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Faculty of physical education and sport

DIPLOMA THESIS

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Faculty of physical education and sport

Analysis of selected gene variants among athletes – runners at 400m

DIPLOMA THESIS

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Abstrakt

Název: Rozbor vybraných genových variant u atletů – běžců na 400 m

Cíle: Hlavním cílem této práce je zjištění genového profilu u čtvrtkařů pracujícím v laktátovém režimu

Metody: Průřezová studie zahrnovala 203 východoevropských mužů. Výzkum byl prováděn ve spolupráci mezi FTVS UK a Univerzitou tělesné výchovy Józefa Piłsudského ve Varšavě (AWF Varšava). Sportovci se po krátkém zahřátí zúčastnili jednoho Wingate testu trvajícího 30 sekund. DNA vzorky byly zajištěny neinvazivní metodou pomocí výtěru slin. Hladiny laktátu byly měřeny invazivním způsobem.

Výsledky: Ve variantě rs4646994 genu *ACE* byl zaznamenán významný rozdíl v genotypových ($p = 0{,}001$, $x2 = 14{,}90$) a alelických ($p = 0{,}004$, $x2 = 8{,}52$) frekvencích mezi celkovou skupinou sportovců a kontrolními jedinci. Výrazný rozdíl v genu *ACE* byl pozorován při srovnání genotypů ($p = 0{,}001$, $x2 = 13{,}66$) a alel ($p = 0{,}003$, $x2 = 8{,}89$) mezi kontrolní skupinou a elitními sportovci. Poslední statisticky významný rozdíl byl identifikován mezi genotypy ($p = 0{,}03$, $x2 = 6{,}79$) sub elitních sportovců a kontrolní skupiny. Při hodnocení genotypových a alelických frekvencí polymorfismů rs1815739 v genu *ACTN3* jsme nenalezli žádné významné rozdíly, a to ani v genotypové ($p = 0{,}33$, $x2 = 2{,}25$), ani v alelické frekvenci ($p = 0{,}82$, $x2 = 0{,}05$).

Klíčová slova: Genetika, genomika, *ACE, ACTN3*, atleti, čtvrtkaři, vytrvalostní výkon, rychlostně silový výkon

Abstract

Title: Analysis of selected gene variants in athletes – runners at 400 m

Objectives: This work aims to find out what the genetic profile of athletes – runners at 400 meters, who are working in lactate mode, looks like.

Methods: A cross-sectional investigation involved 203 East European Caucasian males and was conducted collaboratively at the FTVS UK and Józef Piłsudski University of Physical Education in Warsaw (AWF Warsaw). The athletes, after a brief warm-up, participated in a single 30-second Wingate test. DNA samples were collected non-invasively through saliva swabs. Lactate levels were measured using an invasive approach.

Results: In the polymorphisms rs4646994 gene *ACE* was found to have a significant difference in genotypic ($p = 0.001$, $x2 = 14.90$) and allelic ($p = 0.004$, $x2 = 8.52$) frequencies between the overall athlete group and the controls. A notable difference in the *ACE* gene showed the comparison of the genotypes ($p = 0.001$, $x2 = 13.66$) and between the alleles ($p = 0.003$, $x2 = 8.89$) between the control group and elite athletes. The last statistically significant difference was observed among the genotypes ($p = 0.03$, $x2 = 6.79$) between the sub-elite athletes and the control group. When assessing the genotypic and allelic frequencies for the polymorphisms rs1815739 in the *ACTN3* gene, we identified no significant differences in either genotypic frequency ($p = 0.33$, $x2 = 2.25$) or allelic frequency ($p = 0.82$, $x2 = 0.05$).

Keywords: Genetic, *ACE, ACTN3,* athletes, runners at 400 meters, endurance performance, power and strength performance

CONTENT

LIST OF ABBREVIATIONS

ACE - angiotensin-converting enzyme

ACTN3 - alpha-actinin 3

ADH - antidiuretic hormone

AnC - anaerobic capacity

ATP - adenosine triphosphate

AWF - Akademie tělesné výchovy Józefa Piłsudského

CMJ - Countermovement Jump

CP - creatine phosphate

D - deletion

DNA - deoxyribonucleic acid

GWAS - Genome-Wide Association Studies

HA - high altitudes

HWE - Hardy-Weinberg Equilibrium

I - insertion

K - potassium

LA - lactic acid

Max - maximum values

Min - minimum values

Na - sodium

Na^+-K^+-ATPase - sodium-potassium-adenosine triphosphate

No. - number

P - p-value

RAAS - renin-angiotensin-aldosterone system

RNA - ribonucleic acid

SD - standard deviation

VO_2max - maximal aerobic capacity

Ø - mean

INTRODUCTION

Throughout human history, the question of whether nature (genetic influence) or nurture (environmental effects) has a greater impact on the human body has been a subject of significant interest. This enduring question has motivated numerous scientific studies, particularly those examining differences among twins or siblings.

Ribas et al. (2020) suggest that genetic factors can contribute to up to 50% of the phenotype characteristics associated with the performance, training, and physical fitness of high-performance athletes. Notably, the Human Genome Project has revealed that humans share approximately 99% of their genetic information, suggesting that the perceived differences among individuals are primarily linked to a small fraction of DNA and its interactions with other factors (Green, 2023). It is widely accepted in the scientific community that both nature and nurture play crucial roles in shaping an individual's characteristics.

Akkoc et al. (2020) have highlighted that sports performance results from a combination of genetics and phenotype characteristics. Furthermore, these characteristics encompass a range of factors, including speed, power, endurance, flexibility, coordination, and psychological traits (Akkoc et al., 2020; Kishita et al., 2020). These aspects hold particular interest to ambitious parents, coaches, and professionals who are invested in the success of athletes, whether they are children or proteges.

The identification of specific genetic polymorphisms, as indicated by previous research, offers insights into predispositions for particular sports. This information not only aids in talent identification but also facilitates the tailoring of training programs and other factors that impact athletic performance.

The identification of these genetic polymorphisms is carried out through genetic tests. Many genetic companies advertise commercial genetic testing for sports predispositions. However, not all of these companies offer recognition of the genes angiotensin-converting enzyme (*ACE)* or α-actinin 3 (*ACTN3*).

In light of these insights, our research centers around two key genes in the field of sports genomics: angiotensin-converting enzyme (*ACE*) and α-actinin 3 (*ACTN3*). Our study aims to profile the genotypes of 400-meter runners and determine whether they are

characterized by a greater potential for speed, as indicated by a higher occurrence of the RR or RS genotype (allele F in *ACTN3*), or if they predominantly exhibit endurance characteristics, reflected by the II genotype in *ACE*.

THEORETICAL PART

This master's thesis explores the pivotal role of two genes in sports genomics: angiotensin-converting enzyme (*ACE*) and α-actinin 3 (*ACTN3*). These genetic factors significantly influence an athlete's performance. The *ACE (Angiotensin-Converting Enzyme*) and *ACTN3 (A-actinin 3*) genes are extensively studied and tested in the field of sports genomics (Williams et al., 2016). The *ACE* gene is associated with advantages in endurance exercises, making it particularly relevant for endurance sports such as long-distance running or cycling (Ahmad Yusof & Che Muhamed, 2021; Petr, 2015). In contrast, the *ACTN3* gene confers benefits for strength and speed-oriented sports, providing advantages in activities that require explosive power and speed, such as sprinting and jumping (John et al., 2020; Wei, 2021).

The *ACE* gene is a crucial component of the renin-angiotensin-aldosterone system (RAAS), which regulates factors like the body's water balance, blood pressure, and mineral excretion by the kidneys, including sodium and potassium levels. The *ACE* gene influences these processes through the angiotensin-converting enzyme pathway (Petr, 2015).

On the other hand, the *α-actinin 3 (ACTN3*) gene encodes a structural protein found in the Z-zone of the sarcomere, anchoring myofibrillar actin filaments within the sarcomere (Petr, 2015). ACTN3 plays a crucial role in generating sarcomeric force and, in conjunction with other proteins, regulates both contractional and signaling processes within human skeletal muscles (Baltazar-Martins et al., 2020; Petr, 2015).

However, the crucial determinant of an athlete's success lies at the intersection of genetic predisposition and environmental influences. Research involving Olympic champions suggests that their superiority over fellow elite athletes is attributed to heightened levels of intrinsic motivation, determination, dedication, persistence, and creativity (Issurin, 2017). These extraordinary expressions of personality traits have the potential to be accurately identified even during the early phases of their training regimen (Issurin, 2017). Notably, gifted individuals tend to accelerate learning and a significant increase in their athletic prowess. Additionally, prospective champions were distinguished by their exceptional approach to training, combined with a readiness to undertake more extensive and carefully designed training protocols (Issurin, 2017).

Moreover, the theoretical part of this thesis consists of four chapters. The initial chapter explains key terminology associated with genetics. Subsequently, the third chapter focuses on the physiological aspects of athletes competing in the 400-meter event. The fourth chapter delves into the renin-angiotensin-aldosterone system to enhance understanding of the *ACE* gene's role. Lastly, the concluding chapter examines the explored genes *ACE* and *ACTN3* and their impact on the human body and sports performance.

1.1 Genetic terms

1.1.1 Genetic and genomics

Although genetics as a science is relatively young, its origins can be traced back to ancient times. Throughout history, humanity has been accompanied by questions about the variability among individuals and the heritability of traits within all groups. As mentioned earlier, genetics is a science focused on individual genes and their heritability. These genes have profound effects on various aspects of human bodies, influencing biomechanical pathways and determining characteristics such as eye and hair colour (Petr, 2015).

A more recent scientific approach is genomics. The term "genomics" was first used by Hansen Winkler in 1920 to refer to every gene in germ cells (Yadav, 2007). The primary subject of genomics is the genome, which encompasses the entire genetic information in DNA. It represents the complete set of all genes within one body (Green, 2023). Once the genome is known, the next step is understanding the phenotype, which refers to the expression of all genes within an individual. This determines how and if the genes manifest themselves. These pieces of information lead to the realization that the significant difference between genetics and genomics is that genomics aims to understand the entire genome, whereas genetics explores individual genes (Petr, 2015).

1.1.2 Gene and DNA

Deoxyribonucleic Acid (DNA) consists of four distinct chemical bases, represented by the letters A, C, G, and T within the human genome (Green, 2023). Genes are specific segments of DNA that carry instructions for the production of particular proteins or the regulation of specific functions within an organism. They are the fundamental units of inheritance, transferred from parents to offspring, and play a crucial role in determining physical and biological traits by synthesis of proteins (Collins, 2009).

According to the Green (2023), "most genes code for specific proteins, or segments of proteins, which have differing functions within the body." Humans possess approximately 20,000 protein-coding genes (Green, 2023).
Interestingly, all of the information for the entire 20,000 protein-coding genes is encoded by a mere 1.5% of the entire human genome (Green, 2023).

1.1.3 Genotype

While discussing genomics and heredity, it is important to notice two concepts that play a significant role in genetics and biology. These two concepts are genotype and phenotype.

According to the (Adams, 2023) the genotype is defined as „*the entire genetic makeup of an individual, including all of its genes and DNA variations"*. An individual's genotype is determined by the combination of genes inherited from its parents and plays an essential role in shaping their traits (Adams, 2023).

A better understanding of the genotype is fundamental in various fields, such as medicine, for improving health and developing new treatments for genetic disorders (Venter et al., 2022). It also has a crucial role in determining the sports performance of athletes, evolving new training methods, and preventing injuries (Collins, 2009). Massidda et al. mentioned in their study (2019) that football players with XX genotype in the *ACTN3* gene had a 2.66 higher chance for an injury prevalence than their *ACTN3* RR counterparts.

Numerous research studies have shown that certain genes can influence an athlete's endurance, speed, and strength, among other factors (John et al., 2020; Niemi & Majamaa, 2005). According to John et al. (2020) and Niemi & Majamaa (2005)

strength/power and sprinting ability are associated with the gene *ACTN3*. On the other hand, endurance performance is highly linked with the gene *ACE*, which has a significant influence on regulating blood pressure (Ahmad Yusof & Che Muhamed, 2021; Petr, 2015).

1.1.4 Phenotype

Phenotype, as opposed to genotype, refers to the observable physical and behavioural traits resulting from the interaction of an individual's genes and environment (Pierce, 2017). It involves the expression of the genotype under the influence of the environment. As underscored in prior discussions, an athlete's actual performance, or phenotype, is highly influenced by a range of factors beyond their genetic makeup. These include environmental factors such as training, coaching, nutrition, and recovery, as well as psychological factors like motivation and mindset (Gucciardi et al., 2012).

An athlete's muscle fiber type, a phenotype trait, can be influenced by their training regime. Research has shown that endurance training can lead to an increase in the proportion of slow-twitch muscle fibers, while power training can lead to an increase in the proportion of fast-twitch muscle fibers (Bassett et al., 2000). Therefore, optimizing an athlete's muscle fiber type can be achieved by tailoring the training to their specific needs (Bassett et al., 2000).

In a similar vein, an athlete's phenotype is intricately linked to various factors, notably their nutritional status, which profoundly affects body composition, energy levels, and overall health (Burke et al., 2018). Emphasizing the pivotal role of nutrition in sports performance, Morton et al. (2017) underscore the significance of optimizing an athlete's nutritional intake to enhance both physical and mental prowess. Moreover, an athlete's phenotype extends beyond the physical realm, encompassing the influence of their mental state on aspects such as focus, motivation, and resilience during training and competitions (Jones et al., 2005). Notably, a study by Stambulova (2019) found that athletes exhibiting high levels of grit and perseverance are more likely to achieve success in their respective sports.

The comprehensive understanding of an athlete's phenotype, in conjunction with their genotype, serves as a crucial foundation for developing personalized training

programs aimed at optimizing performance. As highlighted by Ribas et al. (2020), genetic factors can account for up to 50% of phenotypic characteristics related to elite athletes' performance, training, and physical fitness. This underscores the intricate interplay between genetic predispositions and environmental influences in shaping the multifaceted aspects of athletic achievement.

1.1.5 Heredity and heritability

According to Downes & Matthews (2022), heredity is a process responsible for the transmission of traits from one generation to the next while heritability is a measurement of genetic influence on variation. Heritability is typically expressed as a value between 0 % to 100 % (Downes & Matthews, 2022).

The percentage effect of heredity versus environment on athletic performance is a topic of ongoing debate and research. Both genetics and the environment are acknowledged as significant contributors to athletic ability and success, but quantifying their specific impacts remains challenging. Collins (2009) highlighted the substantial role of genetics in various aspects of athletic performance, encompassing strength, power, endurance, muscle fiber size and composition, flexibility, neuromuscular coordination, temperament, and other characteristics. Recent studies propose that genetic factors may explain up to 50% of the variation in sports performance, while ethnicity, lifestyle, and environmental factors like training, nutrition, and mindset could account for the remaining 50% or potentially even more (Ribas et al., 2020). Wang et al. (2016a) noticed that the heritability of athletic status, trainability, and exercise behaviour is predicted to be 66, 47, and 62 % respectively. Most research quantifying the effects of heredity and environment relies on familial studies, often involving twins or siblings.

As pointed out by Quijada (2016), several studies have investigated the heritability of particular physical characteristics or fitness parameters to determine the probability of certain traits that are essential for athletic performance being passed down through genetics.

In Quijada's study (2016), we find an intellectually stimulating table of the inheritance level of different functional and conditional parameters.

Table 1: Inheritance level of different functional and conditional parameters, adaptation of García Manso & Campos Granell (2003)

Parameter	**Heritability**	**Author of the Study**
Maximum consumption of O_2	93 % 79 % 77 % 73 %	Klissouras (1971) Shawartz (1972) Venerando (1973) Sergienko (1975)
Aerobic productivity	70 – 80 %	Platonov & Fesenko (1984)
Lactate production	81 %	Klissouras (1976)
Anaerobic productivity	70 – 80 % 90 – 99 %	Platonov & Fesenko (1984) Svart (1972); Margaria (1973)
Maximum heart rate	86 – 97 %	Klissouras (1976)
Endomorphy	69 %	Kovar (1980)
Mesomorphy	88 %	Kovar (1980)
Ectomorphy	87 %	Kovar (1980)
Simple reaction time	70 – 80 %	Platonov & Fesenko (1984)
Maximum static force	50 – 60 %	Platonov & Fesenko (1984)
Flexibility	91 %	Sergienko (1975)
Coordination	40 – 50 %	Platonov & Fesenko (1984)

However, according to Maes et al. (1996), the range of heritability for performance-related fitness is estimated between 23-74%, while the range of heritability for health-related fitness is higher (51-90%). Cerit et al. (2020) mentioned that under ideal circumstances, the heritability was found to be 48% in flexibility, 32% in grip strength, 41% in sit-ups, and 52% in push-ups, with sit-ups and push-ups representing muscular endurance. Another study by Puthucheary et al. (2011) mentioned that *"genetic factors account for approximately 50-80% of inter-individual variation in lean body mass, with impacts detected on both 'training-naive' muscle mass and its growth response".*

Likewise, it is important to note that the yet-mentioned estimates can vary depending on the sport and individual factors, as well as the complex ways in which genetic

and environmental factors can interact to influence athletic performance. As Quijada (2016) stated: *"The future of research in sports identification, training, and coaching calls for an approach that stresses the interaction between genes, the process of sports training, and environmental, nutritional, and psychological factors*".

1.1.6 Environment

Environment interaction is a great topic in current genetic studies. The environment can influence an individual's traits, development, and gene expression, playing a crucial role in shaping an organism's phenotype.

Research findings suggest that athletes develop their personality, performance, and sports achievements under the influence of environmental factors, especially during their childhood and adolescence (Trninic, et al., 2018).

The data related to Olympic champions submit that their superiority over their fellow elite athletes can be attributed to elevated levels of intrinsic motivation, determination, dedication, persistence, and creativity (Issurin, 2017). These extraordinary expressions of personality traits have the potential to be accurately identified even during the early phases of their training regimen. Moreover, according to Issurin (2017), exceptionally gifted individuals showed a remarkable tendency for accelerated learning and demonstrated a notable increase in their athletic prowess. Furthermore, prospective champions were distinguished by their exceptional approach toward training, coupled with a readiness to undertake more extensive and carefully created training protocols (Issurin, 2017).

As mentioned earlier environmental factors contain a wide range of influences, including nutrition, exposure to toxins, physical activity, climate and social interactions. These factors can affect gene expression, mutation rates, and overall health. According to Sandoval-Motta et al., (2017), these facts are also closely associated with the "missing heritability" problem. This problem asserts that the genetic variations identified in Genome-Wide Association Studies (GWAS) are insufficient to completely account for the heritability of complex traits (Sandoval-Motta et al., 2017). Gene-environment interaction is one of the potential candidates for unveiling the missing heritability problem. Other potential candidates include gene-gene interactions, rare genetic variants,

epigenetic modifications, RNA influences, overestimations of heritability, small-effect genetic variants, and limitations in GWAS, among others (Sandoval-Motta et al., 2017).

A study by Sandoval-Motta et al. (2017) also focuses on the genetic and functional diversity of the microbiome as a factor related to the existing gap between heritability measured by GWAS and familial studies.

Nevertheless, while the significance of this subject is widely acknowledged, the quantity of studies addressing it is very limited (Ueki et al., 2021).

1.2 400 meters run

The “diaulos”, also known as race around two marks was one of the original events of the Ancient Olympics. However the 400m or “one-lap race” has its place in modern Olympic games since 1896 (*400 Metres*, 2021).

The 400-meter race, classified as a “sprint distance”, is widely known for its demanding speed endurance challenges in athletics. Elite sprinters, as highlighted by Batra & Krzyszkowski (2020), exhibit the ability to apply greater force to the ground, resulting in increased stride lengths, quicker stride frequencies, and ultimately, faster sprint times compared to less experienced sprinters. This suggests that athletes competing in the 400-meter race may possess enhanced strength-speed abilities.

Currently, the men's 400-meter record is held by Wayde van Niekerk, achieved at the 2016 Olympic Games in Rio de Janeiro. He completed the 400 meters in 43.03 seconds, securing the gold medal (*400 Metres*, 2021). On the women's side, Marita Koch set a record by winning the 400-meter gold medal at the IAAF World Cup in Canberra, Australia, in 1985 with a time of 47.60 seconds (*400 Metres*, 2021). However, until 1983, the women's 400-meter record was held by Jarmila Kratochvílová, who completed the race in 47.99 seconds (*400 Metres*, 2021).

1.3 Physiology and energetic threshold of athletes at 400 meters

The 400-meter race, classified as a “sprint distance”, is widely recognized as one of the most challenging speed endurance events in athletics. Its distinctiveness doesn't just arise from the extended distance beyond the typical sprint length but also from the unique energy system employed by athletes during the sprint. As highlighted by Batra & Krzyszkowski (2020), the 400m run necessitates the utilization of both aerobic and anaerobic, encompassing alactic and lactic mechanisms, to generate the required energy. This demand places a premium on a high level of anaerobic glycolysis, buffering capacity, and aerobic processes, essential for sustaining peak velocity throughout the race (Batra & Krzyszkowski, 2020).

Furthermore, the 400-meter race introduces specific demands on an athlete's neuromuscular and technical skills, differentiating it significantly from the challenges posed by the 100-meter and 200-meter races (Batra & Krzyszkowski, 2020). This nuanced understanding of the physiological intricacies and skill requirements underscores the race's complexity, making it imperative for athletes, coaches, and researchers to comprehend the multifaceted nature of this distinguished sprint distance.

1.3.1 Anaerobic alactic (ATP – CP) system

The anaerobic alactic system is supported by the presence of stored adenosine triphosphate (ATP) and creatine phosphate (CP). The foundational basis for physical exertion within the alactic zone resides in the activation of rapid glycolytic fibers present in skeletal muscles. Prevalent in individuals specializing in sprinting, these specific fibers play a pivotal role in enabling high-intensity contractions. It is essential to note that their dominance also contributes to the propensity for rapid fatigue (Havlíčková, 1999).

Contraction correlates with a consumption of adenosine triphosphate (ATP). The majority of energy is used for the movement of actin and myosin filaments. (Kittnar, 2011)

ATP is a nucleotide compound composed of adenosine and three phosphates. It is extracted from the sarcoplasm, where its concentration reaches approximately 4 mmol/l. It maintains its concentration in muscles for one to two seconds coinciding with

the initial sprint of an athlete from the starting block. Hence, there is a need for a constant resynthesis of ATP within the muscle through other sources, such as carbohydrate and lipid metabolism (Kittnar, 2011). To sustain further concentration, additional sources of energy are used.

Firstly, creatine phosphate (CP) serves as an available reserve of energy. CP is a phosphorylated form of creatine, a molecule present in muscles with slightly higher energy content than ATP. When ATP levels decrease rapidly, CP quickly provides energy for muscle contraction by transferring its phosphate group to adenosine diphosphate (ADP) and converting it to ATP (Kittnar, 2011).

Because the amount of CP is only about 5 times greater than the amount of ATP, it can sustain energy production for approximately 10 seconds which is important for the initial sprint (Kittnar, 2011). However, even this energy source needs to be substituted.

1.3.2 Lactic system

During physical exertion of submaximal intensity lasting 45-90 seconds, or in longer activities with insufficient oxygen supply, the predominant energy source is the lactate non-oxidative (anaerobic) system. This system is characterized by an increase in the concentration of lactic acid and its salts in the bloodstream as a result of anaerobic glycolysis (Havlíčková, 1999).

The system operates by breaking down muscle glycogen, resulting in the production of ATP. In the lactate threshold zone, the rate of ATP production is half as fast as in the alactic metabolic phase. As a result, this leads to a decrease in the intensity of physical activity, which is also linked to the presence of released lactic acid (LA) (Havlíčková, 1999).

The representative indicator of the organism's anaerobic lactate capacity is the concentration of LA in the bloodstream (Havlíčková, 1999).

1.3.3 Anaerobic glycolysis

Anaerobic glycolysis plays a significant role in meeting the energy demands of athletes during the 400-meter event. It is one of the primary mechanisms of energy production during high-intensity, short-duration activities. This system primarily relies on the breakdown of muscle glycogen into glucose. When glucose levels decrease, glucose, glycogen undergoes glycogenolysis to produce glucose (Kittnar, 2011).
Subsequently, glucose enters glycolysis, where it is converted into pyruvate while producing adenosine triphosphate (ATP). Glycolysis maintains efficient concentrations of both creatine phosphate and ATP directly through the production of pyruvate and lactic acid (Kittnar, 2011).

The advantage of glycolysis lies in its ability to occur even without sufficient oxygenation of the muscle tissue, i.e., if muscle effort persists for longer than 10 seconds but comes at the expense of accumulating an oxygen debt.

It's important to note that lactic acid is a byproduct of glycolysis and can accumulate during intense exercise. This accumulation contributes to muscle fatigue and the athlete's perception of exertion. The final accumulation of pyruvate and lactic acid leads to the acidification of the sarcoplasm, resulting in a depletion of capacity after one minute (Kittnar, 2011).

1.3.4 Oxidative system

Athletes at 400m use the aerobic energy system to support their performance. This oxidative metabolism is the only possible final energy source for ATP synthesis (Kittnar, 2011).

In shorter muscle performances lasting 2 - 4 hours, the main source of energy acquisition is carbohydrates (initially glucose from the bloodstream and later glucose from muscle glycogen). However, in the case of extremely prolonged muscle work and gradual depletion of carbohydrates, fatty acids play a significant role in energy transformation. After finishing intense muscle exertion, it becomes essential to break down accumulated metabolites, especially lactic acid. This process requires an adequate amount of oxygen (Kittnar, 2011).

Fats possess a high energy content. The burning of 1 kg of fat yields 37.7 kJ for the body, twice the energy produced by burning carbohydrates. However, energy production from fats demands more oxygen. Using 1 liter of oxygen, fat combustion yields only 19.7 kJ, approximately 8% less than the energy produced during ATP synthesis from carbohydrates. When the same amount of oxygen is used, less energy is released during fat utilization, leading to a lower level of physical stress (Havlíčková, 1999).

According to Arcelli, et al. (2008), sprint-type athletes (such as those who excel in the 200m) rely more on anaerobic mechanisms compared to athletes with higher aerobic capabilities due to genetics or training. The study also noted that women tend to utilize the aerobic mechanism more than men. Interestingly, in this study, none of the athletes reached their maximum value of VO_2max, and the VO_2max slightly decreased towards the end of the race (Arcelli et al., 2008).

Another study, by Plevnik (2013), investigated physiological factors among male and female athletes and declared that male athletes showed a statistically significant variation in the parameters used to assess aerobic energy capacity, favoring them over female athletes. Additionally, male sprinters exhibited significantly higher levels of anaerobic capacity (5.7 km/h) (Plevnik, 2013).

However, there is a difference between runners specialized in 400m flat and 400m hurdles. The study by Zouhal et al. (2010) mentioned that the calculated aerobic contribution exhibited a notable increase during the 400m hurdles in contrast to the 400m flat (43.0 ± 2.0% versus 37.4 ± 2.7%, $p < 0.05$, respectively). These findings indicate that the aerobic contribution is higher in a 400m hurdles race than in a 400m flat race.

1.3.5 Renin-angiotensin-aldosterone system

The renin-angiotensin-aldosterone system (RAAS) is a system of hormones, proteins, enzymes, and reactions that regulate blood pressure, fluid volume, and electrolyte balance (Kittnar, 2011; Zhang et al., 2017). The history of the RAAS began with the discovery of renin by Robert Tigerstedt in 1897 when he first demonstrated that an extract from the renal cortex of rabbits increases systemic blood pressure when injected intravenously into recipient rabbits (Tsukamoto & Kitakaze, 2013).

The renin-angiotensin-aldosterone system (RAAS) is one of the leading regulators of homeostasis. This system is associated with the water economy of the body, blood pressure, and excreted minerals by the kidneys. Its function begins by releasing the enzyme renin in the bloodstream when the blood flow in the kidneys starts to decrease. During the next step, renin splits angiotensinogen into angiotensin I, which is by angiotensin-converting enzyme (ACE) split into angiotensin II (Kittnar, 2011). Angiotensin II leads to vasoconstriction of the vessels and helps to release aldosterone and antidiuretic hormone (ADH). Antidiuretic hormone induces the resorption of water in the kidneys (Kittnar, 2011). Aldosterone causes the excreting of potassium and together with antidiuretic hormone induces retaining of sodium. (*Renin-Angiotensin-Aldosterone System (RAAS)*, n.d.; Kittnar, 2011)

1.3.6 Renin

Renin is a hormone with a quality enzyme made by the cortex of the adrenal gland. It is activated in situations when the body suffers from low blood pressure or low blood flow rate. Renin splits angiotensinogen during the formation of angiotensin I. Angiotensin I is converted by angiotensin-converting enzyme (ACE) into angiotensin II which ensures vasoconstriction of vessels. (Kittnar, 2011)

1.3.7 Angiotensin II

Angiotensin II is a peptide with a very powerful vasoconstrictive effect on the vessels, particularly arterioles that leads to increased blood pressure (hypertension). Together with sympaticus activate adrenaline that decreases the blood flow rate in the kidney and so reduces the glomerular filtration and its surface. Another of angiotensin II's tasks is to release the catecholamines and aldosterone and allow the back resorption of sodium (Na^+) and water in the proximal tubule. (Kittnar, 2011)

1.3.8 Aldosterone

Aldosterone is a hormone created by the cortex of an adrenal gland. By Kittnar (2011) its function is to stimulate the resorption of sodium (Na^+) in Henley's loop and in the distal tubule and collection duct where it supports the secretion of potassium (K^+) ions. It also increases the number of Na and K canals and Na^+-K^+-ATPase pumps. Aldosterone reacts particularly to increased concentrations of angiotensin II and potassium. In summary, the main duty of aldosterone is to maintain the regulation of potassium ions and the volume of extracellular liquid through the renin-angiotensin-aldosterone system (RAAS), which allows the back resorption of sodium and water in situations with decreased volume of plasma. (Kittnar, 2011)

1.4 Explored "sports genes"

The fact that humans share 99% of their genetic information according to the human genome project suggests that the differences between individuals are mainly caused by a small percentage of DNA and other factors interacting with it. Human DNA has about 35,000 genes, out of which more than 200 are believed to be directly linked to athletic performance, and the number is still increasing. (Green, 2023; Williams, et al., 2016) A study by Bray et al. (2009) suggests that the number of genes connected to athletic performance is around 230. Additionally, Williams & Folland (2008) identified 23 genetic variations associated with a high level of endurance sport performance, and the probability of all these variations occurring in one individual is 1 in 20 million.

Power/strength and endurance phenotypes can have the same candidate genes. It is based on the assumption that the alleles disadvantageous for endurance can be advantageous for power-strength-orientated phenotypes (Petr, 2015, str. 42).

According to Williams et al. (2016), the R577X variant in the gene *ACTN3* is the most commonly tested. Based on this knowledge, *ACTN3* R577X is currently the polymorphism with the strongest scientific evidence supporting its association with athlete phenotypes. Wang et al. (2016a), mentioned that among the genes investigated, the angiotensin-1-converting enzyme insertion/deletion (*ACE* I/D) polymorphism and the α-actinin-3 (*ACTN3*) R577X polymorphism have received the most comprehensive

attention. Overall, these genetic variations have consistently shown associations with elite endurance (*ACE* I allele) and sprint performance (*ACTN3* R allele) (Wang et al., 2016a).

1.4.1 Gene *ACE*

The *ACE* (*Angiotensin-Converting Enzyme*) gene is a crucial component of the renin-angiotensin-aldosterone system (RAAS), which plays a pivotal role in regulating the body's water balance, blood pressure, and the excretion of minerals by the kidneys. This gene directly influences these processes through the action of the angiotensin-converting enzyme, responsible for constricting blood vessels. Consequently, genetic variations within the *ACE* gene have been closely linked to the performance and physiological state of elite athletes (Petr, 2015).

According to Orysiak et al. (2017), an insertion/deletion (I/D) in intron 16 is the most studied polymorphism of gene *ACE* which is characterized by the presence (I allele) or absence (D allele) of a 287 bp Alu repeat element (rs4646994). The I allele initiates lower enzyme activity, reducing skeletal muscle vasoconstriction, while the D allele is associated with higher ACE activity and increased angiotensin II levels, leading to skeletal muscle hypertrophy (Ahmad Yusof & Che Muhamed, 2021; Eider et al., 2013). This results in three possible combinations: II, ID, or DD. Individuals with the II genotype have the lowest levels of the ACE enzyme among all mentioned genotypes. The ID genotype, with one insertion and one deletion allele, exhibits intermediate levels of the ACE enzyme, while individuals with the DD genotype possess the highest levels of the ACE enzyme in their bloodstream.

Several studies confirm that II polymorphism is generally associated with endurance performance while the D allele seems to be presented more amongst power and strength-orientated athletes (Ahmad Yusof & Che Muhamed, 2021). Another study by Moran et al. (2006) presented that II homozygotes performed the best of all polymorphisms. This result also confirms a study by Ma et al. from year 2013.

A study by Eider et al. (2013) investigated 100 Polish power athletes and found that the D allele was overrepresented among 'elite' and 'top elite' athletes (68.1% and 77.8%, respectively), whereas among 'sub-elite' athletes, it had the lowest frequency (53.3%). However, in this study, they found a significant positive association between top-level Polish power athletes and the *ACE* D allele (Eider et al., 2013).

Nevertheless, some studies, like the one conducted by Orysiak et al. (2017), have shown that *ACE* may not significantly influence power and strength performance among elite athletes. Additionally, several studies found no prevalence of the D allele (or a low proportion of the II genotype) in endurance-oriented athletes when compared to control groups (Ahmetov & Fedotovskaya, 2015). Last but not least, Ahmetov & Fedotovskaya (2015) mentioned that Japanese endurance runners with the DD or ID genotypes had significantly higher average running speeds than those with the II genotype.

ACE also plays significant roles in the physiology and pathophysiology of individuals living at high altitudes (HA) and the development of HA sickness. These functions of *ACE* are likely achieved through its effects on blood pressure regulation by generating the vasoconstrictor angiotensin II and reducing the production of the vasodilator bradykinin. (Wang et al., 2016b)

The research findings, reported by Wang et al. (2016b), indicated that the frequency of the DD genotype in individuals at high altitudes (0.31) was significantly lower compared to those living at lower altitudes (0.52). Therefore, possessing a lower frequency of the characteristic DD genotype of the *ACE* I/D polymorphism in a population could be advantageous for high-altitude residents, potentially enhancing their ability to adapt to hypoxic conditions at elevated altitudes. The authors concluded that the DD genotype could be considered a risk factor for maladaptation to high altitudes. (Wang, et al., 2016b)

In addition to performance factors in sports, *ACE* is also associated with several health issues, including heart problems. As mentioned earlier, *ACE* plays a role in converting Angiotensin I to Angiotensin II, which is implicated in the pathophysiological process of left ventricle hypertrophy, independent of hemodynamic factors. (Mocan et al., 2021) *ACE* accelerates the progression of cardiac fibrosis and the increase in left ventricle mass. Consequently, hypertensive individuals with the DD allele, homozygous carriers, face an elevated risk of left ventricle hypertrophy and ischemic heart disease. It is important to note that the extent of this increased risk varies depending on geographical location and race, with Caucasians being most susceptible. (Mocan et al., 2021) Moreover, athletes with the DD genotype and normal blood pressure experience a more significant increase in heart muscle mass compared to athletes with the I/I genotype. The I/D *ACE* polymorphisms are associated with an increase in left ventricle mass in both hypertensive and normotensive individuals, with the D allele being most commonly linked to left ventricle hypertrophy. (Mocan et al., 2021)

Another study by Di Mauro et al. (2010) evaluated the role of *ACE* on left ventricular hypertrophy amongst healthy male endurance athletes. In this research, they found a significant association between left ventricular mass index and DD polymorphism than among the ID polymorphism ($p = 0.29$). Also, group DD showed a higher prevalence of left ventricular hypertrophy (62.9%) than group ID (44.4%). The last discovery was that subjects with left ventricular hypertrophy had longer left ventricular isovolumetric relaxation time and higher end-systolic wall stress, which is responsible for early impairment of left ventricle filling. (Di Mauro et al., 2010) That would mean that DD polymorphism has a greater risk of heart damage.

1.4.2 Gene *ACTN3*

On the other side, the gene *ACTN3* (*A-actinin 3*) is a structural protein that occurs in the Z-zone of the sarcomere to anchor the myofibrillar actin filaments with sarcomere (Petr, 2015). The key role of *ACTN3* is the production of sarcomeric force, in combination with other proteins it regulates contractional and signalizing processes in human skeletal muscles (Baltazar-Martins et al., 2020). The *ACTN3* gene encodes the protein α-actinin-3, which is mostly expressed in the sarcomere of the fast glycolytic type II fibers (Ma et al., 2013). In the gene, *ACTN3* is distinguished by two alleles. First arginine-coding codon (R allele) and termination codon (X allele) (Wei, 2021). A common stop-codon polymorphism (rs1815739; R577X) in the gene *ACTN3* produces individuals with the XX genotype responsible for the lack of expression of a functional α-actinin-3 and also individuals with the R-allele (i.e., RX vs. RR genotypes), polymorphism that can express α-actinin-3 (Baltazar-Martins et al., 2020). According to Petr et al. (2015), the muscle fibers with α-actinin-3 are capable of faster contraction, higher absorption, and transport of force potential in Z-lines. Another study by Baltazar-Martins et al. (2020) describes the *ACTN3* gene as fundamental for the production of forceful contraction and fast and explosive movements.

Many studies confirm that the R allele is advantageous in power sports and is overrepresented among power/strength-oriented athletes (Ahmetov & Fedotovskaya, 2015; John et al., 2020; Petr, 2015; Petr et al., 2022; Wei, 2021). According to Ahmetov & Fedotovskaya (2015), 69.3% of Polish power-oriented athletes possess the R allele, while controls have a frequency of 59.6%. On the other hand, the X allele is associated

with lower muscle strength and lower sprinting ability (Baltazar-Martins et al., 2020; John et al., 2020; Petr, 2015). However, the findings regarding the X allele have led many scientists to a presumption that it may be advantageous for endurance sports. Although numerous studies have reported on the *ACTN3* gene, no association between the *ACTN3* R577X polymorphism and endurance athletes has been observed (Ahmetov & Fedotovskaya, 2015).

As mentioned earlier, there are many studies confirming findings that the R allele is over-represented in power/strength-orientated athletes (John et al., 2020; Wei, 2021). Some studies report no significant association between the R allele and power/strength and sprint-orientated athletes (Ma et al., 2013; Pasqualetti et al., 2022).

According to Wei (2021), the *ACE* ID and *ACTN3* RR genotype is associated with higher VO2 max that reflects good endurance and explosive force among soccer players. However, some studies, like the study by Orysiak et al. (2017), demonstrate no influential role of *ACE* and *ACTN3* in power/strength performance among elite athletes.

The gene *ACTN3* is also associated with higher risks for some injuries linked to deficiency or high levels of α-actinin-3. One cross-sectional experimental study Gutierrez-Hellin et al. (2021) investigates the influence of *ACTN3* R577X polymorphism in the injury epidemiology amongst elite endurance athletes. In this research, it was observed that elite endurance runners with the RR genotype had a higher injury rate (3.2 injuries/1000 h of running) compared to those with the RX (2.0 injuries/1000 h of running) and XX genotype (2.2 injuries/1000 h of running; $p = 0.030$) (Gutiérrez-Hellín et al., 2021). Additionally, in this study by Gutierrez-Hellin et al. (2021) they found that RR runners were more likely to experience injuries in the Achilles tendon, while RX runners tended to have knee injuries, and the XX genotype showed a higher proportion of injuries located in the groin ($p = 0.025$). However, no significant association was observed between the *ACTN3* genotype and the severity of the injury, the distribution of training and competition injuries, the proportion of recurrent injuries, or the mode of onset (Gutiérrez-Hellín et al., 2021)

On the other hand, Baltazar-Martins et al. (2020) reported that approximately 18% of the world population possesses the XX genotype in the *ACTN3* R577X polymorphism, which can negatively impact sports performance through structural, metabolic, or signaling alterations.

The study by Ahmetov & Fedotovskaya (2015) observed that the *ACTN3* R allele was associated with high levels of testosterone in male and female athletes. They also

suggested that connection might explain the association between the *ACTN3* RR genotype, skeletal muscle hypertrophy, and power athlete status. Furthermore, they presume that the deficiency in α-actinin-3 has an unfavourable impact on the power aspect of competitive performance among endurance athletes. (Ahmetov & Fedotovskaya, 2015)

1.5 Commercial genetic testing

Hand in hand with genetic research, genetic companies, and laboratories are advancing. The popularity of genetic testing is on the rise, with consumers expressing a growing interest in their genetic information (Patel & Varley, 2019). As a result, laboratories are expanding globally, this expansion has led to an increasing demand for genetic counselors (Wolff & Wolff, 2018).

According to Williams et al. (2016), the most commonly tested polymorphism R577X in the *ACTN3* gene was examined by 89% of 18 companies, and the gene *ACE* was tested by 61% of the companies. The Society of Medical Genetics and Genomics has identified only one laboratory (Genomac výzkumný ústav, s.r.o.) that independently examines this gene. (SLG.Cz, n.d.) Additionally, two other institutes test this gene as part of a panel of several genes in the Czech Republic. It is important to note, that nine laboratories in the Czech Republic test the *ACE* gene, and ten organizations offer it as part of a panel of several genes. (SLG.Cz, n.d.)

However, Williams et al. (2016) caution that while genetic tests are becoming more prevalent, their predictive power for various variables is limited. They emphasized that current genetic tests and analysis methods may not provide sufficient information to make significant decisions in sports.

Furthermore, more than half of the genetic tests commercially available for exercise and sports fail to publicly disclose the genetic variants they assess, preventing scrutiny by academic scholars and consumers (Williams et al., 2016). The scientific community is expressing concern that the existing level of knowledge is being subtly misrepresented for commercial motives (Webborn et al., 2015). Furthermore, Collins and September (2023) mentioned that due to the uncertainty surrounding the biological and clinical relevance of genetic markers, and based on existing evidence, it is premature to endorse

any commercial genetic test for determining susceptibility to exercise-associated musculoskeletal injuries.

METHODOLOGY

1.6 Type of the study

The investigation is methodically designed as a cross-sectional study, an observational research approach celebrated for its meticulous data collection methodology. This design is characterized by its thorough gathering of data, extending beyond a representative subset to encompass the entirety of the population under examination at a specific, singular point in time.

The comprehensive research initiative unfold through a collaborative partnership with the distinguished Józef Piłsudski University of Physical Education in Warsaw, commonly abbreviated as AWF Warsaw.

1.7 Research problem

The genetic factors influencing performance in the 400-meter race, renowned for its demanding speed endurance challenges in athletics, remain unclear. This study aims to address this gap by profiling the genotypes of 400-meter runners, with a specific focus on the *ACE* and *ACTN3* genes. The primary objective is to discern whether these athletes show a predisposition toward speed, indicated by a higher occurrence of the RR or RS genotype (allele R in *ACTN3*), or if their characteristics predominantly align with endurance, as reflected by the II genotype in *ACE*. The outcomes of this research may have the potential to provide valuable insights into the genetic influences shaping athletic performance in the 400 meters race.

1.8 Aim of the study

The study aims to compare genotypic and allelic frequencies for the polymorphisms rs4646994 in the *ACE* gene and rs1815739 in the *ACTN3* gene. This comparison is made between three distinct groups: elite and sub-elite quarter-mile runners, as well as a control group.

1.9 Research question

1. Question

Is there a significant difference ($p < 0,05$) in the genotypic or allelic frequencies for the polymorphism rs4646994 in the gene *ACE* among the groups of elite athletes, sub-elite athletes, and controls?

2. Question

Is there a significant difference ($p < 0,05$) in the genotypic or allelic frequencies for the polymorphism rs1815739 in the gene *ACTN3* among the groups of elite athletes, sub-elite athletes, and controls?

1.10 Research hypothesis

1. Hypothesis

There will be a significant difference ($p < 0.05$) in the genotypic or allelic frequencies for the polymorphisms rs4646994 in the gene *ACE* among the groups of elite athletes, sub-elite athletes, and controls.

2. Hypothesis

There will be a significant difference ($p < 0.05$) in the genotypic or allelic frequencies for the polymorphism rs1815739 in the *ACTN3* gene among the groups of elite athletes, sub-elite athletes, and controls.

1.11 Subjects

From official statistics provided by the Czech Athletics Federation (ČAS), 157 active athletes specializing in middle-distance and endurance disciplines met the designated performance criteria for the 400m run. Subsequently, 23 athletes were excluded from consideration due to their non-participation in races during the summer season.

Therefore, the research sample was composed of 103 East European Caucasian males who were active members of the Czech Athletics Federation (ČAS) and had demonstrated exceptional performance in the 400m run. The study cohort included ice hockey players, athletes competing in 200-400m races (including hurdles), and MMA fighters, providing a diverse representation of sports. These individuals had an average age of 20.2 ± 3.3 years, an average body weight of 73.8 ± 7.8 kg, and an average height of 182.5 ± 5.9 cm.

The athletes were classified into two groups based on their best 400m run times. Athletes classified as elite were chosen by considering the average times of the best 100 Czech 400m runners between 2018 and 2021. The selection process was informed by the continually updated tables for the specific seasons, as recorded in the official statistics of the Czech Athletics Federation (ČAS). These tables have consistently tracked the top 100 athletes each season since 2018 and can be accessed at www.atletika.cz.

Table 2: Times of the best 100 athletes in the Czech Republic in the 400 m run

SEASON	1. PLACE (s)	100. PLACE(s)	Ø 1.-100. PLACE
2018	45,59	50,85	49,40
2019	46,37	50,61	49,30
2020	46,00	50,65	49,32
2021	45,41	50,67	49,23
Ø	45,84	**50,70**	49,31
SD	0,43	0,11	0,07
max	46,37	50,85	49,40
min	45,41	50,61	49,23

The elite athlete group comprised 52 participants, with an average age of 21.2 ± 3.4 years, average body weight of 75.1 ± 8.5 kg, and average height of 182.6 ± 6.6 cm. Additionally, 47 athletes were selected for the sub-elite group, which had an average age of 19 ± 2.8 years, average body weight of 72.3 ± 6.6 kg, and average height of 182.5 ± 5.1 cm

To compare allele and genotype frequencies, a control group (n=100; average age 20.5 ± 1.2; average body weight 77.5 ± 9.47 kg; average height 181.5 ± 6.35 cm) was selected. This group consisted of physically active individuals who were not engaged in speed and strength sports. Furthermore, they had not participated in highly intense competitions or training in the last 72 hours and had not experienced any injuries in the past 3 months.

Table 3: Profile of the tested sample.

n = 103	**AGE (years)**	**Height(cm)**	**Body weight (kg)**	**Fat (%)**	**Lean body mass (kg)**	**Time at 400 m (s)**
Ø	20.2	182.5	73.8	9.20	66.17	49.68
SD	3.3	5.93	7.8	3.10	8.75	1.56
max	33	196	94.50	16.2	80.5	53.94
min	16	168.5	57.40	1.9	5.3	45.73

The selected athletes, along with their coaches, were personally contacted and invited to participate in the study. In the case of athletes under 18 years old, their legal guardians were also contacted. After this initial screening, a total of 91 athletes were included in the study. Each participant provided signed informed consent, as documented in the attached forms (Informed Consent - Adults; Informed Consent - Minors).

The experiment took place during August, September, and October 2021, at the laboratory in the Faculty of Physical Education and Sport Charles University (FTVS UK). This time frame coincided with the later stages of the summer athletic season and the immediate post-competition period, during which high athletic performance was anticipated.

The testing protocol remained the same for every participant. Ethical approval (attached in the appendices) from the Ethics Committee at FTVS UK was obtained,

necessitating informed consent for genetic analysis from all participants. A fundamental project declaration stated that the genetic analysis results would be kept strictly confidential, with disclosure limited to the examined individuals and the project investigators. Detailed information was provided to each proband about the genetic examination process. Their informed consent confirmed that the results of their genetic analysis would be utilized for statistical assessment within the study and for the anonymous presentation of findings in publications.

1.11.1 Inclusion criteria

- Active participation in races in the current season
- At least 4 running training sessions per week
- Attendance at 80% of club training sessions
- Personal record in the 400m run below 50.70 seconds

1.11.2 Execution criteria

- High-intensity competition or training in the last 72 hours
- Injuries in the last 3 months
- An invalid medical examination according to the regulation of the Ministry of Health of the Czech Republic No. 391/2013 Coll. on medical fitness for physical education and sports

1.12 Research design

The research was structured as a cross-sectional investigation and was conducted at the FTVS UK and in collaboration with the Józef Piłsudski University of Physical Education in Warsaw (AWF Warsaw).

Participants who met the predetermined criteria were included in the research.

The first step involved participants completing a survey questionnaire, which is attached in the appendices.

Next, DNA samples were obtained using a non-invasive buccal swab from the oral cavity during the testing days at the FTVS UK laboratory. The swabbing procedure was conducted under the supervision of a trained individual using specialized collection kits, specifically the Copan-Flocked Swabs (Interpath, Australia). These kits are equipped with a sampling brush and a tube, following a standardized protocol outlined in the manufacturer's instructions. To ensure the highest sample quality, rinsing the oral cavity with water was essential before collection. After rinsing, the participant opened the tube and swabbed the buccal mucosa with a rotating motion for approximately 15 seconds. Following the successful collection of an adequate sample, the brush was securely reinserted into the tube, which was subsequently sealed.

All samples were meticulously labelled and stored in a freezing device until transportation to the laboratory. The analysis and preservation of genetic material (saliva swabs) were performed by the laboratory of the Institute of Biology and Medical Genetics, 1st Faculty of Medicine, Charles University, as part of their long-standing collaboration.

Following this, all participants underwent an initial screening of lactate levels. Lactate samples were collected from the fingertip or earlobe through a puncture performed by a trained professional at specified intervals. The collection of capillary blood, subsequent analysis, and proper removal of biological samples were all conducted under standard conditions. Samples were collected at various time points throughout the testing procedure: immediately upon arrival at the laboratory, after a 2-minute warm-up on the cycle ergometer, in the 3rd minute after completing the 1WT, immediately after completing the 2WT, and subsequently at the 3rd, 6th, 9th, 20th, and 30th minute following the 2WT. Lactate level analysis was performed using a Biosen C-line Clinic device (EKF Diagnostic, Barleden, Germany) with a 20 µl capillary blood sample obtained using an end-to-end capillary and transferred into a micro test tube containing a haemolytic solution.

Finally, it's important to note that the device was properly calibrated before initiating the analysis.

After the screening of lactate levels, participants underwent measurements of anthropometric characteristics, including height, weight, and body composition. Body height was measured using a portable wall-mounted anthropometer A213 with an accuracy of 0.5 cm. Body weight and composition were assessed using a Tanita BC 418 body composition analyser. Bioelectrical impedance analysis was conducted

at least three hours after eating and without engaging in intense exercise before the measurement. The obtained body weight data, measured with a precision of 0.1 kg, was used to calculate individual resistance during WT30.

Subsequently, a warm-up exercise was conducted, which included two Squat Jumps (SJ), two Countermovement Jumps (CMJ), and five maximal jumps from two different positions with arms positioned at the sides. After these dynamic stretching exercises, the participants were ready for evaluations of their jumping ability. The evaluation consisted of two types of vertical jumps. The first was the Squat Jump, initiated from a static stance with a knee angle set at 90° (measured with a manual goniometer), with no additional lowering of the body's center of gravity permitted before the jump. The second was the Countermovement Jump (CMJ), which involved a dynamic descent of the center of gravity, allowing for the utilization of muscle pretension before the actual jump.

To measure explosive strength, we used the Kistler Multicomponent Force Plate Type 9286B. These force plates were placed on a flat and stable surface and calibrated before each use. The signals recorded from these plates were captured using BioWare software (Kistler, Winterthur, Switzerland) and subsequently processed with MATLAB software (Mathworks, United States).

For assessing anaerobic capabilities, the athletes participated in a single 30-second Wingate test to exhaustion (WT30) using a stationary bicycle ergometer after a brief warm-up. The primary parameters of interest were maximum power output and anaerobic capacity. Maximum power was determined by analyzing the best 1-second test (peak power per second). The absolute value of maximum power (Pmax) indicates an individual's strength-speed abilities and indirectly reflects muscle fiber typology (Heller, 2018).

Another measured parameter was anaerobic capacity (AnC), quantifying the total anaerobic work performed, expressed in both absolute terms (kJ) and relative terms (J/kg). This measure aligns with the assumptions related to strength-speed endurance. The repetition of these tests during the second round offers insights into an individual's ability to recover quickly after a maximal anaerobic workload and assesses their tolerance to anaerobic fatigue and the accumulation of metabolic by-products in the body.

The WT30 test was conducted on a calibrated friction-loaded cycle ergometer (Monark 894E Peak bike, MONARK, Sweden) connected to a microcomputer. The test involved two maximal 30-second sprints against constant resistance, adjusted according to the subject's body weight (0.075 kg/kg body weight). A four-minute rest interval

separated the first and second WT30 sprints. The test began with a flying start, with participants exerting maximum effort against minimal resistance. When a cadence of 120 revolutions per minute was achieved, the test officially started as the resistance basket was dropped, indicating the braking force. Before the test started, participants were instructed to pedal as rapidly as possible for 30 seconds.

The research took place from August to October 2021, aligning with the late summer athletic competition season or the immediate post-competition period. Participants were advised to avoid intensive training or competitive activities for three days before the testing sessions and to maintain their regular dietary routines. The last meal before testing was to be consumed 2-3 hours prior, and while fluid intake was not restricted, excessive consumption was discouraged.

The Czech research received approval from the Ethical Committee of the UK Faculty of Physical Education and Sport on November 13, 2018, under file number 232/2018.

The Polish research was approved by the Committee for Bioethics in the Regional Medical Chamber in Gdańsk, with resolution number KB-2/21 issued on February 3, 2021.

1.13 Statistical analysis

The statistical analysis of the data employed the chi-square test, a method designed to discern associations between two categorical (nominal) variables. The chi-square test belongs to nonparametric statistics, a methodology that doesn't assume data adherence to predefined models with a limited number of parameters. Nominal data, where categories are mutually exclusive, is the focus of this statistical approach. In this methodology, ratios are examined to determine their statistical significance. The observed ratios, representing the collected data, are then compared against proportions expected if no differences existed between the groups, aligning with the concept of the null hypothesis. (Connelly, 2019)

The chi-square test was used to assess the frequencies of genotypes and alleles and all analyses were conducted at a significance level of $p < 0.05$.

The last employed method for the statistical evaluation of the data was the Hardy-Weinberg Equilibrium (HWE). This test is commonly used for random samples of unrelated individuals. (Graffelman, 2010) It identifies genotyping errors in large-scale

genotyping investigations. (Graffelman & Weir, 2016) Divergences from the Hardy-Weinberg equilibrium (HWE) may signal inbreeding, population stratification, and potential issues in genotyping. Within samples of affected individuals, these variations can additionally serve as indications of association. Assessments of HWE typically involve the application of a straightforward $\chi 2$ goodness-of-fit test. (Wigginton et al., 2005) It is important to note that the critical values for chi-square tests can vary based on the degrees of freedom and the chosen significance level.

Therefore, when considering the critical value for a chi-square test with a significance level of 0.05 and one degree of freedom, it was set at approximately 3.84.

RESULTS

The control group exhibited a distribution of 2 % for genotype II, 59 % for ID, and 39 % for DD in the polymorphisms rs4646994 of the *ACE* gene. This corresponds to 31.5 % I alleles and 68.5 % D alleles among the control group participants.

In contrast, the overall athlete's group (comprising both elite and sub-elite) consisted of 2.9 % genotype II, 32.0 % ID, and 65.0 % DD. This resulted in 18.9 % I and 81.1 % D alleles.

Breaking it down, elite athletes showed 1.8 % genotype II, 28.6 % ID, and 69.6 % DD. Hence 16.1 % I alleles and 83.9 % D alleles.

On the other hand, the sub-elite group had 4.3 % II genotype, 36.2 % ID, and 59.6 % DD, aligning with 22.3 % I alleles and 77.7 % D alleles.

Table 4: Genotype and allele frequencies in the gene *ACE* among the groups

	II	ID	DD	Overall I	Overall D
All athletes	3	33	67	39	167
Sub elite	2	17	28	21	73
Elite	1	16	39	18	94
Controls	2	59	39	63	137

Table 5: Genotype and allele frequencies in the gene *ACE* among the groups in %

	II	ID	DD	Overall I	Overall D
All athletes	2.9 %	32.0 %	65.1 %	18.9 %	81.1 %
Sub elite	4.3 %	36.2 %	59.6 %	22.3 %	77.7 %
Elite	1.8 %	28.6 %	69.6 %	16.1 %	83.9 %
Controls	2 %	59 %	39 %	31.5 %	68.5 %

The distribution of genotypes and alleles in the rs1815739 polymorphism of the *ACTN3* gene varied between the control group and the overall athlete group, encompassing both elite and sub-elite athletes. The control group comprised 39% RR, 49% RX, and 12% XX genotypes, resulting in 63.5% R alleles and 36.5% X alleles.
Additionally, the overall athlete group demonstrated a distribution of 35.9% RR, 57.3% RX, and 6.8% XX genotypes, corresponding to 64.6% R and 35.4% X alleles.
Among elite athletes, the distribution was 39.3% RR, 53.6% RX, and 7.1% XX genotypes, with 66.1% R and 33.9% X alleles.
However, the sub-elite group exhibited a distribution of 31.9% RR genotype, 61.7% RX, and 6.4% XX, aligning with 62.8% R alleles and 37.2% X alleles.

Table 6: Genotype and allele frequencies in the gene *ACTN3* among the groups

	RR	RX	XX	Overall R	Overall X
All athletes	37	59	7	133	73
Sub elite	15	29	3	59	35
Elite	22	30	4	74	38
Controls	39	49	12	127	73

Table 7: Genotype and allele frequencies in the gene *ACTN3* among the groups in %

	RR	RX	XX	Overall R	Overall X
All athletes	35.9 %	57.3 %	6.8 %	64.6 %	35.4 %
Sub elite	31.9 %	61.7 %	6.4 %	62.8 %	37.2 %
Elite	39.3 %	53.6 %	7.1 %	66.1 %	33.9 %
Controls	39 %	49 %	12 %	63.5 %	36.5 %

For the statistical analysis of the data among the groups, we employed the chi-square test. This test revealed a notable difference between the overall athlete group and the controls in terms of genotypic ($p = 0.001$, $x^2 = 14.90$) and allelic ($p = 0.004$, $x^2 = 8.52$) frequencies for the polymorphisms rs4646994 in the gene *ACE*. Additionally, a significant difference in the *ACE* gene was found among the genotypes ($p = 0.001$,

$x^2 = 13.66$) and between the alleles ($p = 0.003$, $x^2 = 8.89$) when comparing the control group and elite athletes. In the gene *ACE*, a statistically significant difference was observed among the genotypes ($p = 0.03$, $x^2 = 6.79$) between the sub-elite athletes and the control group. However, the chi-square test indicated no substantial variation among the alleles ($p = 0.11$, $x^2 = 2.63$) in this gene. There was no significant difference observed between the elite and sub-elite groups in the *ACE* gene, neither among the genotypes ($p = 0.50$, $x^2 = 1.39$) nor between the alleles ($p = 0.25$, $x^2 = 1.31$).

Table 8: p-value among the groups in the gene *ACE*

Gene ACE	p value among genotypes	p value among alleles
All athletes vs. Controls	0.001	0.004
Sub elite vs. Controls	0.03	0.11
Elite vs. Controls	0.001	0.003
Elite vs. Sub elite	0.50	0.25

When assessing the genotypic and allelic frequencies for the polymorphisms rs1815739 in the *ACTN3* gene, we identified no significant differences in either genotypic frequency ($p = 0.33$, $x^2 = 2.25$) or allelic frequency ($p = 0.82$, $x^2 = 0.05$) between the overall athlete group and the control group. Similarly, the comparison between the control group and elite athletes yielded comparable results, with no substantial variation observed in either genotypes ($p = 0.61$, $x^2 = 0.98$) or alleles ($p = 0.65$, $x^2 = 0.21$). Furthermore, no significant differences were observed between the sub-elite athletes and the controls in the *ACTN3* gene, neither among the genotypes ($p = 0.30$, $x^2 = 2.40$) nor between the alleles ($p = 0.90$, $x^2 = 0.02$). Finally, when comparing the elite and sub-elite groups, no significant differences in the polymorphisms rs1815739 in the *ACTN3* gene were found among the genotypes ($p = 0.70$, $x^2 = 0.70$) or between the alleles ($p = 0.62$, $x^2 = 0.24$).

Table 9: p-value among the groups in the gene *ACTN3*

Gene ACTN3	p value among genotypes	p value among alleles
All athletes vs. Controls	0.33	0.82
Sub elite vs. Controls	0.30	0.90
Elite vs. Controls	0.61	0.65
Elite vs. Sub elite	0.70	0.62

Last but not least, the Hardy-Weinberg Equilibrium was utilized to assess genetic variation among groups and populations. Firstly, we applied it to determine the frequencies of alleles and genotypes in the *ACE* and *ACTN3* genes within the entire athlete group. Secondly, we employed the same approach with the control group.

In our analysis of the entire group of Czech athletes, we applied the Hardy-Weinberg Equilibrium to assess the rs4646994 polymorphism in the *ACE* gene. The obtained p-value of 0.66 and a χ^2 value of 0.20 suggested a concordance between observed and expected frequencies of alleles and genotypes. This outcome reinforced the notion that our research sample effectively represents the broader population.

However, when examining the rs1815739 polymorphism in the *ACTN3* gene within the same athlete group, we observed a p-value of 0.01 and a χ^2 value of 6.53. The p-value of 0.01, being less than the conventional threshold of 0.05, indicated a significant difference between the frequencies of alleles and genotypes, in which an endurance foundation is necessary for elite performance. Therefore, a higher frequency of the endurance-form gene can be observed.

In summary, our findings affirmed the representativeness of the Czech athlete population in the context of the *ACE* gene's rs4646994 polymorphism. Conversely, the observed differences in the *ACTN3* gene's rs1815739 polymorphism underscored the impact of deliberate selection from specific disciplines, contributing to a refined understanding of genetic variations within this athlete cohort.

The p-value for the rs4646994 polymorphism in the *ACE* gene within the control group was 0.00, indicating a significant difference between observed and expected frequencies of alleles and genotypes. The χ^2 value of 13.48 further supported the evidence of departure from the Hardy-Weinberg Equilibrium.

Regarding the rs1815739 polymorphism in the *ACTN3* gene among the control group, a p-value of 0.57 suggested that there might not have been a significant difference between observed and expected frequencies. The χ^2 value of 0.33, while low, supported the conclusion from the p-value, indicating no strong evidence of departure from the Hardy-Weinberg Equilibrium.

Table 10: p-value and χ^2 value in the gene *ACE*

Gene ACE	p value	χ^2 value
All athletes	0.33	0.82
Controls	0.00	13.48

Table 11: p-value and χ^2 value in the gene *ACTN3*

Gene ACTN3	p value	χ^2 value
All athletes	0.66	0.20
Controls	0.57	0.33

DISCUSSION

The 400-meter race is widely acknowledged as one of the most demanding speed endurance events in athletics. Its challenge doesn't just stem from the extended distance, surpassing the typical sprint length, but also from the distinctive energy system athletes engage during the sprint. Genetics and genomics play pivotal roles in influencing various processes within organisms. They not only impact human development but also determine an individual's energy system and genetic predispositions. These genetic predispositions wield significant influence over an individual. They can determine susceptibility to genetic diseases, propensity for muscle injuries, or whether an individual possesses exceptional talent for endurance or speed-strength sports. Especially genes *ACE* and *ACTN3* are associated with athletic performance. Studies suggests that endurance genotype is linked to the gene *ACE* rs4646994 polymorphism (Ipekoglu et al., 2022; Ahmad Yusof & Che Muhamed, 2021). Primarily the II genotype can be advantageous for the endurance athletes. Studies by Moran et al. (2006) and Ma et al. (2013) presents that II homozygotes performed the best of all polymorphisms. In our study, only 3 (2.9 %) out of 203 athletes exhibited the II genotype for the *ACE* gene polymorphism rs4646994, while the majority had 67 (65.1 %) DD and 33 (32.0 %) ID genotypes. In the control group of 200 probands, there were 2 (2 %) II genotypes and 39 (39 %) DD genotypes. It's important to note that the distribution of the *ACE* gene and its genotypes may vary across ethnicities, as highlighted in a study by Ahmad Yusof & Che Muhamed (2021), which reported that the D allele is most prevalent in the Caucasian population, with a frequency of 0.77. Additionally, Papadimitriou et al. (2016) reported that Caucasian sprinters with the *ACE* DD genotype achieved a faster best 400-meter sprint time compared to their counterparts with the *ACE* II genotype (46.94 ± 1.19 s vs. 48.50 ± 1.07 s, $p = 0.003$).

On the other hand the polymorphism rs1815739 in the *ACTN3* gene is associated with speed-strength sports. Many studies confirm that the R allele is advantageous in power sports and is overrepresented among power/strength-oriented athletes (Ahmetov & Fedotovskaya, 2015; John et al., 2020; Petr, 2015; Petr et al., 2022). According to Wei (2021), the *ACE* ID and *ACTN3* RR genotype is associated with higher VO2 max that reflects good endurance and explosive force among soccer players. In our study the overall athlete group demonstrated a distribution of 37 (35.9 %) RR, 59 (57.3 %) RX, and 7 (6.8 %) XX genotypes, while the arrangement among elite athletes was 22 (39.3 %)

RR, 30 (53.6 %) RX, and 4 (7.1 %) XX genotypes. The control group comprised 39 (39 %) RR, 49 (49 %) RX, and 12 (12 %) XX genotypes.

This study aimed to compare genotypic and allelic frequencies for the polymorphism rs1815739 in the *ACTN3* gene and polymorphism rs4646994 in the *ACE* gene among elite and sub-elite quarter-mile runners, as well as a control group. The objective was to investigate whether the cohort predominantly possesses an endurance genotype or a speed-strength genotype.

The results of the rs4646994 polymorphisms in the *ACE* gene revealed statistically significant differences between the overall athlete group and the control group in terms of genotypic ($p = 0.001$, $x2 = 14.90$) and allelic ($p = 0.004$, $x2 = 8.52$) frequencies. Significant differences in genotypes ($p = 0.001$, $x2 = 13.66$) and alleles ($p = 0.003$, $x2 = 8.89$) were also observed when comparing the control group with elite athletes. Likewise, a significant difference in genotypes ($p = 0.03$, $x2 = 6.79$) emerged between sub-elite athletes and the control group. These findings align with outcomes from some other studies.

Eider et al. (2013) discovered a significant association between top-level Polish power athletes and the *ACE* D allele. Akkoc et al. (2020) observed a higher prevalence of the D allele than the I allele among mid-long distance runners (67% and 33%, respectively) but an equal distribution among sprinters (50% and 50%). Another study noted a significant difference in genotypes (II vs. ID + DD: $p = 0.001$) in the *ACE* gene between endurance runners and controls (Ipekoglu et al., 2022).

However, no statistically significant differences were observed among alleles ($p = 0.11$, $x2 = 2.63$) between the groups. Furthermore, there were no significant differences among genotypes ($p = 0.50$, $x2 = 1.39$) or alleles ($p = 0.25$, $x2 = 1.31$) when comparing elite and sub-elite athletes. This result might be influenced by the fact that the difference in performance between certain elite and sub-elite athletes in our study is only a matter of a few seconds. Notably, studies such as the one conducted by Orysiak et al. (2017) have indicated that *ACE* may not significantly influence power and strength performance in elite athletes.

Even though some studies, such as the ones by Petr et al. (2022) and Wei (2021), confirm that the R allele in the *ACTN3* gene is advantageous in power sports and is overrepresented among power/strength-oriented and speed-oriented athletes, we found no significant differences when assessing genotypic and allelic frequencies between overall athletes and controls. Similar results were observed in comparisons between controls and elite athletes, as well as among other groups. Thus, only the first hypothesis we can confirmed. The second hypothesis we must rejected based on the obtained results.

These findings may implicate the outcome of the Hardy-Weinberg Equilibrium (HWE) test, signalling a notable disparity in allele and genotype frequencies within the group of all athletes while the HWE test of the genotype frequencies in the controls showed no significant difference. Our results align with some studies that report no significant association between the R allele and power/strength and sprint-oriented athletes (Ma et al., 2013; Pasqualetti et al., 2022). Similarly, Orysiak et al. (2017) demonstrate no influential role of ACTN3 in power/strength performance among elite athletes. Additionally, Cankli et al. (2022) found no significant association in RR and RX genotypes between the athletes and the controls. Finally, Ribas et al. (2020) noticed no statistically significant difference in *ACTN3* among the genotypes ($p > 0.05$) between the athletes and the controls, while the HWE of the genotype showed no difference from the values observed in the general population ($p = 0.45$).

Nevertheless, while our study identified significant differences exclusively in the *ACE* gene, the commercial market encompasses numerous laboratories primarily specializing in the examination of the *ACTN3* gene. Williams et al. (2016) highlighted that the most frequently evaluated polymorphism, R577X, in the *ACTN3* gene was scrutinized by 89% of 18 companies, with the *ACE* gene ranking as the second most commonly tested gene.

The scientific community is expressing concern that the existing level of knowledge is being subtly misrepresented for commercial motives (Webborn et al., 2015). Even the genetic testing can be useful to predict the risk of injury. Using genetic testing to detect injury risks among elite middle/long-distance runners can be premature (Gutiérrez-Hellín et al., 2021). Nevertheless, Williams et al. (2016) warn that despite the increasing prevalence of genetic tests, their capacity to predict various variables is limited.

Also according to Gutiérrez-Hellín et al. (2021) the primary aim of sports genomics should not solely focus on conclusively determining an individual's genetic

predispositions for athletic performance (talent identification). Rather, the basic goal should be tailoring the training process and other associated lifestyle factors to suit each individual.

Considering the obtained results, it is necessary to note that, the expression of genes and genotypes is not always entirely clear as their manifestation. In this study we observed only two commonly tested genes linked to athletic performance. The uncertain expression of the genes is influenced by various factors, including environmental conditions, lifestyle, diet, and other genetic interactions. The combination of multiple genes can play a pivotal role in determining how genes are expressed and what the resulting phenotype of an individual will be.

1.14 Limits of the study

Although the sample size was considerable, there are some notable aspects. Firstly, even the control group comprised of 100 participants, it was comparatively smaller than the group of all athletes (103). Secondly, the Hardy-Weinberg Equilibrium detected a significant difference between observed and expected frequencies of alleles and genotypes for the rs4646994 polymorphism in the *ACE* gene within the control group. The χ^2 value, while low, supported the conclusion from the p-value, indicating no strong evidence of departure from the HWE when comparing the *ACTN3* genotypes within the same group. These outcomes raised doubts about whether the research sample accurately represents the broader population.

CONCLUSION

The research sample was composed of 103 East European Caucasian males demonstrating exceptional performance in the 400m run. On the other side the control group (n = 100) consisted of physically active individuals who were not engaged in speed and strength sports.

The highest statistically significant difference was noticed when comparing the elite athletes and the control group in terms of genotypic ($p = 0.001$, $x^2 = 13.66$) and allelic ($p = 0.003$, $x^2 = 8.89$) frequencies for the polymorphisms rs4646994 in the gene ACE. We observed a statistically significant difference in genotypes and alleles when comparing the controls with overall athletes and genotypes between sub-elite athletes and the control group. However, the chi-square test indicated no substantial variation among the alleles between this group. There was no significant difference observed between the elite and sub-elite groups in the ACE gene, neither among the genotypes nor between the alleles. Based on these obtained results we confirmed the first hypothesis.

The results of the genotypic and allelic frequencies for the polymorphisms rs1815739 in the ACTN3 gene showed no significant differences between overall athletes and controls, as well among the other groups.

While we made every effort to construct this research diligently, it is important to acknowledge certain limitations. However, we hope that future studies can overcome these challenges.

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APPENDICES

1.15 The survey questionnaire

Dotazník

A. Osobní data			
Jméno			
Email		Telefon	
Datum narození		Age	
Výška [cm]		pohlaví	□ Muž □ Žena
Hmotnost [kg]			
Etnická skupina	□ Afričan/Černoch □ Běloch □ Indian □ Smíšený původ □ Asiat □ Jiné		
Etnický původ	Otec:		□ Neznám
	Matka:		□ Neznám
Země narození			

B. Sportovní detaily			
Zapište sportovní aktivity dle pořadí jejich důležitosti			
Typ sportovních a pohybových aktivit	Hlavní sport 1	Jiný sport 2	Jiný sport 3
Doba systematického tréninku (v letech)			
Množství tréninkových jednotek týdně			
Délka jedné tréninkové jednotky			
Soutěžil jsi v této sezóně?	□ ANO □ NE		
Kolik tréninků máš v týdnu?			
	200 m		400 m
Jaký je váš osobní rekord na 200 and 400 metrů?			
Jaké je vaše letošní maximum na 200 and 400 metrů?			
Kolik klubových tréninků jsi se letos zúčastnil?	□ Méně než 80% □ 80% a více		
Měl jsi za posledních 72 hodin intenzivní trénink nebo závod?	□ Ano □ NE		
Zranění za poslední 3 měsíce	□ Ano □ NE Pokud ano, jaká? ……		

Máte platnou sportovní prohlídku?	□ Ano □ NE

E. Zdravotní historie		
Trpíte některými z následujících zdravotních komplikací?		
□ Arteriální hypertenze □ Dušnost □ Neoplastická nemoc Jiné …… …..	□ Kardiovaskulární choroby □ Artritida □ Zvýšený cholesterol □ Diabetes □ Poruchy funkce ledvin	□ Astma □ Osteoporóza □ Poruchy nadledvinek □ Thyroidální poruchy □ Amyloidosis

Máte aktuální revmatoidní onemocnění nebo onemocnění pojivové tkáně?	□ ANO □ NE Pokud ano, jaká? ……
Prodělal jste COVID 19?	□ ANO □ NE Pokud ano, datum infekce ……
Jste vakcinován proti COVID 19?	□ YES □ NO Typ vakcíny: První dávka, datum …….. Druhá dávka, datum……..

1.16 The ethics committee's approval

UNIVERZITA KARLOVA
FAKULTA TĚLESNÉ VÝCHOVY A SPORTU
José Martího 31, 162 52 Praha 6-Veleslavín

Žádost o vyjádření Etické komise UK FTVS

K projektu výzkumné, kvalifikační či seminární práce zahrnující lidské účastníky

Název projektu: Genetické faktory ovlivňující utilizaci laktátu během zatížení

Forma projektu: výzkumná práce

Období realizace: leden 2019 – prosinec 2020

Předkladatel: Pavlína Vostatková, Mgr.

Hlavní řešitel: Pavlína Vostatková, Mgr.

Místo výzkumu (pracoviště): katedra fyziologie a biochemie

Spoluřešitel (é): Miroslav Petr, doc. PhDr., Ph.D., Zdeněk Ledvina, Valdemar Moska

Vedoucí práce (v případě studentské práce): ----

Finanční podpora: Grantová agentura Univerzity Karlovy (GAUK)

Popis projektu: Projekt je experimentální studií, jež je zaměřen na posouzení vlivu polymorfismů v genech kódujících monokarboxylové transportéry MCT1 a MCT4, které se významně podílejí na distribuci laktátu v lidských tkáních včetně kosterního svalu. K použitým metodám patří laboratorní zátěžové testy a analytické molekulárně-genetické metodiky vedoucí k určení příslušných genotypů. Konkrétně budou u vybrané skupiny vytrvalostních sportovců testovány následující genové varianty: *MCT1* 1470T>A (rs1049434), *MCT1* 2197C>T (rs7169), *MCT4* 44C>T (Ala15Val), *MCT4* 55G>A (Gly19Ser), *MCT4* 574G>A (Val192Met), *MCT4* 916G>A (Gly306Ser), *MCT4* 641C>T (Ser214Phe) a další. Cílem předkládaného projektu je ověřit pomocí testu anaerobních schopností WT30, zda se jednotlivé polymorfismy v genech, které ovlivňují utilizaci laktátu během zatížení, projevují rozdílně na hladinách laktátu po intenzivním zatížení.
Charakteristika účastníků výzkumu: Sportovci zahrnutí do testované kohorty (n = 60) jsou v užším výběru reprezentace (hokejisté, atleti 200-400 m včetně překážek, MMA bojovníci s výkonností širší reprezentace). Každý ze sportovců podstupuje každoroční povinnou lékařskou prohlídku u sportovního lékaře. V případě nedoporučení lékaře, proband nebude zařazen do studie. Věk probandů se pohybuje od 15 do 30 let. Do projektu nemůže být zařazen proband, který bude mít zranění či akutní onemocnění nebo proband s jakýmkoliv onemocněním či omezením pohybového aparátu ani s kardiovaskulárním onemocněním.
Zajištění bezpečnosti: Výzkum je garantován katedrou fyziologie a biochemie UK FTVS. Sportovci postoupí po krátkém rozcvičení jednorázový test anaerobních schopností – 30-ti sekundový Wingate test do vita-maxima (WT30) na bicyklovém ergometru. Tento test spočívá v 30 s sprintu proti konstantnímu brzdícímu odporu, jehož hodnota se odvíjí od tělesné hmotnosti (0,091 kg.kg-1 tělesné hmotnosti) v souladu s optimalizačními tabulkami. Stěry slin z dutiny ústní pro získání vzorku DNA (neinvazivní metoda) budou probíhat v rámci testovacích dnů v laboratořích UK FTVS. Všechny činnosti související s analýzou a uchováváním genetického materiálu (setřených slin z dutiny ústní) budou realizovány laboratořemi ústavu biologie a lékařské genetiky, 1. lékařské fakulty UK v rámci dlouhodobé spolupráce. Bude využitá invazní metoda pro stanovení laktátu. Laktát bude odebírán z prstu nebo ušního lalůčku na základě vpichu zaškolenou osobou ve stanovených časech v souladu s doporučenými standardy. Odběr kapilární krve, její analýza a likvidace bude provedena za standardních podmínek.
Rizika související s testováním jsou pouze běžně očekávaná rizika u tohoto typu testování. Rizika prováděného výzkumu nebudou vyšší než běžně očekávaná rizika u aktivit a testování prováděných v rámci tohoto typu výzkumu.
Etické aspekty výzkumu: Pokud budou do výzkumu zahrnuty i osoby nezletilé, v jejich případě je podmínkou participace ve studii souhlas zákonného zástupce.
Ve všech těchto případech se bude jednat o jedince s vrcholnou sportovní výkonností v rámci své věkové kategorie, kteří periodicky podstupují laboratorní zátěžové testy v souladu s procesy nastavenými zaštiťujícím Českým atletickým svazem. Získání dostatečného počtu probandů pro následné statistické analýzy vyžaduje zahrnutí i právě nezletilých osob. Výzkum zahrnuje vulnerabilní skupinu nezletilých osob, aby bylo možné získané výsledky zobecnit i na skupinu nezletilých. Výsledky bude možné využít pro individualizaci tréninkových postupů u sportující mládeže.
Ochrana osobních dat: Získaná data budou zpracovávána a bezpečně uchována v anonymní podobě a publikována v odborných časopisech a prezentována na konferencích, případně budou využita při další výzkumné práci na UK FTVS. Po anonymizaci budou osobní data smazána. V rámci výzkumu nebudou pořizovány žádné fotografie ani video materiál. V maximální možné míře zajistím, aby získaná data nebyla zneužita.
Text informovaného souhlasu: přiložen

Povinnosti **všech účastníků výzkumu na straně řešitele** je chránit život, zdraví, důstojnost, integritu, právo na sebeurčení, soukromí a osobní data zkoumaných subjektů, a podniknout k tomu veškerá preventivní opatření. Odpovědnost za ochranu zkoumaných subjektů leží vždy na účastnících výzkumu na straně řešitele, nikdy na zkoumaných, byť dali svůj souhlas k účasti na výzkumu. Všichni účastníci výzkumu na straně řešitele musí brát v potaz etické, právní a regulační normy a standardy výzkumu na lidských subjektech, které platí v **České** republice, stejně jako ty, jež platí mezinárodně.

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Potvrzuji, že tento popis projektu odpovídá návrhu realizace projektu a že při jakékoli změně projektu, zejména použitých metod, zašlu Etické komisi UK FTVS revidovanou žádost.

V Praze dne: 13. 11.2018 Podpis předkladatele:

Vyjádření Etické komise UK FTVS

Složení komise: **Předsedkyně:** doc. PhDr. Irena Parry Martínková, Ph.D.
Členové: prof. PhDr. Pavel Slepička, DrSc.
doc. MUDr. Jan Heller, CSc.
PhDr. Pavel Hráský, Ph.D.
Mgr. Eva Prokešová, Ph.D.
MUDr. Simona Majorová

Projekt práce byl schválen Etickou komisí UK FTVS pod jednacím číslem: 202/2018

dne: 13. 11. 2018

Etická komise UK FTVS zhodnotila předložený projekt a **neshledala žádné rozpory** s platnými zásadami, předpisy a mezinárodní směrnicemi pro provádění výzkumu zahrnujícího lidské účastníky.

Řešitel projektu splnil podmínky nutné k získání souhlasu Etické komise.

razítko UK FTVS

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podpis předsedkyně EK UK FTVS