European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 10, 2020

Optimization Of Molecular- Genetic Methods For The Determination Of Resistance Markers Using Genotyping Of Actn3 And Ace Genes

Berdieva Dilnavoz Toshkan kizi[1], Bakieva Gulnoza Habibullayevna[1], Abdusattarova Sagdiana Sobitjanovna[2], professor Abdullaeva Muborak Makhmusovna[2], professor assistent Gazieva Zebunniso Yusubjanovna[3]
Department of Sports Medicine and Biochemistry, Uzbek State University of Physical Culture and Sports[1], Faculty of Biology, Department of Biochemistry National University of Uzbekistan
dilnavoz_dbt@mail.ru[1]

Abstract: Recently parents want their children to be busy with a particular kind of sport. Adults often decide on this issue, based on their own desires. But still, we must remember that, first of all, it is necessary to focus on the child's abilities and capabilities. Physical culture and sports are different things, and they pursue different goals: they are engaged in physical culture in order to strengthen their physical condition and be healthy, and sports are also for the sake of victories, achievements, and results. In humans, more than two hundred genes are known that are associated with endurance, speed and strength. Usually, the reason for differences (polymorphism) of genes is the replacement of individual nucleotides in the DNA molecule, leading to a change in the properties of the gene.

The main advantage of molecular genetic methods is that a person's behavioral activity is shown to be hereditary, and early diagnosis reveals a highly informative assessment of the possibility of physical development. A distinctive feature of this diagnosis is the ability to identify hereditary trends, the development of factors of occupational diseases, determining the physical performance of a person and reducing the quality of his life.

Keywords: sport biochemistry, gene, ACTN3, ACE, Polymorphism, allele endurance, DNA

The relevance of the research: specialists will be able to select young athletes for sports, determining the right direction in sports, the appropriate load, using genetic testing. They can be used as stages in the development of sports genetics in Uzbekistan.

Purpose of research: Practical introduction of genetic methods in the selection of athletes for sports. Also, molecular genetic study of the endurance marker using genotyping of the ACTN3 gene in R577X polymorphism in athletes. So, molecular genetic study of the endurance marker using genotyping of the ACE gene in I/D polymorphism in athletes

Organization of research: this research is based on molecular genetic analysis that will allow an athlete to choose a sport based on his / her genetic, physiological status. This improves performance in sports. Therefore, at the Department of Sports Medicine and Biochemistry of Uzbek State University of Physical Culture and Sports, a theoretical study of molecular genetic analysis was started.

1. INTRODUCTION :

In the late 80s of the last century, within the framework of the Human Genome project, data on genes associated with the manifestation and development of human physical qualities began to appear. As of 2009, 239 marker genes for human physical activity have already been found, and progress in the discovery of new genes is obvious[1,3].

In February 2001, the two most respected scientific journals in the world, Nature and Science, published reports from two scientific groups that deciphered the human genome. The journal "Nature" dated February 12, 2001 provides detailed data on the structure of the human genome, obtained by an international consortium led by Francis Collins, in which scientists from England, Germany, China, USA, France and Japan worked in the framework of the international program "Human Genome" with attracting government funding. This group isolated special markers in DNA, easily recognizable regions, and determined the nucleotide sequences of the human genome from them [2,5].

Due to the fact that many genes have been identified, a new direction is gradually being formed, which can be attributed to functional genomics, since it reveals the links between the activity of individual genes and various human functions. Among them, an important place is occupied by the identification of the relationship of specific genes with the development of human motor function.

So, in the course of the research, various forms of the same gene (alleles) were identified, associated with the characteristics of carbohydrate or lipid metabolism and, accordingly, determining the predisposition of the athlete's body to aerobic (endurance sports) or anaerobic (sports of speed-power character) mechanisms of energy supply.

For example, playing football develops the quality of strength, but not so much static strength as the so-called "explosive" strength, which is especially important for the development of speed-strength abilities. The physical quality of strength quite noticeably depends on the innate characteristics of a person. The development of maximum static force is 55% determined by heredity and 45% by environmental influences, i.e. various external influences throughout life, including training influences. To an even greater extent, the development of explosive strength depends on the genetic factor, where about 68% of this indicator is determined by heritability, and only 32% can undergo changes under the influence of directed training and other environmental influences[3,4,5,7].

The manifestations of genetic influences depend on age (they are more pronounced in young players) and work power (they increase with increasing work power). Having reached maximum values by the age of 20, muscle strength begins to decline at the age of 45 and older, and speed-strength capabilities deteriorate from the age of 35. Morphological parameters are also most susceptible to hereditary influence. The hereditary dependence is especially pronounced in the longitudinal dimensions of the player's body and much less in the volumetric ones.

There are also alleles that restrict a person's physical activity by decreasing or increasing the intensity of gene turn-on. The consequence of such a limitation, at best, is the cessation of the growth of sports results, at worst - the development of pathological conditions, for example, excessive hypertrophy of the left ventricular myocardium. In humans, more than two hundred genes are known that are associated with endurance, speed and strength. Usually, the reason

for differences (polymorphism) of genes is the replacement of individual nucleotides in the DNA molecule, leading to a change in the properties of the gene[8,9,11].

By structure and function, two types of muscle fibers are distinguished: fast, capable of developing high speed and strength of muscle contraction, but not adapted to prolonged load, and slow, capable of working in a long mode.

The fast muscle fibers synthesize the protein alpha-actinin-3, which is encoded by the ACTN3 gene. Alpha-actinin-3 stabilizes the contractile apparatus of skeletal muscles and is involved in various metabolic processes. The replacement of the nitrogenous base of cytosine (C) with thymine (T) in the coding sequence of the gene at position 18705 results in the absence of alpha-actinin-3 protein in the muscles. On this basis, it is assumed that the ACTN3 gene polymorphism is one of the reasons for the decrease in the development of speed-strength qualities in humans. But since the function of the alpha-actinin-3 protein can be performed by another protein, alpha-actinin-2, which is also present in fast muscle fibers, no pathology is observed in people with an altered version of the ACTN3 gene[1,2,8,9,11].

The main (functional) variant of the ACTN3 gene is designated as R, the minor (more rare and non-functional) allele is X. The X / X genotype is found in approximately 18% of people (this figure is relevant for Europeans) and is the only reason for the complete absence of alpha protein in their muscles -actinin-3.

The presence of the R gene variant gives its owner an advantage in explosive strength and speed, and the X allele contributes to the development of endurance.

In Russian population, the frequencies of genotypes for the ACTN3 gene are distributed as follows: R / R - 36.5%, R / X - 49%, X / X - 14.5%[8,9,12,13,15].

In Uzbekistan, the association and frequency of occurrence of these genes have not yet been studied. Therefore, we have introduced molecular genetic methods at the Uzbek State University of Physical Culture and Sports to identify markers of these genes, primarily for the selection of athletes for sports

The frequencies of the R and X alleles in professional athletes of speed-strength sports significantly differ from their distribution in the normal human population.

The R / R genotype offers a significant advantage in sports that require explosive strength and speed (eg, shot throw, sprint, soccer). There is a tendency - the higher the qualifications of an athlete in a speed-strength sport, the higher the likelihood of having the R / R genotype.

Carriers of the R / X genotype are able to achieve high results at medium distances and in sports where a combination of speed, strength and endurance is required. The X / X genotype provides advantages in those sports in which endurance is primarily important (race walking, long-distance swimming, marathon running). Thus, the identification of this genetic marker allows you to choose a sport, for example, speed-strength or requiring endurance. The ACE gene (21 kb) is located on chromosome 17q23 and consists of 26 exons. Polymorphism in the 16th intron of the ACE gene is due to the presence (insertion or I allele) and the absence (deletion or D allele) 287 bp. plot. Allele I is associated with low activity of the ACE gene and increased athletic endurance. Allele D, on the contrary, is associated with a higher activity of the ACE gene and the manifestation of speed, strength and coordination abilities in athletes[1,2,8,9].

The ACE gene encodes angiotensin converting enzyme (ACE), a protein circulating in the extracellular space (carboxypeptidase) that plays an important role in the regulation of blood

pressure and electrolyte balance, catalyzing the breakdown of inactive angiotensin I to active angiotensin II.

Genetic marker Alu Ins / Del

In the 16th intron of the ACE gene, an insertion-deletion (I / D) polymorphism was revealed, consisting in the insertion (insertion, I) or loss (deletion, D) of an Alu repeat of 289 base pairs. Deletion of the Alu repeat leads to increased expression of the ACE gene.

Possible genotypes: I / I, I / D, D / D

Association of the marker with diseases; Myocardial infarction, coronary heart disease, ischemic stroke, Alzheimer's disease, chronic renal failure, osteoporosis, age-related macular degeneration, atherosclerosis[16,17,18].

2. MATERIALS

Reagents used in the research: 3.8% sodium citrate, Diatom TM DNA Prep 200 (manufactured in IzoGen Laboratory) reagent kit, agarose, bromphenol-cookie, 121g tris for TBE buffer, 55g boric acid, 7.55g EDTA, etidium bromide, Nuclease Free Water, 76% and 96% ethyl alcohol, ddH2O, primers, marker- 100 bp DNA Ladder RTU (Ready-to-Use) GeneDireX, Taq-pol polymerase (enzyme)), 10x PCR Buffer, $MgCl_2$, ddNTP SYBR-Green. Tools and equipment: Step One Real-Time PCR - amplifier (Applied Biosystems, Singapore), Transillyuminator - Wise Doc, Korea, Centrifuge - Eppendorf 5417 C, Vortex - FVL - 2400 N, Combi-Spin BIOSAN, Electrophoresis equipment - Helicon, Power supply Elf-4 , 1.5 ml eppendorph solutions and 100 μl optical eppendorf solutions., Distiller - Thermo Scientific, thermostat - Termite, DNA technology, Thermostat.

3. METHODS:

In order to study the endurance gene, 50 adolescent athletes and 50 non-athletes were selected. DNA samples were isolated from their venous blood by nucleosorption. When DNA was isolated from venous blood by the colon method, the DNA was not clearly visible on electrophoresis. In the nucleosorption method, however, it was clearly visible.

The nucleotide sequence of the ACTN3 and ACE gene was searched using the Genbank database. Primers and probes were designed using the online software http://biotools.umassmed.edu/bioapps/primer3 to match the ACTN3 gene and Ace gene. The temperatures of the primers and probes were selected using the programs www.bio.bsu.bu / molbiol / oligocol.html, http://www.basic.northwestern.edu. Current RT (Real-Time RT) reaction on the ACTN3 gene was performed and optimized on DNA samples, and based on the results obtained, the R genotype of the ACTN3 gene was found to be higher and associated in weightlifters than the control group.

Real-time PCR is an amplification reaction. The nucleotide sequence of the ACTN3 gene was searched using the Genbank database. Primers and probes were designed using the online application http://biotools.umassmed.edu/bioapps/primer3 to match the ACTN3 gene.

The temperatures of the primers and probes were selected using www.bio.bsu.bu / molbiol / oligocol.html, http: //www.basic.northwestern.eduwww.www.inSilico.ehu.es/PCR/ electronic program to theoretically add PSR amplification 'year. We diluted the primers and probes accordingly. The primers were lyophilized and added ddH2O as shown in the passport. Because the ACTN3 gene forward primer has a volume of 190 (picomol / μl) per ml, we

added 190 µl of bi-distilled water (concentrated). We diluted the concentrated primers 20 times. That is, we took 5 µl of the concentrated primer and dissolved it in 95 µl of bidistilled water, and it can be used. The primers and probes were also diluted in the order specified in the passport. DNA samples isolated using the current PCR (Real-time PCR) amplifier were genotyped according to the R337X polymorphism of the ACTN3 gene. Primers and probes consisting of specific oligonucleotides were used for genotyping (Table 1) (http: //sportstati.rf/geneticheskiye-markery-sportivnyx-zad/, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3420864/). A reaction kit was also used. The kit used thermostable Taq DNA polymerase, deoxynucleosode triphosphates and magnesium chloride, and 10x buffer for PCR resection. Sterile and optical solutions were filled with primers with a concentration of 10 pkM / µl, 10 µl with a 2.5x reaction mixture, 0.1-0.5 µl with a fluorescent probe with a concentration of 10 pkM / µl, 1.5 µl with magnesium chloride, and 1.2 µl with DNA being tested. The current PCR amplification was carried out according to the standard protocol. A 96-cell GeneAmp® PSR - Real Time PCR 7500 amplifier was used to perform the current PCR amplification. The temperature regime for simultaneous PCR amplification of the ACTN3 gene was as follows: 5 minutes at 95 ° C before denaturation, followed by 44 cycles: 95 ° C for 15 seconds and 54 ° C for 60 seconds .

Table 1 Nucleotide sequence of primers and probes.

Gene	Polymorphism	Nucleotide sequence of primers and probes (5→3)
ACTN3	R577X	forward –5'- *CTGTTGCCTGTGGTAAGTGGG* -3' reverse- 5'- *TGGTCACAGTATGCAGGAGGG* -3' FAM -5'-CAATACTCACATTTCT-3'-BHQ1 ROX-5'-AATACTCACGTTTCTC-3'-BHQ2

Primers F and R, two different probes FAM and ROX were used. The FAM probe corresponds to the R allele for the R577X polymorphism of the ACTN3 gene, while the ROX probe corresponds to the X allele. For this reason, each probe is placed in a separate test tube.

4. RESULTS:

A comparative analysis of the ACTN3 gene R577X polymorphism genotypes for the distribution frequency of 50 people from 25 weightlifters and 25 football athletes (50 in total) and 50 people in the control group (not regularly engaged in sports) was performed. The R577X genotype of the ACTN3 gene was identified in all (100%) of the 25 weightlifters. No other genotypes were identified in either of the two different groups of athletes. Normal R577X genotype was detected in 37 (74%) of the 50 people in the control group (non-athletes).

When the R577X S genotype of the ACTN3 gene is detected, the ascending line of the R graph in the Real-Time (PCR) amplifier rises 5-7 cycles before the X graph. In this case, we can interpret the ACTN3 gene as the X genotype according to the R577X polymorphism (Figure 1).

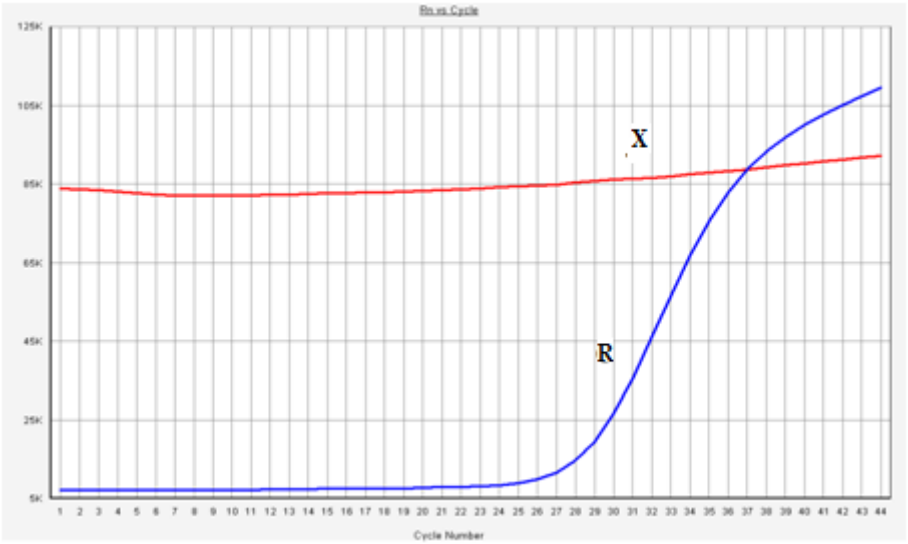

Figure 1 The result of the current PCR amplification. ACTN3 gene R577X genotype. While the R graph rose from the 24th cycle, the X graph 34 began to rise.

Table 2. Occurrence of ACTN3-gene polymorphism R577X in soccer players and weightlifters

ACTN3 gene	**Soccer players (25)**	**weightlifters (25)**	**Control group (50)**
RR genotype	100 %	100 %	74 % (37 та)
RX genotype	0 %	0 %	24 % (12 та)
XX genotype	0 %	0 %	2 % (1 та)

5. DISCUSSION:

A-actinin-3 (ACTN3, actinin alpha 3), localized on the long shoulder of chromosome 11 (11q13-q14), was first studied in athletes. There are two isoforms of α-actinin in skeletal muscle: the α-actinin-2 (ACTN2) isoform and the α-actinin-3 (ACTN3) isoform, which are localized in different parts of the muscle fibers. All muscle fibers hold α-actinin-2, α-actinin-3 is localized only in rapidly contracting skeletal muscle fibers. a-actin-3 deficiency is an indicator of a person's slowing down of physical activity. Such ACTN3 protein deficiency is caused by the replacement of a single nucleotide cytosine with thymine at 577th place in DNA (point mutation, SNP, R577X). As a result of the mutation, a stop codon is formed on the arginine shell, and the synthesis of the polypeptide chain of the α-actin-3 protein is stopped. There are three different genotypes in the polymorphism of the ACTN3 gene: normal allele RR-homozygous, heterozygous RX, mutant XX-homozygous alleles. Muscles of the homozygous genotype X-alleles do not contain the α-actin-3 protein. A-actin-2 in muscle fibers, which rapidly contract muscle pathology in such people, compensates for its absence. In the presence of a normal 577R-allele, skeletal muscle contains the α-actinin-3 protein, which gives individual preference to physical properties such as speed and strength (Rubio J.C 2005).

M.A. Mills, together with co-authors, studied the distribution of different genotype variants of the ACTN3 gene among athletes and the population. The first was dominated by low-

frequency homozygous XX (7%) mutant alleles. Athletes of genotype XX do not have the α-actin-3 protein in rapidly contracting muscle fibers, and their ability to achieve high results in speed and strength in sports is limited. In addition, athletes of the XX genotype train for a long time. High results in sports are more likely to be achieved by people with RR homozygotes and RX heterozygotes for the ACTN3 gene.

Our research shows that the percentage of RR genotype encounters in athletes is higher than in non-athletes. In particular, the RR genotype was 100% found in both 25 athletes and 25 weightlifters. The RX and XX genotypes had a matching percentage of 0. In non-athletes, 74% had the RR genotype, 24% had the RX genotype, and 2% had the XX genotype.

6. CONCLUSION:

The fast muscle fibers synthesize the protein alpha-actinin-3, which is encoded by the ACTN3 gene. Alpha-actinin-3 stabilizes the contractile apparatus of skeletal muscles and is involved in various metabolic processes.

As a result of nonsense mutation, the codon encoding the amino acid arginine is converted into a stop codon and the synthesis of the polypeptide chain of the protein stops, which entails the absence of alpha-actinin-3 protein in the muscles.

On this basis, it is assumed that the polymorphism of the ACTN3 gene is one of the causes of changes in metabolism (metabolism) in muscle tissue and a decrease in the level of development of speed-strength qualities in humans. But since the function of the alpha-actinin-3 protein can be performed by another protein, alpha-actinin-2, which is also present in fast muscle fibers, no pathology is observed in people with an altered version of the ACTN3 gene.

The main (functional) allele of the ACTN3 gene is designated as R, the minor (more rare and non-functional) allele is designated as X.

The X / X genotype is found in approximately 2% of people and is the only reason for the complete absence of the alpha-actinin-3 protein in their muscles.

The occurrence of R and X alleles in professional athletes of speed-strength sports differ from their distribution in the general population.

Allele R is more often detected in high-class athletes in types that require explosive speed and strength, such as sprint running, weightlifting, etc.

Allele X is predominant in athletes who need endurance to achieve high results, such as marathon runners

7. REFERENCES

[1] Ahmetov I.I. Genes, athlete status and training – An overview / I.I. Ahmetov, V.A. Rogozkin // In: Genetics and Sports, ed.: Collins M. – Medicine and Sport Science.Basel, Karger, V.54. 2009- P.43–71.

[2] I.I. Ahmetov, A.M. Druzhevskaya, I.V. Astratenkova, D.V. Popov, O.L. Vinogradova, V.A. Rogozkin. The *ACTN3* R577X polymorphism in Russian endurance athletes // British Journal of Sports Medicine. V.44. 2010- P.649–652.

[3] Sharp A.J., Locke D.P., McGrath S.D., et al. Segmental duplications and copy-number variation in the human genome //Am J.Hum Genet, vol. 77. 2005-P.78—88.

[4] Wolfarth B.. Bray M.S., Hagberg J.M., et al. The human gene map for performance and health-related fitness phenotypes: the 2004 update // Med. Sci. Sports Ex. vol. 37, №6. 2005- P. 881-903.

[5] Nazarov I.B., Woods D.R., Montgomery H.E., et al. The angiotensin converting enzyme I/D polymorphism in Russian athletes // Eur. J. Hum. Genet, vol. 9. 2001-P. 797-801.

[6] Woods D., Hickman M., Jamshidi Y., et al. Elite swimmers and the D allele of the ACE I/D polymorphism // Hum. Genet, vol. 108. 2011- P. 230-232.

[7] Yang N., MacArthur D.G., Gulbm J.P., et al ACTN3 genotype is associated with human elite athletic performance // Am. J. Hum. Genet, vol. 73, № 3. 2013- P. 627-631.

[8] Rubio J.C., Martin M.A., Rabadan M.. et al. Frequency of the C34T mutation of the AMPD1 gene in world-class endurance athletes: does this mutation impairperformance? // J. Appl. Physiol. vol. 98, № 6. 2005- P. 2108-2112.

[9] Mills M.A., Nan Yang, Weinberger R.P. Differential expression of the actinbindind proteins, a-actinin-2 and-3, in different species: implications for the evolution of functional redundancy // Human Molecular Genetics, vol. 10. № 13. 2011- P. 1335-1346.

[10] Montgomery H., Clarkson P., Bornard M. et al. Angiothensin-converting enzyme gene insertion/deletion polymorphism and response to physical training // Lancet, vol. 53. 2009-P. 541-545.

[11] Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Pérusse L, Bouchard C // Genes and Endurance Performance. 25 NOV 2010-P. 54-59.

[12] Weyand P.G., Davis A.J, Running performance has a structural basis // The Journal of Experimental Biology, 2005- vol. 208- P. 2625-2631.

[13] Chen S, Yan W., Huang J, et al. Peroxisome proliferator-activated receptor-gamma coactivator-1 alpha polymorphism is not associated with essential hypertension and type 2 diabetes mellitus in Chinese population // Hypertens Res, 2004- vol. 27, №11. P. 813-820.

[14] Lucia A., Gomez-Gallego R, Barroso I., et al. PPARGCI Agenotype (Gly482Ser) predicts exceptional endurance capacity in European men. // J. Appl. Physiol, 2005- vol.99, №1. P. 344-348.

[15] Braissant O.. Foufelle R, Scotto C, et al. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, — beta, and —gamma in the adult rat // Endocrinology, 2006- vol. 137. P.354—366.

[16] Morisaki T, Gross M, Morisaki H, Pongratz D, Zöllner N, Holmes EW. Molecular basis of AMP deaminase deficiency in skeletal muscle //Proc Natl Acad Sci U S A. Jul 2012- P. 6457-6461.

[17] Juan C. Rubio, Miguel A. Martrın, Manuel Rabadarn, Frelix Gromez-Gallego, Alejandro F. San Juan. Frequency of the C34T mutation of the AMPD1 gene in world-class endurance athletes: does this mutation impair performance? // J Appl Physiol 98: 2015- P. 2108–2112.

[18] Philip F. Binkley, Alex Auseon, Glen Cooke. A Polymorphism of the Gene Encoding ACTN3: ClinicalImpact and Proposed Mechanisms in Congestive Heart Failure //Congestive Heart Failure. September 21, 2014- P. 274–280.