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Two mutations in one dystrophin gene

Dwie mutacje w jednym genie dystrofiny

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Abstract

Background and purpose: Duchenne/Becker muscular dystrophies (DMD/BMD) lead to progressive irreversible muscle deterioration caused by recessive mutations in the dystrophin encoding gene (Xp21.1). Approximately 60% of mutations are deletions, 10% are duplications and the remaining 30% are point mutations. The aim of the study is to present the rare occurrence of two pathogenic mutations (deletions or duplications) in one allele of the dystrophin gene.
Material and methods: DNA of patients from 1364 DMD/BMD families was tested. Two techniques – PCR-multiplex and multiplex ligation-dependent probe amplification – were used to search for mutations in the dystrophin gene.
Results: Deletion was detected in 648 families and duplication was found in 74 families (analysis in progress). In two families, presence of two mutations in one gene was documented – in the first family two deletions were found (exons 45-49 and 60-61), and in the second family two duplications were detected (exons 2-7 and 50-59). One of the deletions disrupted the reading frame, and the other deletion retained the reading frame. Both duplications also retained the reading frame of the gene but in both families the disease took a severe course (DMD). In the family with two duplications prenatal diagnosis was also carried out, and carriership of both mutations was discovered in the female fetus.
Conclusions: In the analyzed group of DMD/BMD families, the frequency of combined occurrence of two mutations in one gene was 2 per 722 (0.3%). The phenomenon of detected non-contiguous deletions and duplications is presented together with 31 similar cases published so far.

Streszczenie

Wstęp i cel pracy: Dystrofia mięśniowa Duchenne'a/Beckera (DMD/BMD) jest związana z postępującym i nieodwracalnym zanikiem mięśni wywołanym recesywnymi mutacjami w genie dystrofiny (Xp21.1). Szacuje się, że 60% mutacji stanowią delecje, a 10% – duplikacje; pozostałe 30% mutacji ma charakter punktowy. Celem pracy jest przedstawienie rzadkich przypadków współwystąpienia w jednym allelu genu dystrofiny dwóch chorobotwórczych mutacji – delecji lub duplikacji.
Materiał i metody: Badano DNA pacjentów z 1364 rodzin skierowanych z podejrzeniem DMD lub BMD. Mutacji poszukiwano, używając dwóch technik: PCR-multiplex i MLPA (*multiplex ligation-dependent probe amplification*).
Wyniki: W 648 rodzinach wykryto delecję, a w 74 rodzinach – duplikację (badania w toku). W dwóch rodzinach udokumentowano łączne wystąpienie w jednym genie dystrofiny dwóch mutacji – w pierwszej rodzinie w jednym allelu wykryto dwie delecje (eksony 45-49 i 60-61), a w drugiej rodzinie dwie duplikacje (eksony 2-7 i 50-59). Jedna z delecji naruszała fazę odczytu, druga zaś ją zachowywała; obie duplikacje zachowywały fazę odczytu, jednak w obu rodzinach choroba przybierała ostrą postać (DMD). W rodzinie, w której wykryto dwie duplikacje, wykonano diagnostykę prenatalną, stwierdzając u płodu płci żeńskiej nosicielstwo obu mutacji.
Wnioski: W analizowanej grupie rodzin z delecją lub duplikacją częstość łącznego wystąpienia dwóch mutacji wyniosła 2 na 722 (0,3%). Zjawisko wykrytych nieciągłych delecji i duplikacji przedstawiamy w zestawieniu z opisanymi dotychczas 31 podobnymi przypadkami.

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Key words: non-contiguous deletions, non-contiguous duplications, Duchenne muscular dystrophy, Becker muscular dystrophy, multiplex ligation-dependent probe amplification.

Słowa kluczowe: nieciągłe delecje, nieciągłe duplikacje, dystrofia mięśniowa Duchenne'a, dystrofia mięśniowa Beckera, *multiplex ligation-dependent probe amplification*.

Introduction

Duchenne muscular dystrophy (DMD) (MIM 310200) and Becker muscular dystrophy (BMD) (MIM 300376) are caused by mutations in the dystrophin gene localized at Xp21. Incidence of DMD is estimated to be 1 : 3500 of living male births, while the incidence of less severe BMD is five times lower [1]. The dystrophin gene, the largest human gene, consists of 79 exons. Mutations causing DMD/BMD include deletions (60%), duplications (10%), and point mutations (30%). Deletions can involve a various extent of the gene, from one exon to all exons. Duplications are of similar size. Point mutations are alterations of one, several or a dozen nucleotides within exons or at the intron-exon border [2].

According to Monaco *et al.* [3], mutations leading to a stop codon, either due to the substitution of one nucleotide with another, or due to a frame shift, result in complete lack of dystrophin and produce the severe disease form, i.e. DMD. Mutations that do not interfere with the reading frame (deletions or duplications that are multiplications of complete trinucleotide codons) lead to the synthesis of a truncated protein that might partially fulfill its role within the muscle fiber, resulting in a milder form of the disease, i.e. BMD. The theory of frame shift or its preservation is appropriate in 90% of cases. In another 10%, deletions may not include the whole exons or may affect the mRNA splicing in such a way that mRNA does not exactly match other exons within the gene. It is also theoretically possible that the deletion with the preserved reading frame coexists with the point mutation leading to the stop codon. The compliance of duplications with Monaco's theory is lower and may reach only 70% [4].

We present the coexistence of two different mutations in one allele of the dystrophin gene leading to DMD/BMD. This phenomenon is very rare and in Poland it was documented for the first time in our laboratory.

Material and methods

Between 1991 and 2011, the Department of Genetics (Institute of Psychiatry and Neurology) collected DNA samples of members of 1364 families, referred with suspected DMD/BMD. Mutations were sought initially with hybridization and PCR-multiplex, and since 2009 with the method of multiplex ligation-dependent probe amplification (MLPA). PCR-multiplex used the set of primer pairs for the following exons: 1, 2, 3, 4, 6, 7, 8, 11, 12, 13, 17, 19, 20-22, 29, 34, 42-55, 60 (www.dmd.nl/DMD_mPCR.html), while the MLPA method used two sets of reagents allowing for the qualitative and quantitative assessment of all 79 exons of the dystrophin gene: SALSA P034 and SALSA P035 (MRC-Holland, Amsterdam, The Netherlands).

Patients

The mutations presented below were detected in DNA of subjects from two families: D166 – three boys affected with DMD were tested; women, obligatory carriers, were not tested with molecular methods (Fig. 1A); and D1299 – four healthy women suspected of being mutation carriers were tested and additionally prenatal testing was performed (trophoblast sampled at 12 weeks of pregnancy); a DNA sample was not available in the case of a deceased male member of the family in whom DMD was diagnosed clinically (Fig. 1B).

Results

Among studied DNA samples, deletions in the dystrophin gene were detected in 648 families, and duplications in 74 families. Among those 722 families there were two families with coexistence of two mutations in one allele in affected men and in female carriers. Those families (pedigrees D166 and D1299) are described below (Figs. 1A-B).

In D166 pedigree (Fig. 1A), two deletions in exons 45-49 and 60-61 (c.[6439-?_7200+?del; 8938-?_9163+?del]) were detected in the dystrophin gene of the proband (IV-2) (the extent of deletions in the adjacent introns in unknown). Figure 2A shows the result of MLPA testing (control testing – Fig. 2C). The first deletion did not disrupt the reading frame, while the second one shifted the frame by one nucleotide, lead-

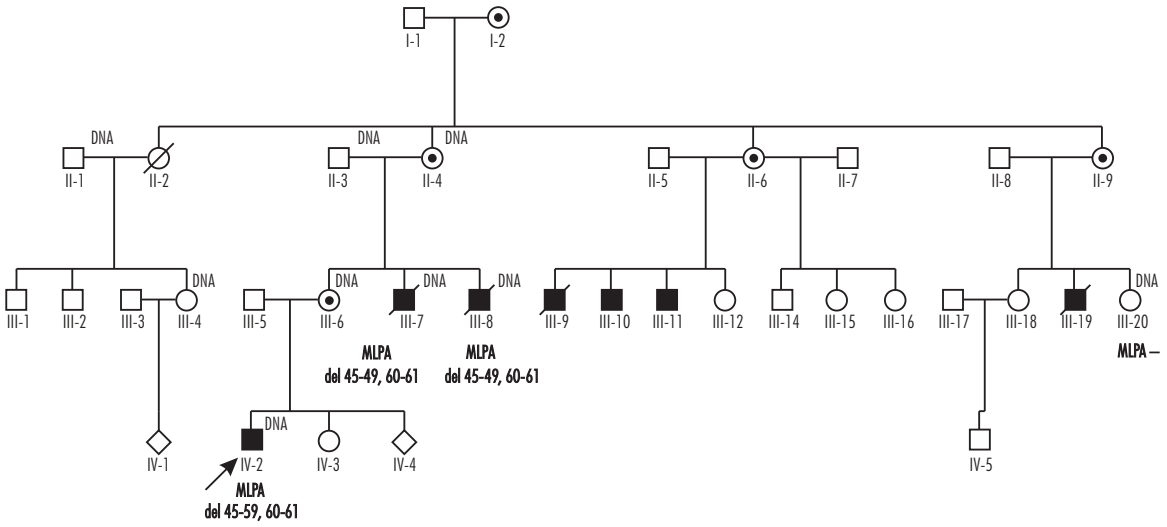

Fig. 1. A) Pedigree of the D166 family. Affected boys are marked in black, the proband is indicated by an arrow. The following male members of the family were analyzed: III-7, III-8 and IV-2

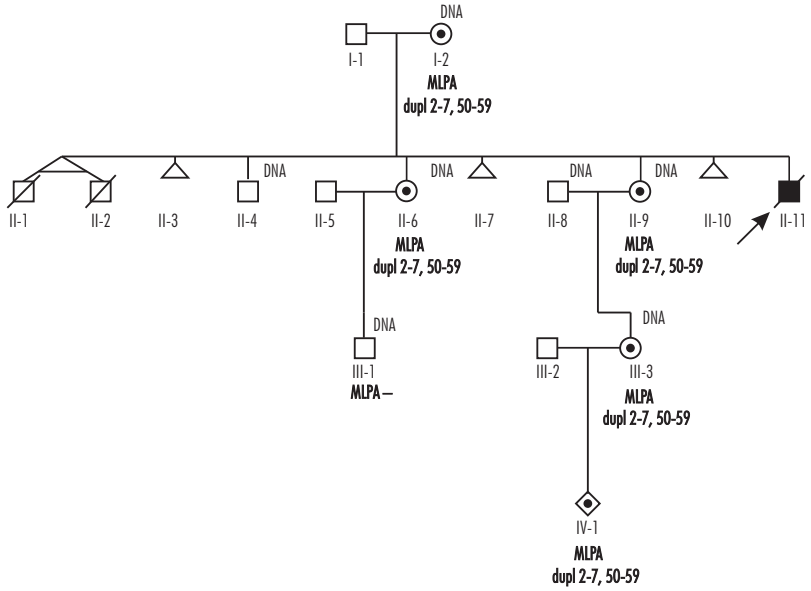

Fig. 1. B) Pedigree of the D1299 family. The affected boy is marked in black; the proband is indicated by an arrow. The following members of the family were analyzed: I-2, II-6, II-9, III-3 and IV-1 (prenatal test). DNA of the proband was not available

ing to the change of 33 amino acids coded by exon 62 and part of exon 63; it also resulted in a stop codon in exon 63 (proband IV-2). The same two deletions were detected in the two other affected boys (III-7 and III-8). All three patients were affected with the acute form of the disease (DMD). The mothers of the affected boys (II-4 and III-6), who were obligatory carriers, were not tested with molecular methods.

In D1299 pedigree (Fig. 1B), two duplications affecting exons 2-7 and 50-59 (c.[32-?_649+?dup; 7201-?_8937+?dup]) were detected in the dystrophin gene of the tested female subject (II-9) who was the sister of a boy who died 20 years ago because of DMD (the extent of duplications in the adjacent introns is unknown). Figure 2B shows the result of MLPA testing (control testing – Fig. 2C). Those duplications did not disrupt the open reading frame. The same duplications were found in the mother (I-2), sister (II-6) and daughter (III-3) of the female patient. Patient III-3 decided to be tested prenatally because of the risk of giving birth

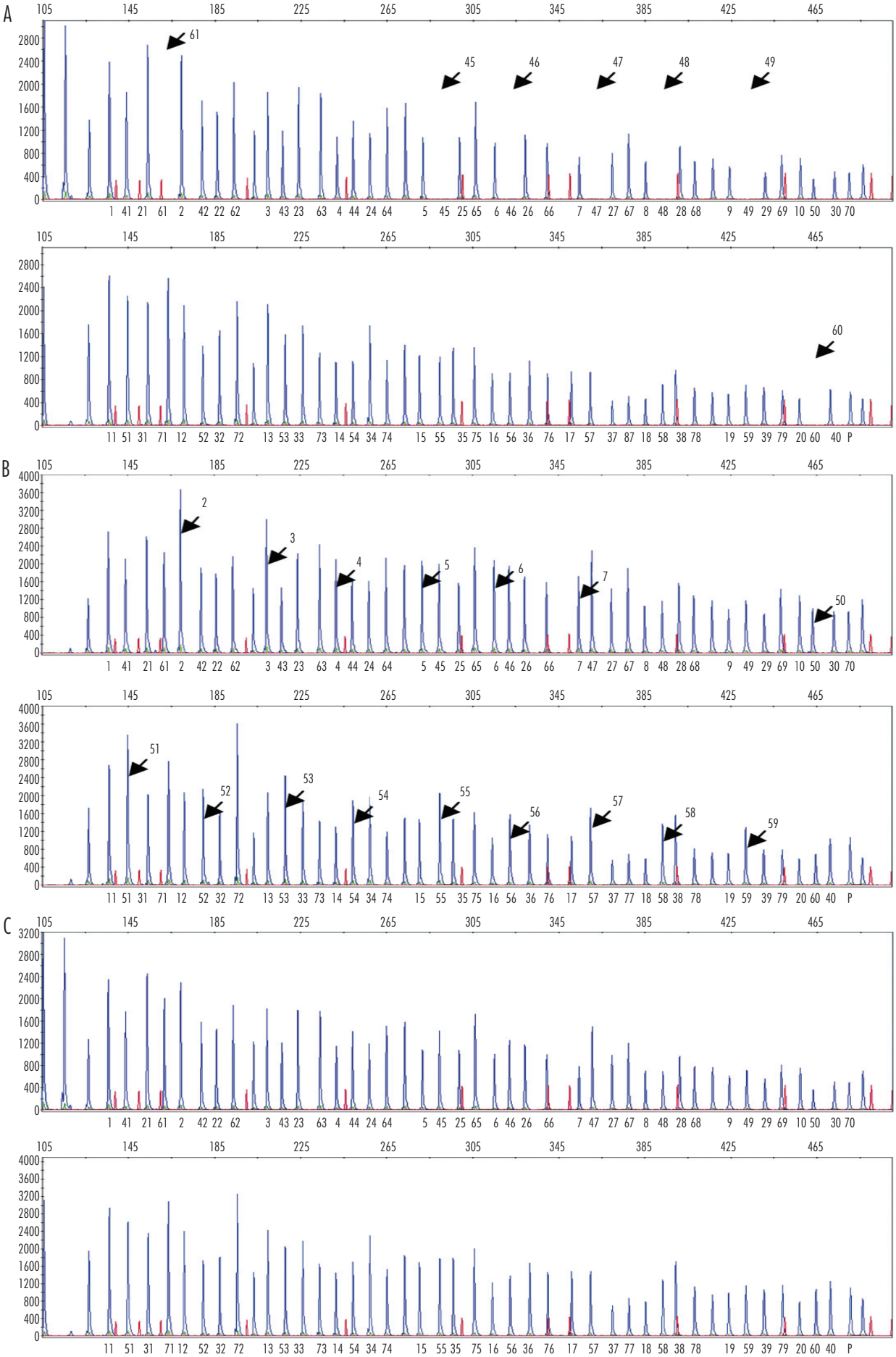

Fig. 2. Results of DNA analysis carried out by MLPA method: (A) DMD patients from D166 family (III-7) – deleted exons: 45-49 and 60-61; missing peaks due to deletion marked by arrows; (B) prenatal diagnosis in D1299 family – female fetus (IV-1), a carrier of duplicated exons: 2-7 and 50-59; elevated peaks due to duplication are marked by arrows; (C) control DNA – unaffected male

to a child affected with DMD. DNA testing in the female fetus (IV-1) found the same duplications.

Discussion

The occurrence of two mutations in one dystrophin gene is a rare finding; it was demonstrated for the first time by Hoopa *et al.* [5], who found the co-occurrence of two deletions involving exons 12-17 and 50-51. One year later, Florenti *et al.* [6] found two deletions (19-21 and 26-29) in one gene. Numerous studies performed in the 1990s, involving the search for deletions in patients with DMD/BMD using either PCR-multiplex or very laborious hybridization, did not reveal any further cases of double mutations. It was the introduction of MLPA technique that enabled fast and simultaneous testing of presence and quantity of all 79 exons and resulted in reports on two mutations in one allele of the dystrophin gene.

In 2004, the coexistence of deletions in exons 1 and 3-11 was reported [7, www.dmd.nl] but subsequently it was corrected – finally, the duplication of exon 1 and exons 3-9 was established. One year later, the co-occurrence of two duplications of exons 45-48 and 54-55 was reported [8]. In the following year, White *et al.* [9] described four cases of complex duplications, including two with the fragments in a triple dose (i.e. triplication). Then, several papers on other duplications, termed by the authors non-contiguous duplications, were published [10-15].

The results of two large studies showed that the presence of more than two mutations in one gene, although extremely rare, is also possible [16,17]. The largest database of complex deletions and duplications can be found at www.dmd.nl, although not all cases are included. Table 1 provides a list of 33 cases of double deletions and duplications, including two families reported here.

The data compilation presented in Table 1 and Figure 3 suggests that double duplications are the most prevalent complex mutations; double deletions are infrequent and coexistence of duplication with deletion is even more rare, similarly to cases with duplications associated with tri- or quadruplications.

The case of two deletions detected by us results in the acute form of the disease. The first deletion (exons 45-49) does not disrupt the reading frame and could result in a relatively mild course of the disease, but the other deletion (exons 60-61), disrupting the reading frame and leading to the stop codon in exon 63, leads

Table 1. Reported non-contiguous deletions and duplications in the dystrophin gene according to www.dmd.nl (Fig. 3 illustrates the schema of non-contiguous mutations)

Mutations detected	Reference and year of publication
P-2 del; 44 del	ESHG* 2004
P dupl; 2 dupl	[17] 2009
P-1 dupl; 2-7 dupl	[14] 2008
P-1 dupl; 3-9 dupl	[7] 2004
P-1 dupl; 45-55 dupl	www.dmd.nl**
1-8 dupl; 45-52 del	[12] 2008
2-7 dupl; 50-55 dupl	[15] 2008
3-12 dupl; 38-47 dupl	www.dmd.nl**
5-7 del; 10-11 del	[17] 2009
5-13 dupl; 45-52 dupl	[10] 2007
5-18 dupl; 19-41 tripl; 42 dupl; 43-44 tripl	[9] 2006
5-19 dupl; 38-41 dupl	[9] 2006
9-16 dupl; 31-32 dupl; 38-42 dupl	[12] 2008
10-16 dupl; 22-44 dupl	[16] 2009
13-19 del; 31-45 quadr; 46-48 dupl; 51-52 dupl	[12] 2008
19-21 del; 26-29 del	[6] 1995
29 dupl; 45 dupl	[16] 2009
29-33 dupl; 34-36 tripl; 37-51 dupl	[12] 2008
30-35 dupl; 48-50 del	[12] 2008
43-44 dupl; 45-47 del	ESHG 2004
43-45 dupl; 56-74 dupl	www.dmd.nl**
43-47 dupl; 60-67 dupl	[4] 2008
43-51 dupl; 53-60 dupl	[14] 2008
44 dupl; 51-55 dupl	[7] 2004
45-48 dupl; 51-55 dupl	[12] 2008
45-48 dupl; 54-55 dupl	[8] 2005
45-48 dupl; 55-63 dupl	[13] 2008
45-55 dupl; 65-79 dupl	[9] 2006
50-60 dupl; 63-79 dupl	[16] 2009
52-55 dupl; 63-67 dupl; 68-79 tripl	[9] 2006
54-59 dupl; 63-79 dupl	[17] 2009
45-49 del; 60-61 del	Our own data
2-7 dupl; 50-59 dupl	Our own data

**Data presented at the meeting of the European Society of Human Genetics*

***Website of Leiden University Medical Center, the Netherlands*

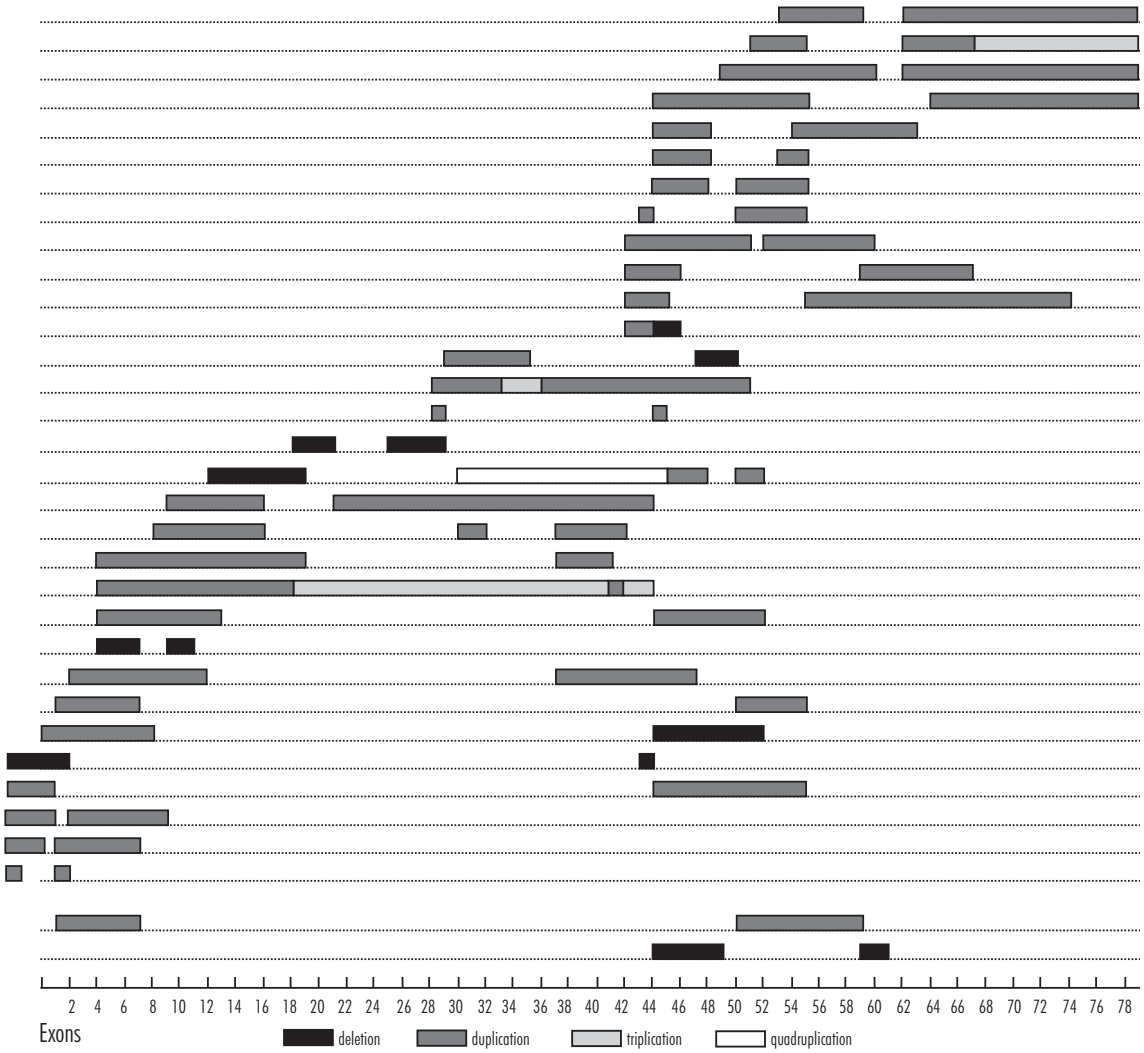

Fig. 3. A schematic representation of the distribution of published noncontiguous mutations listed in Table 1

to the marked shortening of the protein, deprives it of two very important domains (cysteine-rich and *C*-terminal ones) and produces the acute form of the disease (DMD).

Our second case, with two duplications in the dystrophin gene, is very similar to that reported by Spanish authors [15]. In both cases, duplication at the 5'-terminal involves exons 2-7, and duplication in the hotspot of the 3'-terminal involves exons 50-59 in our case and exons 50-55 in the Spanish case. Despite the preservation of the reading frame in both those deletions, the introduction of two important changes to the dystrophin protein, especially the one related to the *N*-terminal domain responsible for the binding of dystrophin with the cytoskeleton of the muscle fiber, may result in the same effect as the single mutation which disrupts the reading frame. We are not aware, however, of the location and orientation of the replication of the gene fragment; also we do not know how it translates into the sequence present in the mRNA. The result of DNA testing only reports the summarized number of particular exons.

Families in whom the reported deletions and duplications were found, as in the case of other families affected with DMD/BMD, are managed by the out-patient genetic clinic and have access to prenatal diagnosis. To date, this opportunity has been used by one of the abovementioned carriers. The healthy daughter is a carrier of

the double duplication in the dystrophin gene, similarly to her mother and grandmother.

Disclosure

Authors report no conflict of interest.

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