DOI 10.26724/2079-8334-2020-3-73-130-134
UDC 616.858-008.6:575

K.A. Tarianyk, N.V. Lytvynenko, V.K. Oliinychenko[1], T.V. Byslik[1], K.F. Pochernyaev[1]
Ukrainian Medical Stomatological Academy, Poltava
[1]Institute of Pig Breeding and Agro-Industrial Production of NAAS, Poltava

ADAPTATION OF POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM METHOD FOR POLYMORPHISM (RS2583988) ANALYSIS IN ALPHA-SYNUCLEIN GENE

e-mail:: tkapolt@gmail.com

The article suggests the polymerase chain reaction-restriction fragment length polymorphism method for a single nucleotide polymorphism (rs2583988) detection for further prediction of Parkinson's disease risks. Based on the meta-analysis results for the single nucleotide polymorphism rs2583988 (C>T) in αlpha-synuclein gene; current polymorphic variant was chosen by analyzing bioinformatical and genetic studies. We have designed a pair of primers and conditions for restriction analysis to detect different alleles in rs2583988. As a result, there was developed a simple and affordable method for detecting single nucleotide polymorphism rs2583988 alleles. The method is expected to be suitable for clinic laboratories, due to affordable cost and equipment, and to be used in further association studies.

Key words: Parkinson's disease, αlpha-synuclein gene, single nucleotide polymorphism, polymerase chain reaction-restriction fragment length polymorphism.

The work is a fragment of the research project "Clinical, molecular genetics and neurophysiologic features of the course in various forms of Parkinson's disease", state registration No. 0119U102848.

Most of non-infection diseases develop as a result of complex gene interaction and the environmental influence. Parkinson's disease is a prototypical chronic condition and its risk increases with age [1, 11] that results to progressive disability [3], impairs driving ability [2]. A person, who was not born with the disease, may have a high risk of its further development. This is known to be the genetic predisposition or susceptibility. The genetic predisposition to a particular disease is associated with the presence of mutations in one or more genes and / or combinations of their alleles. Development of methods in molecular genetics has made it possible to detect a predisposition to one or another disease long before its clinical manifestations. Currently, it is believed that the key reason of Parkinson's disease pathogenesis is the presynaptic alpha-synuclein protein aggregation [8, 12]. Based on the meta-analysis results for the α-Synuclein Gene (*SNCA*) rs2583988 (C>T) polymorphic variant, significant associations with the development of Parkinson's disease have been established [4, 5], and the T-allele of the polymorphic variant of the *SNCA* gene rs2583988 is significantly spread in patients with cognitive impairments [7, 14]. The rs 2583988 is located in the promoter region of the *SNCA* gene [6, 9].

The purpose of the work was to apply known methods for the polymorphism analysis of *SNCA* gene (rs2583988), though application of PCR-RFLP method hasn't been suggested yet.

Materials and methods. Samples of buccal epithelium were collected from patients with PD, hospitalized in the Neurological Department, M.V. Sklifosovsky Poltava Regional Clinical Hospital. The agreements for using biological materials for further analysis were taken from each individual.

Genotyping was performed at the LLC "Gentris LTD" Poltava laboratory. DNA was extracted using ion exchange resin Chelex-100 [13]. Selected samples were placed in labeled polypropylene tubes with a lid with a capacity of 1.5 cm3. To the contents of the tubes was added 0.1 cm3 of 20% Chelex-100 suspension and incubated for 6 h at +56º C. After shaking the vortex tubes, they were placed in the thermostat and incubated for 8 mins at +96º C. Samples of the DNA solution were stored at -20º C.

The allele determination of SNCA gene (rs2583988) was performed using PCR-RFLP. DNA amplification was performed using recombinant Taq DNA polymerase (Thermo Fisher Scientific), according to the manufacturer's recommendations. We have designed the following primers, using the Blast primer tool [10]: forward – CCATGACCTCCTTGAGACCT and reverse – TGCCAAAGGACTAACAAATTACC (Metabion international AG, Germany). Amplification was performed on programmable thermostat "Tercik-2" (DNA Technologies, RF) under the conditions of annealing oligonucleotide primers at 60 °C. The components of the PCR mixture used for DNA amplification in polymerase chain reaction were stored in a freezer at -20 ºC. Before use, they were thawed at room temperature. The required quantity of clean polypropylene microtubes with a lid with a capacity of 0.5 cm3 was marked with serial numbers.

The Rsa I restriction of PCR products was carried out in accordance to the manufacturing instructions (Thermo Fisher Scientific). The hydrolyzed PCR products were separated by 8% polyacrylamide gel (SDS) electrophoresis in 1 × TBE buffer. To the tubes with samples of hydrolyzed PCR

products was added 6 × sample buffer and mixed thoroughly. 0.005 cm3 of molecular weight marker was added to the first well, and 0.01 cm3 of hydrolyzed PCR products were added to the following wells. DNA pUC19 / MspI (HpaII) (Thermo Fisher Scientific, USA) was used as a molecular weight marker. Electrophoresis was performed for 3 h at a current of 50 mA in a vertical electrophoresis chamber 20 × 20 cm (Cleaver Sci.Ltd omniPAGE Maxi, UK). After electrophoresis, the gel was stained with ethidium bromide solution (10 mg / cm3) for 3–6 min, the gel was washed three times with distilled water and the electrophoresis results were documented using a gel imaging system (MicroDOC with UV transilluminator UVTS312, UK).

Results of the study and their discussion. First of all, it should be noted that the Parkinson's disease (PD) is one of most common neurodegenerative diseases. One of the key reasons of Parkinson disease pathogenesis is the presynaptic alpha-synuclein protein aggregation. Based on this, the polymorphism of the gene encoding the presynaptic alpha-nucleic protein – SNCA gene, could affect the development of PD.

The SNCA gene was discovered to provide coding information to create a small size protein, called alpha synuclein. Alpha-synuclein was found to be a member of the synuclein family, which also included beta- and gamma-synuclein. It was detected in high amounts in the brain, and lower amounts in the heart, other muscles. In the brain, alpha-synuclein was found primarily at the ends of neurons in specialized structures called presynaptic endings. SNCA peptides happened to be major components of amyloid plaques in the brains of patients with Alzheimer's disease. Alternatively, spliced transcripts encoding different isoforms had been identified for this gene.

SNPs could act as biological markers, helping scientists find genes associated with disease. When SNPs occurred within a gene or in a regulatory gene, they might play a more direct role in disease by affecting gene function. Most SNPs didn’t seem to affect health, however, some of these genetic differences had proven to be very important for the study of human health. Researchers had found SNPs that could help predict a person's response to certain medications, susceptibility to environmental factors such as toxins, and the risk of developing certain diseases. SNPs could also be used to track the inheritance of disease genes in families.

Current SNP rs2583988 was detected in the promoter region of the *SNCA* gene significant associations with the development of Parkinson's disease have been established. The rs2583988-*T* allele had significant associations with the development of Parkinson's disease. The rs2583988-*T* allele is more common in patients with cognitive impairments.

There had been relieved methods for the polymorphism analysis of *SNCA* gene (rs2583988):

Polymerase chain reaction in real time (Real-time PCR). Real-time qPCR has widely been used in many diagnostic areas, including the analysis of foodborne pathogens. There are certain benefits of real-time PCR, such as ability to monitor the progress of PCR, ability to accurately measure the amount and amplification and detection occurs in one tube excluding post-PCR manipulation;

DNA sequencing. In chain-termination PCR, the user mixes a low ratio of chain-terminating dNTPs in with the normal dNTPs in the PCR reaction. dNTPs lack the 3'-OH group required for phosphodiester bond formation; therefore, when DNA polymerase incorporates dNTP at random, extension ceases. The result of chain-termination PCR was found to be millions to billions of oligonucleotide copies of the DNA sequence of interest, terminated at a random lengths (n) by 5’-dNTPs. In manual Sanger sequencing, four PCR reactions are set up, each with only a single type of dNTP mixed in;

Sequenom MassArray iPLEX platform. The method for SNP genotyping described in this unit is based on the commercially available Sequenom MassARRAY platform. The assay consists of an initial locus-specific PCR reaction, followed by single base extension using mass-modified dideoxynucleotide terminators of an oligonucleotide primer which anneals immediately upstream of the polymorphic site of interest. Using MALDI-TOF mass spectrometry, the distinct mass of the extended primer identifies the SNP allele.

All the mention methods turned out to have several limitations, such as high budget of equipment and reagents, due to which they are hard to be implemented in Ukrainian laboratories for the temporary research. Polymerase chain reaction with further restriction enzyme analysis was chosen for the current SNP studies due to affordable cost and the equipment. Using PCR, specific sequences in a DNA was copied or "amplified" from many thousands to a million times using sequence-specific oligonucleotides, heat-resistant DNA polymerase and thermal cycling. Traditional (end point) PCR, detection and quantification of amplified the sequence is executed at the end of the reaction after the last PCR cycle, and include post-PCR analysis, such like gel electrophoresis and image analysis.

PCR theoretically was discovered to amplify DNA exponentially by doubling the number of target molecules for each amplification cycle. When it was first developed, scientists believed that the number of cycles and the amount of the final PCR product could be used to calculate the initial amount of genetic material compared to a known standard. To address the need for reliable quantification, a quantitative real-time PCR and endpoint PCR technique has been developed used mainly to amplify specific DNA for sequencing, cloning and use in other methods of molecular biology.

Among known methods the polymorphism analysis of *SNCA* gene (rs2583988) was not performed by PCR-RFLP yet. The *SNCA* gene sequence data (GenBank accession number AY079082) for designing the primers for SNP (rs2583988) was taken. Current SNP was analyzed for restriction site of any suitable enzyme. For detecting rs2583988 allele variants *Rsa* I enzyme was used; also, it is appropriate to use *Rsa* I isoshizomers (*Afa* I, *Csp* 6I, *Cvi* QI).

Primer-BLAST tool was used to design specific primers [6]. The program was developed at NCBI to help users make primers that are specific to the input PCR template. It used the program to design PCR primers and then submits them to BLAST search against user-selected database. The blast results were then automatically analyzed to avoid primer pairs that can cause amplification of targets other than the input template. From different primer variants the one with small amplification product, (173 bp) was chosen.

DNA oligonucleotides appeared to be essential components of PCR reaction. The key event of each oligonucleotide-based assay was the specific binding between oligonucleotides and their target DNA. However, single-stranded DNA molecules also tended to bind to unintended targets or themselves. The probability of such unspecific binding could increase with the complexity of an assay.

The received primers revealed no unexpected fragments, so the primers were picked up the right way. We expected that the digesting of the amplified DNA product with restriction enzyme *Rsa* I would allow to evaluate the cut of 173 bp DNA into 110 bp and 63 bp fragments.

Since a restriction enzyme might lose activity due to improper storage or handling, it is important to check the expiration date, verify that the enzyme had been stored at -20°C. The enzyme for activity by setting up a control reaction with 1 µg of standard control DNA, where you know that the DNA quality was high and the expected banding pattern. Enzyme had to be kept in frost-free freezers that undergo temperature fluctuations. It is also recommended to keep the enzymes in a cold rack in the freezer, as this helps to stabilize the storage temperature

The chosen conditions for PCR-RFLP method of SNP (rs2583988) analysis allowed determining the different alleles of the gene *SNCA* (fig. 1).

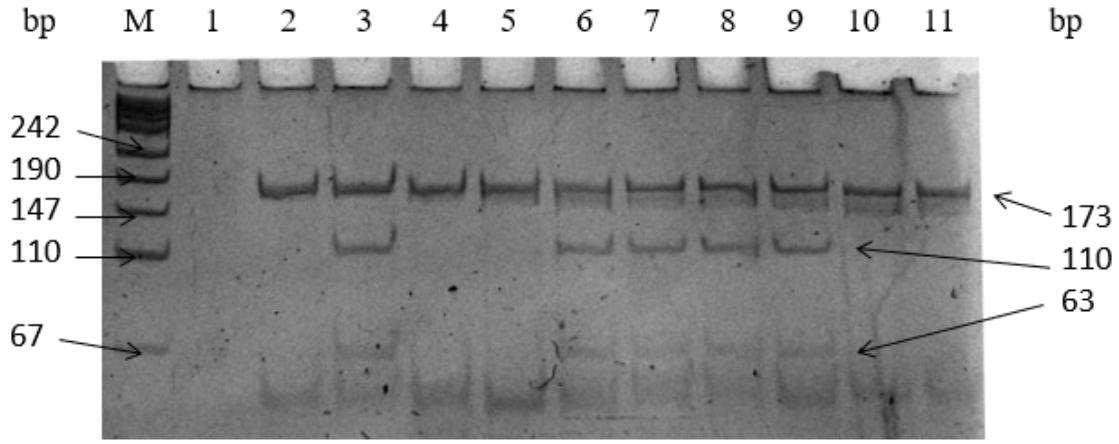

Fig. 1. M – *pUC*19/*Msp* I molecular weight marker, 1 – negative control (PCR without DNA), 2-11 – human DNA.

The resulting PCR amplification product sizes and enzymed DNA fragments completely coincided with the expected. It was shown that the detection of reaction products could be performed using electrophoresis in both 6% PAAG and 1.5% agarose gel (stained with ethidium bromide) using Tris-borate buffer (1 × TBE). The suggested conditions for PCR-PRLRF could be implemented to PCR laboratories.

It was essential to do SNP (rs2583988) the meta-analysis of previous studies [1, 2, 7, 14] to see the importance of its detection by comparing to the results in another populations. We found current SNP to be highly polymorphic in Brazilian, Canadian and American populations.

The first step for sample analysis should include DNA isolation. The isolation method including ion exchange resin Chelex-100, 20% was found to be the most convenient, comparing to DNA isolation methods including lysis solution and wash buffers [13]. For the convenient SNP analysis negative and positive controls were used during the experiment.

In order to achieve successful DNA amplification, we followed its importance to start off with the right primers. We took into account GC content to be 50%. The G and C bases have stronger hydrogen bonding and help with the stability of the primer. We avoided runs of 4 or more of one base, or dinucleotide repeats. Apparently, the designed primers in the Blast primer design program [10] met the expectations of the successful detection.

The developed method for detecting SNP (rs2583988) includes the primers and the PCR conditions. Not following the protocol could lead to wrong concentration of reagents and the reaction failure. We managed to complete the optimal reaction conditions, which were revealed in the methods.

Conclusion

After performing informational studies *SNCA* gene has been chosen as a candidate gene for further correlation studies with PD. Its SNPs are known to be influencing on PD development among the American population. From various SNPs of *SNCA* gene it has been selected SNP rs2583988 (C>T), which is located in the promoter region of the current gene. After the development of the primers and optimizing the conditions of PCR-RFLP method, the detection of SNP rs2583988 (C>T) turned out to be available. As a result of bio informational and experimental studies, we were able to implement a PCR-RFLP method for analyzing the *SNCA* gene polymorphism (rs2583988). Due to the affordable cost of reagents and equipment, the method can be applied in clinical and research studies in laboratories. Further associative studies with SNP genotypes and PD development should be conducted in the Ukrainian population to approve the correlation statistically. We assume that the method will allow predicting risks of PD and taking measures in a timely manner, preventing its development or facilitate its course. Also, it will allow taking into account the individual characteristics for providing the therapy. Further other SNPs in different regions of *SNCA* gene might be considered as relevant for the researches.

References

1. Campelo CLC, Cagni FC, de Siqueira Figueredo D, Oliveira LG. Variants in *SNCA* gene are associated with Parkinson's disease risk and cognitive symptoms in a Brazilian sample. Front aging neurosci. 2017; 9: 198-204.
2. Caslake R, Taylor K, Scott N, et al. Age-, gender-, and socioeconomic status-specific incidence of Parkinson's disease and parkinsonism in northeast Scotland: the PINE study. Parkinsonism Relat Disord. 2013; 19(5): 515-521.
3. Elbaz A, Ross OA, Ioannidis JP, Soto-Ortolaza AI, Moisan F, Aasly J, Annesi G, Bozi M, Brighina L, Chartier-Harlin MC. Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium. Independent and joint effects of the MAPT and SNCA genes in Parkinson disease. Ann Neurol. 2011; 69(5): 778-92.
4. Emelyanov A, Kulabukhova D, Garaeva L. SNCA variants and alpha-synuclein level in CD45+ blood cells in Parkinson's disease. J Neurol Sci. 2018; 395: 135-140.
5. Han W, Liu Y, Mi Y, Zhao J, Liu D, Tian Q. Alpha-synuclein (SNCA) polymorphisms and susceptibility to Parkinson's disease: a meta-analysis. Med Genet. 2015; 168 (2): 123-34.
6. Homo sapiens synuclein alpha (SNCA), transcript variant 4, mRNA - Nucleotide – NCBI https://www.ncbi.nlm.nih.gov/nuccore/NM_007308.
7. Iakovenko EV, Abramycheva NY, Fedotova EY, Illarioshkin SN. The SNCA-Rep1 Polymorphic Locus: Association with the Risk of Parkinson's Disease and SNCA Gene Methylation. Acta Naturae. 2020; 12(2): 105-110.
8. Linnertz C, Lutz MW, Ervin JF, Allen J, Miller NR, Welsh-Bohmer KA, Roses AD, Chiba-Falek O. The genetic contributions of SNCA and LRRK2 genes to Lewy Body pathology in Alzheimer's disease. Hum Mol Genet. 2014; 23(18): 4814-21.
9. McCarthy JJ, Linnertz C, Saucier L, et al. The effect of SNCA 3' region on the levels of SNCA-112 splicing variant. Neurogenetics. 2011; 12(1): 59-64.
10. Primer designing tool https://www.ncbi.nlm.nih.gov/tools/primer-blast/
11. Szigeti E. Nursing care of patients with Parkinson's disease. Neurosci Biobehav Rev. 1988 Fall-Winter; 12(3-4): 307-309.
12. Toffoli M, Dreussi E, Cecchin E, et al. SNCA 3'UTR genetic variants in patients with Parkinson's disease and REM sleep behavior disorder. Neurol Sci. 2017; 38(7): 1233-1240.
13. Walsh PS, Metzger DA, Higushi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques. 2013; 10(4): 506-13.
14. Zhang Y, Shu L, Sun Q, Pan H, Guo J, Tang B. A Comprehensive Analysis of the Association Between SNCA Polymorphisms and the Risk of Parkinson's Disease. Front Mol Neurosci. 2018; 11:391-95.

Реферати

РОЗРОБКА МЕТОДУ ПОЛІМЕРАЗНОЇ ЛАНЦЮГОВОЇ РЕАКЦІЇ ДОВЖИН РЕСТРИКЦІЙНИХ ФРАГМЕНТІВ ДЛЯ АНАЛІЗУ ПОЛІМОРФІЗМУ (RS2583988) ГЕНА АЛЬФА-СІНУКЛЕЇНА

Таряник К.А., Литвиненко Н.В., Олійниченко Є.К., Буслик Т.В., Почерняєв К.Ф.

У статті запропоновано метод полімеразної ланцюгової реакції довжин рестрикційних фрагментів для виявлення однонуклеотидного поліморфізму (rs2583988) для подальшого прогнозування ризику хвороби Паркінсона. На підставі результатів мета аналізу

РАЗРАБОТКА МЕТОДА ПОЛИМЕРАЗНОЙ ЦЕПНОЙ РЕАКЦИИ ДЛИН РЕСТРИКЦИОННЫХ ФРАГМЕНТОВ ДЛЯ АНАЛИЗА ПОЛИМОРФИЗМА (RS2583988) ГЕНА АЛЬФА-СИНУКЛЕИНА

Таряник Е.А., Литвиненко Н.В., Олейниченко Е.К., Буслик Т.В., Почерняев К.Ф.

В статье предложен метод полимеразной цепной реакции длин рестрикционных фрагментов для выявления однонуклеотидных полиморфизмов (rs2583988) для дальнейшего прогнозирования риска болезни Паркинсона. На основании результатов цель анализа для

для однонуклеотидного поліморфізму rs2583988 (C> T) в гені альфа-синуклеїна. Поліморфний нуклеотид був обраний шляхом аналізу біоінформатичних та генетичних досліджень. Ми розробили пару праймерів та умови рестрикційного аналізу для виявлення різних алелів у rs2583988. Як результат, був розроблений простий і доступний метод виявлення однонуклеотидного поліморфізму алелів rs2583988. Очікується, що метод виявиться зручним для клінічних лабораторій, завдяки доступній вартості та обладнанню, а також використовуватиметься в подальших асоціативних дослідженнях.

Ключові слова: хвороба Паркінсона, ген альфа-синуклеїна, однонуклеотидний поліморфізм, полімеразна ланцюгова реакція довжин рестрикційних фрагментів.

Стаття надійшла 18.09.2019 р.

однонуклеотидных полиморфизма rs2583988 (C> T) в гене альфа-синуклеина. Текущий полиморфный вариант был выбран путем анализа биоинформатичних и генетических исследований. Мы разработали пару праймеров и условия рестрикционного анализа для выявления различных аллелей в rs2583988. Как результат, был разработан простой и доступный метод выявления однонуклеотидных полиморфизма аллелей rs2583988. Ожидается, что метод окажется удобным для клинических лабораторий, благодаря доступной стоимости и оборудованию, а также использоваться в дальнейших ассоциативных исследованиях.

Ключевые слова: болезнь Паркинсона, ген альфа-синуклеина, однонуклеотидный полиморфизм, полимеразная цепная реакция длин рестрикционных фрагментов.

Рецензент Дельва М.Ю.

DOI 10.26724/2079-8334-2020-3-73-134-139
UDC 616.728.2-089.28:612.76

O.A. Tyazhelov, O.D. Karpinska, L.D. Goncharova[1], Klymovytskyi F.V.[1]
SI "Sytenko Institute of Spine and Joint Pathology, NAMS of Ukraine", Kharkiv
[1]Research Institute of Traumatology and Orthopedics of DonNMU, Lyman

FEATURES OF THE ABDUCTION MECHANISM WORK BEFORE AND AFTER TOTAL HIP JOINT ARTHROPLASTY

e-mail: alzhar3001@gmail.com

Increased tone of the flexors and adductor muscles of the hip in combination with a decrease in elasticity and contractile function of abductors and protractors of the hip due to contracture of the hip joint leads to imbalance in the muscles of the pelvic girdle, which negatively affects the positional adaptation of endoprosthesis components during the surgery and complicates the postoperative period in patients. The treatment results analysis of 114 patients with diseases of the hip joint showed that in most patients the manifestations of pelvic girdle muscle deficiency after hip arthroplasty decreased, but did not disappear for a long time. The study of vertical posture in patients with normal and reduced size of the total femoral offset after hip arthroplasty showed that the reduction of the abductor shoulder leads to a significant change in the main indices of the stabilogram, namely to increased energy expenditure to maintain vertical posture, therefore energy depletion occurs faster. It is clinically manifested by symptoms of pelvic girdle muscle insufficiency - lameness, positive symptom of Trendelenburg, Duchenne, the need to use additional support, etc.

Key words: endoprosthetics, hip joint, vertical posture, statography, femoral offset.

The work is a fragment of the research project "To study the biomechanical features of the standing and walking function in humans after hip arthroplasty", state registration No. 0118U006950.

Owing to the classic works by Yanson Kh.A. [2], Kizilova N. [7] it is generally accepted that maintaining the horizontal balance of the pelvis in the frontal plane is achieved by the work of the pelvic girdle muscles, namely the ability to dynamically balance the gravitational, reactive and muscular forces acting on the joint components in the frontal plane in single-support standby.

The main condition for stable single-support standby is the balance (relative to the center of the hip joint rotation) of the abductor muscles force moments and gravitational and reactive forces, which is determined by the so-called abduction mechanism - the work of pelvic girdle muscles, which provide constant dynamic restoration of the pelvis horizontal balance [11]. To counteract the gravity force, muscles must develop significant efforts. This is especially true of dynamic loads, because in these conditions great effort is required to maintain a horizontal balance of the pelvis. Given that the arm of the abductor muscles is by 2.2-2.5 times shorter than the arm of gravity, to ensure the operation of the abduction mechanism, the muscular effort must exceed the body weight by 1.5 to 3 times [2, 5].

This makes it clear what an important factor in the normal functioning of the hip joint is the functional state of the abductor muscles, i.e. the work of the abduction mechanism of the hip joint to ensure the horizontal balance of the pelvis [4].

The work efficiency of the hip joint is determined by two main factors: the anatomical parameters of the joint elements and the condition of the pelvic girdle muscles, which are closely related. Thus, the change in the anatomical parameters of the hip joint: deformation of the femoral head, its protrusion, elongation of the trochanter major, impairment of the proximal femur torsion, which are caused by various orthopedic diseases and injuries, lead to a change in the force arm of abductor muscles, which adversely effects the abduction mechanism's operation and significantly slows down the process of the patient's