USING THE MOLECULAR GENETICS METHODS FOR STUDYING THE PECULIARITIES OF MUSCLE ACTIVITY AND INHERITED PREDISPOSITION IN THE SPORT

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Annotation. Analyzes of the methods used in sports genetics, and molecular genetics of muscle activity in particular are considered. The historical retrospective review of sports genetics methods was created. Contemporary level sports genetics methods were determined by the scientific literature analysis. The most common molecular genetics techniques that are used for practice and for research into sports were highlighted. It is shown that the main method, which has the greatest practical application in sport, is the method of polymerase chain reaction in various versions. These methods allow genes diagnostic and determine the genetic predisposition to perform of different kinds of physical exercises. The advantages of using these methods in sport, their features and shortcomings, prospects for further research were established.

Key words: sport, genetics, methods, chain, reaction.

Introduction

Molecular level of researches of modern genetics conditioned appearance of new branch of knowledge – genomics, which study genes' structure and functions, realization of ancestral information and is the basis of proteomics and mitabolomics. The possibilities of these sciences are unlimited: they permit to solve great number of practical tasks, including health and environment protection, sports. Their actual fundamental character lies in possibility of practical realization.

Impetuous development of molecular genetics in the second half of 20th century, connected with discovery of deoxyribonucleic acid (DNA) structure, decryption of genetic code, discovery of genes' transcription and translation mechanisms, opened broad prospects for solution both fundamental and applied tasks, facilitated using of its methods in applied disciplines , in particular, in sports genetics. As on to day, two strategies of genetic analysis have been formed in genetics: "classical" – research of genetic stipulation of sign (way from sign to gene) and "reverse" – research of phenotypic effects of separate genes (way from gene to sign). [5]. If at first stages of development sports genetics used "classical" approach, at the present stage of science development "reverse" approach is used oftener in sports genetics.

Sports genetics arose in 50th- 60th years of the past century and was originated from the works by Grebe H., 1956, Gedda L., 1960, Mosser H., 1960 on the base of analysis of highly qualified sportsmen's genealogy. [18, 19]. The main methods, which were used, were the following: ontogenetic (longitudinal), genealogical, family and twins methods. [1, 9]. In the first attempts of genetics methods' usage for searching of distinctions between persons, who have bents to sports and those, who have no bents, steady phenotypic markers, closely connected with genotype, were used. Among them there were: morphological signs, which included body proportions, shape of skeleton muscles, their topological content, level of adipopexis, serological signs (blood group by different systems), dermatoglyphic markers, composition of skeleton muscles, level of hormones in blood and etc. [1, 7-9].

Official recognition of sports genetics occurred in 1980 at Olympic Games scientific congress "Sports in modern society", which was held in Tbilisi, by mean of creation of sports genetics and somatology scientific association. For the first time term "genetics of physical activity" was offered by Claude Busharre (Canada, USA) in 1983.

Large-scale long-term international project "Human genome", which started in 1986 under the guidance of James Watson and later - Fransis Collins, significantly influenced on the development both the whole medical/biological science and sports genetics in particular. The object of researches were studied on the level of genome – ancestral mechanism of a cell, which contains all information, required for development of organism, its existence in environment, evolution and transmitting of ancestral information to generations. [3].

The work has been fulfilled as per subject 2.22 "Development of complex system of sportsmen's individual-typological features' determination on the base of genome's manifestation" and subject 2.25 "Monitoring of qualified sportsmen's adapting, considering their individual peculiarities" of combined plan of scientific & research work in the field of physical culture and sports for 2011-2015.

Purpose, tasks of the work, material and methods

The purpose of the work is to review modern methods of molecular genetics, which can be used in sports activity; to characterize molecular-genetic methods, which are used in sports genetics in the whole and molecular genetics of muscular activity in particular.

The methods of the research: analysis, comparisons, generalization of scientific and scientific-practical literature.

Results of the researches

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As per the modern conceptions of molecular genetics it is considered that individual peculiarities of development level of any human physical or psychic features are conditioned by DNA polymorphisms, which are more than 21 million (as per polymorphisms data base of EMBL). DNA polymorphisms are genetic variants of nucleotides' succession of one and the same DNA part of different people, which are found in one population with frequency not less than 1%. Some polymorphisms are able to influence on the level of genes' expression, activity of functional products (proteins, RNA – ribonucleic acid) and structure of proteins.

Now, more than 214 genes are known, the polymorphisms of which are associated with the development and manifestation of human physical abilities, as well as with morpho-functional signs and bio-chemical indicators, which are changed under the influence of different physical loads. [16]. The method of polymerase chain reaction (PCR) belongs to molecular genetic methods, which are widely used in sports genetics and permit to determine one-nucleotide polymorphisms (ONP). It permits to quickly obtain the studied parts of DNA in pure form and unlimited quantity. This method was offered by Kary Mullis , the officer of "Cetus" company, in 1983. This method implicates repeated copying (amplification) of certain DNA parts in test tube, in the process of repeated temperature cycles. [6]. At every amplification cycle the synthesized earlier fragments again are being copied by ferment, DNA polymerase. Owing to it, repeated amplification of the quantity of determined DNA fragments occurs. PCR is fulfilled in special device – amplificatory, which, with high accuracy, ensures periodical cooling and heating of reacting mixtures. DNA polymerase, which is used for PCR, is a ferment of thermophile micro-organisms, most frequently Thermus aquaticus (*Taq)*, and preserves biological activity after heating up to 100°C. Except *Taq*- polymerase, reacting mixture includes: DNA matrix, primers, dNTP (desoxyribonucleotide tri-phosphates, ions of magnesium.

For fulfillment of PCR, as a rule, two primers are used, which complementary interact with opposite DNA chains and restrict the sector of matrix molecule, which must be amplified. For introduction of primers, it is necessary to, previously, disconnect two chains of matrix DNA molecule (denaturation), for this purpose reacting mixture is to be heated up to 93-96°C. After that, the mixture is cooled up to temperature, at which primers can interact (hybridization) with one-chain matrix DNA (40-75°C). DNA polymerase synthesizes complementary DNA chain (elongation) by elongating of primers (at temperature of 60-75°C). With repeating of reaction cycle, earlier created DNA molecules will be the matrixes for synthesis of new molecules. In order to receive sufficient, for detection, quantity of DNA, from 20 to 50 PCR cycles are required, depending on initial matrix concentration and efficiency of reaction.

PCR result can be registered either after completing of amplification ("by final point") or in the process of the reaction ("in real time"). For PCR evaluation "by final point", a number of methods is used, among which the most frequent is electrophoresis of DNA molecules in gel, colored by bromide ethidium, hybridization-ferment analysis (HFA), fluorescent detection FLASH.

For receiving of DNA for conducting PCR different methods are used. All they implicate extraction of DNA from bio-preparation and elimination or neutralization of foreign impurities for obtaining of nucleic acids (NA) sample of purity, which is required for amplification. All modern methods of NA purification can be divided into two big groups: methods of stage-by-stage extraction of impurities from NA water solution and methods, which are based on NA sorption in solid phase. The most known and commonly used methods of the first type include phenol-chloroform extraction, enzyme proteolysis with following de-proteining and deposition by spirit, application of ion-exchangers of Chelex™ type (Bio-Rad, USA). The second group is based on application of silicate sorbates, which effectively tie NA in solution with high ion force. The most widespread is the method, based on guanidine thiocyanate (GuSCN) cells' lysis [14] and the following sorption of DNA on solid carrier (based on silicon oxide). After washing of sorbate pure NA remains on it and it is easily washed away by distilled water. One of widespread methods in sports genetics is the method of DNA sample obtaining by scraping of epithelial cells from oral cavity with the help of reagents kit Diatom™ DNA Prep (Biokom).

Sampling of DNA is carried out with the help of universal probes. Before scraping oral cavity shall be washed previously by 0,9% solution of NaCl. The applied method is based on using of lysing reagent with guanidine cyanate, which is stipulated for cells' lysis, solubilization of cells debrisas well as for denaturation of cells' nucleases. In the presence of lysing reagent DNA is actively sorbed on *NucleoS*™- sorbate, then, it is easily purified from proteins and salts by spirit solution. Then, DNA is extracted from sorbate and carried in sterile, free from DNA and RNA micro test tubes. The received DNA can be directly used for conducting of polymerase chain reaction. The reagent kit permits to extract highly molecular DNA from fresh biological material.

For quantitative evaluation of DNA amplification "real time" polymerase chain reaction is used, because it gives information about kinetics of reaction. PCR "in real time" permits to estimate DNA accumulation directly in the process of PCR with the help of fluorescent registration. Reaction is to be conducted in special device, which is an amplificatory, connected with fluorescent meter. In this method either fluorescent dyes are used, which interact with two-chain DNA molecules, or modified primers, which become fluorescent after hybridization with complementary DNA parts. All methods of PCR products' detection are divided into specific and not specific for certain succession of DNA. Not specific systems detect any DNA, which is created in the course of reaction. Not specific methods include systems with interacting dyes and systems with marked fluorescent dyes – primers. As interacting dye, cyanic dye SYBR Green I is used most often. Increase of two chain DNA quantity will result in intensification of fluorescence.

There are several variants of registration of accumulated product with the help of fluorescently marked primer. In the simplest variant, primer contains additional succession at 5'- end, which can create pin (of "stem-loop" type, with it stem includes 5-6 nucleotides, most of which are G-C). The loop can cover a part of target or can be completely

independent. Primers carry fluorescent marker (phosphor) and damper of fluorescence, which is located so, that with creating of pin, phosphor and damper are close to each other. In solution primers preserve the structure of pin, which has low level of fluorescence. In the process of reaction the pin of marked primer opens (the marked primer becomes a part of two chain product) and it results in increasing of signal. The other variant of this method offers [24] usage of standard marked hybridized samples, which are complimentary to special 5' – ends adapter succession.

Specific systems permit to authentically register accumulation of DNA fragment. All specific systems of PCR products' detection differ by the presence of marked oligo-nucleotide or some of them in reacting mixture, which are not able to be a primer and are complimentary to unique amplified succession. Marked oligo-nucleotide can be joined to primer or can be in solution in free form. There are different variants of specific detection, among which, the so called, primer tests ("scorpions") , where primer and hybridization samples are joined into one molecule, "displacing tests", linear disintegrated tests (Tag Man), tests with inverted end repeating (IER), adjoining tests.

In linear disintegrated tests oligo-nucleotide, which is complementary to PCR product, is marked by phosphor and fluorescence damper (it is possible to mark both the end and inner oligo-nucleotides). When target is absent, phosphor and damper are close to each other and fluorescence is weak. With accumulation of appropriate reaction product the sample is hybridized on apmlicon, that causes its disintegration, owing to 5' – exo-nuclease activity of *Tag*-polymerase. Intensity of signal increases with every PCR cycle in proportion to accumulation of amplificons.

As on to day influence of physical loads on genes activity level is an undoubted fact, which has been confirmed by a great number of experimental data. It has been established that physical loads can change expression of more that five hundred genes in muscles of hip, in diaphragm, myocardium, liver, hematopoietic system, human brain. [22, 23]. Affecting on genes expression, physical work results in change of RNA molecules in cells. At present, for quantitative evaluation of transcription level two main approaches are widely used: hybridizing (Northern-blot hybridizing, hybridizing in-situ, "RNA protection", micro-chips or hybridizing matrixes) and amplification approach (RT-PCR) and etc. PCR method "in real time" in combination with the method of reverse transcription (RT) has a lot of advantages over other methods. This technology can work in wide range of transcript concentrations and does not require additional manipulations after amplification. The first stage of RNA study is reverse transcription reaction (reaction of DNA synthesis on RNA matrix with using of special ferment – reverse transcriptase). Reverse transcription reaction can be conducted either in separate test tube (two stage PCR) or in the same test tube that the following PCR (one stage PCR). One stage PCR "in real time" reduces probability of experimenter's mistakes and minimizes occasional scattering of reaction parameters, but it has less sensitivity and efficiency of reaction. The disadvantages of two-stage approach are increased probability of samples' cross contamination and high complexity.

Modern methods of molecular genetics permit to simultaneously genotype dozens of polymorphisms, to determine genes expression on transcription level and sequent genome. For this purpose the method of hybridizing on micro-chips is applied. Micro-chip is a solid carrier (glass, plastic or silicon) with collection of probes fixed to it. Oligo-nucleotides, kDNA or small fragments of genes, which corresponds to coding sectors, are used as probes. There are different variants of micro-chip hybridizing, among them there is comparative genome hybridizing on chips, which permit to simultaneously carry out genetic analysis on transcription level of the whole genome. Some firms «"Illumina", "Affymetrix" propose micro-chips, which can contain hundred thousands of cells with specific oligo nucleotides for detection of separate polymorphisms on a surface of approximately 1,5 cm^2. Comparative hybridizing on chips, which is the most widely used method of transcription analysis, permits to simultaneously analyze expression of tens of thousands of genes. But this method is rather expensive, though it opens prospects for development of sports genetics. For example, system OpenArray™ (Applied Biosystems) with the help of Taqman® OpenArray™ Plate permits to simultaneously determine from 64 polymorphisms for 48 persons to 256 polymorphisms for 12 persons, depending on the type of plate.

For determination of different genetic factors' role in skeleton muscles' regulation researchers use different methods of genetic engineering and functional genomics – the science, which study features and peculiarities of human genome, while human genetics – is the science about heredity (transmitting of features to generations**).** [15.]. The most frequently used are: gene knockout, chimeric gene construction, plasmid constructions, siRNA- induced silencing. [12, 13, 17, 20, 21]. These methods belong to "reverse" approaches; they are used, most often, in experiments on animals.

Gene functional analysis can be carried out with the help of its inactivation with following analyzing of loss of gene's function influence on organism. The simplest way of gene inactivation is homological re-combination between gene's chromosome copy and inactivated copy of gene. First, the researched gene must be marked out, then, it is cloned as a component of plasmid vector in cells of bacteria Escherichia coli. If gene succession is too big for cloning in plasmid vector, then gene fragment is cloned. Selective marker (for example, gene of resistance to antibiotics) is introduced into the composition of the cloned gene. Then, recombinant construction is introduced in embryonic cells of mice (*in vitro*), and transgenic cells are sampled. For sampling of homozygous by mutation cells, PCR analysis is conducted. Cells, which are resistant to antibiotics, are injected to a mouse embryo; after it the embryo is implanted in the womb of specially prepared mouse female. The embryo initiates chimera, which consists of mutated and normal cells. Crossing of two chimeras can probably result in receiving of progeny: homozygous mouse with inactivated gene, if mutation manifested in the cells of chimera's genital tracts. Analysis of "knockout" mouse's phenotype brings to understanding of inactivated gene's function. If gene is of vital importance, than inactivation of it will be lethal. [5]. Disadvantage of this method is that gene inactivation not always results in clear phenotypic distinctions. There are methods of genomes' inactivation with the help of transposons (plasmids).

SiRNA- induced silencing - is a process of gene's expression inactivation on post-transcription level by mean of mRNA inhibition, which is launched by two-chain short interfering RNA of 20-30 n length. SiRNA- induced silencing implicates complete excluding of gene, involved in one or other mechanism of adapting to physical loads. For this purpose, most often the molecules of short hairpin-shaped RNAs, shRNAs, which are connected with appropriate parts of RNA of gene-target and cause its destruction, are used.

Sequent of RNA or DNA is the process of determination of their amino acid or nucleotide succession (from Latin sequentum –succession). After sequent, researchers obtain description of primary structure of linear macro molecule in the form of monomers' succession. For sequent Senger's method with didezoxynucleozidtriphoshpates (ddNTR) are used most frequently.

The sense of genome's sequent implies its disjunction into small parts and each of them it is possible to sequent by method «shotgun». Sequent process can be conventionally divided into three stages: obtaining of genome "library", sequent of occasional clones and joining of successions, received as a result of sequent. To day there are DNA sequent systems, which have been by companies created Roche Applied Sciences, Illumina, Applied Biosystems.

One more aspect of molecular-genetic methods' application in sports is considering of pharmacological genetics' foundations with using of permitted pharmacological means in order to increase sportsmen's physical workability and their recreation. Genetic dependence of medicines effect, which was studied long before discovering of genome, has been become effectively considered and applied for using of pharmacological preparations, considering individual response. It was stated that 50% of unfavorable pharmacological responses (progressing of unwanted reactions, insufficient effectiveness) depend on genetic features of an individual. [11]. After discovering of genome, it was found, that all known pharmacological phenomena, which determine individual responses to medicines, depend on ancestrally determined peculiarities of bio-transformation, on interaction with receptor creatures and ferment systems. Pharmacological genetics is the science, which studies influence of heredity on effect of medicines. [10]. Sports pharmacological genetics is a section of sports pharmacology and genetics of physical activity, which studies sportsmen's genetic features, influencing on pharmacological response. [2]. Sports pharmacological genetics permits to individualize selection of pharmacological preparations, their dozing on the base of certain sportsman's genotype.

Both, main methods of classical genetic analysis and new genetic methods are based on the methods of mathematical statistics. Genetic population methods permit to determine the frequency of meeting of different alels and genotypes in a population, to research the factors, which influence on the ales' frequencies, to prognosticate the number of individuals with mutated manifestation of gene's action.

The processing of huge number of data, obtained as a result of genome sequent, "decoding" of genome DNA successions, is fulfilled by new branch – bio-informatics. The main tasks of bio-informatics is studying of genomes, analysis and prognostication of proteins' structure, of protein molecules' interaction with each other and with other molecules.

PCR method is widely used for DNA identification of man for health protection and sports purposes. More than 16 large research centers, which deal with the problems of sports genetics and physical activity, have been created in the world. In Russia, there are several scientific laboratories, among which the largest are: group of sports genetics of sports bio-chemistry sector of St. Petersburg SRI of physical culture; laboratory of sports reserves' preparation technologies of Povolzhskiy state academy of physical culture, sports and tourism; Olympic sports SRI of Ural state university of physical culture and other. In Byelorussia such researches are conducted in 4 laboratories, among which there is human genetics laboratory DNA bio-technologies Center. [4]. Sports genetics in Ukraine has rather long and interesting history. The work of L.P. Sergiyenko and his disciples in the field of sports genetics, which lasted for many years, resulted in publication of a number of scientific monographs and manuals "Research of ancestral and environmental factors' influence on development of human motion abilities" (1975), "Principles of sports genetics" (2004), "Dermatoglyphics, health, sports" (2012). Great contribution into understanding of problem of bent to physical loads was made by the scientists of physiology institution, named after O.O. Bogomolets at National Academy of Science of Ukraine, who, already in 80-s years of 20^{th} century studied oxygen transport systems of human and animals organisms in conditions of hypoxia of different genesis, including hypoxia of loading. The works by academician V.A. Berezovskiy, professors T.V. Serebrovskaya and M.M. Fhilipov laid the foundations for modern molecular-genetic researches. In Ukraine, scientific researches, which are connected with molecular-genetic markers in sports, are conducted biological department of National University of physical education and sports of Ukraine (NU PESU) and by laboratory of sports training methodology's theory and sportsmen's reserve capabilities of SRI of NU PESU together. Some researches are conducted in Mykolayiv National university, named after V.O. Sokhomlinskiy (A.V. Kozyriev, 2011).

Using of molecular-genetic methods has both advantages and disadvantages. Advantages are as follows: as far as genotype of every individual temains unchaged during all life, the future abilities can be revealed even in childhood, that reduces time and efforts expenses, required for reaching of high sports results; genetic testing permits to preserve the health of a sportsman, showing his bents to diseases and pathologies, which can appear with intensive physical loads. Using of genetic information can serve for creating of multi-year training process of sportsmen. One of the most important problems of application of genetic information is ethic problem, because there is possibility of its using for different types of discrimination (at employment, at marriage), of violation of this information confidentiality.

The results of sportsmen's genetic testing are often published only for administrative use; new technologies of sportsmen's training on the base of genetic information are developed, considering national interests; that is why every

country creates its own DNA bank. Besides, with evaluation of ancestral bent to physical work, it is necessary to consider ethnic characteristics in distribution of genes variants in populations. That is why, creation of own DNA bank of Ukrainian sportsmen is an important problem. For analyzing of ancestral bent it is very important to know the quantity of analyzed genes, which is connected with the fact that unfavorable alel of one gene can be compensated by other genes at the cost of metabolism ways change. The probability of favorability of one or other alel must be proved on large sample of elite sportsmen of a certain kind of sports. This is the first challenge to the researches, because creating of such sample is very difficult due to economical and social circumstances. In spite of all above mentioned facts, application of molecular-genetic technologies promotes development of sports science, permits to raise it on new, modern, highly technological level.

Conclusions

Heavy growth of molecular biology methods for the last 2 decades, permits to carry out researches in the sphere of sports genetics on molecular level. Application of modern molecular-genetic methods in scientific achievements and in sports practice will result in increase of fundamental knowledge and will have great practical significance, i.e. rising of sports results, reduction of financial expenses for preparation of sportsmen and risk of chronic diseases and pathological state appearance.

The prospects of further researches. The conducted analysis of modern molecular-genetic methods will permit to adequately use them for evaluation of ancestral bents to physical loads and to conduct on molecular level scientific researches of human organism's adapting to physical loads.

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