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Polimorfizm I/D genu ACE i jego związek z siłą mięśni oraz stanem zdrowia osób po 60. roku życia

ACE I/D polymorphism associated with muscle strength and its relation to the health status of people over 60 years of age

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Abstract

Among older people, a big problem is the decrease in both muscle strength and general physical fitness. This often prevents the proper functioning of a person, which makes him or her dependent on their guardians or family. It is believed that the ACE gene is associated with better results achieved in strength sports. It may also be related to the health condition of older people. For this purpose, a study was conducted on a group of 60 people over 60 years of age. The study was conducted with the consent of the bioethical commission and under the supervision of physiotherapists. The relationship

between overall fitness and muscle strength of older people with ACE genotypic variants was checked. For this purpose, a questionnaire on physical activity of IPAQ was used. On its basis, the values of MET and BMI coefficients were determined. Fitness tests were also carried out, i.e. SPPB, DGI and Up & Go. The ACE gene was tested by PCR. The insertion-deletion polymorphism of the Alu sequence in this gene was examined. It was found that: DD gene genotype ACE is associated with higher values of BMI and shorter standing time on the left leg ($p<0.05$), genotype II is associated with lower overall physical activity determined on the basis of MET ($p<0.05$), genotype ID is associated with better results in the left leg test ($p<0.05$). Based on obtained results it is impossible to determine if ACE gene has clear impact on muscle strength and health of the older adults.

Keywords: ACE, older adults, physical performance tests, sport genetics

Streszczenie

Wśród osób starszych dużym problemem jest spadek zarówno siły mięśniowej jak i ogólnej sprawności fizycznej. Często uniemożliwia to prawidłowe funkcjonowanie danej osoby, przez co staje się ona zależna od swoich opiekunów lub rodziny. Uważa się, że gen ACE ma związek z lepszymi wynikami osiąganymi w sportach siłowych. Może być on związany również ze stanem zdrowia osób starszych. W tym celu przeprowadzono badanie na grupie 60 osób po 60. roku życia. Badanie przeprowadzono za zgodą komisji bioetycznej i pod nadzorem fizjoterapeutów. Sprawdzono związek ogólnej sprawności oraz siły mięśniowej osób starszych z wariantami genotypowymi ACE. W tym celu wykorzystano kwestionariusz dotyczący aktywności fizycznej IPAQ. Na jego podstawie określono wartości współczynników MET oraz BMI. Przeprowadzono również testy sprawnościowe, tj. SPPB, DGI oraz *Up&Go*. Gen ACE zbadano metodą PCR. Zbadano polimorfizm insercyjno-delecyjny sekwencji Alu w tym genie. Ustalono, że: genotyp DD genu ACE powiązany jest z wyższymi wartościami współczynnika BMI oraz krótszym czasem stania na nodze lewej ($p<0,05$), genotyp II powiązany jest z niższą ogólną aktywnością fizyczną określoną na podstawie współczynnika MET ($p<0,05$), genotyp ID powiązany jest z lepszymi wynikami w teście stania na lewej nodze ($p<0,05$). Nie udało się określić jednoznacznego związku pomiędzy genem ACE a siłą mięśniową i stanem zdrowia u osób starszych.

Słowa kluczowe: ACE, osoby starsze, testy sprawnościowe, genetyka sportowa

Introduction

The course of aging processes is an individual feature. Different people obtain various level of limitation of movement. It depends on many factors, for example the environment of life, work, lifestyle, completed drug therapies, psychological condition and probably genetics. Genetic factors have a big influence on phenotypic traits as muscle building, motor coordination or flexibility. De Moor et al. determined that the features associated with good performance in various sports disciplines are 66% heritable.[1][2]Physical fitness is not clearly defined and therefore difficult to measure. [3] To assess individual components of physical fitness different tests were developed, among which the most popular is International Physical Activity Questionnaire (IPAQ). Due to the efficiency of functioning in various types of exertion, skeletal muscles are divided into "strength" and "endurance" type muscles. This division is a results from the observation of muscle work in athletes practicing various types of sports. The group of strength sports includes an intensive but short-time effort. This group of sports include short-distance running, short-distance swimming or weight lifting. In contrast, endurance sports requirelong-lasting but less intense effort. This disciplines include running, swimming and long-distance cycling.[4]

ACE

The Angiotensin-Converting Enzyme (ACE) gene belongs to the group of protein coding genes. It is located on the short arm of chromosome 17 (loci: 17q23.3) andits protein product is angiotensin convertase enzyme.[5] The angiotensin convertase is an enzyme belonging to the group of hydrolases (EC 3.4.15.1). It is a metalloenzyme and requires zinc ions (Zn^{2+}) to properly function. The main role of ACE is to regulate blood pressure. Its activity causes the blood vessels to shrink, which leads to an increase in blood pressure.[6]ACE Insertion/Deletion polymorphism (ACE I/D) rs4646994belongs to the group of non-LTR transposons. It is a fragment of 280pz, which in the human genome exists in the form of more than a million copies. These fragments are often expressed under stress conditions and stimulate the translation process. ACE activity is

strongly correlated with the isokinetic and isometric strength of the quadriceps muscle. [7] This effect may depend on ACE-dependent activation growth factor of angiotensin II and the increased degradation of growth inhibitors called bradykinin. Studies have shown that the ACE D allele is associated with higher strength, grater muscle volume and a higher rate of rapidly contracting muscle fibers.[8],[9]Determining whether an ACE gene insertion or deletion is related to muscle strength is a discussing issue. The positive result of the relationship between the presence of the D allele and muscle strength was obtained in 7 studies including 385 athletes in total. Another 7 studies in which 618 athletes were tested showed that this allele was not related to sport predispositions.[4]

Finding the relationship between different gene variants and ailments in old age patients can help reduce severity of those ailments by using the right set of exercises or medications before the discomfort occurs. The aim of this workis to check the association of the ACE gene I/D polymorphism with muscular strength and the impact of its various variants on the physical fitness of people over 60 years of age.

Materials and methods

Subjects

The study was conducted on a group of 60 people over 60 years of age. It was divided into two parts. The first part was the survey in which IPAQ was filled by patients. The second part was fitness tests: one leg standing test, Tinetti test, *Up&Go Test*, Dynamic Gait Index (DGI), Short Physical Performance Battery (SPPB). Due to the older age of the subjects, the tests were carried out under the control of qualified persons. After completing the questionnaire and fitness tests, a buccal swab was taken from each patient using Sigma-Aldrich's Whatman's OmniSwab. The swab was stored for further analysis. Each respondent was informed about the purpose of the research and their anonymity and expressed their consent in writing. The research was carried out with the permission of the relevant bioethical committee.

Genetic analysis

DNA isolation was performed using a DNA extraction kit from biological traces GeneMATRIX Bio-Trace DNA Purification Kit (version 3.1, cat. nr E3510EURx). The ACE gene polymorphism was tested using PCR reactioncarried out according to the

modified Klaus Lindpainter's protocol.[10] The primers used for the reaction were: R 5'- GCC CTG CAG GTG TCT GCA GCA TGT-3' and F 5'-GGA TGG CTC TCC CCG CCT TGT CTC -3' (synthesized by Sigma-Aldrich). Final concentration of reagents: Green GoTaq Flexi Buffer 1x, primer R and F 1 µM, dNTPs 0,2mM, $MgCl_2$ 1,5mM, GoTaq Hot Start polymerase 0,35U/reaction (Promega).The reaction was carried out in a volume of 50µl. Amplification was performed using the GeneAmp 2720 Applied Biosystems thermocycler. The program consisted of 35 cycles of denaturation at 94°C for 30s, annealing at 58°C for 45s, elongation at 72°C for 2min, and final elongation at 72°C for 7 minutes. The results of the PCR reaction were visualized on a 3% agarose gel using TAE buffer (Sigma Life Science).

Results

The list of traits with division into genotypes is presented in Table I.

Table I List of features divided into genotypes. The values in the table are average values for a given feature. The values in brackets are standard deviations.The bold scores are showing statistically important results.

Trait/test	ACE		
	II	ID	DD
Numer of people	9	37	13
Age	77,2 (±5,6)	74,3 (±7,9)	74,5 (±5,4)
BMI	29,1 (±6,56)	28,17 (±4,02)	**30,87 (±4,87)**
SPPB	12,5 (±3,6)	**13,5 (±2,9)**	12,38 (±2,57)
DGI	20,55 (±3,71)	21,38 (±4,02)	20,46 (±3,33)
Tinetti	26 (±1,58)	25,86 (±3,94)	25,38 (±2,53)
Right leg[s]	8,39 (±6,7)	10,24 (±7)	8,78 (±7,5)
Left leg [s]	9,76 (±9,22)	10,45 (±11,47)	**5,19 (±3,68)**
Up&Go [s]	10,53 (±3,75)	10,13 (±4,27)	10,71 (±3,7)
MET	**1531 (±1356)**	4128 (±3763)	6169 (±6130)

Patients with DD genotype hadon average higher BMI than people with different gene variants. We have also observeda difference in the results of the SPPB test - higher

scores were achieved by people with the genotype ID. In the case of standing on the left leg test, people with the DD genotype also achieved lower results. Patients with genotype II were on average less active and achieve lower values of MET.

Discussion

Currently, researchers are seeking to understand the interaction between genes and the relationship between the occurrence and severity of the individual characteristics of an organism and its habitat.[11] A popular subject of research is the identification of relationships between the occurrence of specific gene alleles and the results in strength sports carried out on professional groups of athletes. One of the most frequently studied genes is ACE. The actual relationship between the polymorphisms of this gene and the results achieved in strength sports and mobility in the elderly is a matter of dispute. Papadimitriou et al. carried out research on a group of 101 Greek sprinters. Obtained results did not suggest an association of the ACE gene with the results achieved in sport, although the DD genotypedominated in the group of sprinters in comparison to the control group.[12] The association of the D allele with better results in strength sports was also confirmed by the studies of Costa et al. and Wang et al.[7],[13] Analysis of ACE genotypes showed that the genotype ID is most common in the population studied (62.7%). Genotypes II and DD occur less frequently (15.3% and 22% respectively). A similar distribution of genotypes was obtained by Buford et al. in their studies.[14] The association of ACE genotypes with gender has not been demonstrated. None of the genotypes was significantly more likely to occur in a given group because the ACE gene is not found on the sex chromosome. Lack of relationship with gender is confirmed by studies conducted by Lemes et al. on a group of boys and girls. In the group of subjects, the occurrence of a specific genotype was not found to be different depending on the sex.[15] In the study of links between the BMI index and the ACE gene variant, it was found that in the study group the DD genotype was more common in patients with obesityof I, II and III degree and this was a statistically significant difference($p<0.05$). Similar results have also been published by a group led by J. Ho Kim. The study was conducted on a group of Korean ballerinas. Among subjects, people with a higher weight and a higher percentage of fat in the body more often had the DD genotype ($p<0.05$).[16] The group led by Lemes carried out research on children aged 7-16 with obesity. In this case, the results also suggest the association of the DD gene genotype ACE with increased body mass and a higher fat content.[15] A group of

people with normal weight and overweight more often had the genotype ID. This feature was on the verge of statistical significance ($p = 0.0588$). In this group, the I allele was significantly more frequent (53%), whereas in the obese group it was only 37%. Other studies did not show a significant association of this allele with the BMI index.[15], [16] In the case of SPPB test results, there were no statistically significant differences in the ACE gene variants ($p>0.05$) in our study. Also, no association was found in a study conducted by a group led by Thomas Buford. This study was conducted on a group of 283 people over 60 years of age.[14] The lack of correlation between the results of the SPPB test and the genetic variant of the ACE gene is also confirmed by Delmonico et al. After examining 1367 people aged 70-79, there were no statistically significant differences in the occurrence of genotypes in groups of different efficiencies[17] Carried out MET index analysis suggests that genotype II correlates with less or no activity (lower MET) ($p <0.05$). In a study conducted by Kritchevsky et al., people with genotype II were less active than people with the DD or ID genotype. People with genotype II spend less time on both strength (weight lifting) and endurance (aerobic exercise) sports. The difference was not statistically significant .[18] There was no association between any of the ACE genotypes on the results of the *Up&Go* test. Both in the low-risk group and in the medium-high risk of falling group, the frequency of genotypes and alleles was comparable. Pereira et al. have studied the relationship of ACE genotypes with results of the *Up&Go* test. The research was carried out on a group of women over 57 years old. The association of any of the genotypes with the results achieved in this test was not observed ($p>0.05$).[19] Analysis of the relationship between standing time in the right leg standing test and genotypic variants suggests that the ACE gene is not related to this feature. Checking the correlation between standing time on the left leg standing test and genotypic variants suggests that the genotype ID of the ACE gene is significantly associated with better results, while the DD gene genotype ACE with worse results ($p<0.05$). The relationship between the genotype and the results of the equilibrium tests was also examined (Tinetti, DGI). In this case, none of the features was statistically significant. On the basis of the conducted research it is impossible to clearly determinethe relation of the I/D polymorphism of the ACE gene with sports predispositions.

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