	744	275	37.0		Isol. and Fam.

Abbreviations: Fam., familial; Isol., isolated.

^aA cohort in which only the deletions were considered.

Larger cohorts from Dutch and Australian studies considering only families with isolated cases yielded results of 48.1% (91/189) and 54.2% (109/201), respectively, of the new mutations.^{18,19} The respective percentage in the largest analyzed group of France was only 24.5% (361/1472).²⁰ Closest to our result—37.0% (275/744)—was the Japanese one, 38.6% (61/158).⁷ The inflated value of the frequency of *de novo* mutations in some publications (Table 3) was probably partly due to the fact that the authors took only into account the available cases caused only by deletions.^{13–16} Our study shows that deletions among new mutations are significantly most frequent.

According to our data, the percentage of *de novo* mutations reached 37.0% (Table 3) and was therefore close to the expected 33.33%. It should also be mentioned that among the 275 mothers with excluded carrier status, based on analysis of DNA extracted from peripheral blood leukocytes, 9 persons are with germinal mosaicism. The true proportion of germinal mosaicism is difficult to verify, however, it was estimated that the percentage of such cases is ~5–10%.²¹

Higher frequency of *de novo* deletions in the dystrophin gene than the frequency of duplications and small mutations indicates the existence of responsible mechanisms and causative factors that require explanation and basic studies on the level of oogenesis and spermatogenesis.^{22,23}

To conclude, our study shows that the frequency of the mutation carriers in the dystrophin gene does not differ significantly between mothers of DMD and BMD patients, and in the subgroup of patients having deletions in the dystrophin gene, the frequency of new mutations is significantly higher than that in other subgroups exhibiting other types of mutation, namely duplications and small mutations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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