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Žurnalas „Sporto mokslas“ išleidžiamas keturis kartus per metus.

Gene-profiling power supply for athletes of high qualification. The example of biathlon

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Summary

In the presented article, the possibilities of carrying out the gene-profiling of the power supply process in biathlon, as an example, are considered. The results of genotyping of 31 biathlon-athletes of high qualification on a panel of six polymorphic markers of genes, involved in energy supply processes (ACE, PPARA, PPARGC1A, PPARD, PPARGC1B, and PPARG2), was given. The research was conducted in compliance with necessary ethical criteria: obtaining informed consent from athletes and ensuring the confidentiality of personal information.

In the examined group of biathlon-athletes, the prevalence of allele frequencies was observed: 67.74% D allele of the ACE gene; 77.42% G allele of the PPARA gene; 56.45% Ser allele of the PPARGC1A gene; 74.20% T allele of the PPARD gene; 95.16% C allele of the PPARG2 gene; 100.00% C allele of the PPARGC1B gene.

Establishing the genetic potential of each athlete allows determining or refining the molecular mechanisms of inheritance and expanding the theoretical and methodological basis of the process of sports training. At the same time, having determined the differences in the distribution of genotypes in groups of highly qualified athletes, engaged in various sports, it is possible to carry out with confidence the genetic prognosis of success in the group of reserve athletes.

Based on the results of earlier conducted research, analysis of the world experience – an algorithm for managing the selection process of the reserve group athletes and subsequent optimization of their training process – is proposed.

Keywords: *gene-profiling, power supply, athlete of high qualification, biathlon.*

Introduction

Modern approaches to the selection of athletes and the individualization of their training process must necessarily take into account their genotypic characteristics. The identification of genetic marker allows predicting the patterns of development of the physical qualities of athletes, and the investigation of the influence of genetic determinacy on training process helps to define approaches to the development and correction of training programs for athletes taking into account their genetic polymorphisms (Elferink-Gemser et al., 2011; Tucker, Collins, 2011; Рыбина, 2013; Ahmetov et al., 2015; Yevdaliuk et al., 2015). Genetic researches of contenders, before the beginning of sports activity, provide possibility to receive recommendations for choosing specialization in sports. Genetic testing makes possible to determine the perspectives of active athletes at the stages of the improvement and enhance athletes' selection process for representative teams of different levels as it serves as an objective scientific argument in disputable situations. Moreover, genetic analysis

data aim to predict possible changes in health status of future athletes and their potential for sport longevity (Tucker, Collins, 2011; Ahmetov et al., 2015; Кручинский et al., 2015; Рыбина, 2016). Genetic research data can also be useful in correction of training process in relation to the direction and correlation of the types of training loads, particularly in giving recommendations, based on the identified physiological risk factors of athletes in accordance with genotype (Rankinen et al., 2010; Ahmetov et al., 2015; Bouchard, 2015). At the same time, the exploration of the dynamic changes of physical qualities at different stages of athletes' training process with different variants (polymorphisms) of gene(s) showed the need for a differentiated approach to athletes of different polymorphic groups (Рыбина, 2013; Шепелевич et al., 2013; Ahmetov et al., 2015).

As a result of the analysis of an international experience and data of our research, a control algorithm of the selection process of athletes has been developed. Let us consider these opportunities on the example of biathlon.

Biathlon is one of the most popular winter sports in the Republic of Belarus. Specificity of biathlon is the combination of running on skis and shooting from two positions. The particularities in the movements and muscle activity are feature superiors of the individuality of biathlon-athletes. The same speed of movement can be achieved with a different combination of length and frequency of steps. The best option of technique is the one, characterized by the least energy costs. The energy potential of an athlete and the efficiency of its realization are the main limiting factors in the level of sport achievements in skiing races and biathlon (Манжосов, 1986; Мищенко, 1990; Wilkison et al., 2008; Рыбина, Ширковец, 2015).

It is well known that the most significant factors, affecting sport results, are the energy capabilities of athletes (their aerobic and anaerobic productivity); speed-strength qualities; morpho-functional indicators and inherited abilities (skeletal body size, morphotype, muscular fibre composition, joint flexibility, heart rate in working out the standard submaximal load, maximum aerobic productivity, some elementary demonstration of speed, and the results of a series of motor tests) (Безуглая, 2016; Рыбина, 2016).

The aerobic way of energy supply (metabolism) is the main energy source for biathlon athletes, and its importance is growing with an increase of the length of the competitive distance. Anaerobic metabolic way is necessary to overcome the hills as well as for the development of high speed in various sections of the route (Мищенко, 1990; Drozdovska, Tyrtushnyk, 2015; Рыбина, Ширковец, 2015).

In the view of foregoing, the achievement of high results in any kind of motor activity depends on many factors, mainly, the maximum correspondence of the individual characteristics of a personality to the requirements of the selected sport. Consequently, the most important condition for the effective selection of advanced athletes is the awareness of particular sport and their requirements for athletes of high qualification.

The effectiveness of training top-level athletes is determined not only by the organization of the training process, moreover, the strategy of increasing the intensity of training loads to improve their technical and physical training, is now almost exhausted and requires the search and development of knowledge about individual limits of physical and

reserve abilities of the athlete, in other words, its genetically pre-deterministic qualities (Мищенко, 1990; Ахметов, 2009; Рыбина, 2013; Ahmetov et al., 2015; Drozdovska, Oleshko, 2016). In our opinion, the way out from this situation is a focus on parameters and indicators with a high degree of inherited determinacy that have the stability of individual developmental ranks and do not comply with generally organized influence in the training process (Шепелевич et al., 2013; Кручинский et al., 2015, 2017).

Let us consider the features of polymorphisms of some genes that influence the processes of energy supply of training and competitive activity as the most important.

Receptors that activate peroxisome proliferation (*PPARs*) are a family of nuclear receptors, belonging to the superfamily of steroid receptors (Tucker, Collins, 2011; Ahmetov, 2015; Кручинский et al., 2015). They play an important role in the regulation of energy supply for athletes as well as ensure the interaction of nervous, humoral, and energy processes during change of environmental factors or homeostasis parameters. The range of biological functions of *PPARs* is very wide. *PPAR* regulates the expression of genes, involved in the process of steroidogenesis, angiogenesis, tissue remodelling, regulation of the cell cycle, apoptosis, and metabolism of lipids and carbohydrates. Thus, the *PPARA* gene is located in chromosome 22 at the locus q13.31 and is expressed in tissues, where the enhanced metabolism of fats takes place, notably, in the muscles, liver, heart, and brown fat. *PPARAs* act as activators of oxidation of fatty acids. Expression of *PPARA* is controlled by stress stimulus, glucocorticoids, and insulin. It is also activated by fatty acids, eicosanoids, carbaprostacyclin, nonsteroidal anti-inflammatory drugs, and leukotriene B. The main function of the *PPARA* protein is the regulation of lipid metabolism, glucose, and energy homeostasis as well as body weight by regulating the expression of genes, involved in peroxisomal and mitochondrial oxidation. *PPARA* regulates genes, responsible for the metabolism of fatty acids, and mediates the balance between cellular fatty acids and glucose metabolism, especially in metabolic or physiological stresses. During physical activity of aerobic nature, the growth of utilization of fatty acids takes place due to an increase of the *PPARA* gene expression and a cascade of regulated genes that ultimately improve the oxidative capacity

of skeletal muscles. It is known that, with a low expression of the *PPARA* gene, the capacity of tissues of efficient β -oxidation of fatty acids decreases and tissue metabolism moves on a glycolytic method of getting the energy. Among the examined *PPARA* polymorphisms, the G/C polymorphism of the seventh intron G2528C can be distinguished. The prevalence of rare C allele in the European population is 20%. This replacement of guanine by cytosine leads to a decrease in the expression of the *PPARA* gene, as a result, the regulation of lipid and carbohydrate metabolism is impaired.

The *PPARG* gene is localized on chromosome 3p25 locus (Ahmetov et al., 2015; Кручинский et al., 2015). As a result of alternative splicing from this, the gene of four transcripts is formed, differing at the 5-end with a different number of untranslated exons: *PPARG1*, *PPARG2*, *PPARG3*, and *PPARG4*. The functions of this transcription factor are the regulation of genes, connected with fat accumulation, the differentiation of adipocytes and myoblasts, sensitivity to insulin, and osteoblast and osteoclasts activity. The most studied polymorphism of the *PPARG* gene is Pro12Ala that causes the replacement of nucleotide C by G at the 34th position of exon B that leads to the replacement of proline by alanine at amino acid position 12 of the *PPARG2* isoform. The Pro12Ala polymorphism leads to a decrease of the affinity and reduce in *PPARG* activation by ligands. The rare Ala allele's frequency varies from 1% Chinese to 25% Europeans. With the Ala/Ala genotype, a lower level of insulin resistance and a lower risk of hyperglycaemia in type 2 diabetes than the Pro/Pro genotype carriers as well as a lower risk of myocardial infarction were observed. Moreover, the homozygotes (Pro/Pro genotype) have a higher risk of developing type 2 diabetes in cases of misbalancing glucose tolerance. A meta-analysis of 30 different researches with a reference set of 19,136 people showed that the carriers of the Ala allele have a larger body mass index than the Pro/Pro homozygotes, i.e., they lose weight harder in passing to a hypocaloric diet, but they quickly gain weight after the finishing diet.

The *PPARD* gene is located on chromosome 6p21.1-p21.2 and is actively expressed in fat tissue and in slow muscle fibres of skeletal muscles (Elfernik-Gemser et al., 2011; Ahmetov et al., 2015). The gene product – *PPARD* protein that regulates the expression of genes, involved in fatty acid oxidation

and cholesterol metabolism, – is an important factor in insulin sensitivity. The target genes of the transcriptional factor *PPARD* in muscle tissues are the genes of oxidative metabolism, mitochondrial respiration, and thermogenesis genes, determining the functions of slow muscle fibres, brown and white fat tissues. Among the allelic variants of the *PPARD* gene, T294C polymorphism of the untranslated part of the fourth exon is the most interesting. The frequency of minor allele C in the European population is 21.7%. The *PPARD* transcriptional activity formed by 39% of the mutant allele C is higher than the T allele. In addition, the replacing of nucleotide T to C leads to the formation of a new determinant point with transcriptional factors, intensifying *PPARD* expression. It is shown that the presence of the C allele of the *PPARD* gene promotes greater fat catabolism and reduces the risk of obesity to a certain extent. The homozygotes CC have an increased level of low density lipoproteins and a low level of high density lipoproteins in the blood.

The peroxisome proliferator-activated receptor gamma coactivators (encoded by *PPARGC1A* and *PPARGC1B*) are transcriptional coactivators of PPAR family that regulates genes, involved in energy metabolism. *PPARGC1A* interacts and regulates the activity of the cAMP-dependent transcriptional factor (CREB) and nuclear respiratory factors. This provides a direct link between external physiological stimuli and the regulation of mitochondrial biogenesis, and this is the main mechanism that regulates the differentiation of muscle fibres. *PPARGC1A* also takes part in the control of blood pressure, regulates cellular cholesterol metabolism and the development of obesity. *PPARGC1B* gene stimulates the activity of transcription factors and nuclear receptors, including the estrogen alpha-receptor, nuclear respiratory factor 1, and glucocorticoid receptors. The encoded protein may be involved in fat oxidation, a non-oxidative metabolism of glucose as well as the regulation of energy usage and the development of pre-diabetes and type 2 diabetes. The Arg292Ser (+102605C>A) allelic variation in exon 5 of this gene increases the risk of obesity.

The *ACE* gene is localized in chromosome 17; it encodes the amino acid sequence of the angiotensin converting enzyme that catalyses the proteolytic cleavage of angiotensin I into angiotensin II (Elfernik-Gemser et al., 2011; Рыбина, 2013; Шепелевич et al.,

2013; Ahmetov et al., 2015). The ACE gene contains a I/D polymorphism in intron 16. In the case of the DD genotype, the concentration of the c-AMF is increased, which causes the participation of the allele D in vasoconstriction, blood pressure, association with arterial hypertension, degradation of bradykinin, the main vasodilator. It has been found that the allele D is associated with the prevalence of fast muscle fibres and with physical qualities such as speed, strength, rapidity as well as the increase in explosive strength and velocity qualities in response to anaerobic loads. The increase of fast glycolytic muscle fibres is accompanied by powerful short-term reductions, which ensure the performing of high-intensity exercises. The homozygous genotype DD, generating angiotensin II in elevated levels, is a factor in the synthesis of structural proteins in the heart cells, which provoke cardiac hypertrophy under prolonged loads.

At the moment, the sports activity is a complex of training process and medical-biologic support of athletes at all stages of a long-term preparation (Maron, Klues, 1994; Corrado et al., 2003; Безуглая, 2016).

In the view of foregoing, the purpose of this research is to analyse the frequencies of the polymorphic markers of the genes, involved in the energy supply of high qualification biathlon-athletes.

Material and methods

The research involved 31 biathletes of high qualification (sport masters and international sport masters).

The selection of biological material for subsequent research and the analysis of the material were preceded by an information procedure for the research and a written confirmation agreement for participation. Polymerase chain reaction (PCR) was used to detect the I/D of the ACE gene. The genotyping of the PPARA gene G2528C, PPARGC1A gene Gly482Ser, PPARD gene +294T/C, PPARGC1B the Arg292Ser, and PPARG of the Pro12Ala variants were carried out by PCR, followed by the restriction endonuclease (TaqI, MspI, BslI, ApaI, BstUI). The visualization of the results of genotyping was carried out using a UV trans-illuminator of the gel-documentation system (Vilberlaurmat, France). Fragments of DNA and DNA marker appeared in the form of luminous bands, when the gel was irradiated with a UV lamp. DNA markers were verified by the presence of amplified fragments as well as their size (Ахметов, 2009).

Statistical evaluation of the genotyping was carried out by computer program *Statistica v. 8.0* for Microsoft Excel 2007. The χ^2 criterion with the Yates correction or the exact Fisher test was used in a pairwise comparison of the frequencies of alleles and genotypes between analysed groups. The difference between two comparative values was considered statistically significant when $p < 0.05$.

To analyse the results of genotyping, the following genotypes of biathlon-athletes were graded by the most favourable genotypes for the process of energy supply: II genotype of ACE, GG genotype of PPARA, CC genotype of PPARD, CC genotype of PPARG2, GlyGly genotype of PPARGC1A – 2 points; heterozygous genotypes – 1 point; homozygous unfavourable genotype – 0 points.

Results and discussion

The distribution of genotypes and alleles of researched polymorphisms are presented in Table 1.

Table 1

The genotype and allele frequencies of the biathlon-athletes

Polymorphisms	Genotype/allele	Frequencies, n (%)
1	2	3
I/D ACE	DD	14 (45.16)
	ID	14 (45.16)
	II	3 (9.68)
	D	42 (67.74)
	I	20 (32.26)
G2528C PPARA	GG	20 (64.52)
	GC	8 (25.80)
	CC	3 (9.68)
	G	48 (77.42)
	C	14 (22.58)
Gly482Ser PPARGC1A	Gly/Gly	1 (3.23)
	Gly/Ser	25 (80.64)
	Ser/Ser	5 (16.13)
	Gly	27 (43.55)
	Ser	35 (56.45)
+294T/C PPARD	TT	17 (54.84)
	CT	12 (38.71)
	CC	2 (6.45)
	T	46 (74.20)
	C	16 (25.80)
Arg292Ser PPARGC1B	CC	31 (100.00)
	CA	-
	AA	-
	C	62 (100.00)
	A	-
Pro12Ala PPARG2	CC	28 (90.32)
	CG	3 (9.68)
	GG	-
	C	59 (95.16)
	G	3 (4.84)

In the researched group of biathlon-athletes, the prevalence frequency of minor alleles was: the *ACE* allele D – 67.74%, *PPARA* allele G – 77.42%, *PPARGC1A* allele Ser – 56.45%, *PPARD* allele T allele – 74.20%, *PPARG2* allele C – 95.16%, and *PPARGC1B* allele C – 100.00%.

Next, the genetic profile of the energy supply process was constructed from tested polymorphisms

(Fig. 1). In this case, the results of genotyping of the *PPARGC1B* gene polymorphism were not taken into account, because only one genotype was identified during the research.

In principle, the results, obtained by us, do not contradict published studies of foreign authors (Corrado et al., 2003; Elfernik-Gemser et al., 2011; Tucker, Collins, 2011).

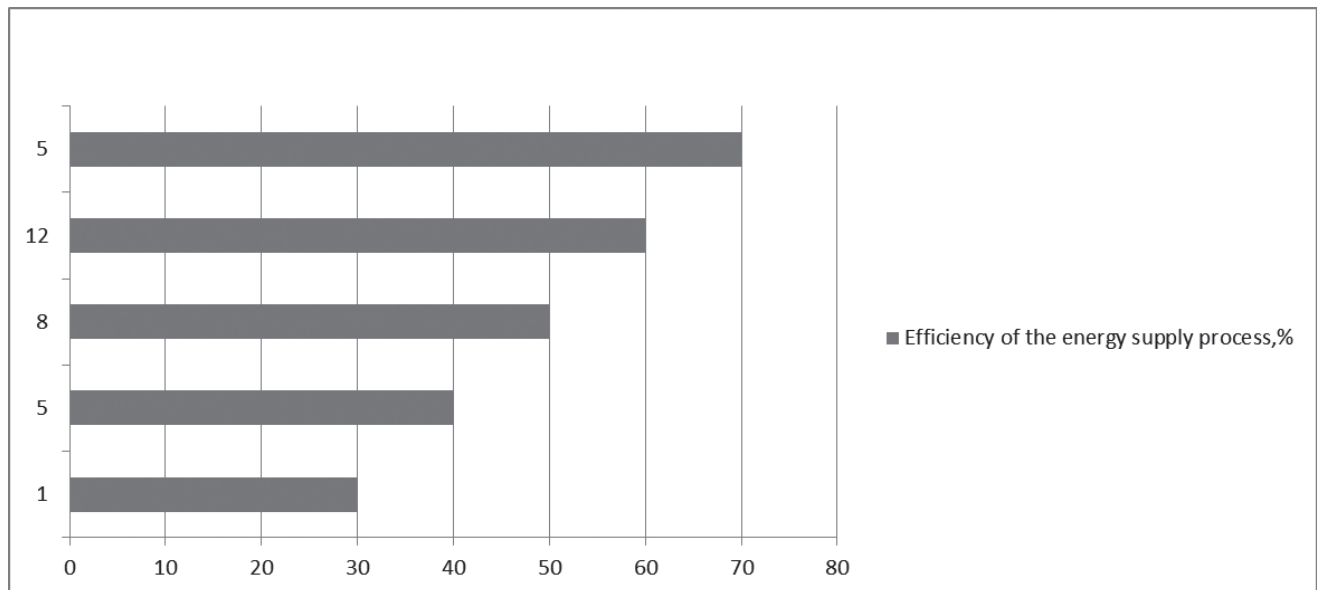


Fig. 1. Distribution of genetic profile results in the studied group of biathlon-athletes

Obviously, the majority of athletes in the researched group showed the effective energy supply processes that are quite understandable by their high level of sports qualification.

For the research, we have considered polymorphisms of genes that were involved in cardiovascular system, carbohydrate and lipid metabolism, energy metabolism, affecting the muscle and fat mass, and, therefore, associated with physical activity. Obtained results in the research of the genetic predisposition of biathlon-athletes demonstrated the possibility of this scientific and methodical approach to optimization and individualization of training. The presented results lead us to conclude that the gene-profiling allows to choose a biathlon specialization for beginners as well as to improve the training of highly qualified athletes.

The effectiveness in sport activities is a symbiosis of environmental influences and genetically determined abilities as well as qualities of the athletes. Having information on genetically

determined characteristics within the reaction norm and information on anthropometry and component composition of the body and their dynamics under the influence of high physical loads, the trainer and athlete are able to achieve high results.

Consequently, the determination of the genetic potential of each athlete makes it possible to define or refine the molecular mechanisms of inheritance and expand the theoretical and methodological basis of the process of sport training. At the same time, having determined the differences in the allocation of genotypes in groups of highly qualified athletes, engaged in various sports, it is possible to carry out with confidence the genetic prognosis of success in the group of reserved athletes.

The presented results of genotyping of highly qualified biathlon-athletes for estimating the effectiveness of their energy supply for training and competitive activities demonstrated the possibility of influencing practically on all stages of training of high-class athletes. This example is an illustration of the analysis of both world experience and our

research. Based on the results, we developed an algorithm for managing the selection process of the reserve athletes group and subsequent optimization of their training process (Рыбина, 2015; Безуглая, 2016).

At the first stage, it is recommended to perform genotyping to obtain a basic information on the predisposition of athletes to speed-strength training or endurance training as well as for the estimation of the nervous system potential. This process includes the following operations: a questioning; a buccal test (epithelium of inner surface of cheek) for sampling of biomaterial for investigation (an extract DNA from buccal epithelium); an identification of polymorphisms according to selected (depending on the task) gene panel by polymerase chain reaction and other molecular genetic methods.

As a result, the coach receives the primary information that is the basis for further selection and orientation work. In this case, genetically examined athletes are divided into three groups. For example, the *ACE* gene polymorphism allocation is: the first group consists of applicants, who by their genotype fully correspond to the sport, for example, athletes with a II homozygous genotype are appropriate to ski races and biathlon; the second group includes athletes, who have found a complete discrepancy, i.e., applicants with a homozygous variant *DD*; and the third group is composed of all other athletes, who have a heterozygous *ID* genotype. Accordingly, the athletes of the second group are considered as applicants after all the others and the athletes of the first group – as primal.

It should be emphasized that the results of genotyping are not a strict contraindication to selection as success in various activities since it is determined by a complex of abilities and even a number of indicators, negatively affecting the results, as well as can be compensated by a high level of development of other qualities. In this context, the results of genotyping should help coaches in identifying potentially advanced athletes, who should not be “lost” at early stages.

At the second stage, athletes are estimated according to medical and biological criteria. Their health status, anthropological features of development (accelerated-delayed), functional capabilities of aerobic energy supply system, data of muscle biopsy (is not performed in the Republic of Belarus), level of specialized perceptions – complex

psycho-physiological (sense of time, sense of pace, feeling of developed efforts, feeling of snow in skiers and biathletes, etc.) characteristics, efficiency of work, capacity to transfer loads and efficient recovery, availability of reserve capabilities of the body to maintain and possibly enhance previously achieved level of adaptation are taken into account.

At the third stage, athletes are estimated according to psychological and pedagogical criteria. We have taken into account the state of the art of performing various special preparatory exercises.

These aspects were settled as criteria: the effectiveness of movements that, in most cyclic sports, is evidenced by their low tempo with a long step length and high speed when passing short segments; the level of development of physical qualities (speed-strength, various types of endurance, flexibility, and coordination abilities) with particular attention paid to the rate of their growth from one stage of preparation to another; and personality-mental qualities (mental reliability, motivation, will, aspiration for leadership, resistance to stressful situations during competitions, the ability to tune into an active competitive struggle, the ability to mobilize forces in acute competition, the mental stability in performing volumetric and intense training work, the ability to control effort, pace, speed, direction of movement; distribution of strength in the process of competition as well as the ability to show the highest results in the most important competitions, surrounded by strong competitors).

The final decision to involve a child in sports is to be based on a comprehensive assessment of all the listed selection criteria, rather than taking into account one or two characteristics.

Conclusion

The effectiveness in sport activities is a symbiosis of environmental influences and genetically determined properties as well as the qualities of a person. Athlete's body is able to achieve high results with the knowledge of its size, its proportions, genetically determined within the norm of reaction, and under the influence of high physical loads.

Our results can be used to design better selection and training processes in order to allow athletes achieving their full potential.

This significantly expands the theoretical and methodological base of the sports training and

allows making the transition from the recruitment system in sport sections to the targeted selection of potentially successful athletes already at the stage of improving sports skills.

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DIDELIO MEISTRISKUMO BIATLONININKŲ ENERGIJOS GAMYBOS GENŲ PROFILIAVIMAS

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SANTRAUKA

Darbo tikslas – ištirti didelio meistriskumo biatlonininkų energijos gamybos proceso genų profiliavimo galimybes. Darbe pateikiami 31 didelio meistriskumo biatlonininko 6 polimorfinių markerių sistemos rinkinys, veikiantis energijos gamybos procese (*ACE*, *PPARA*, *PPARGCIA*, *PPARD*, *PPARGCIB* ir *PPARG2*). Tyrimai atlikti laikantis reikiamų etikos reikalavimų, asmeninės informacijos konfidencialumo.

Tyrimo rezultatai parodė, kad dažniausiai randami aleliai yra: *ACE* geno D alelis (67,74 proc.), *PPARA* geno G alelis (77,42 proc.), *PPARGCIA* geno Ser alelis (56,45 proc.), *PPARD* geno T alelis (74,20 proc.), *PPARG2* C alelgeno alelis (95,16 proc.), *PPARGCIB* geno C alelis (100 proc.).

Atlikti šios sportininkų grupės DNR tyrimai parodė pakankamai efektyvią energijos gamybą – tai patikimai patvirtinama aukštu jų sportinės kvalifikacijos lygiu. Kiekvieno sportininko genetinio potencialo tyrimas leidžia nustatyti arba patikslinti paveldimumo molekulinį mechanizmą ir papildo teorinę ir metodinę sportinio rengimo duomenų bazę. Nustačius įvairių sporto šakų sportininkų genotipų pasiskirstymo skirtumus, galima atlikti patikimą jaunųjų sportininkų genetinę prognozę.

Remiantis kitų pasaulio šalių mokslininkų tyrimų rezultatų analize ir šio darbo autorių atliktų tyrimų duomenimis, buvo sukurtas jaunųjų sportininkų atrankos algoritmas, leidžiantis geriau optimizuoti jų treniruočių procesą.

Raktažodžiai: genų profiliavimas, energijos gamyba, didelio meistriskumo sportininkas, biatlonas.