Original Article

REVEALING FINDINGS FROM A GENETIC STUDY OF CHILDREN AFFECTED BY DUCHENNE MYODYSTROPHY IN KAZAKHSTAN

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ABSTRACT

Duchenne muscular dystrophy is an inherited neuromuscular disorder that follows an X-linked recessive pattern of inheritance. It manifests as a severe condition characterized by progressive muscle degeneration due to mutations in the DMD gene, which is responsible for encoding the dystrophin protein.

Objective

To assess clinical changes in children with Duchenne myodystrophy from the type of DMD gene mutation.

Material and methods

The study involved 97 boys aged from 3 to 15 years, the average age of onset of the disease was 3.2±0.18 years. To study patients, laboratory research methods were used, such as a biochemical blood test (creatinine phosphokinase level), and a search for a genetic mutation in the DMD gene was carried out using MLPA and NGS. Clinical manifestations of this disease were assessed using functional status scales.

Results

Among the children studied, MLPA revealed deletions in 56 (57.5%), large duplications in 13 (13.2%), and point mutations in 28 (29.3%). The remaining mutations were situated closer to the terminal part responsible for the linkage of the dystroglycan-protein complex within the muscle framework.

Conclusion

In our investigation, we explored the correlations between primary clinical data and different mutation characteristics. Our analysis revealed a significant and reliable association between early loss of independent movement, as well as early disease onset, and mutations capable of disrupting the translational reading frame of the dystrophin protein. Given that neuromuscular diseases, including genetic disorders, represent a pressing issue in clinical neurology, these findings underscore the importance of understanding such correlations in managing these conditions.

Keywords

neuromuscular disorders, Duchenne myodystrophy, genetic research, mutations, sequencing.

RELEVANCE

Duchenne muscular dystrophy (DMD) is a fatal X-linked recessive disorder marked by gradual muscle degeneration caused by mutations in the DMD gene, which encodes the dystrophin protein. These mutations result in the absence of dystrophin beneath the sarcolemma in individuals with DMD. Dystrophin is crucial for preserving muscle fiber integrity and membrane stability. Without dystrophin, muscle fibers sustain damage during contractions [1]. Between the ages of 1 to 3 years, individuals with Duchenne muscular dystrophy (DMD) typically exhibit noticeable symptoms such as delayed walking, frequent falls, and challenges in activities like running and climbing stairs. During this stage, DMD patients often develop enlarged muscles around the calf, pelvis, and thigh, which is termed pseudo muscle hypertrophy. Muscle weakness and loss of ambulation usually commence around age 8 and steadily worsen

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over time. Unfortunately, DMD patients typically have a life expectancy ranging from 18 to 30 years, with most succumbing to cardiorespiratory complications [2,3]. The clinical picture depends on the type of mutation, on the location of mutation in the gene, on the size of mutation, and on therapy. Historically, there is no treatment for DMD, with the exception of a syndromic approach, where the death of muscle fibers slows down; for this, glucocorticoid therapy is often used, as well as rehabilitation measures [4]. In more than half of cases, there is a deletion of one or several exons, only in 10% of cases there is a duplication of gene regions, in other cases a point mutation occurs. Today, when modern methods of pathogenetic therapy are being developed and implemented, the early and accurate diagnosis of changes in the *DMD* gene is of the greatest interest. With regard to the diagnosis of DMD, the method of molecular ligase-dependent amplification (MLPA) allows testing all 79 exons of the dystrophin gene at once for the presence of deletions and duplications [5,6,7]. Genetic methods are not routine, therefore, in order to optimize the diagnostic search, only MLPA is indicated in the Kazakhstan DMD protocol. The disadvantage of the MLPA method is that point intradermal and/or foreign mutations cannot be detected by this method. Taking into account the prevalence of point mutations in the range of 10-15%, the importance of new generation sequencing (NGS) becomes obvious [8]. Prior to genetic testing, a clinical assessment of the condition is carried out based on specific symptoms, the main of which are progressive muscle weakness in males, increased CK in the blood, "duck" gait, difficulty climbing stairs, pseudohypertrophy of the calf muscles. This symptom is caused by muscle weakness and degeneration of muscle tissue [9, 10]. An increase in CK is a preclinical symptom in burdened families. The variety of clinical symptoms dictates the need to monitor this contingent of children by a multidisciplinary team. Medical and genetic consultation is the basis of secondary prevention, for which it is necessary to know the types of mutations, and also to determine the status of the mother's carrier [11].

The aim of the study was to identify the clinical features of the course of DMD, depending on the type of mutation.

MATERIAL AND METHODS

The study enrolled 97 boys aged between 3 and 15 years, with an average age of 8.5±0.42 years. All participants presented complaints of muscle weakness, fatigue, and various gait abnormalities. Diagnostic methods employed included laboratory analysis to determine creatine kinase levels using biochemical assays, as well as MLPA and NGS analysis of the DMD gene. Genealogical and clinical assessments were conducted using the P.J. Vignos functional scale for lower extremity motor activity and the 6-minute walking test (6MWT). Diagnosis was confirmed based on the prevailing clinical diagnostic and treatment protocol for Progressive Duchenne/Becker muscular dystrophy. Each patient (or their parent, if applicable) provided informed consent before undergoing a thorough clinical and neurological assessment. During the data collection process, comprehensive information was gathered regarding the patient's family history, disease onset, progression, clinical manifestations throughout the entire duration of the illness, and the rate of disease advancement.

The genealogical approach involved constructing pedigrees to examine familial relationships. Motor abilities of the boys were assessed using Vignos scales and the 6-minute walking test. Standard biochemical methods were utilized to determine the level of creatine kinase (CK) in blood serum. For gene mutation analysis, all children underwent multiplex-ligase-dependent probe amplification (MLPA) using SALSA 034 and SALSA 035 probe reagents. The genetic material was processed using the ABI PRISM 3100 genetic analyzer. For children with a negative MLPA result, an NGS analysis was performed to search for point mutations. The *DMD* gene is usually analyzed by next-generation amplicon-based sequencing. Amplicons cover the entire coding region and highly conserved Exon-Intron junctions. Minimum coverage >20x for each amplicon and technical sensitivity (SNV/InDels) 99.9%. CNV (variation of the number of copies included) allows the detection of deletions and duplications using the NGS methodology.

RESULTS

The primary method for identifying genetic mutations was through MLPA, which confirmed large mutations in the form of deletions and duplications in 69 cases (70.7%). In patients with negative MLPA results, further investigation for point mutations was conducted via gene sequencing, identifying such mutations in 28 children (29.3%). As a result, Duchenne muscular dystrophy was genetically confirmed in all 9patients.

The identified mutation types were categorized as follows: major deletions in 40 cases (57.5%), major duplications in 29 cases (13.2%), and point mutations encompassing various types such as microdeletions, microduplications, nonsense mutations, single nucleotide substitutions, intron mutations, insertions, missense mutations, and splicing site mutations in 28 cases (29.3%). The distribution of mutations is shown in Figure 1.

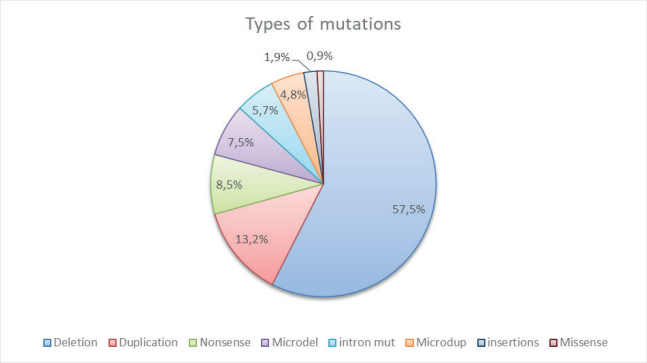

Figure1. Distribution of mutations

In numerous instances, the severity and progression of the disease are influenced by the specific mutation type and whether the translational reading frame is preserved. In our study, the assessment of this reading frame was conducted in 93 cases, revealing a shifted reading frame in 55 cases (59.1%) and a preserved translational frame in 38 cases (40.9%). In our study, we noted an average disease onset age of 3.98 ± 0.12 years, which falls within a range different from the commonly reported debut around 5 years according to existing literature. Additionally, our research identified various clinical features based on different criteria. The average age of non-outpatient is 9.5 ± 0.25 years, the average level of CK is 8063.54 units / l, which is almost 50 or more times higher than the standard values. The indicator of a 6-minute walk averaged 390.97 ± 16.8 meters. The clinical characteristics of the assessment of the functional status of motor function were different, more dependent on the age of the onset of the disease, on the type of mutation and on the localization of the mutation.

Evaluation of clinical data showed various correlations from many characteristics of mutations. When comparing the results of the 6MWT test, the average figures of the distance of independent walking are significantly lower in the group of children with the type of mutation shifting the reading frame. All children with this disease have a 6MWT test score lower than 500 meters. To assess the role of mutation with a shift in the translational framework, 37 children were evaluated, whose indicator was 2 or more times less than the norm, that is, 250 meters. Table 1 – Comparative characteristics of the distribution of children from the reading frame and 6MWT (%)

reading frame and 6MWT (%)	X	±m	[95% Confidence interval]	
Negative				
250 meters or less	34,38	8,40	19,94	52,42
Positive				
250 meters or less	57,14	8,70	22,48	85,98

The analysis of Table 1 shows that a significant low indicator of 6MWT was detected in the group of children in whom the mutation led to a shift in the translational frame in 57.14% of cases, whereas this indicator in children without a shift in the reading frame was within 34.38% of cases (a difference of 1.66 or more cases).

The Vignos Scale, where the assessment is carried out at the level of grade 10, where the level of grade 5 is characterized by a significant loss of independent movement, which corresponds to the definition of early non-ambulatory. The distribution of children with Duchenne myodystrophy in the context of the reading frame and the Vignos scale showed that, while maintaining the translational reading frame, the average values varied at the level of 20.0%±17.8% in class 5, in contrast to children of this class with a violation of the translational reading frame of 80.0%±17.8%, which is almost 4 times higher ($p \leq 0.05$). Table 2 – Comparative characteristics of the distribution of children from the reading frame and the Vignos scale (%)

Reading frame and Vignos scale	X	±m	[95% Confidence interval]	
Negative				
1	32,36*	8,40	18,17	50,53
2	72,73*	13,43	41,01	91,10
3	44,44	16,56	17,43	75,20
4	50,00	17,68	19,71	80,29
5	20,00*	17,89	2,64	69,72

Reading frame and Vignos scale	X	±m	[95% Confidence interval]	
6	0,00	н/о	н/о	н/о
7	35,71*	12,81	15,50	62,71
8	62,50	17,12	28,10	87,67
9	50,00	35,36	5,69	94,31
10	0,00	-	-	-
Positive				
1	67,74	8,40	49,47	81,83
2	27,27	13,43	8,90	58,99
3	55,56	16,56	24,80	82,57
4	50,00	17,68	19,71	80,29
5	80,00	17,89	30,28	97,36
6	100,00	0,00	-	-
7	64,29	12,81	37,29	84,50
8	37,50	17,12	12,33	71,90
9	50,00	35,36	5,69	94,31
10	100,00	0,00	-	-

Analysis of the Vignos scale indicators were higher by class in the group of children with various types of mutations in the context of assessing the presence or absence of a shift in the translational reading frame, where in the group of children with a shift in this frame, the Vignos scale score showed higher grades, which means loss of the ability to move independently.

Each patient has a different experience of the disease, which is due from the onset of the disease to the assessment of the functional status was different. In a group of children (n=74) with an experience of no more than 7 years, an assessment was executed on the Vignos scale. Correlation analysis of the deterioration of motor functions depending on the length of the disease showed a significant dependence (r=0.78) (Figure 2).

Figure 2 - Correlative dependence by Vignos classes depending on the length of service.

The distribution of children with Duchenne myodystrophy in the context of the age of the debut and the reading frame found that in the age intervals of the debut – 1.5 – 3.5 years, 3.6 – 5.6 years and 5.7 – 7.3 years and the negative reading frame, the average values fluctuated at the levels of 29,41%±11,05%; 46,43%±6,66% and 54.55%±15.01% accordingly, for a positive reading frame in the same age ranges of the debut – 70,59%±11,05%; 53,57%±6,66% and 45.45%±15.10%, respectively (Table 3).

Table 3 – Comparative characteristics of the distribution of children from the reading frame and the age of debut (%)

Reading frame and age of debut	X	±m	[95% Confidence interval]	
Negative				
1,5-3,5 years	29,41	11,05	12,63	54,57
3,6-5,6 years	46,43	6,66	33,71	59,62
5,7-7,3 years	54,55	15,01	26,46	80,01
Positive				
1,5-3,5 years	70,59	11,05	45,43	87,37
3,6-5,6 years	53,57	6,66	40,38	66,29
5,7-7,3 years	45,45	15,1	19,99	73,54

As can be seen from Table 3, the early onset of the disease depended on a violation of the translational framework in 70.59% (the value exceeds 2.4 times), whereas at the age of early debut, this indicator in the group of children without a violation of the translational framework was in 29.41% of cases.

In our study, the functional regions of the *DMD* gene were divided into 3 regions according to exon numbering: actin-binding (2-10,32-45), hot spots covering 19-20 exons, mainly located in the central part of the gene (hot spots 45-55), dystroglycan-binding (64-70) and syntrophin-binding (71-79). Such a variety of mutation types obtained in our study allows us to speak about the proposed concept that for this gene it is impossible to safely talk about major mutations, since the percentage of mutation detection in hot spots (45-55 exons) in our study was 34.9%, while the proximal part of the gene and the distal part of the gene were 39.6% and 25.5% respectively. Therefore, the interpretation of mutation types depending on the simple localization distribution is not informative, and therefore, the prediction of pathogenicity by localization depending on the functional regions of the gene is a necessary tool. Out of 97 patients, mutations located in the 70-79 exons included in the dystroglycan complex in 31 children were reliably associated with cognitive impairment

with various degrees of severity, which did not progress despite the deterioration of motor functions. That is, it can be assumed that mutations located closer to the promoters responsible for the isoforms of dystrophin in the brain are responsible for the absence or incomplete synthesis of dystrophin in the brain.

In 4 cases of mutations located in the actin-binding site without shifting the translational reading frame, the course and progression of the disease based on the assessment of the functional status using clinical scales, both at the time of diagnosis verification and in catamnestic observation, were comparable to the severe DMD phenotype, similar to mutations with a shift in the translational reading frame (by predicting pathogenicity). This fact can be explained by the fact that regardless of the characteristics of the type of mutations, the location in a highly functional actin-binding site was a clinically significant criterion.

DISCUSSION

According to the results of molecular diagnostics, large deletions were detected in 56 cases (57.5%), large duplications in 13 cases (13.2%), point mutations (microdeletions, microduplications, nonsense mutations, single nucleotide substitutions, intron mutations, insertions, missense mutations, splicing site mutations)– in 28 patients (29.3%). The identified mutations in the hot spots (45-55 exons) in our study amounted to 34.9%, while in the proximal part of the gene and in the distal part of the gene amounted to 39.6% and 25.5%, respectively. The reading frame was evaluated in 93 cases, where in 55 cases (59.1%) the reading frame is shifted, in 38 cases (40.9%) the translational frame is preserved. According to the results of the 6MWT test, in children (37) mutations with a shift in the translational frame, the indicator was 2 or more times less than the norm, that is, 250 meters. The assessment of the Vignos scale and the reading frame showed that while maintaining the translational reading frame, the average values varied at the level of 20.0% ±17.8% for class 5, in children of this class with a violation of the translational reading frame of 80.0% ± 17.8%, which is almost 4 times higher. The early onset of the disease depended on a violation of the translational framework in 70.59% (the value exceeds 2.4 times), in children without a violation of the translational framework was in 29.41% of cases.

CONCLUSION

In our research, we investigated the correlation between key clinical data and different mutation characteristics. Our analysis revealed a significant and reliable association between early loss of independent movement, as well as early disease onset, and mutations that disrupt the translational reading frame of the dystrophin protein. Considering those neuromuscular diseases, including those of genetic origin, represent a critical challenge in clinical neurology, these findings emphasize the importance of understanding such associations.

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