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Clinical Characteristics of Patients With Becker Muscular Dystrophy Having Pathogenic Microvariants or Duplications

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Abstract

Background and Objectives

Becker muscular dystrophy (BMD) is an allelic disorder of Duchenne muscular dystrophy (DMD) in which pathogenic variants in *DMD* cause progressive worsening of motor dysfunction, muscle weakness and atrophy, and death due to respiratory and cardiac failure. BMD often has in-frame deletions that preserve the amino acid reading frame, but there are some cases with microvariants or duplications. In recent years, the importance of therapeutic development and care for BMD has been emphasized. Therefore, the purpose of this study was to understand the clinical characteristics of BMD patients with microvariants or duplications and to determine the genotype-phenotype relationship.

Methods

The study focused on patients with pathogenic microvariants or duplications in *DMD* who were ambulatory after 16 years of age or had specific muscle biopsy results between June 13, 2017, and March 31, 2023. Informed consent was obtained from the patients or their surrogates. Data concerning *DMD* variants, muscle biopsy findings, skeletal muscle, respiratory and cardiac function, and CNS involvement were collected and analyzed statistically.

Results

Thirty-three patients with BMD had pathogenic microvariants (missense variants, nonsense variants, splice site variants, and other microvariants), and 16 patients had in-frame duplications in *DMD*. Many patients with microvariants had abnormal ECG findings. The effect of variant type on patient outcomes varied. Regardless of the type of microvariant, skeletal muscle and respiratory dysfunction was more severe in mutants of the cysteine-rich/C-terminal domain than in rod domain mutants. On the other hand, there was no significant difference in the complication rate of CNS disorders among the 3 domains of dystrophin.

Discussion

Microvariant forms, in particular, tend to vary in clinical severity according to the site of the dystrophin protein mutation rather than the type of pathogenic variant. The results of this study may be useful for genetic counseling, care, and treatment of patients with BMD.

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Glossary

BMD = Becker muscular dystrophy; **CK** = creatine kinase; **DMD** = Duchenne muscular dystrophy; **FVC** = forced vital capacity; **LVEF** = left ventricular ejection fraction; **MDCTN** = Muscular Dystrophy Clinical Trials Network.

Introduction

Becker muscular dystrophy (BMD) is an allelic disorder of Duchenne muscular dystrophy (DMD) in which pathogenic variants in *DMD* cause progressive motor dysfunction, muscle weakness and atrophy, and death due to respiratory and heart failure. Unlike DMD, which is deficient in the sarcolemma protein dystrophin coded by *DMD*, BMD is milder than DMD because it produces a small amount of truncated or dysfunctional dystrophin. However, the severity of the disease varies widely.[1,2] Recently, we reported the natural history and genotype-phenotype relationships of 225 patients with BMD with in-frame deletions.[3] We observed an association between genotype and phenotype, especially in patients with the most frequent in-frame deletions, and we discussed the clinical significance of this association. Although there are several reports describing DMD/BMD variants at a large scale,[4-6] patients with BMD generally have more in-frame deletions and fewer duplications and microvariations, and detailed clinical information in these variants is scarce. Recently, the development of novel therapies, such as fast myosin inhibitors,[7] steroidal anti-inflammatory agents (e.g., vamorolone),[8] and a histone deacetylase (HDAC) inhibitor (givinostat),[9] have been proposed for BMD and are expected to be developed actively in the future. Therefore, it is necessary to collect and analyze the clinical profile of patients with BMD with variants other than in-frame deletions. In this study, we report clinical characteristics of patients with BMD having microvariants or duplications collected by the Muscular Dystrophy Clinical Trials Network (MDCTN) in Japan.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the Institutional Ethical Review Boards of the National Hospital Organization Matsumoto Medical Center and participating institutes. Consent to participate in the study was obtained from the patient (≥16 years old) or a surrogate, if patient was <16 years old. The MDCTN secretariat handled the registration of cases. Some of the *DMD* variants involved in the study have been mentioned in previous publications. The purpose of the study, the use of patient information, and the name of the information manager were continuously displayed on the website of the study institution and MDCTN so that patients could withdraw from the study if desired.

Patients

The inclusion criteria for eligible patients were as follows: (1) in-frame duplication of *DMD* identified by multiplex ligation-dependent probe amplification or micromutations identified by the method described previously[5] for cases diagnosed with dystrophinopathies through dystrophin immunostaining. In addition, missense mutation determination and in silico analysis of the effect of amino acid changes were performed as described previously[5]; (2) received medical care at a representative or cooperating research institution between June 13, 2017, and March 31, 2023, with data extractable from medical records; (3) able to walk after the age of 16 years; and (4) if younger than 16 years of age, having undergone a muscle biopsy and confirmed to express dystrophin, or have a relative with the same variant diagnosed with BMD.

Survey Items

DMD variants and clinical information obtained at the time of the initial and final medical record survey were collected from the medical record by the muscular dystrophy specialist. The survey items included *DMD* pathogenic variants (microvariants or duplications), muscle biopsy findings, cardiac and CNS comorbidities, family history, initial symptoms, ambulatory status, serum creatine kinase (CK) levels measured with a spectrum altimeter at each site, percentage of forced vital capacity (%FVC) by age (>55%, normal), ventilator use, heart failure symptoms, ECG findings, and left ventricular ejection fraction (LVEF) measured by an echocardiogram using the Teichholz method (>55%, normal).

Data Analysis

Survey items were analyzed by descriptive statistical analysis. Clinical characteristics recorded at the time of the initial and final medical record survey were extracted. For between-group comparisons, *t* tests were performed between microvariants and duplications. For testing between the 3 groups, ANOVA analysis was performed followed by the Bonferroni test. In addition, the Pearson χ^2 test was used to test the crosstabulation table. Data are presented as mean ± SD and median. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using R (v.3.5 or later; R Foundation for Statistical Computing, Vienna, Austria) and SPSS (v.23 or later; IBM Corp., Chicago, IL).

Data Availability

Anonymized data not published within this article will be made available by reasonable request from any qualified investigator. The data supporting the findings of this study are available from the corresponding author on reasonable request.

Results

Pathogenic *DMD* Variants of 49 Patients With BMD

There were 33 patients with pathogenic microvariants and 16 patients with duplications (eTable 1). Microvariants included 5 missense variants with amino acid substitutions, 6 nonsense variants, 12 splice site variants, and 6 other microvariants mapped in *DMD* (eFigure 1). All duplications were in-frame.

Overall Characteristics of Patients With BMD

Table 1 presents the overall characteristics of patients with microvariants and duplications, as well as the 225 patients with BMD with in-frame deletions reported previously.[3] Patients with microvariants had a higher frequency of diagnostic muscle biopsy compared with the other groups, but this may be due to diagnostic uncertainty. In addition, many patients with microvariants seem to have abnormal ECG findings (axial deviation, PR shortening, conduction abnormalities, ST/T wave abnormalities, abnormal Q waves, and R/S >1 in V1 lead).[3]

Point Variants Involving Amino Acid Substitutions

Table 2 presents the 5 patients with variants involving amino acid substitutions. Of the 5 patients, c.481A>C (no. 2) and c.10864G>A (no. 5) used wheelchair before 20 years of age and are considered relatively severe outcomes. The c.2096C>T patient (no. 4) with febrile convulsions was characterized by the absence of skeletal muscle symptoms until around 20 years old. The overall muscle biopsy rate was 60%.

Nonsense Variants

Eight patients with nonsense variants are given in Table 3. Six patients (nos. 6–11) had rod domain variants, and 2 patients (nos. 12 and 13) had C-terminal domain variants (eFigure 1). The overall muscle biopsy rate was 50%. Two patients with c.5407C>T were no. 10 and his grandson (no. 11). Patient no. 11 had a serum CK level over 20,000 IU/L in childhood and could have been diagnosed with DMD. However, he was diagnosed as having BMD because his muscle biopsy showed dystrophin expression, and his grandfather (no. 10) was able to walk at older than 70 years of age. In this case, the mutated exon 38 is skipped, which may be the result of retention of the amino acid reading frame. However, siblings with c.10320T>A (nos. 12 and 13) showed relatively severe symptoms because both patients used wheelchair at around 20 years old and had similar respiratory and cardiac dysfunction.

Pathogenic Splice Site Variants

BMD with splice site variants was the most common microvariants with 14 patients (Table 4). c.3603+3A>T in 2 patients (nos. 23 and 24) were paternity cases, and C.9650-2A>G in 2 patients (nos. 26 and 27) were sibling cases. The overall muscle biopsy rate was 51%. Wheelchairs were introduced in 2 patients (nos. 15 and 25) at around 40 years of age. No patient was placed on a ventilator. CNS disorders were present in 5 patients, and 4 had intellectual and developmental disabilities.

Other Microvariants

Four of the 6 patients with other microvariants (single-nucleotide deletions, double-nucleotide deletions, etc.) underwent muscle biopsy (Table 5). c.160_162del (no. 31) and c.10453_10454del (no. 33) were in their 20s and used wheelchairs. Patients with other variants had impaired respiratory and cardiac function but were considered to have mild skeletal muscle impairment. Both c.93+5590T >A (no. 28) and c.265-463A>G (no. 29) were deep intronic single-nucleotide variants.

Comparison of Microvariants in the Actin-Binding Domain, Central-Rod Domain, and Cysteine-Rich/C-Terminal Domains of Dystrophin

We compared the phenotypes of the different sites of microvariants (actin-binding domain, central-rod domain, and cysteine-rich/c-terminal domains) (Table 6). There were no significant differences in age at survey, serum CK levels, and age at measurement. Significant differences were observed in wheelchair adoption rates among the 3 groups, especially in the c-terminal domain compared with the central-rod domain. There were also significant differences among the 3 groups in the rate of ventilator use, with a higher rate in the c-terminal domain compared with the actin coupled and rod regions. The rate of FVC <80% was also higher in the c-terminal domain than in the central-rod domain. On the other hand, there were no significant differences in the rates of ECG abnormalities, LVEF <50%, heart failure, or CNS disorders among the 3 groups.

Duplications

There were 16 duplicate variants, all of which were in-frame duplications (Table 7). Muscle biopsies were performed in 2 patients showing hyperCKemia and CNS involvement (one was bipolar disorder, and the other was intellectual developmental disorder). Three patients (nos. 39, 40, and 49) used wheelchairs, 2 of them after 30 years of age. Serum CK levels were significantly lower than those of the microvariants, as presented in Table 1. HyperCKemia was the initial symptom or finding in approximately half of the patients. The CNS complication rate was >40%.

Discussion

Microvariants in *DMD* of patients with BMD were mostly in the actin-binding domain, the first half of the central-rod domain (exons 11–38), and the cysteine-rich/C-terminal domain, but not within exons 45–55, the hotspot for deletion variants. No major differences in clinical characteristics were observed between the microvariant groups, although some cases with variants in the C-terminal region showed severe respiratory and CNS disorders. The high rate of muscle biopsy in small variants may be due to the delay in insurance coverage of the sequencing method and the influence of practice guideline for DMD[10] in Japan.

Table 1 Comparison of Clinical Characteristics of BMD Between With Microvariants and Duplications

	Microvariants	Duplications	In-frame deletions[a]
Number of patients	33	16	225
Mean age (y) at examination (median)	28.9 ± 19.2 (25.0)	24.5 ± 14.8 (20.5)	31.5 ± 17.9 (29.0)
Mean age (y) at diagnosis (median)	18.8 ± 17.6 (12.0)	15.9 ± 13.2 (13.5)	21.6 ± 16.6
Wheelchair use (%)	27.3	25.0	27.1
Mean age (y) at introduction of wheelchair (median)	27.6 ± 13.1 (20)	32.7 ± 10.1 (34)	36.5 ± 15.8 (35)
Asymptomatic hyperCKemia (%)	42.4	50.0	32.4
Mean serum CK level (U/L) at initial survey (median)	4,113 ± 6,471 (1,271)	2,059 ± 1,805 (1,417)	3,582 ± 5,308 (1,480)
Muscle biopsies performed (%)	54.5	12.5	17.8
Ventilator use (%)	6	12.5	6.7
Mean age (y) at introduction of ventilator (median)	27.6 ± 13.1 (20)	32.7 ± 10.1 (34)	36.6 ± 14.2 (34)
%FVC at initial survey (median)	76.6 ± 28.5 (76.6)	80.5 ± 23.2 (86.1)	91.5 ± 17.7 (91.5)
ECG abnormalities (%)	90.9	31.3	59.5
LVEF at initial survey (%) (median)	56.0 ± 16.6 (60.4)	54.0 ± 21.5 (61.0)	56.9 ± 13.7 (60.4)
Concomitant heart failure (%)	9.1	18.8	15.1
Concomitant CNS disorders (%)	30.3	43.8	11.1
Receiving steroid therapy (%)	15.2	18.8	13.1

Abbreviations: BMD = Becker muscular dystrophy; CK = creatine kinase; FVC = forced vital capacity; LVEF = left ventricular ejection fraction.
Values shown are mean ± SD.
[a] Data from Nakamura, et al., 2023.[4]

Cases of BMD with nonsense variants have been reported in exons 25,[11-14] 27,[15] 29,[14,16] 31,[14,17-19] 37,[20] 38,[14,19,21] and 72.[14,22] In addition, 11 patients with BMD have been reported with exon 9, 25, 27, 31, 37, 38, 41, 72, and 74 nonsense variants. Nonsense variants that result in BMD are selectively associated with a subset of *DMD* exons where exon skipping is likely to be induced by the variant.[23] In addition, a mini-gene plasmid containing mutated/nonmutated exons and their flanking intron sequences was introduced into HeLa cells, and the transcript skipping rate was examined. The exon skipping rate correlated well with dystrophin expression, suggesting that accurate quantification of the skipping rate is important in interpreting the phenotype of patients with BMD.[24]

In our study, splice site variants, which accounted for 45.5% of all microvariants, occurred in positions that would not cause a frameshift if the exons near the variant site were spliced out. Nonsense and splice site variants that occur in patients with BMD often result in exon deletions at or near the site of the variant and may resemble the pathogenesis of BMD with in-frame deletions. On the other hand, read-through therapy has been considered for nonsense variants, but, for BMD with nonsense variants, the efficiency of exon skipping in skeletal muscle and clinical severity should be considered.[24] For this purpose, the accumulation of clinical information on patients with BMD with microvariants of *DMD* is necessary.

Single-nucleotide substitutions caused by missense variants may affect the structure and function of long dystrophins; however, the pathomechanism is unclear. In our case, these variants were observed frequently in the actin-binding or C-terminal domains. Among them, nos. 28 and 29 had a deep intronic variant, which have been reported.[25,26] The mutation in no. 28 (c.93+5590T>A) created a novel consensus sequence as a splice acceptor site, and 2 cryptic splice donor sites at 132 bp or 46 bp downstream were reported to form 2 novel exon structures. The former was predicted to maintain the dystrophin reading frame and insert 44 amino acids into the N-terminal domain of dystrophin.[25] The mutation in no. 29 (c.265-463A>G) created a donor-motif mutation, resulting in a recursive splicing regulation.[26] The case was diagnosed with BMD based on the results of muscle biopsy, and there were other patients diagnosed as BMD.[26] On the other hand, another patient was diagnosed with DMD due to dystrophin deficiency by muscle biopsy, and a sibling of no. 29 was unable to walk at 14 years of age and had DMD. Thus, it is interesting to note that the phenotypes are different even for the same variant. Up to 7% of DMD variants are deep intronic, which suggests that analysis of skeletal muscle RNA is important for the identification of variants.[27]

Regardless of the type of microvariant, phenotypes were examined in 3 major locations: the actin-binding domain, central-rod domain, and cysteine-rich/C-terminal domain.

Table 2 Clinical Characteristics of Patients With BMD Having Point Variants Involving Amino Acid Substitutions

No.	Pathogenic variants	Location	Dystrophin immunoreactivity in muscle biopsy	Age at examination (y)	Family history	Initial symptoms or findings	Serum CK value at initial survey (age in years)	Wheelchair usage (introduction age in years)	Respirator introduction	FVC <80%	ECG abnormality	LVEF <55%	Heart failure	CNS disorders	Steroid therapy Dosage Dosing period
1	c.152T>G p.L51R	Exon 3	Faint and patchy	>60	(+)	Easy fatigability	3,430 (42)	+, >50	(−)	(−)	(+)	(+)	(−)	(+)[b]	(−)
2	c.481A>C p.T161P	Exon 6	Faint and patchy	>30	(+)[a]	Slow to gait	7,705 (13)	+, <20	(−)	(−)	(+)	ND	(−)	(−)	(−)
3	c.1318G>A p.E440K	Exon 11	ND	>30	(+)	HyperCKemia	2,868 (31)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)
4	c.2096C>T p.A699V	Exon 17	ND	>20	(−)	Febrile seizure	979 (5)	(−)	(−)	(−)	(+)	(−)	(−)	(+)[c]	(−)
5	c.10864G>A p.D3622N	Exon 76	Faint & patchy	>30	(−)	NA	922 (27)	+, <20	(−)	(−)	(+)	(−)	(−)	(−)	(+, PSL) 5 mg/every other day 1 y

Abbreviations: BMD = Becker muscular dystrophy; CK = creatine kinase; FVC = forced vital capacity; LVEF = left ventricular ejection fraction; NA = data not available; ND = not done; PSL = prednisolone.
[a] His sibling was unable to walk at 17 y of age.
[b] Maladjustment.
[c] Febrile seizure.

Table 3 Clinical Characteristics of Patients With BMD Having Nonsense Variants

No.	Pathogenic variants	Location	Dystrophin immunoreactivity in muscle biopsy	Age at examination (y)	Family history	Initial symptoms or findings	Serum CK value at initial survey (age in years)	Wheelchair usage (introduction age in years)	Respirator introduction (age)	FVC <80%	ECG abnormality	LVEF <55%	Heart failure	CNS disorders	Steroid therapy Dosage Dosing period
6	c.3304C>T p.Q1102*	Exon 25	ND	>10	(−)	HyperCKemia	17,509 (6)	(−)	(−)	(+)	(+)	(−)	(−)	(−)	(−)
7	c.3337C>T p.Q1113*	Exon 25	Faint and patchy	>30	(−)	Slow to settle down	4,586 (26)	(−)	(−)	(−)	(+)	ND	(−)	ND	(−)
8	c.4294C>T p.Q1432*	Exon 31	Faint and patchy	>10	(−)	hyperCKemia	3,955 (5)	(−)	(−)	NA	(+)	(−)	(−)	(+)[a]	(−)
9	c.4303G>T p.E1435*	Exon 31	Faint and patchy	>10	(−)	HyperCKemia	2,567 (2)	(−)	(−)	NA	(+)	(−)	(−)	(−)	(−)
10	c.5407C>T p.Q1803*	Exon 38	ND	>70	(+)	Calf hypertrophy, Easy fatigability	187 (75)	(−)	(−)	(−)	(+)	(+)	(−)	(−)	(−)
11	c.5407C>T p.Q1803*	Exon 38	Faint and patchy	>10	(+)	HyperCKemia	24,010 (7)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)
12	c.10320T>A p.T3440*	Exon 72	ND	>40	(+)	Prone to falling over	3,700 (10)	+, <20	33	(+)	(+)	(+)	(−)	(−)	(−)
13	c.10320T>A p.T3440*	Exon 72	ND	>40	(+)	Slow to run	924 (8)	+, >20	34	(+)	(+)	(+)	(−)	(−)	(−)

Abbreviations: BMD = Becker muscular dystrophy; CK = creatine kinase; FVC = forced vital capacity; LVEF = left ventricular ejection fraction; NA = data not available; ND = not done.
[a] Intellectual and developmental disorder.

Table 4 Clinical Characteristics of Patients With BMD Having Splice Site Variants

No.	Pathogenic variants	Location	Dystrophin immunoreactivity in muscle biopsy	Age at the examination (y)	Family history	Initial symptoms or findings	Serum CK value at initial survey (age in years)	Wheelchair usage (introduction age in years)	Respirator introduction	FVC <80%	ECG abnormality	LVEF <55%	Heart failure	CNS disorders	Steroid therapy Dosage Dosing period
14	c.31+1G>T	Intron 1 splice donor site	Faint and patchy	>40	(−)	Myalgia	3,691 (31)	(−)	(−)	(−)	(+)	(+)	(−)	(−)	(−)
15	c.94-9dupT	Intron 2 splice acceptor site	Faint and patchy	>70	(−)	Calf hypertrophy	1,309 (45)	+, >40	(−)	(+)	(+)	(+)	(+)	(−)	(−)
16	c.94-3_95del + c.94-9delT	Exon 3 splice acceptor site	ND	<10	(+)[a]	hyperCKemia	7,885 (0)	(−)	(−)	ND	(−)	(−)	(−)	(+)[b]	(−)
17	c.264+1G>T	Intron 4 splice acceptor site	Faint and patchy	<10	(−)	hyperCKemia	7,639 (2)	(−)	(−)	ND	(−)	(−)	(−)	(−)	(−)
18	c.264+1G>A	Intron 4 splice acceptor site	Faint and patchy	<10	(−)	hyperCKemia	12,535 (2)	(−)	(−)	ND	(+)	(+)	(+)	(−)	(−)
19	c.264+1G>A	Intron 4 splice acceptor site	Faint and patchy	>40	(+)	Dyspnea	4,283 (29)	(−)	(−)	(−)	(+)	(−)	(−)	(+)[c]	+, PSL 20 mg/every other day >1 y
20	c.650-3C>G	Intron 7 splice acceptor site	ND	<10	(−)	hyperCKemia	4,603 (5)	(−)	(−)	ND	(−)	(−)	(−)	(−)	(−)
21	c.1483-2A>C	Intron 12 splice acceptor site	Faint and patchy	>10	(−)	Delayed motor development	4,247 (1)	(−)	(−)	(−)	(+)	(−)	(−)	(+)[b]	(−)
22	c.3276+2T>A	Intron 24 splice donor site	ND	>10	(−)	hyperCKemia	14,089 (8)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)
23	c.3603+3A>T	Intron 26 splice donor site	Faint and patchy	<10	(−)	hyperCKemia	5,948 (1)	(−)	(−)	ND	(+)	(−)	(−)	(−)	(−)
24	c.3603+3A>T	Intron 26 splice donor site	Faint and patchy	>20	(+)	Difficulty to climb stairs	3,142 (19)	(−)	(−)	(−)	(+)	(−)	(−)	(−)	(−)
25	c.4071_ 4071+9del	Intron 29 splice donor site	NA	>40	(−)	Difficulty to climb stairs	622 (36)	+, >30	(−)	ND	(+)	(+)	(−)	(−)	(−)
26	c.9650-2A>G	Intron 66 splice acceptor site	ND	>20	(+)	Difficulty to climb stairs	4,864 (11)	(−)	(−)	(+)	(+)	(−)	(−)	(+)[b]	(+, PSL) 25 mg/every other day >19 y
27	c.9650-2A>G	Intron 66 splice acceptor site	Negative	>20	(+)	Difficulty to climb stairs	1,183 (24)	(−)	(−)	(+)	(+)	(−)	(−)	(+)[b]	(+, PSL) 20 mg/every other day, >17 y

Abbreviations: BMD = Becker muscular dystrophy; CK = creatine kinase; FVC = forced vital capacity; LVEF = left ventricular ejection fraction; NA = data not available; ND = not done; PSL = prednisolone.
[a] His sibling with same variant was unable to walk at 20 y of age.
[b] Intellectual and developmental disorder.
[c] Convulsion.

Table 5 Clinical Characteristics of Patients With BMD Having Other Microvariants

No.	Pathogenic variants	Location	Dystrophin immunoreactivity in muscle biopsy	Age at the examination (y)	Family history	Initial symptoms or findings	Serum CK value at initial survey (age in years)	Wheelchair usage (introduction age in years)	Respirator introduction	FVC <80%	ECG abnormality	LVEF <55%	Heart failure	CNS disorders	Steroid therapy Dosage Dosing period (y)
28	c.93+5590T>A	Intron 2	ND	>30	(−)	HyperCKemia	1,241 (9)	(−)	(−)	ND	(+)	(−)	(−)	(−)	(−)
29	c.265-463A>G	Intron 4	Faint and patchy	>20	(+)	NA	6,140 (14)	(−)	(−)	(+)	(+)	(−)	(−)	(−)	(−)
30	c.40_41del	Exon 2	Faint and patchy	>40	(−)	ECG abnormality	1,016 (45)	(−)	(−)	(−)	(+)	(+)	(+)	(−)	(−)
31	c.160_162del p.L54del	Exon 3	Faint and patchy	>30	(−)	HyperCKemia	1,669 (34)	+, >20	(−)	(+)	(+)	(−)	(−)	(−)	(−)
32	c.3882_3883insTT	Exon 28	Faint and patchy	>10	(+)	Malleability	ND	(−)	(−)	(−)	(+)	(+)	(−)	(+)[a]	(−)
33	c.10453_10454del	Exon 74	ND	>30	(−)	HyperCKemia	2,157 (25)	+, >20	(−)	(+)	(+)	(+)	(−)	(+)[b, c]	(+, PSL) 15 mg/every other day 8 y

Abbreviations: BMD = Becker muscular dystrophy; CK = creatine kinase; FVC = forced vital capacity; LVEF = left ventricular ejection fraction; NA = data not available; ND = not done; PSL = prednisolone.
[a] Personality disorder.
[b] Intellectual and developmental disorder.
[c] Maladjustment.

Table 6 Comparison of Phenotypes Among Patients With BMD Having Microvariants in the Actin-Binding Domain, Rod Domain, or C-Terminal Domain of Dystrophin

Dystrophin domain (corresponding exon no.)	Actin-binding domain (exon 1–8)	Central-rod domain (exon 9–61)	Cysteine-rich and C-terminal domains (exon 62–79)	*p* Value
Pt. no. (total number)	1, 2, 14, 15, 16, 17, 18, 19, 20, 28, 29, 30, 31 (13)	3, 4, 6, 7, 8, 9, 10, 11, 21, 22, 23, 24, 25, 32 (14)	5, 12, 13, 26, 27, 33 (6)	
Age at examination (y)	32.0 ± 22.8 (32, 3–72)	23.7 ± 18.3 (15.5, 5–76)	34.2 ± 9.8 (35.5, 21–45)	ns[a]
Serum CK value at initial survey (IU/L) **Age at initial survey (y)**	4,857 ± 3,416 (4,283, 1,016-12,535) 20.8 ± 17.3 (14, 0–45)	6,546 ± 7,336 (3,955, 198-24,010) 17.1 ± 21.1 (7, 1–75)	2,292 ± 1,649 (1,670, 922-4,864) 17.5 ± 8.7 (17.5, 8–27)	ns[a] ns[a]
Wheelchair introduction rate (%) **Mean introduction age in years [mean ± SD (median, range)]**	30.8 20.8 ± 17.8 (14, 17–55)	7.1 35	66.7 19.3 ± 1.0 (19.5, 18–20)	<0.05[b] ns[a]
Respirator introduction rate (%) **Age (y)**	0 NA	0 NA	33.3 33.5	<0.01[b] ns[a]
FVC <80% rate (%)	37.5	10.0	83.3	<0.05[b]
ECG abnormality rate (%)	77.0	100	100	ns[b]
LVEF <55% rate (%)	38.5	23.1	50	ns[b]
Complication rate of heart failure (%)	23.1	0	0	ns[b]
Complication rate of CNS disorders (%)	23.1	30.8	50	ns[b]

Abbreviations: BMD = Becker muscular dystrophy; CK = creatine kinase; CNS = central nervous disorders; ECG = ECG; FVC = forced vital capacity; LVEF = left ventricular ejection fraction; NA = not available; ns = not significance. Age and values are shown by mean ± SD (median, range).
[a] After test by analysis of variance (ANOVA), a multiple group comparison test was performed with Bonferroni analysis.
[b] Pearson χ^2 test was performed.

Table 7 Clinical Characteristics of Patients With BMD Having Duplications

No.	Duplications	Dystrophin immunoreactivity in muscle biopsy	Age at the examination (y)	Family history	Initial symptoms or findings	Serum CK value at initial survey (age in years)	Wheelchair usage (introduction age in years)	Respirator introduction	FVC <80%	ECG abnormality	LVEF <55%	Heart failure	CNS disorders	Steroid therapy Dosage Dosing period (y)
34	Exon 1 of Dp427c dup	ND	>30	(−)	Bipolar disorder	1,002 (30)	(−)	(−)	(+)	(+)	(+)	(+)	(+)[a]	(−)
35	Exons 3–9 dup	ND	<10	(+)	hyperCKemia	7,415 (0)	(−)	(−)	ND	(−)	(−)	(−)	(−)	(+, PSL) dosage unknown 1 y
36	Exons 3–10 dup	ND	>20	(−)	hyperCKemia	2,471 (23)	(−)	(−)	(−)	(+)	(−)	(+)	(−)	(+, PSL) unknown Unknown
37	Exons 3–12 dup	ND	>40	(−)	ECG abnormality myalgia	1,058 (24)	+, >20	(+)	(+)	(+)	(+)	(−)	(+)[b]	(−)
38	Exons 3–18 dup	ND	>20	(+)	Calf hypertrophy Walking on tiptoe	2,820 (17)	(−)	(−)	(+)	NA	(+)	(+)	(−)	(+, PSL) unknown Unknown
39	Exon 5 dup	ND	>40	(−)	Slow to run	1,154 (45)	+, >30	(−)	ND	(+)	(+)	(−)	(−)	(−)
40	Exons 5–16, 26–42 dup	ND	>50	(+)	Weakness of lower legs	4,356 (31)	+, unknown	(+)	(−)	(+)	(+)	(−)	(+)[c]	(−)
41	Exon 16 dup	Faint and patchy	>30	(−)	Mental and motor retardation	1,377 (1)	(−)	(−)	(−)	(−)	(−)	(−)	(+)[d]	(−)
42	Exons 17–18 dup	ND	>10	(+)	hyperCKemia	394 (4)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
43	Exons 17–18 dup	ND	>10	(+)	hyperCKemia	381 (4)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
44	Exons 19–25 dup	ND	>10	(+)	hyperCKemia	835 (9)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
45	Exons 21–30 dup	ND	>10	(−)	hyperCKemia	552 (10)	(−)	(−)	ND	(−)	(−)	(−)	(−)	(−)
46	Exons 21–30 dup	ND	>10	(−)	Myalgia	2,566 (8)	(−)	(−)	(−)	(−)	(−)	(−)	(+)[d]	(−)
47	Exons 28–29 dup	ND	>10	(+)	hyperCKemia	2,165 (17)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
48	Exons 48–51 dup	Faint and patchy	<10	(−)	hyperCKemia	1,456 (3)	(−)	(−)	ND	(−)	(−)	(−)	(+)[e]	(−)
49	Exons 56–67 dup	ND	>40	(−)	Slow to run	2,946 (29)	+, >40	(−)	(+)	(−)	(−)	(+)	(+)[d,e]	(−)

Abbreviations: BMD = Becker muscular dystrophy; CK = creatine kinase; dup = duplication; FVC = forced vital capacity; LVEF = left ventricular ejection fraction; NA = not available; ND = not done; PSL = prednisolone.
[a] Bipolar disorder.
[b] Anxiety neurosis.
[c] Personality disorder.
[d] Intellectual and developmental disorder.
[e] Convulsion.

The results showed that variants in the cysteine-rich/C-terminal domain, in particular, had more severe skeletal muscle and respiratory dysfunctions than those in the rod domain. Various dystrophin-associated proteins bind to the cysteine-rich/C-terminal domain,[28] and variants in this region may be more severely impaired in muscle function.

CNS disorders have been suggested to be associated with variants in the regions encoding dystrophin isoforms, Dp140[29] and Dp71.[30] However, a negative report[31] and our natural history study of BMD with in-frame deletions[3] do not support this conclusion. Since in-frame deletions/duplications or microvariants in the region encoding Dp140 and Dp71 in BMD may not cause complete loss of function, it may be difficult to define the relationship between this region of variants and CNS disorders. In our cases, other than the gene regions encoding Dp140 and Dp71, some cases with variants in the actin-binding domain had CNS disorders, and a comparison of the 3 domains of dystrophin showed no significant differences in the complication rate of CNS disorders (Table 6). Therefore, it is possible that factors other than molecular species may be involved in CNS damage.

Regarding duplications, there may be no association between length of duplication and severity of disease because the 3 patients with single-exon duplications (nos. 34, 39, and 41) were severely affected. In addition, no site specificity of *DMD* was observed in duplications with CNS involvement. Recently, an attempt to restore full-length dystrophin by exon-skipping therapy for DMD with a single-exon duplication was reported.[32] Exon skipping therapy may be a promising treatment for patients with severe BMD, but it will be interesting to determine how the restoration of full-length dystrophin affects CNS abnormalities.

The number of cases treated with steroid was small, but patient nos. 26, 27, and 33 (all with mutations in cysteine-rich and C-terminal domains) were treated long-term and the treatments were considered effective. For other patients treated with steroids, assessment of their efficacy was difficult because the duration of treatment was as short as 1 year or because no information was available. Corticosteroids have been reported to increase Becker dystrophin levels.[33] However, given the need for long-term administration, a steroid with fewer side effects, such as vamorolone,[8] may be a better choice.

Our results summarize the clinical characteristics of BMD cases with variants in the same region of *DMD* in terms of BMD treatment and clinical findings. In cases with nonsense or splice site variants, muscle biopsy is required to determine expression of dystrophin in the specimen and spontaneous exon skipping efficiency may be useful for treatment.

Since patient information for this study was provided by several institutions, data entry errors, missing or incomplete data, or other errors may have affected the results. In addition, a wide age range of patients were included, and phenotypes may have been influenced by a variety of factors, including exercise, corticosteroids, rehabilitation, and obesity.

In this study, we analyzed the clinical presentation of patients with BMD with microvariants and in-frame duplications. Microvariants, in particular, tend to vary in severity depending on the site of variant rather than the type of variant. Currently, treatment for BMD is under investigation, but, to develop effective therapies, clinical data from a large number of patients with BMD with microvariants and duplications are required, as well as analysis of their genotype-phenotype profile. This information will also be useful for genetic counseling.

Author Contributions

A. Nakamura: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. T. Matsumura: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. K. Ogata: major role in the acquisition of data; study concept or design. M. Mori-Yoshimura: major role in the acquisition of data; study concept or design. E. Takeshita: study concept or design. K. Kimura: study concept or design. H. Arahata: major role in the acquisition of data. Y. Takeshima: major role in the acquisition of data. T. Takahashi: major role in the acquisition of data. K. Ishigaki: major role in the acquisition of data. H. Awano: major role in the acquisition of data. K. Sugie: major role in the acquisition of data. T Fujii: major role in the acquisition of data. H. Oi: study concept or design. H. Komaki: study concept or design.

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Disclosure

The authors report no conflicts of interest regarding this study. Dr. Madoka Mori-Yoshimura is deceased; to the best of our knowledge, the relevant disclosures are none. Go to Neurology.org/NG for full disclosures.

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