NJSC «West Kazakhstan Marat Ospanov medical university»

ANNOTATION of the dissertation work
for the Degree of Doctor of Philosophy (PhD)

"Clinical and genetic characteristics of Duchenne muscular dystrophy in the Republic of Kazakhstan"

Specialization: «6D110100 – Medicine»

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ANNOTATION

Ainur Ontalapovna Umurzakova on the topic “Clinical and genetic characteristics of Duchenne myodystrophy in the Republic of Kazakhstan”, submitted for the degree of Doctor of Philosophy (PhD) on specialty 6D110100 - “Medicine”

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Relevance of the research. At present, the problem of neuromuscular diseases is one of the urgent tasks of modern medicine, which is due to the tendency to increase their number (Venugopal et al. 2023). The more common form of hereditary neuromuscular diseases is progressive Duchenne myodystrophy (DMD), which is registered with a frequency of 1 case per 3500/6000 newborn boys (Shuaiwei et al. 2024). In many countries of the world, data on the widespread prevalence of this disease have been accumulated, while in Kazakhstan it is not possible to determine the exact frequency of occurrence of these diseases due to the lack of an official register.

Duchenne myodystrophy is a severe inherited neuromuscular disease characterized by X-linked recessive inheritance and a progressive course based on mutations in the gene encoding the dystrophin protein (DMD; locus Chr21.2). The main function of dystrophin in muscle is to provide mechanical strength to the sarcolemma (Childs A.M. et al. 2024). The dystrophin protein is also found in heart muscle and in small amounts in the brain (Zhou Y. et al. 2024). The length of the dystrophin gene is 2.2 million nucleotide pairs and contains 79 exons, 78 introns (Aldharee H. 2024).

The disease affects mainly boys; in approximately 2/3 of cases, the son receives a chromosome with a damaged gene from a carrier mother who is phenotypically healthy. In the remaining cases, the disease results from a de novo mutation in maternal and paternal germ cells (Min et al. 2019).

Currently, extensive data have been accumulated regarding deletion spectra in different populations of the world, and certain population differences have been established (Kong et al. 2019). According to recent data, it is known that the nature of mutations in *DMD* are different, may include deletions, duplications and point mutations. The different nature of mutations including nonsense mutations, missense mutations and splice site mutations occur unevenly in different populations. The study of point mutations allows for a more detailed functional analysis of the dystrophin protein, similar to the analysis of the correlation between mutation localization and clinical manifestations in patients with Duchenne myodystrophy (M. Neri et al. 2020, Shuyu et al. 2018). The study of mutation spectra in the dystrophin

gene with the exact localization of the mutation relative to the exon and their extent represents a unique opportunity to elucidate the mechanisms at the molecular level. The effect of different mutations on the translational reading frame of the genetic code allows us to analyze the correlation with clinical manifestations of DMD (Hart C.C.et al. 2024).

To date, the basis of therapy for Duchenne myodystrophy has been symptomatic treatment only. There is only a small experience in the management of children on pathogenetic therapy, conditioned on a narrow range of mutations. It is relevant to identify mutations of *DMD* gene, which diagnoses the disease and provide accurate genetic counseling and early prenatal diagnosis of patients with DMD. In the last 3-5 years, pathogenetic therapies for this pathology are available and used, for the precise selection of which new knowledge about the patterns of gene alterations is needed (Min et al. 2019, D. Mias-Lucquin et al. 2020).

For the application of new therapies, such as exon skipping and drug application with restoration of the reading frame of the genetic code, determination of the nature of mutations becomes necessary and relevant (Greigess et al. 2020). The study of data on the prevalence of DMD with issues of diagnosis and treatment in Kazakhstan is of priority importance for the development of neurology, medical genetics of practical health care.

Purpose of work: on the basis of mutation type analysis in DMD gene to study clinical features and the effect of therapy on the clinical course of Duchenne myodystrophy.

Objectives of the study:

1. To study the frequency of occurrence and inheritance of Duchenne myodystrophy in the Republic of Kazakhstan
2. To analyze the spectrum of mutations in DMD gene in Duchenne myodystrophy.
3. To determine the peculiarities of the clinical course of DMD taking into account the type of mutations and therapy.

Scientific novelty of the study:

- The frequency of occurrence and inheritance of DMD (carrier) was studied for the first time in Kazakhstan;
- a wide range of mutations in *DMD* gene with evaluation of their functional characteristics was analyzed;
- based on the study of the spectrum of mutations in the DMD gene, data on the peculiarities of the clinical course of DMD were obtained.

Theoretical and practical significance.

The results of thisscientificwork will serve as the basis for thedevelopment of a National Registry and may serve as a basis for making additions to the clinical protocol for the diagnosis and treatment of Duchenne myodystrophy. The results of this work will allow physicians to timely initiate genetic diagnosis of Duchenne myodystrophy in case of serum creatine phosphokinase (CPK) elevation in boys with

gait disorders by MLPA method, and in case of negative results - to perform gene sequencing.

Assessment of the translational reading frame status of the genetic code will help to predict disease progression, which is a key criterion for timely initiation of glucocorticosteroid (GCS) therapy.

The results of the dissertation work are implemented in the educational process of the Department of Neurology, psychiatry and narcology of JSC “WKMU named after Marat Ospanov”, as well as in practical health care.

Main points to be defended

1. In the studied regions of the republic, the number of male children under 5 years of age according to statistical data amounted to 350991; we diagnosed 106 cases of Duchenne myodystrophy for the first time, which corresponds to a frequency of 1 case per 3311 boys. In 64, 4% of cases of sick boys, the mutation in the *DMD* gene was inherited from their mothers, whereas in 35,6% of cases the mutation was spontaneous (de novo).

2. In the analysis of mutations in the *DMD* gene, the deletion spectrum 59,3% (95%CI: 51,9-68,9)was more prevalent than duplications and point mutations, with extended deletions accounting for 81,5% (95%CI: 77,4-86,1). Mutations were predominantly located in the high-functioning actin-binding site of the gene(60,4%; 95%CI: 51,5-70,1), which were represented by all types of mutations, whereas the central site was predominantly deletions, and the site responsible for dystroglycanprotein complex attachment was represented by point mutations (p=0,015). Translational reading frame disruption was observed in 82,4% (95%CI: 72,3-92,1) of cases and was predominantly located in the actin-binding 76,4% (95%CI: 66,5-85,9) region of the gene.

3. Reading frame condition influenced clinical characteristics: impairment was associated with early disease debut (2,95±0.25 years), a low NSAA score of 15±5,6, 292,4±11,3 meters on the 6MWT with a 1-year decline to 285,8±3.12. For loss of ambulation, impaired reading frame was a risk OR of 12,36 (95% CI: 2,51-22,18), with an observed duration of independent walking of 3,9±0,26 years.

In outpatient children receiving GCS therapy, 6MWT improved from 317,4±12,57 meters to 348,1±9,65 meters over one year, whereas in non-users from 319,8±13,07 to 324,4±8,23 meters. In non-ambulatory children, the “rise from the supine position” test lengthened from 12±2,45 seconds to 17±2,18 seconds and from 13±3,54 seconds to 24±3,68 seconds over a year, in those receiving and not receiving GCS therapy, respectively. Against the background of therapy with Ataluren (Translarna), the 6MWT test score improved from 327,4±2,57 to 349,1±2,65 meters in 12 months.

Approbation of the work. The results of the study were reported at:

1. IX International Scientific and Practical Conference “Actual Issues of Medicine” Baku, Azerbaijan, May 6-8, 2020. Report: “Preliminary results of clinical and genetic study of Duchenne myodystrophy in the Western region of Kazakhstan”.

2. International Scientific and Practical Conference “XXIV University Neurological Readings. “Neurology today” Ufa, Bashkortostan, 29.10.-31.10.2020. Report: “Cognitive disorders in patients with Duchenne myodystrophy”.

3. LXI International Scientific Conference of Young Scientists “Science: Yesterday, Today, Tomorrow” Aktobe, Kazakhstan, 27.04.2022 Report: “Duchenne Muscular Dystrophy. Discription of clinical case”.

4. International scientific and practical conference "young researcher: challenges and prospects for the development of modern Pediatrics and pediatric surgery". Almaty, 2022 Report:"Clinical and genetic features of Duchenne myodystrophy in the Western region of Kazakhstan".

5. LXII International Scientific Conference of Young Scientists “Science: Yesterday, Today, Tomorrow” Aktobe, Kazakhstan, 27.04.2023 Report: “Pathogenetic therapy of Duchenne myodystrophy”.

6. X Annual International Scientific-Practical Conference«Medicine Pressing Questions» and «Satellite Forum on Public Health and Healthcare Politics»Baku, Azerbaijan, 27-28.04.2023. Report: “Clinical features of Duchenne myodystrophy in the Western region of Kazakhstan”.

Publications on the subject of the thesis: 8 scientific works have been published on the subject of the dissertation: 3 articles in the editions indexed in the information base Scopus; 2 articles in the journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Education and Science of the Republic of Kazakhstan; 3 abstracts in the collections of international conferences.

1. Umurzakova A, Ayaganov D, Mannapova A, Dzhumasheva B, Dzhaksybayeva A. Preliminary results of a genetic study of children with Duchenne myodystrophy in the Aktobe region. Achieves of Razi Institute 2023, Vol.78 No.3, p.949-954;

2. Ainur Umurzakova, Dinmukhamed Ayaganov, Roza Nurgalieva. Results of genetic study of children with Duchenne myodystrophy in Kazakhstan. Journal of Neurosciences in Rural Practice 2023, Vol. 14 Issue 2, p. 389-390;

3. Ainur Umurzakova, Dinmukhamed Ayaganov, Roza Nurgalieva, Kamalzhan Nadyrov, Sarkyt Kozhantayeva, Gulnara Sakhipova, Aniya Seypenova, Akzhunus Mannapova, Azat Chinaliyev, Ainur Donayeva. Revealing finding from genetic study of children affected by Duchenne myodystrophy in Kazakhstan. Bangladeshjournalofmedicalscience 2025 Vol. 24 No. 01, p. 279-284.

4. Umurzakova A., Ayaganov D. Clinical and genetic characteristics of Duchenne myodystrophy. Astana medicine journal 2020 issue 4 (106), p. 82-87;

5. A.O. Umurzakova, D.N. Ayaganov, M.B. Dzhumagalieva. The role of multidisciplinary team in the management of patients with progressive Duchenne muscular dystrophy. Journal of Pharmacy of Kazakhstan 2022 issue 3(242), pp.96-100.

6. Analysis of the spectrum of mutations in Duchenn’s muscular dystrophy. D.

Ayaganov, A. Umurzakova, A. Mannapova. Recent advances in rare disease. June 20-22.2019. Bogota. Colombia, p.59;

7. UmurzakovaA.O, AyaganovD.N., JumagaliyevaM.B. Duchenne Muscular Dystrophy. Discription of clinical case. Collection of abstracts “LXII International Scientific Conference of Young Scientists ‘Science: Yesterday, Today, Tomorrow’” 27.04.2023 Aktobe, Kazakhstan, pp. 47-48;

8. Clinical features of Duchenne myodystrophy in the Western region of Kazakhstan Umurzakova A.O., Ayaganov D.N., Nurgalieva R.E. Collection of abstracts “The IX Annual International Scientific - Practical Conference ‘Medicine Pressing Questions’”May 27-28, 2023 Baku, Azerbaijan, pp. 22-23;

Implementation of the research results

3 acts of implementation in practical healthcare and 1 act of implementation in the educational process were received:

1. “Medical and genetic counseling of patients with Duchenne myodystrophy and their families to assess the genetic risk of disease recurrence (genetic prognosis), to determine the ways of diagnosis and prevention, to explain the meaning of the collected and analyzed medical and genetic information” in the conditions of the Center for Family Planning and PHC of Aktobe city #134 from 06.04.2021.

2. “Application of the “6MWT test”, “Vignos scale”, “Time for Govers symptom” for functional assessment of children with Duchenne myodystrophy in PHC settings during initial examination” #135 dated 06.04.2021.

3. An act of implementation was received for educational and methodological work: "Modern strategies for diagnosis and treatment of neuromuscular diseases". No1 dated 29.10.2021

4. Assessment of upper and lower extremity function using the Brooke, P.J. Scales. Vignos in patients with advanced Duchenne muscular dystrophy to analyze the level of disease progression. No. 265 dated 03.11.2022. SCP “City Polyclinic No. 5” on PCV.

The dissertation research was carried out within the framework of the intra-university grant of the NJSC West Kazakhstan Marat Ospanov medical university "Genetic characteristics of Duchenne muscular dystrophy in the Republic of Kazakhstan" No0119RKI0240 dated 06.17.2021.

Personal contribution of the author. The author made trips to 9 regions of Kazakhstan to collect materials. The initial examination, the formation of a sample, the filling out of individual registration cards were carried out by the author personally. Knowledge in the field of molecular genetic analysis and scientific management has been improved as part of a foreign scientific internship. The author analyzes the results of genetic analyses and the interpretation of pathogenicity prediction from databases. The main provisions and conclusions are formulated.

Scope and structure of the thesis. The thesis is presented on 99 pages without annexes, consists of 3 chapters, conclusions and conclusions, practical

recommendations, a list of references, appendices, contains 22 tables, 20 figures. The list of references contains 155 titles in Russian and English.

2 MATERIALS AND METHODS OF RESEARCH

The present study was conducted with field visits to different regions of Kazakhstan: Aktobe region, West Kazakhstan region, Mangistau region, Atyrau region, Karaganda region, Akmola region, Kostanay region, Astana city and Petropavlovsk.

Examination of patients, blood sampling for molecular genetic testing was carried out in medical institutions of these regions. DNA samples obtained from patients with suspected Duchenne myodystrophy were used in the study. After genetic verification of the mutation in the child, blood was collected from the mothers to determine the inheritance variant.

Inclusion criteria:

- Male children;
- muscle weakness on the MRS scale≤2;
- any gait and posture disorders;
- presence of children with DMD in the family.

Exclusion criteria:

- Genetically verified other neuromuscular diseases;
- organic lesions of the CNS (central paralysis).

In accordance with the goals and objectives of the dissertation work, a research design was developed, determining the choice of patients and research methods.

The study is a combined study investigating the incidence of Duchenne myodystrophy, issues of disease inheritance (maternal examination for mutation carriage), clinical features depending on mutation characteristics and on the basis of standard basic glucocorticosteroid (GCS) and targeting (in case of nonsense mutation by Ataluren (Translarna)) therapy received.

The design of the first and second objectives was a single-institution, cross-sectional study; the third objective was a prospective cohort study. The third objective describes the functional status of children with DMD at the time of initial evaluation and after 12 months to assess disease progression.

Clinical characterization was based on the determination of age of disease debut, age of loss of ambulation, functional status, the results of which are described according to mutations and their location in the gene with functional impact on the reading frame of the genetic code, and the effect of GCS therapy and target therapy with Ataluren (Translarna) on clinical outcomes was evaluated.

Glucocorticosteroid therapy forms the basis of DMD therapy (according to the clinical protocol) and is the basic standard therapy. Prednisolone or Deflazocort is used. Standardly GCS therapy is started at the age of 4 years, it can be started at an earlier age if necessary, it is decided on an individual basis. The dose of Prednisolone should be 0.75 mg/kg, Deflazacort - 0.9 mg/kg for ambulatory children, for non-ambulatory children half dose and if necessary a lower dose. GCS preparation is

taken daily once orally, washed down with milk..

The drug Ataluren (Translarna) is used exclusively for nonsense mutation in outpatient children over 2 years of age, according to the instructions (Annex E). The drug TranslarnaTM is available in 125, 250, and 1000 mg sachets. The recommended dose is 10 mg/kg body weight in the morning, 10 mg/kg body weight at lunchtime, and 20 mg/kg body weight in the evening (so that the total daily dose is 40 mg/kg body weight). They are available in the form of granules for preparation of suspension for oral administration, pre-mixed in liquid or semi-solid food.

Continuous consecutive sampling was used. With an alpha error correction of 5% and a beta threshold of 20% (power of 80%), to achieve a predictive power of 0.95 and in accordance with our selection criteria and according to the statistical data of the RK, a representative sample should be 64 children. A total of 269 children with muscle weakness were examined, of whom 127 met the inclusion criteria, for whom genetic analysis was performed and the diagnosis of DMD was confirmed in 106 children. To determine the inheritance variant of the mutation in 75 children with large mutations, 45 mothers were genetically verified for carriage.

Verification of the diagnosis was carried out according to international recommendations and standards, as well as the clinical protocol of RCHD of MH RK for protocol №63 dated 19.04.2019.

The principles of the Declaration of Helsinki of the World Medical Association (World Medical Association of Helsinki, 1964, updated in October 2013 at the 64th General Assembly of the WMA, Fortaleza, Brazil) were followed.

Permission to conduct the study was obtained from the Local Bioethics Commission at NJSC «West Kazakhstan Marat Ospanov medical university» #24 dated 17.06.2019 Meeting #4.

The following functional scales were used to describe the clinical condition of patients: to assess muscle strength – the MRC scale (Medical Research Council Paralysis Scale, 1976); the P.J. Vignos 1963 and NSAA (North Star Ambulatory Assessment) scales were used to measure motor abilities. Temporary tests (6-minute walk test (6MWT) and "rise from a lying position" test were also used.

Genetic research. The biological material was DNA isolated from the blood. In a test tube with EDTA, it was transported with the preservation of all requirements to Astana, to the genetic laboratory of the Corporate Foundation "University Medical Center" - the National Scientific Center for Motherhood and Childhood. DNA was isolated by the standard Phenol-Chloroform method using ready-made kits from the manufacturer. MLPA analysis was performed after DNA isolation from the blood according to the manufacturer's instructions (MRC-Holland, Amsterdam, Netherlands). The material was processed on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystem, USA). NGS (Next Generation Sequencing) analysis was carried out in the Centogene laboratory (Germany). This method identifies point mutations, single nucleotide substitutions, which are not identified in MLPA. The principle of NGS is based on mass parallel sequencing of specially prepared single-strand

libraries of fragmented DNA of the studied samples. In our study, this method was carried out in the Centogene laboratory, Germany. Financial support and analysis of sequencing data was provided by PTC Therapeutics Kazakhstan.

In the work, information methods were used that include the use of the NCBI, OMIM, Genbank, UMD Treat, UCSC Genome Browser, Clin Var database, the pathogenicity prediction services were carried out according to Polyphen, Sift, Mutation Taser, Align-GVGD, GNOM Ad, Gnom Ad, Gnom Ad, Gnom Ad, Gnom Ad, Gnom Ad, 1000g, Cento MD.

Statistical data processing methods. Statistical data processing was carried out using the Statistica 10.0 program StatSoft, Inc. USA. Statistical analysis: checking the zero hypothesis about the absence of differences between the observed distribution of the attribute and the theoretical expected normal distribution was performed using the criterion of Kolmogorov-Smirnov. Assessment of the differences between the samples was carried out: with the normal distribution of paired variables using the T-criterion of the Student and ANOVA in the case of multiple independent; in the absence of normal distribution and in the case of paired independent aggregates using the Mann-Withney U-criteria and a ranking dispersion analysis of the Kruskal-Wallis.The average arifmatic values of the quantitative indicators presented in the text in the form of M ± SD were calculated, where M was the arithmetic mean, SD - a standard deviation, for qualitative indicators in the form of %, CI for a share. To determine the likelihood of non-ambulator status in children with Duchenne myodystrophy, depending on the factors, the binary logistic regression method was used and indestructive survival assessment using the Kaplan Meier curve. In all procedures of statistical analysis, the level of significance was accepted $p \leq 0, 05$.

Objective 1**: to study the frequency of occurrence and inheritance of Duchenne myodystrophy in the Republic of Kazakhstan.** A simultaneous transverse study was conducted. To study the frequency of occurrence of the DMD, exits to the regions of the republic, a fence of blood for genetic analysis was made; the determination of the type of inheritance was carried out by the genetic verification of the mother with the search for the already known mutation from her son (sick DMD).

Objective2: **to analyze the range of mutations in the *DMD* gene in Duchenne myodistrophy.** A simultaneous transverse study was conducted. On the basis of genetic results, 106 different mutations are identified; the types of mutations, their localization and analysis of the state of the broadcasting frame for reading the genetic code are identified.

Objective 3: **to determine the features of the clinical course of DMD, taking into account the type of mutation and therapy.** A prospective cohort study was conducted. The functional status of the children was determined at the time of initial examination and 12 months later to assess disease progression. Clinical characterization was based on the determination of age of disease debut, age of loss of ambulation, and motor skills through the use of functional scales, with results

described according to the characteristics of the mutations and taking into account the therapy received. Within this task, the effect of GCS therapy and Ataluren (Translarna) targeted therapy on clinical outcomes was evaluated.

THE RESULTS OF THEIR OWN RESEARCH

The frequency of occurrence and inheritance of DMD. 106 cases of Duchenne myodistrophy are diagnosed, which corresponds to the frequency as 1 case for 3311 boys. In 64.4% of cases, sick boys inherited a mutation from mothers, while in 35, 6% of cases there was a spontaneous mutation (de novo).

Characterization of mutations in the *DMD* gene.The deletion spectrum of mutations was more prevalent (59,3%; 95%CI: 51,9-68,9) than duplications and point mutations. Extended deletions accounted for 81,5% (95%CI: 77,4-86,1). Point mutations presented: nonsense mutation in 14 (13,2%) cases; microdeletions in 4 (3,8%) cases; intronic mutations in 5 (4,7%)cases; microduplications in 4 (3,9%) cases; insertion in 2 (0,9%) cases and missense mutation in 1 (0, 9%) case, combined mutation in the form of presence of nonsense and missense mutation in 1 (0, 9%) and deletion with insertion in 1 (0,9%) case. Whenanalyzing the structure of pointmutationtypes, sibling mutation analysis was excluded, namely 2cases of nonsensemutations,1case each of microdeletion and intronmutation.

Disruption of the translational reading frame was noted in 82,4% (95%CI: 72,3-92,1) of cases, with 14 cases out of 91 having a conserved translational frame and 2 analyses out of 106 failing to predict.

In the highly functionalactin-binding region of the gene, mutations were locatedin55(60,4%;95%CI;51,5–70,1)cases,in the centralregion of the gene-29(31.9%;95%CI;23.9–40.1)andin the region connecting DHA-7(7,7%;95%CI;1,3-13,4)($p<0.05$). Localization of mutations with a reading frameshift in 42 (76,4%95%CI:66,5-85,9) cases out of 55 were located in the actin–binding region; in 29 (100%) cases out of 29–in the central region; in 4 (57,2%) out of 7-in the region responsible for attachment in the DHA-binding site ($p<0, 05$).

Analysis of clinical characteristics of children with DMD. The mean age of disease debut was 3,7±0,09 years. The mean age of diagnosis was 5,4±0,09 years, and the mean age of onset of non-ambulatory stage was 9,5±0,51 years. In 71 (67%) children, the functional status was ambulatory, among which 7 (6, 6%) children were in the preclinical stage, and in 28 (26,4%) - non-ambulatory.

Significant differences were obtained between the indicators of the age of onset, clinical characteristics (according to the results of scales) and characteristics of mutations (state of the reading frame of the genetic code, assessment of mutations localization) ($p<0,05$), namely in the group of children with reading frame disorder the mean age of onset was 2,95±0,25 years; mean NSAA 15±5,6 points, 6MWT 292,4±11,3 meters in contrast to 4,77±0,5 years; and NSAA 25±8,7, 6MWT 391,6±16,2 meters in the group of children without reading frame disorder, respectively ($p<0.001$).

Assessment of clinical characteristics of children with DMD after 12 months

(catamnesis). During this period, 18 (25,3%) of 71 ambulatory children became nonambulatory, thus nonambulatory children totaled 46; ambulatory, 53; and in the preclinical stage, 7 children.

Of the 53 outpatients, 45 children were in the early ambulatory stage, among whom 33 had reading frame disruption mutations, whereas 12 had no reading frame disruption. Comparative analysis of 6MWT scores of 45 children in the early ambulatory stage according to reading frame status after 12 months showed: when reading frame was disrupted, 6MWT score changed from 314,2±3,17 to 285,8±3,12 meters, whereas when reading frame was preserved from 450,7±3,09 to 437,5±3,22 meters.

Multivariate logistic regression analysis was performed to assess the impact of mutation reading frame status, patient age, and disease debut on loss of ambulatory care. The highest risk of ambulatory loss of 12,36-fold (95% CI: 2,51 to 22,184) was observed for the factor assessing the reading frame state (frame disruption) of the mutation.

To examine the effect of translational reading frame status on the duration of preservation of independent walking, Kaplan Meier analysis was performed, which showed a longer duration of preservation of independent walking ability of 5,3±0,19 years (95% CI: 4,93-5,67) when translational reading frame was preserved than 3,9±0,26 years (95% CI: 3,38-4,42) when it was disrupted.

Clinical characteristics of Duchenne myodystrophy with regard to the type of therapy. The impact of glucocorticoid therapy on motor functions was assessed at the time of examination and after 12 months, taking into account the stage of the disease. Patient groups corresponding to early ambulatory and early non-ambulatory stages were formed.

At the time of evaluation (after 12 months), there were 45 children in the early ambulatory stage, including 13 children receiving specific targeted therapy with early ambulatory stage, who were not analyzed with respect to the evaluation of the efficacy of GCS therapy. Of the 32 children (mean age 6,14±0,84 years), 25 (78,1%) children were receiving hormone therapy; 7 children (21,9%) were not. The analysis showed improvement in 6MWT scores in the form of an increase in distance traveled from 317,4±12,57 meters to 348,1±9,65 meters in those taking GCS therapy and from 319,8±13,07 meters to 324,4±8,23 meters in those not taking it.

The mean age of the 21 boys at the early non-ambulatory stage was 8,98±0,94 years. Twelve (57,1%) children received hormone therapy; 9 children (42.9%) did not. The results of the time test “lifting from the supine position” showed an increase in the time (worsening) of the test performance in children receiving hormone therapy (from 12±2,45 seconds to 17±2,18 seconds) and a greater increase in time in children not receiving hormone therapy (from 13±3,54 seconds to 24±3,68 seconds).

The effect of Translarna therapy on clinical outcomes was assessed before therapy and after 12 months for 13 children in the early ambulatory stage using the 6MWT test, with mean scores of 327,4±2,57 (95% CI: 319,7 to 334,3) and

349,1±2,65 (95% CI: 345,35 to 354,65) meters, respectively.

CONCLUSION

In this paper we studied the clinical and genetic characteristics of Duchenne myodystrophy, which included the study of Duchenne myodystrophy frequency and inheritance, described the clinical features taking into account the mutations and the therapy received.

Thus, the following conclusions can be drawn **based on the results** of our study:

1. The incidence of Duchenne myodystrophy in Kazakhstan was 1 case per 3311 boys. The frequency of X-linked inheritance and spontaneous new mutations amounted to 64, 4% and 35,6%, respectively.

2. The deletion spectrum prevailed in 59,3%(95%CI:51,9-68,9),with extended deletions in 81,5%(95%CI:77,4-86,1).Mutations were more often detected in the actin-binding region of the gen e(60,4%;95%CI:51,5-70.1) and covered allt ypes. Violations of the translational reading frame were recordedin82,4%(95%CI:72,3-92,1) of cases, mainly in the actin-binding (76,4%;95%CI:66,5-85,9) region of the gene.

3. When the reading frame was impaired, NSAA and 6MWT scale scores were low and worsened over time, there was an early disease debut at 2,95±0,25 years and an increased risk of loss of ambulation (OR 12,36, 95% CI: 2,51-22,18), and the duration of independent walking was 3,9±0,26 years. Standard therapy influenced the lengthening of distance from 317,4±12,57 meters to 348,1±9,65 by 6MWT in ambulatory children, and a loss of 5 seconds in non-ambulatory children, versus 11 seconds (in non-users of standard therapy) on the “rise from supine position” test. Target therapy influenced the improvement in 6MWT test score from 327,4±2,57 to 349,1±2,65 meters at 12 months.

PRACTICAL RECOMMENDATIONS

The frequency of the disease identified in this work will be useful in the creation and maintenance of a national registry of DMD patients, which will provide an opportunity for high-precision epidemiologic monitoring and increase the availability of modern diagnostic procedures.

Within the framework of this work, point mutations, which accounted for one third of cases, were diagnosed by gene sequencing, which emphasizes the need to introduce this method in our country. Calculation of the translational reading frame of the genetic code can serve as a reliable marker in predicting the course of the disease.

The results of our work showed the importance of early initiation of both hormonal and pathogenetic therapy in improving the outcome.

Based on the knowledge of the type and characterization of mutations, prenatal diagnosis and accurate medical and genetic counseling for the prevention of new cases in aggravated families is possible.