



Genotype–phenotype correlation in Becker muscular dystrophy in Chinese patients

Ruiyi Yuan^{1,2} · Junfei Yi¹ · Zhiying Xie¹ · Yimeng Zheng¹ · Miao Han³ · Yue Hou⁴ · Zhaoxia Wang¹ · Yun Yuan¹

Received: 21 November 2017 / Revised: 23 May 2018 / Accepted: 1 June 2018 / Published online: 5 July 2018
© The Author(s) under exclusive licence to The Japan Society of Human Genetics 2018

Abstract

Large deletions and duplications are the most frequent causative mutations in Becker muscular dystrophy (BMD), but genetic profile varied greatly among reports. We performed a comprehensive molecular investigation in 95 Chinese BMD patients. All patients were divided into three subtypes: normal muscle strength (type 1) in 18 cases, quadriceps myopathy (type 2) in 20 cases, and limb-girdle weakness (type 3) in 57 cases. Nineteen cases (20.0%) had small mutations and 76 cases (80.0%) had major rearrangements, including 67 cases (70.5%) of exonic deletions and 9 cases (9.5%) of exonic duplications. We identified 50 cases (65.8%) of in-frame mutations, and 26 cases (34.2%) of frame-shift mutations. The frequency of deletion in exons 13–19 was 30.6% in type 1 patients, 9.7% in type 2 patients, and 10.4% in type 3 patients. The frequency of deletion in exons 45–55 was 28.6% in type 1 patients, 40.8% in type 2, and 50.0% in type 3 patients. All major rearrangements of *DMD* gene in type 1 patients were also observed in type 3 patients. Our study suggested that frame-shift mutation was not rare in Chinese BMD patients. Although no difference was observed on the forms of *DMD* gene mutations among the three types of patients, the mutation in proximal region of *DMD* gene has higher frequency for patients without weakness. Effect of exon skipping for *DMD* depends on the size and location of the mutation. Additional studies are required to determine whether exon-skipping strategies in proximal region of *DMD* gene could yield more functional dystrophin.

Introduction

Becker muscular dystrophy (BMD), caused by mutations on the Dystrophin gene (*DMD* gene) (OMIM: *300377), is an X-linked disease with an incidence of 2.84 per 100,000 males [1]. BMD is clinically more heterogeneous compared to Duchenne muscular dystrophy (DMD), with initial presentation in the teenage years, loss of ambulation beyond the age of 16 years, and a wide spectrum of clinical presentations. Some BMD patients present no muscular

weakness, but with cramp-myalgia syndrome [2], asymptomatic hyperCKemia [3, 4], or myoglobinuria [5]. While other BMD patients exhibit more classical phenotypes: quadriceps myopathy [4, 6] or limb-girdle weakness [4]. Occasionally cardiomyopathy without skeletal muscle weakness is another phenotype caused by *DMD* gene mutation [7].

Since no linear relationship was found between dystrophin levels of muscle fibers and muscle strength or age at different disease milestones in BMD patients [8], the genetic screening is more commonly used in the diagnosis [9] and determination of the disease progress [4, 10]. Furthermore, it is important to find a distinct mutation location of mild or asymptomatic BMD for exon-skipping therapy of *DMD*. Exon-skipping strategy is viewed as a promising therapeutic strategy for muscular dystrophy since it can restore the reading frame of *DMD* gene and allows the production of a shorter but functional dystrophin protein [11]. Previous clinical trials in exon-skipping therapy did not exhibit much effectiveness in converting *DMD* to BMD, and a comprehensive assessment of genotype–phenotype relationship in BMD is needed for future planning of clinical trials, since

✉ Yun Yuan
yuanyun2002@126.com

¹ Department of Neurology, First Hospital, Peking University, 100034 Beijing, China

² Haverford Collage, Haverford, PA 19041, USA

³ Department of Neurology, First Hospital, Henan University, Kaifeng Shi, China

⁴ Department of Neurology, Traditional Medicine Hospital, Guangzhou University, Guangzhou, China

BMD shows a large variety of genetic mutations and clinical phenotypes [12]. Exonic deletion is the major type of mutation in BMD patients, representing 66.7% of all mutations in the Puerto Rico patients with BMD [1]. Previous studies have reported two hotspots for *DMD* gene mutations in BMD, one located between exons 45 and 55, and another located between exons 2 and 19 [4]. The exons in segment 45 to 47 were the most frequently affected in Puerto Rico patients [1], while the deletion of exon 50 was the most common in multiplex ligation-dependent probe amplification (MLPA) confirmed cases in Indian patients [13].

A genetic analysis of *DMD* gene is important in predicting the prognosis for BMD. Early study suggested that no correlation was found between the symptomatology and the location of the mutations [14]. Recent studies suggested that the reading-frame rule held in 82.4% in Spanish BMD patients [10]. Splice site mutations or deletion of exons 3–9 [15, 16] and deletions ending on exon 51 or deletion of exon 48 in the *DMD* gene was detected in mild or asymptomatic BMD [17]. BMD carrying proximal deletions showed a higher degree of cardiac impairment [18]. The intronic splicing mutation [19] and the deletions ending on exon 45 [17] in the *DMD* gene are associated with BMD patients with moderate weakness. Since there are not enough studies to determine which exon is better for exon-skipping therapy, study with large cohort of BMD is needed to determine the genotype–phenotype correlation. Here we looked into the pattern of the genetic screening results of Chinese BMD patients.

Materials and methods

The genetic mutations and clinical phenotypes of 95 male BMD patients from unrelated families were collected in the Neurology Department of Peking University First Hospital. Patients were diagnosed with BMD if his onset age is over 7 years old and/or the patient is able to walk until at least

16 years old [20]. All patients were not treated with steroid therapy. The diagnosis of these patients were all confirmed with *DMD* gene sequencing. Based on the clinical phenotypes, the patients were divided into three clinical subtypes: type 1, patients with normal muscle strength, who experience cramp-myalgia syndrome or asymptomatic hyperCKemia; type 2, patients exhibiting quadriceps myopathy; and type 3, patients experiencing limb-girdle weakness. Clinical manifestation of patients in the present series were summarized in Table 1. Among the 95 patients, 18 patients were classified as type 1, 20 patients were classified as type 2, and 57 patients were classified as type 3. The age at the diagnostic time was 10.4 ± 4.1 years old (7–21 years old) for type 1 patients. The onset age of the disease is 17.7 ± 8.5 years old (7–39.5 years old) for type 2 patients and 11.6 ± 5.8 years old (7–30 years old) for type 3 patients. All clinical investigations were conducted according to the principles of Declaration of Helsinki. The institutional review board and ethics committee at the Peking University First Hospital approved this study. Written informed consent was obtained from all participants.

Genomic DNA was extracted from the blood cells of all patients and their relatives according to standard procedures. Targeted next-generation sequencing of 128 genes associated with known inherited myopathy including the *DMD* gene was performed [21]. The mutations were verified by Sanger sequencing with specific primers. If exonic deletion or duplications was identified, MLPA was used again to confirm the findings. If small mutations were identified, an alternative method was required (QF-PCR or real-time qPCR, Sanger sequencing) to confirm the findings and rule out rare variant or polymorphism. Pathogenicity of amino acid substitutions was evaluated using Polyphen-2 and SIFT algorithms. All substitutions with a Polyphen-2 score below 1 and above 0.908 and a SIFT score below 0.05 were considered damaging substitutions. In addition, we used LOVD database (Leiden Open Variation Database: www.dmd.nl) as a reference to determine if the substitution

Table 1 Summary of clinical manifestation of patients in present series

	Number of patients	Onset age	Diagnostic age	Main clinical phenotype	Blood CK level (IU/L) ^a
Type 1	18	10.4 ± 4.1	11.6 ± 5.0	Without any muscle weakness. Cramp-myalgia syndrome, or asymptomatic hyperCKemia. No cardiac symptoms or respiratory failure.	500–17,976
Type 2	20	17.7 ± 8.5	24.7 ± 10.9	Quadriceps myopathy without respiratory failure or cardiac symptoms. Three cases with cardiac symptoms. No patients have respiratory failure.	866–41,402
Type 3	57	11.6 ± 5.8	15.9 ± 8.0	Limb-girdle weakness. Five cases with cardiac symptoms. No patients have respiratory failure.	514–32,700

Onset age and diagnostic age were presented in the format of “average \pm standard deviation.” Since single patient could have large fluctuation in blood CK level, CK levels were presented in a range

^aOnly 65 patients have documented blood CK level

consisted a missense mutation or a non-synonymous single-nucleotide polymorphism. The nucleotide position was determined according to the DMD reference sequence (RefSeq NM_004006.2) and mutation nomenclature followed the guidelines of the Human Genome Variation Society.

Statistical analysis

All values were calculated using the SPSS statistical package (version 17.0J). Descriptive statistics were carried out when appropriate. Correlations were calculated using Spearman's rank coefficient, and statistical significance was indicated by $p < 0.05$.

Result

The onset age of type 2 patients is significantly older than the diagnostic age of type 1 patients ($p = 0.001$) and the onset age of type 3 patients ($p = 0.003$). There was no significant difference between diagnostic age in type 1 patients and the onset age in type 3 patients ($p > 0.1$).

Table 2 showed an overview of genetic mutations observed in this study. In the 95 independent mutational events, 76 cases (80.0%) were major rearrangements (exonic deletions and duplications) including 16 cases in type 1,

13 cases in type 2, and 47 cases in type 3. Nineteen cases (20.0%) were small mutations (mutations smaller than one exon). Exonic deletions and duplications occurred on 67 patients (70.5%) and 9 patients (9.5%), respectively. Among all mutations of type 1 patients (Fig. 1), 72.2% were exonic deletions, 16.7% were exonic duplications, and 11.1% were small mutations; for mutations of type 2 patients, 60.0% were exonic deletions, 5.0% were exonic duplications, and 35.0% were small mutations; for mutations of type 3 patients, 73.7% were exonic deletions, 8.8% were exonic duplications, and 17.5% were small mutations.

Table 2 Types of genetic mutation in three subtypes of patients in present series

	Type 1	Type 2	Type 3	Total
Deletion				
In-frame	10	7	26	43
Frame-shift	3	5	16	24
Total	13	12	42	67
Duplication				
In-frame	2	1	4	7
Frame-shift	1	0	1	2
Total	3	1	5	9
Small mutation				
Total	2	7	10	19
Total	18	20	57	95

Fig. 1 Frequency of different types of DMD gene mutations in three subtypes of BMD patients. Black: exonic deletion; gray: exonic duplication; shade: small mutations

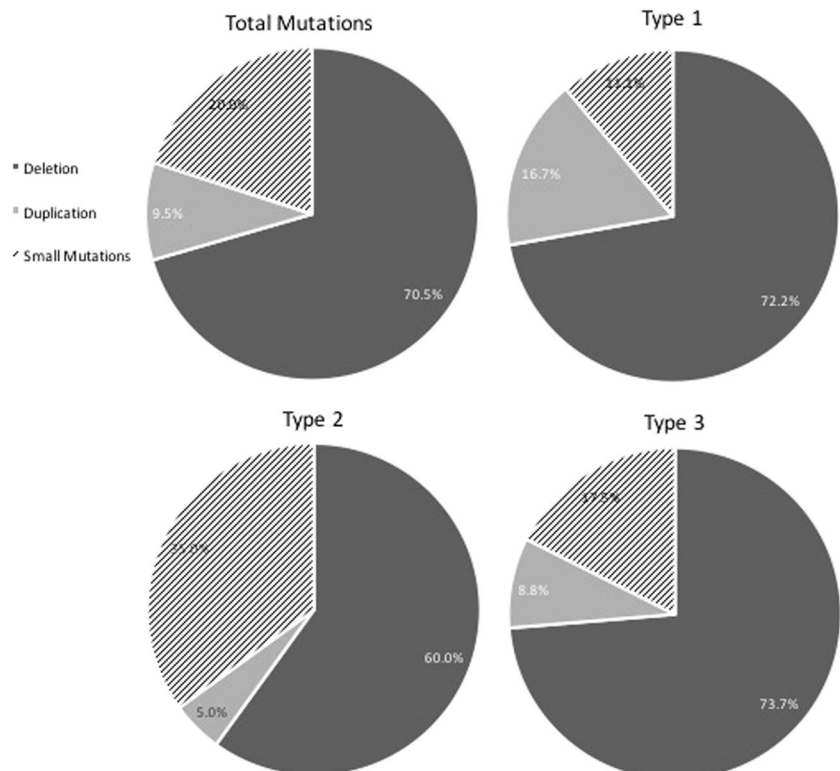
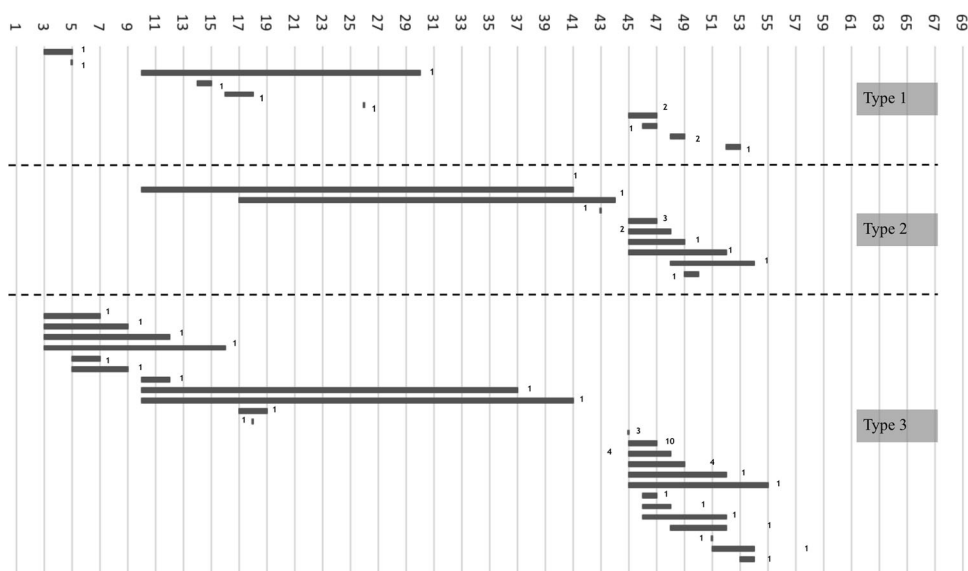


Fig. 2 Patterns of exon deletion in *DMD* gene in BMD patients. The horizontal axis showed the number of exons. The present series had 43 different patterns of exon deletion. One type 1 patient with deletion mutation on exons 13, 17, 19, 43, 45, 47 and one type 3 patient with deletion mutation on exon 13, 17, 19, 43, 44, 45, 47 were not shown. The number on the side indicate the number of cases



were exonic duplications, and 17.5% were small mutations. No statistically significant differences were observed ($p > 0.1$) between different forms of mutations among the three types of patients.

Exonic deletions

According to the Taglia et al.'s report [4], we studied the frequency of deletion mutations on exons 45–55 in our present series of patients. There were 45 patients (67.2%, 45/67) with this deletion mutations, including 6 cases (46.2%, 6/13) with type 1, 9 cases (75%, 9/12) with type 2, and 30 cases (71%, 30/42) with type 3. There was no significant difference among three types ($p > 0.05$). The present series presented 43 different patterns of exonic deletion (Fig. 2). Deletion mutations starting at exon 45 occurred in 32 patients (47.8%, 32/67) and 15 of them on exons 45–47 (22.4%, 15/67). There were 13 cases (19.4%, 13/67) of exonic deletions located on exons 3–19, including 4 cases (30.8%, 4/13) with type 1, 0 cases (0%, 0/12) with type 2, and 9 cases (21.4%, 9/42) with type 3. The significant differences were observed between patients with type 1 and type 2 ($p = 0.03$), but not between type 1 and type 3 nor between type 2 and type 3 of patients ($p > 0.05$).

The frequency of deletions involving exons 45–55 is 28.6% for type 1 patients, 40.8% for type 2 patients, and 50% for type 3 patients. The frequency of deletion involving exons 3–19 is 36.7% for type 1 patients, 12.6% for type 2 patients, and 32.5% of type 3 patients. The histogram of frequency distribution (Fig. 3) of *DMD* gene exonic deletions showed that all three types of patients have a peak at exons 45–49, but only type 1 patients appeared another peak at exons 13–19. The breakpoints of deletions on exons 13–19 have a frequency of 30.6% for type 1 patients, 9.7% for type 2 patients, and 10.4% for type 3 patients.

Exonic duplications

The frequency of exonic duplication of the nine patients was calculated (Fig. 4). Duplications accounted for 9.5% (9/95) of all identified mutations. The duplications were more frequent in the proximal hotspot (66.7%, 6/9). The most frequent breakpoints of duplications were those that started from exon 3 (85.7%, 7/9). Duplications of exons 3–4 were identified three times (33.3%, 3/9). The present series showed eight different patterns of exonic duplications (Fig. 5). Almost all duplication mutations observed in this study started at exon 3.

Small mutations

There were 19 cases (20%, 19/95) with small mutations, 6 of which have been previously reported. There were 7 cases (36.8%, 7/19) with nonsense mutations, 4 cases (21.1%, 4/19) with small deletions, 3 cases (15.8%, 3/19) with splice site mutations, and 5 cases (26.3%, 5/19) with missense mutations. No hotspots were identified because they are distributed throughout all *DMD* exons involving all dystrophin domains. Single base substitutions were more frequent than small deletions or insertions.

Frame-shift mutations

Among 76 cases with major rearrangements, there were 50 cases (65.8%, 50/76) of in-frame mutations and 26 (34.2%, 26/76) cases of frame-shift mutations. We observed frame-shift mutation in 4 cases (25.0%, 4/16) of type 1 patients, 6 cases of type 2 (46.2%, 6/13) patients, and 20 cases (42.6%, 20/47) of type 3 patients. There was no significant difference for in-frame mutation and frame-shift mutation observed among the three subtypes ($p > 0.1$).

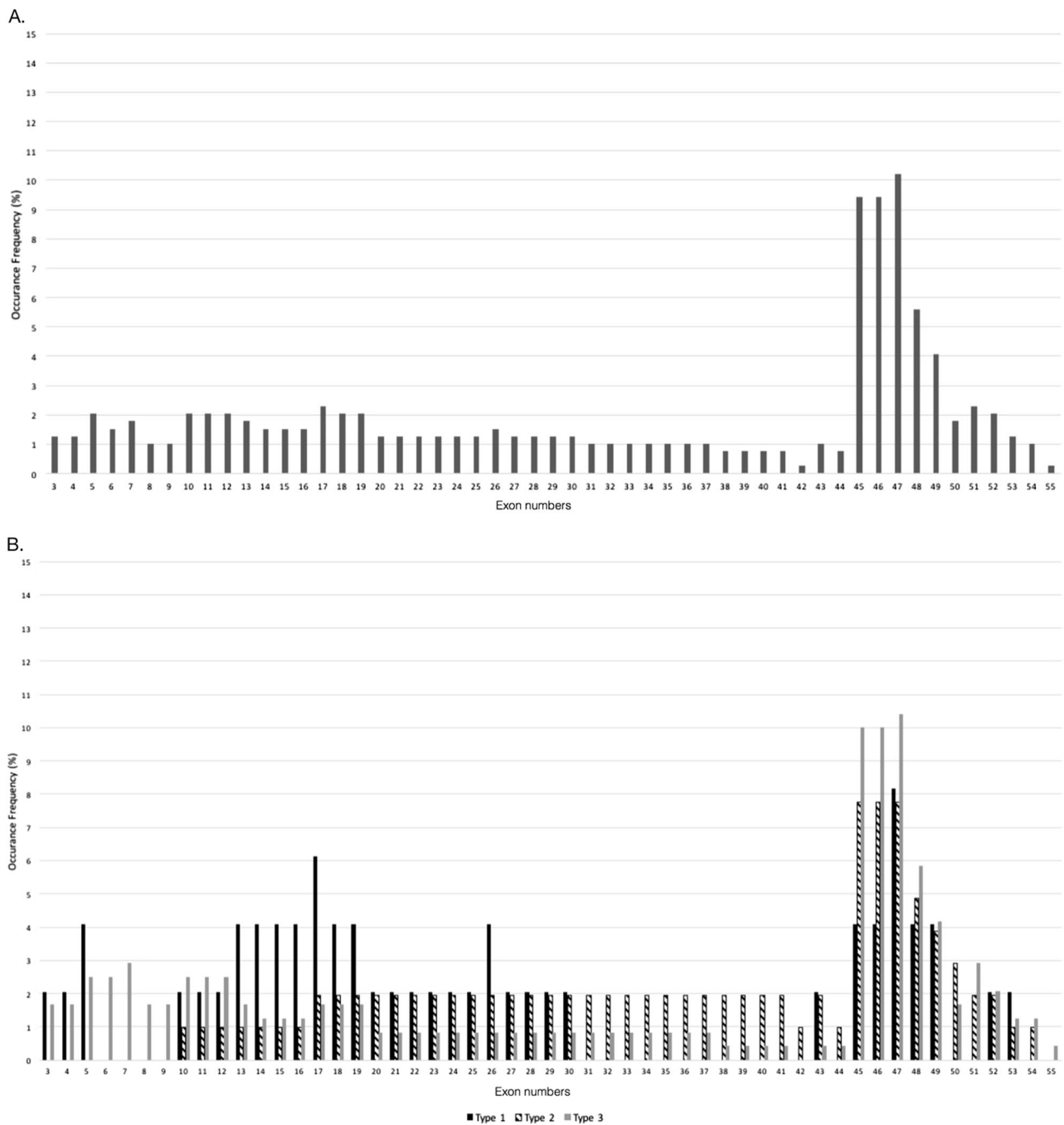


Fig. 3 The frequency distribution of exonic deletions of *DMD* gene in BMD patients. **a** The frequency distribution of exonic deletions of all 67 patients with exonic deletion. **b** The frequency distribution of

exonic deletions of three subtypes of patients. Black: type 1 (no muscular weakness); shade: type 2 (quadriceps myopathy); gray: type 3 (limb-girdle weakness)

Discussion

In the present series of BMD patients, we recruited various phenotypes, including patients with normal muscle strength, quadriceps myopathy, and with limb-girdle weakness. In the present study, we did not include DMD patients with improved phenotypes after steroid treatment, which is considered as BMD patients in present clinical diagnosis

criteria [22]. Patients with normal strength presented with hyperCKemia or cramp-myalgia syndrome were reported frequently before [2–4]. The diagnostic age in present series of BMD patients with normal strength is significantly earlier than the patient with quadriceps myopathy, but not significantly different from that of the patients with limb-girdle weakness. Patients can be free of symptoms till their 50s and cases of late-onset BMD

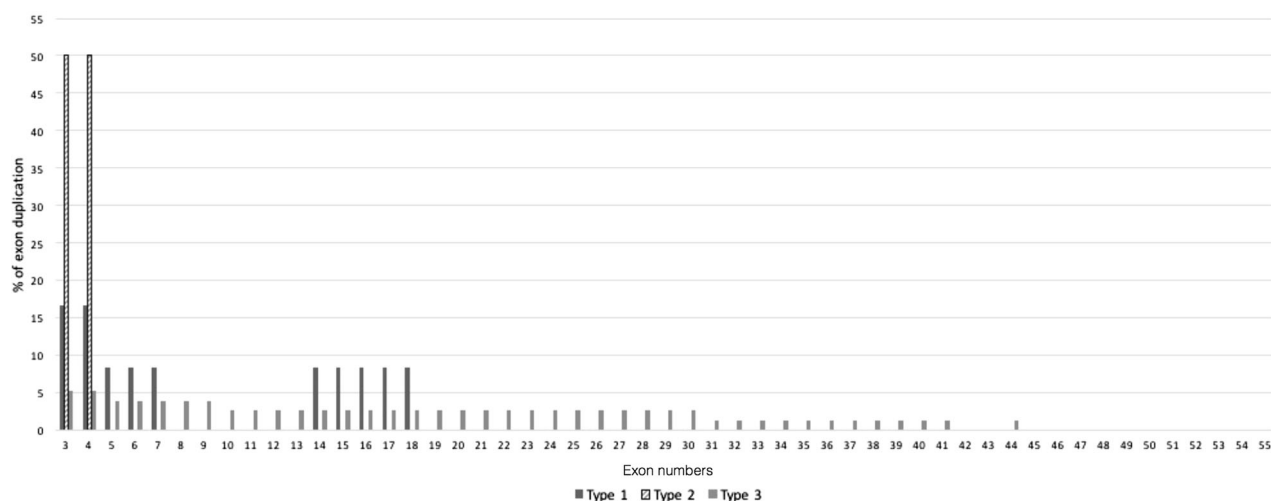
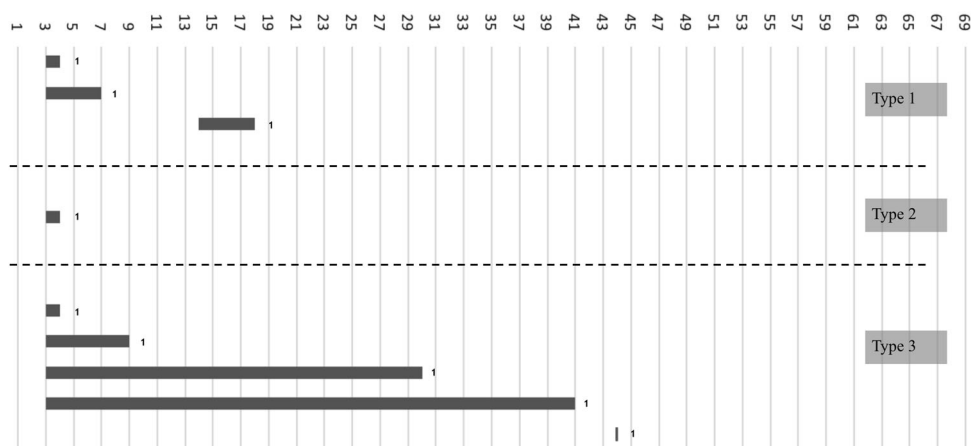


Fig. 4 Frequency distribution of exonic duplication of *DMD* gene of three subtypes of BMD patients. Black: type 1 (no muscular weakness); shade: type 2 (quadriceps myopathy); gray: type 3 (limb-girdle weakness)

Fig. 5 Patterns of exon duplication in *DMD* gene in BMD patients. The horizontal axis showed the number of exons. The present series had seven different patterns of exon duplication. The number on the side indicate the number of cases



have also been described [4]. Familial cardiomyopathy due to mutations of *DMD* gene presented no muscle weakness in adulthood [23]. Some patients with normal strength in present series of BMD patients might be the early stage of patients with quadriceps myopathy or cardiomyopathy. Follow-up study is needed to evaluate disease progression in patients with normal strength. However, phenotypes of most of the patients without muscle weakness did not develop with aging [24, 25]. The genotype characteristics of these patients should be the main study target for multiexon-skipping strategies.

Our study indicated that major rearrangements is the most common type of *DMD* gene mutation in three phenotypes of BMD, which is similar to other reports for BMD patients [10, 13, 25]. Exonic deletions appeared more frequently in patients with normal strength (type 1) and limb-girdle weakness (type 3) than patients with quadriceps myopathy (type 2). Exonic duplications were rare in all patients and more frequent in patients with normal strength

(type 1) than patient with muscle weakness (types 2 and 3). Small mutation is almost twice more frequent in patients with quadriceps myopathy (type 2) than patients with normal strength (type 1) or limb-girdle weakness (type 3). However, such tendency of differences had no statistical significance in present study.

Exonic deletions appeared in 70.5% of the all mutations in our patients, which was very similar to 74.3% reported in Shanghai [26]. However, the frequency of exonic deletions of BMD patients was 80% in Spanish BMD patients [10]. The reading-frame rule was fulfilled in 82.4% of Spanish BMD patients [10], 94% of MLPA identified Indian BMD patients [13], and 93% of France BMD patients [27]; however, only in 72.6% in present series. Usually in-frame mutations result in BMD, including all duplications in our series. The frame-shift mutations accounted for 18.8% Spanish BMD patients [10]; however, 27.4% in our series. Frame-shift mutations are less common in patient with normal strength, more common in patients with quadriceps

myopathy or limb-girdle weakness. The clinical severity is associated with the reading-frame rule for BMD patients, which should be considered in multiexon-skipping strategies in patient with BMD in the future.

Our finding further confirmed that disease progression in BMD patients appears to be dependent on the breakpoints location in *DMD* gene [28]. Deletions between exons 45 and 55 was previously reported as one of the hotspot for *DMD* gene mutations for BMD [4, 29, 30]. We found 67.2% of breakpoints of deletions between exons 45 and 55, mostly in patients with quadriceps myopathy or limb-girdle weakness. A deletion suitable to exon skipping of exons 44, 45, 51, or 53 are currently treated in the context of clinical trials or as commercialized drug in the case for exon 51 skipping in the United States [31]. However, we found that there were different phenotypes of BMD in this breakpoint region of *DMD* gene. We did not confirm that breakpoints of deletion in exons 45–48 and 45–51 led to mild phenotype [32, 33]. Some patients in present series with breakpoints of mutations in this region also showed phenotype of limb-girdle muscular dystrophy. We also did not find that del 45–47 or del 45–49 mutations cause comparatively severe weakness reported in previous studies [4, 17, 32], because some patients with breakpoints of mutations in this region of *DMD* gene shared hyperCKemia.

In general, deletions or duplications in the proximal portion of *DMD* gene tended to cause mild phenotype with cramps and myalgia or hyperCKemia [34]. We confirmed 19.4% cases of exonic deletions on exons 3–19, another deletion hotspots of *DMD* gene in BMD patients [4]. Duplications accounted for 9.5% cases of all identified mutations and more frequent in the proximal hotspot started from exon 3. It appeared only in 3.2% of Indian BMD patients [13]. Patients with mutations in this region had more chance with mild or asymptomatic phenotype. Minority of the deletions was located between exons 2 and 19 in *DMD* [35]. We found deletions on exons 3–19 more common in patients with normal strength than patients with limb-girdle weakness, but no different to patients with quadriceps myopathy. Additional studies are required to determine whether multiexon-skipping strategies in exons 3–19 of *DMD* gene could yield more functional dystrophin, including the exons 3–9 [36].

Small mutations appeared in 20.0% of all mutations in present series, including nonsense mutations, microdeletions, splice site mutations, and missense mutations. The small mutations appeared only in 11% in Spanish BMD patients [10]. The frequency of different small mutations varied from report to report. The nonsense mutations accounted for 36.8% of small mutations in present series and 31.25% in Spanish BMD patients [10]. The splice site mutations accounted for 15.8% of small mutations in present series and 38% in Spanish BMD patients [10].

Missense mutation is 10.5% in present series and 6% of Spanish BMD patients [10]. We found that the same small mutations appeared not only in patients with normal strength but also in quadriceps myopathy or limb-girdle weakness of BMD in present series. We found that single base substitutions were about five times more frequent than small deletions. However, no hotspots were identified because they are distributed diffusely throughout all *DMD* exons involving all domains. It is difficult to determine the location of small mutations as exon-skipping therapies at present time.

Our result showed the genetic mutation spectrum in a large cohort of Chinese BMD patients. Major rearrangements were very common among BMD patients. Exons 45–55 were the hotspot for exonic deletion. Frame-shift mutation was not rare in Chinese BMD patients. The severity of BMD did not depend on the mutation types of *DMD* gene, but the location. The mutations in proximal region of *DMD* gene had higher frequency for patients without weakness. Additional studies are required to determine whether multiexon-skipping strategies in proximal region of *DMD* gene could yield more functional dystrophin.

Acknowledgements We thank all patients and their family members for their participation in this study. We also thank Ms Jing Liu and Ms Yuehuan Zuo for their technical assistance in the preparation of genetic analysis.

Funding This study was supported by the Ministry of Science and Technology of China (No. 2011ZX09307-001-07) and Beijing Municipal Science and Technology Commission (No. Z151100003915126).

Compliance with ethical standards

Conflict of interest The work described here has not been published previously, nor is it under consideration for publication elsewhere. This publication has been approved by all the authors and by the responsible authority where the work was carried out. If this paper is accepted, it will not be published elsewhere in the same form or in any other language, without the written consent of the copyright-holder. The authors declare that they have no conflict of interest.

References

1. Ramos E, Conde JG, Barrios RA, Pardo S, Gómez O, Rodríguez Mas. Prevalence and genetic profile of Duchene and Becker muscular dystrophy in Puerto Rico. *J Neuromuscul Dis.* 2016;3: 261–6.
2. Bushby KM, Gardner-Medwin D, Nicholson LV, Johnson MA, Haggerty ID, Cleghorn NJ, et al. The clinical, genetic and dystrophin characteristics of Becker muscular dystrophy. II. Correlation of phenotype with genetic and protein abnormalities. *J Neurol.* 1993;240:105–12.
3. Melis MA, Cau M, Muntoni F, Mateddu A, Galanello R, Boccone L, et al. Elevation of serum creatine kinase as the only manifestation of an intragenic deletion of the dystrophin gene in three unrelated families. *Eur J Paediatr Neurol.* 1998;2:255–61.

4. Taglia A, Petillo R, D'Ambrosio P, Picillo E, Torella A, Orsini C, et al. Clinical features of patients with dystrophinopathy sharing the 45–55 exon deletion of DMD gene. *Acta Myol.* 2015;34:9–13.
5. Minetti C, Tanji K, Chang HW, Medori R, Cordone G, DiMauro S, et al. Dystrophinopathy in two young boys with exercise-induced cramps and myoglobinuria. *Eur J Pediatr.* 1993;152:848–51.
6. Sunohara N, Arahata K, Hoffman EP, Yamada H, Nishimiya J, Arikawa E, et al. Quadriceps myopathy: forme fruste of Becker muscular dystrophy. *Ann Neurol.* 1990;28:634–9.
7. Tsuda T, Fitzgerald K, Scavena M, Gidding S, Cox MO, Marks H, et al. Early progressive dilated cardiomyopathy in a family with Becker muscular dystrophy related to a novel frameshift mutation in the dystrophin gene exon 27. *J Hum Genet.* 2015;60:151–5.
8. van den Bergen JC, Wokke BH, Janson AA, van Duinen SG, Hulsker MA, Ginjaar HB, et al. Dystrophin levels and clinical severity in Becker muscular dystrophy patients. *J Neurol Neurosurg Psychiatr.* 2014;85:747–53.
9. Bladen CL, Salgado D, Monges S, Foncuberta ME, Kekou K, Kosma K, et al. The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. *Hum Mutat.* 2015;36:395–402.
10. Juan-Mateu J, González-Quereda L, Rodríguez MJ, Baena M, Verdura E, Nascimento A, et al. DMD mutations in 576 dystrophinopathy families: a step forward in genotype–phenotype correlations. *PLoS ONE.* 2015;10:e0135189.
11. Aslesh T, Maruyama R, Yokota T. Skipping multiple exons to treat DMD—promises and challenges. *Biomedicine.* 2018;6:1.
12. Nakamura A. Moving towards successful exon-skipping therapy for Duchenne muscular dystrophy. *J Hum Genet.* 2017;62:871–6.
13. Vengalil S, Preethish-Kumar V, Polavarapu K, Mahadevappa M, Sekar D, Purushottam M, et al. Duchenne muscular dystrophy and Becker muscular dystrophy confirmed by multiplex ligation-dependent probe amplification: genotype–phenotype correlation in a large cohort. *J Clin Neurol.* 2017;13:91–7.
14. Comi GP, Prella A, Bresolin N, Moggio M, Bardoni A, Gallanti A, et al. Clinical variability in Becker muscular dystrophy. Genetic, biochemical and immunohistochemical correlates. *Brain.* 1994;117(Part 1):1–14.
15. Pons R, Kekou K, Gkika A, Papadimas G, Vogiatzakis N, Svingou M, et al. Single amino acid loss in the dystrophin protein associated with a mild clinical phenotype. *Muscle Nerve.* 2017;55:46–50.
16. Nakamura A, Fueki N, Shiba N, Motoki H, Miyazaki D, Nishizawa H, et al. Deletion of exons 3–9 encompassing a mutational hot spot in the DMD gene presents an asymptomatic phenotype, indicating a target region for multiexon skipping therapy. *J Hum Genet.* 2016;61:663–7.
17. Bello L, Campadello P, Barp A, Fanin M, Semplicini C, Sorarù G, et al. Functional changes in Becker muscular dystrophy: implications for clinical trials in dystrophinopathies. *Sci Rep.* 2016;6:32439.
18. Magri F, Govoni A, D'Angelo M, Del Bo R, Ghezzi S, Sandra G, et al. Genotype and phenotype characterization in a large dystrophinopathic cohort with extended follow-up. *J Neurol.* 2011;258:1610–23.
19. Todeschini A, Gualandi F, Trabanelli C, Armadori A, Ravani A, Fanin M, et al. Becker muscular dystrophy due to an intronic splicing mutation inducing a dual dystrophin transcript. *Neuromuscul Disord.* 2016;26:662–5.
20. Bradley WG, Jones MZ, Mussini JM, Fawcett PR. Becker-type muscular dystrophy. *Muscle Nerve.* 1978;1:111–32.
21. Wang Y, Yang Y, Liu J, Chen X, Liu X, Wang C, et al. Whole dystrophin gene analysis by next-generation sequencing: a comprehensive genetic diagnosis of Duchenne and Becker muscular dystrophy. *Mol Genet Genom.* 2014;289:1013–21.
22. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol.* 2010;9:77–93.
23. Obler D, Wu B, Lip V, Estrella E, Keck S, Haggan C, et al. Familial dilated cardiomyopathy secondary to dystrophin splice site mutation. *J Card Fail.* 2010;16:194–9.
24. Ramelli GP, Joncourt F, Luetsch J, Weis J, Tolnay M, Burgunder JM. Becker muscular dystrophy with marked divergence between clinical and molecular genetic findings: case series. *Swiss Med Wkly.* 2006;136:189–93.
25. Ferreira V, Giliberto F, Muñoz G, Francipane L, Marzese D, Mampel A, et al. Asymptomatic Becker muscular dystrophy in a family with a multiexon deletion. *Muscle Nerve.* 2009;39:239–43.
26. Li X, Zhao L, Zhou S, Hu C, Shi Y, Shi W, et al. A comprehensive database of Duchenne and Becker muscular dystrophy patients (0–18 years old) in East China. *Orphanet J Rare Dis.* 2015;10:5.
27. Tuffery-Giraud S, Bérout C, Leturcq F, Yaou R, Hamroun D, Michel-Calemard L, et al. Genotype–phenotype analysis in 2,405 patients with a dystrophinopathy using the UMD-DMD database: a model of nationwide knowledgebase. *Hum Mutat.* 2009;30:934–45.
28. Nicolas A, Raguénès-Nicol C, Ben Yaou R, Ameziane-Le Hir S, Chéron A, Vié V, et al. Becker muscular dystrophy severity is linked to the structure of dystrophin. *Hum Mol Genet.* 2015;24:1267–79.
29. Findlay AR, Wein N, Kaminoh Y, Taylor LE, Dunn DM, Mendell JR, et al. Clinical phenotypes as predictors of the outcome of skipping around DMD exon 45. *Ann Neurol.* 2015;77:668–74.
30. van den Bergen JC, Schade van Westrum SM, Dekker L, van der Kooi AJ, de Visser M, Wokke BHA, et al. Clinical characterisation of Becker muscular dystrophy patients predicts favourable outcome in exon-skipping therapy. *J Neurol Neurosurg Psychiatr.* 2014;85:92–8.
31. Bello L, Pegoraro E. Genetic diagnosis as a tool for personalized treatment of Duchenne muscular dystrophy. *Acta Myol.* 2016;35:122–7.
32. Ameziane-Le Hir S, Paboeuf G, Tascon C, Hubert J, Le Remeur E, Vié V, et al. Dystrophin hot-spot mutants leading to Becker muscular dystrophy insert more deeply into membrane models than the native protein. *Biochemistry.* 2016;55:4018–26.
33. Nakamura A, Shiba N, Miyazaki D, Nishizawa H, Inaba Y, Fueki N, et al. Comparison of the phenotypes of patients harboring in-frame deletions starting at exon 45 in the Duchenne muscular dystrophy gene indicates potential for the development of exon skipping therapy. *J Hum Genet.* 2017;62:459–63.
34. Beggs AH, Hoffman EP, Snyder JR, Arahata K, Specht L, Shapiro F, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. *Am J Hum Genet.* 1991;49:54–67.
35. Basumatary LJ, Das M, Goswami M, Kayal AK. Deletion pattern in the dystrophin gene in Duchenne muscular dystrophy patients in northeast India. *J Neurosci Rural Pract.* 2013;4:227–9.
36. Nakamura A, Fueki N, Shiba N, Motoki H, Miyazaki D, Nishizawa H, et al. Deletion of exons 3–9 encompassing a mutational hot spot in the DMD gene presents an asymptomatic phenotype, indicating a target region for multiexon skipping therapy. *J Hum Genet.* 2016;61:663–7.