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Angiotensin Converting Enzyme (ACE) Gene and Muscle Performance in Muay Thai

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Abstract. At present, Martial art sports are very popular. The muscle strength and muscle power are an important factor to contribute the athlete's performance. It is known that factors that promote physical performances are training, age, sex and genetic differences. Angiotensin converting enzymes (ACE) is the main enzyme in renin angiotensin aldosterone system (RAAS), regulating the circulatory system and water and salts balance in the body. The purpose of this study was to explore the relation between ACE polymorphism and muscle performance in Muay Thai/Thai boxers. The participants were 20 men Thai boxers and 20 controls. The protocols were measure the basic characteristics: weight, height. DNA were extract from buccal cell from mouth-wash method. The polymorphism is a variation of the sequence in the single-nucleotide polymorphisms (SNPs) of ACE gene (insertion or I allele and deletion or D allele) resulting in 3 genotypes: ID, II and DD. The result was showed that the ACE polymorphism ID genotype was highly presented with 55.00% of MuayThai while II and DD genotype was revealed in 45.00% and 25.00% of control groups, respectively. The II genotype in MuayThai showed the significantly different ($p \leq 0.05$) of counter movement jump to ID genotype (50.70 and 46.97, respectively). In contrast with control group that DD genotype presented the highly of CMJ followed by ID and II respectively. This result can be concluded that I allele of ACE genes polymorphism is highly percentage in MuayThai and it was correlated with muscle performance.

Keywords: Polymorphisms, ACE, RAAS, I allele, D allele, MuayThai

1 INTRODUCTION

At present, various sporting events are very popular. In which sporting events play an important and meaningful role, such as the Olympics World Championship Asian Games Competition. In each competition, athletes must have physical and mental readiness, especially physical fitness is very important. The composition of muscle strength and muscle power is a very important factor in the performance of athletes. And it is known that factors that help strengthen physical performance include a variety of exercise, diet, exercise, rest, age and gender, as well as genetic differences. According to studies and researches, it is found that genetic diversity in each

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individual can affect the level of physical performance expressed by 30 - 80 percent (Vincent et al., 2007). The most common polymorphism is caused by a variation of the sequence of the bases in a gene called single nucleotide polymorphisms (SNP). Currently, there are more than 200 gene variants that are related to physical fitness. Both directly and indirectly of these, about 20 genes have been studied and confirmed to be related to muscle strength (Ahmetov et al., 2009; Pitsiladis et al., 2013). Recently, among these genes has received a lot of attention with the genetic diversity that is associated with muscle strength (Kostek et al., 2011). Angiotensin converting enzyme (ACE) is the main body in the renin angiotensin aldosterone system (RAAS) and also performs many functions such as balancing the circulatory system (Coates, 2003), controlling blood pressure, balance of water and minerals in the body. ACE will change Angiotensin I to Angiotensin II. In addition, studies have shown that resistance training increases the ACE levels in the bloodstream and may result in the muscles becoming larger and stronger. The SNP (Single Nucleotide Polymorphism) of the ACE gene is caused by an increase in the number of nucleotides called insertion or I allele, or decreasing deletion or allele. D (D allele) at chromosome 17 . ACE is spread in the muscle muscles and in human tissues. That may have an effect on energy metabolism during exercise (Jone et al., 2002). I allele also has certain relationships with muscle endurance after performance training. (Myerson et al., 1999, Coates, 2003). The D allele is associated with increased muscle strength which is found in power sport athletes. The results of the research in Thai weightlifting athletes have shown that the expression of the ACE gene is DD genotype, which has a high correlation with muscle power (Pimjan et al., 2018), including most short-term runners, with the DD genotype ACE gene (Nazarov. et al., 2001), while endurance and general athletes have the ACE type II genotype (Myerson et al., 1999). However, some research has not found any relationship between the ACE gene and muscle strength, both isometric contraction and concentric contraction (Folland et al., 2008). Studies have shown that genetic variation in ACE (ACE I / D polymorphism) affects cardiovascular disease such as high blood pressure, acute myocardial infarction or thick left ventricular fibrillation (Cambien et al., 1992, Winnicki et al., 2004).

The angiotensin converting enzyme (ACE) gene is the first gene that has been studied and found that There is a relationship with physical performance in both athletes and normal people (Rigat et al., 1992), but knowledge about the relationship or connection between muscle strength and muscle power and the characteristics of the expression of the ACE gene has also been found to have little study, especially with Thai athletes. Therefore, making the researchers interested and want to know the ACE genotype in Thai boxers. According to the above information, it is known that the ACE gene affects the muscles that require and essential for all Muay Thai skills. In this study, the researchers want to investigate the variability of the ACE gene and to study the relationship between ACE gene variations to strength and muscle power in Thai boxing athletes.

Literature Review

The ACE gene is found on the long arm of the chromosome at 17, position q 23.3 at the base position 287 of the Alu repeat element in Intron 16 (Rigat et al., 1992). The ACE gene after the simulation process DNA replication found that there are approximately 27,546 genomic DNA (GenBank: NG_011648.1), consisting of the coding sequence, exon. 26 areas and 25 non-coding sequence, intron regions. After the RNA transcription process (post-transcriptional RNA splicing) the intron region is removed, consisting of 4,194 nucleotide mRNA lines. The mRNA lines are then translated and translation to obtaining a sequence of 1,306 amino acids (GenBank:

NP_000780.1). The ACE gene is no expression of protein that affects the muscle performance (Woods et al., 2000). However, when athletes do physical fitness training it will stimulate the intron area of the Alu repeat element into a new exon area (Myerson et al., 1999). The occurrence of SNP (Single Nucleotide Polymorphism) of the ACE gene is characterized by the presence of alleles, also known as insertion or I allele, and Absence of absence, also known as deletion or D allele, at the Al7 repeat element's 287 base pair in intron 16 results in a gene have 3 types of genotype (Villard et al., 1996; Mafra et al., 2018).

After receiving a muscle training session, the Alu repeat element undergoes a process that results in showing export of ACE gene. After that, resulting in the creation of an enzyme called angiotensin converting enzyme (ACE), which is the main hormone involved in the renin angiotensin aldosterone system (RAAS). The cell area the juxtaglomerular apparatus (JGA) produces a hormone called renin. It will change angiotensinogen produced by the liver into Angiotensin I, angiotensin converting enzyme (ACE) transformed into Angiotensin II, which then stimulates the adrenal glands (adrenal gland) to secrete a hormone called aldosterone. Resulting in reabsorption of Na+ and water in the collecting ducts of the kidneys and also stimulate the posterior pituitary secrete a hormone called antidiuretic hormone (ADH) and also stimulate the contraction of blood vessels (vasoconstriction). Resulting in increased blood volume and blood pressure and causing ACE levels in the bloodstream to increase level that may result in the muscles larger and stronger.

From the study of ACE genes that affect the endurance of human muscles (Jones et al., 2002) and some studies have found that the highest frequency of II and ID genotype in athletes. Use of muscle endurance. In addition, a 10 -week muscle performance training results in an I allele resulting in effective muscle mechanisms. Better when compared to good allele (D allele) (Williams et al., 2000).

Nowadays, combat sport has received worldwide attention (Maffulli et al., 2013). It has a significant impact on many sporting events, such as the international Olympics games, which accounts for around a percentage of 20-25 of all medals (Franchini et al., 2011). The activities of these sports such as judo, wrestling, jujitsu, boxing, karate, taekwondo. Each fighting sport has a specific technique and physical fitness preparation for the affective and the success of that competition (Smith, 2003). Furthermore, the genetics may contribute to athlete performances. For example, a study of 14 wrestlers and fighters found that 47.7% of these athletes were DD genotype (Pruna et al., 2013). Other studies were conducted with 28 judo athletes; it was found that 60.70% of the I allele had an ID genotype of 64.30%. It can indicate that the genetic variation of the ACE gene was one of the factors that influenced the athlete’s performance. In addition, boxers carry the I allele has the highest oxygen consumption and contribute to increasing muscular endurance. The endurance athletes present the highly ID genotype such as highly level climbers (Montgomery et al., 1998), Australian rower cyclists marathon runners (Myerson et al., 1999) and runners (Alvarez et al., 2000; Nazarov et al., 2001; Sevilla et al., 2002; Collins et al., 2004) include athletes boxing. However, the most genotype II is found in general people, but likely to be a muscle endurance athlete (Woods et al., 2000; Ahmetov & Rogozkin, 2009) and DD genotype can be found in muscle athletes such as sprinter (Myerson et al., 1999), weightlifter (Pimjan et al., 2018) and sprinting swimmers (Nazarov et al., 2001, Woods et al., 2000). Moreover, it was found that it could increase the level of enzyme activity in both serum and stripe muscles (Danser et al., 1995). Gene factors may result in differences between individual athletic abilities (Bouchard et al., 1986). Currently, the development of technology for nucleotide analysis sequencing and advanced genotyping monitoring can identify 214 genes that are associated with physical fitness of elite athletes.

From the research report, found that the occurrence of polymorphism of the angiotensin converting enzyme (ACE) is linked to the human physical fitness level (Rigat et al., 1992) and the ACE ID genotype polymorphism affects sprint ability and some studies have found that D allele is more common in fast athletes (Nazarov et al., 2001) While type II genotype is rare in high level athletes. In addition, physical fitness enhancement is influenced by many factors such as environmental conditions, behavior, activities including genetic factors (MacArthur et al., 2007). According to the research, the ACE gene is one of the most physically related (Bouchard et al., 1986) but it also has some research report conflicting reports (Montgomery et al., 1997).

Alu repeat element of 48 pairs of the total of 287 base pairs to use the following sequence 3'-CGGGATGGTCTCGATCTCCTGACCTCGTGATCCGCCCGCCTCGGCCTC-5'- GenBank: X62855.1). SR protein consists of these 1 or 2 amino acids, serine and arginine that can affect Blood flow (Lei et al., 2005). In comparing all the nucleotide sequences of Alu repeat sequence, it is known that there are 9 genes that Alu repeat sequence penetrates into the intragonal area and using I-TASSER program to simulate the structure of ACE I and ACE D proteins respectively (Putri & Lukitasari, 2015). When increasing Alu repeat element, there is a loss of amino acid The C-domain amino, due to the addition of this nucleotide sequence, can induce the process of translating the codon into a stop code, which is called the premature termination codons (PTC), resulting in a shorter protein. (Sorek et al., 2003) which means ACE Lee Angiotensin I to Angiotensin II is a small amount. Resulting in decreased concentration of ACE in the bloodstream. Where II genotype has the lowest concentration of ACE in the bloodstream and ID genotype has moderate level of ACE in the bloodstream (Rigat et al., 1992). Not having enough muscle to feed, but when receiving a performance training session for 10 weeks, the result is appropriate in sports using muscle endurance which is found in athletes with ID and II genotype (Williams et al., 2000), but the absence of Alu repeat element found that both C-domain and N-domain proteins Which has more amino acids than the Alu repeat element increases, resulting in the largest protein and highest concentration of ACE in the bloodstream (Rigat et al., 1992) found in DD genotype, resulting in increased blood flow and blood pressure. These results suggest that DD genotype is at risk for high blood pressure, but patients with this disease can create ACE-inhibitors that is responsible for inhibiting the function of the ACE, resulting in blood pressure to normal levels and sufficient to feed the muscles. Causing the muscles to be strong and have strength in sports using muscle power which can be found in athletes with DD genotype.

Muay Thai is a martial art that is unique and able to protect itself. Which began in ancient times as a way to protect yourself from the attacks of animals and enemies and has become one of the most popular sports in the world. In the past, Thailand was a country with a monarchy. The Thai king supports boxing and Martial arts are firmly established to make Muay Thai become the most important sport in the country apart from being a means of self-defense. After the First World War, when Thailand sent troops to fight with the Allies, Thai boxing began to spread throughout Asia and the West. Today, Muay Thai is known throughout the world. One of the four boxing skills of Muay Thai is the most dangerous weapon to destroy an opponent. The wide kick can be divided into four parts: hip rotation, swinging the feet, supporting the arms swinging and stand out with the shin. Lower or instep which is a force for the thighs, torso, ribs and head. In addition, the linear and angular speeds of the kicking leg are also important to the force from the impact of kick boxing circus (Mohamad et al., 2017). Although the wide kick is a very powerful weapon that do not need to wear protective gear in Thai boxing. Many Thai boxers have suffered brain damage, fractured bones and soft tissue injuries as a result of being kicked. In this study, the average maximum force performed by wide and low kicks was significantly higher than high level kicks.

In addition, the average motivation performed by a wide kick at mid kick is significantly higher than a high kick. However, the maximum power and impulse of a wide kick is not affected by the leg strength. In addition, the average final speed of the ankle is an important factor related to the maximum force exerted by a wide kick. To get the maximum speed of the foot, kick and ankle, consideration should be given to the movements of other parts of the body such as Leg and hip rotation (Sidthilaw, 1996). Thai boxing rules are quite different from boxing rules. The Thai boxing match has five rounds of fight, with three minutes per round and two minutes remaining between rounds (Mohamad, 2017).

The research that will be very useful is biomechanics. It is important for motion analysis, especially the techniques in Muay Thai are Wide kick which is one of the most used techniques among Thai boxers. It consists of four basic components: rotating the hips, turning the feet, supporting, swinging the arms and hitting them below the shin or back of the feet. With a wide kick usually targeting three main parts of the opponent's body, which is the head, body, and thighs. Which kicking can be considered an advantage as it can cause opponents to lose (Mohamad, 2017).

The factors that contribute to be elite Thai boxers is physical fitness such as muscular strength, muscular power is the ability of the muscles to work with maximum effort in the shortest period of time which must have muscle strength and speed as the main component. Muscular endurance is the ability of a particular muscle or bundle of muscles to contract repeatedly, to resist force or the ability to contract once for a long period of time.

There are no genetics studies in Thai boxing. Most of the studies on injury analysis (Maffulli et al., 2013) physiological responses (Crisafulli et al., 2009; Santos et al., 2012), biomechanics (Han, 2018), and psychology (Varillas-Delgado et al., 2022). To find out the new finding, this study will explore the genetics and physical fitness in Muay Thai.

2 METHOD

Population and Samples

There were 20 male Muay Thai athletes with more than 5 years of experience in Thai boxing, aged between 18-25 years, healthy, without any illness or serious illness. Control group: 20 persons, aged between 18-25 years, are healthy, not sick or have serious illness. They are willing to participate in this study and all agree with the consen form. Materials, equipment and chemicals, volume measuring bottle 125 ml, normal saline solution 1 ml, 50 ml centrifuge tube, stopwatch, centrifuge.

Study methods: After collection of buccal cells by mouthwash, pour 25 ml of normal saline solution into a flask. After that, pour 25 ml of normal saline solution into the mouth to harvest the buccal cells for 1 minute by mouthwash. Pour the finished solution from the mouth and put in a small 50 ml centrifuge tube, then rest for 1 minute and repeat 1 time. Bring the solution from item 3 to centrifuge at a speed of 10,000 rpm at 4 ° C for 5 minutes so that the cells become pellet at the bottom of the tube. Extraction of DNA from the buccal cells with extraction solution (Feigelson et al., 2001). DNA extraction from the extraction solution shows highly concentration and pure of the DNA. Analysis of the quantity and quality of DNA extracted from the buccal cells using a Nanodrop spectrophotometer, measuring the absorbance at wavelength 230, 260 and 280 nm respectively, found that the concentration of DNA solution that is pure and sufficient to increase the number of gene by polymerase chain reaction (PCR). Analysis of the quality of DNA extracted

from the cheek lining using the 1% agarose gel electrophoresis. Increasing the amount of ACE from the DNA extracted from the buccal cell by polymerase chain reaction (PCR).

Anthropometry and Physical Fitness Measurement

The weight and height were measured for anthropometry. Leg muscle power test by countermovement jump (CMJ). Subjects perform 3 tests and 1 minute rest between jumps. Record the maximum value by recording the height jump in centimeters.

Data and Statistics Analysis

Statistical data analysis using SPSS program (IBM SPSS Statistics 25) with statistical significance at p-value less than 0.05 and data distribution using Shapiro-Wilk test. Hardy-Weinberg equilibrium was used to test the genetics equilibrium. The Chi square was compared allele frequency between groups. The relationship between the variation of the ACE gene and muscle power using ANOVA (analysis of variance).

3 RESULTS

The qualification of the DNA with an extraction solution as shown below in the figure 1. It was shown the ratio of the absorbance of OD260 / OD280 of Thai boxers and controls.

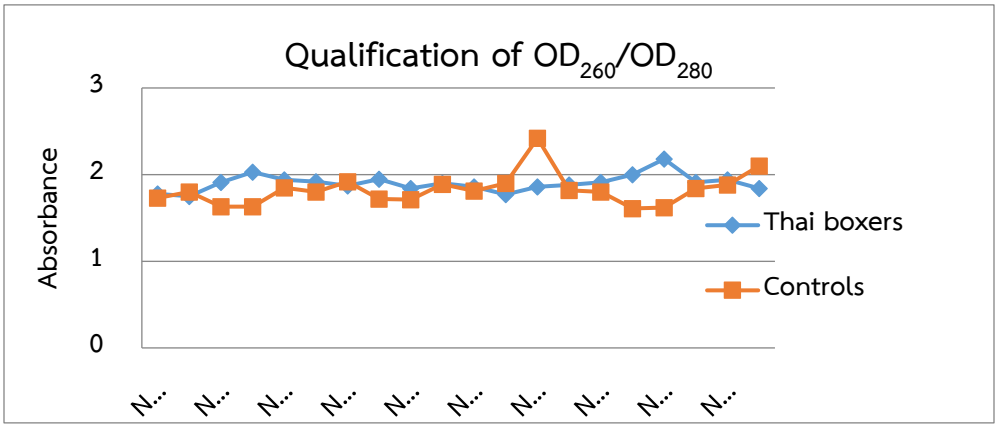

Fig. 1 The Ratio of the Absorbance of OD / 260OD 280of Thai Boxers and Controls.

From the line graph showing the ratio of the absorbance of OD260 / OD280 in Thai boxers and controls, it was found that the average proportion of the absorbance value of OD260 / OD280 of Thai boxers and controls were 1.9020 and 1.8240, respectively. In the range of 1.80-2.0 shows that the extracted DNA solution is pure and the concentration of the extracted DNA solution is large enough for polymerase chain reaction (PCR).

The ACE gene polymorphism and genotype in Thai boxing and control the data shown in figure 2.

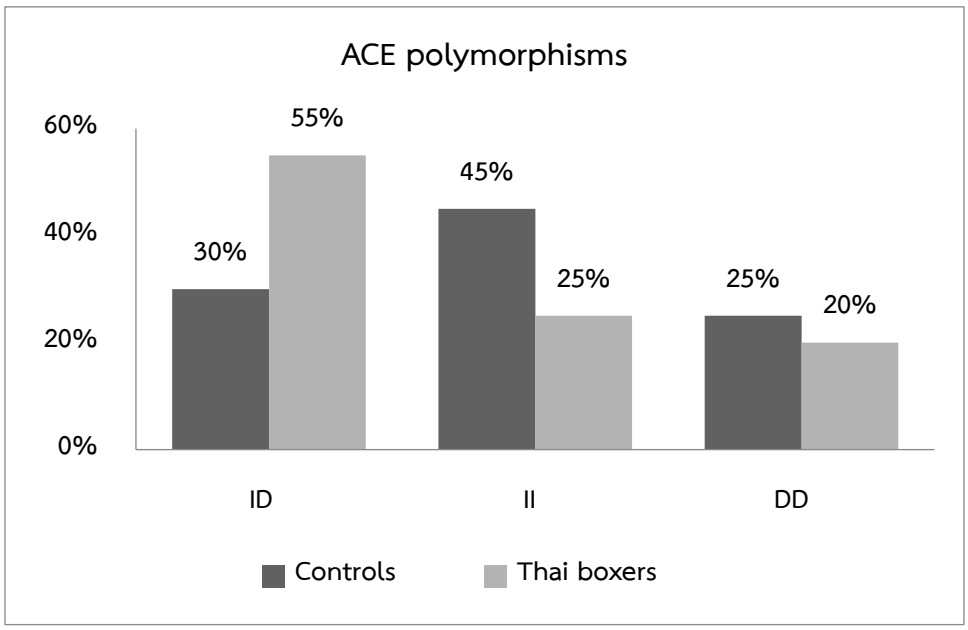

Fig. 2 The ACE Gene Polymorphism in Controls and Thai Boxers.

The data from figure 2 show the correlation between the ACE gene variations between control and Thai boxers. It was founded that the ACE genes in Thai boxers have an ID, II, and DD genotype of 55, 25, 20%, respectively. Compared with the controls group ACE genotypes were ID, II, and DD genotypes of 30, 45, 25%, respectively.

The characteristics of subjects such as age, weight, height and body mass index (BMI) show the detail in table 1 with the statistics significantly different at p-value ≤ 0.05.

Table 1 The Characteristics of Thai Boxers and Controls Groups (Standard Deviation)

Characteristics	Controls (Mean ± S.D)	Thai Boxer(Mean ± S.D)	p-value
Age (Year)	19.33 ± 0.82	21.17 ± 1.47	0.024
Weight (Kg.)	62.67 ± 7.94	57.83 ± 3.06	0.194
Height (m)	1.68 ± 0.03	1.67 ± 0.03	0.538
BMI (kg/m^2)	22.12 ± 2.33	20.70 ± 1.04	0.202

The data of characteristics of both groups showed the mean and standard deviation in table 1. There was no difference in characteristics for weight, height and BMI of both groups. with statistically significant. However, it was found that there were slightly statistically significant differences in age of both groups at p ≤ 0.05

The relationship between the ACE gene genotypes and legs muscle performance that tested by counter movement jump (CMJ) as shown in table 2.

Table 2 shows the mean and standard deviation of legs muscle performance (CMJ) in controls and Thai boxers P ≤ 0.05.

Subjects (n)	Genotype	Counter movement jump (Mean ± S.D)	P-value
Controls (n = 20)	II	40.00 ± 2.83	0.05
	ID	41.80 ± 0.00	
	DD	48.97 ± 6.96	
Thai Boxers (n = 20)	II	50.70 ± 0.00	0.05
	ID	46.97 ± 8.68	
	DD	48.20 ± 2.83	

Testing the equilibrium of the Hardy-Weinberg of both group, there was no significant difference between allele frequencies in both Thai boxers and controls.

From table 2, the results of the legs muscle power for CMJ in control with the DD genotypes was 48.97, which were the highest number, followed by ID genotype 41.80 and II genotype 40.00. There were significantly differences of CMJ with the II and DD genotype in controls group (P ≤ 0.05). The DD genotype presents the highest CMJ followed by ID and II, respectively. In contrast with, the results of the legs muscle power (CMJ) in Thai boxers show that ACE: the II, ID, DD genotypes were 50.70, 46.97, and 48.20, respectively. There were significantly differences in the ACE gene genotype and CMJ in Thai boxers and the I allele shows the highest of counter movement jump in athletes.

4 DISCUSSION AND CONCLUSION

DNA extraction from controls and Thai boxers using the extraction solution found that the average absorbance ratio of OD260 / OD280 was in the range of 1.8-2.0. It shows that the extracted DNA solution has the purity and concentration of the solution. The extracted DNA was very high when compared to the DNA extraction using TNE buffer (Feigelson et al., 2001). The TNE buffer extraction showed the average of the absorbance ratio of OD260 / OD280 is lower than 1.8. This shows that the extracted DNA solution has high protein contamination and the concentration of the extracted DNA solution is also very high. But when analyzing the quality of the DNA extracted by 1% agarose gel electrophoresis It was found that DNA extraction using an extraction solution does not contain any protein contamination, but the DNA extraction using the TNE buffer contains protein contamination and the DNA band obtained from the extraction of both DNA.

Both methods of DNA extraction are close to the DNA extraction using the TNE buffer. Therefore, the DNA extraction using the Extraction solution produces pure more DNA solution than TNE buffer extraction, resulting in DNA extraction. Using extraction solution is the best way when increasing the amount of ACE from the DNA extracted from the cheek lining by polymerase chain reaction of the two groups extracted using Extraction solution, annealing temperature at 58 ° C is the right temperature for increasing the amount of ACE gene. Then, when the results of the ACE gene multiplication were examined by 2% agarose gel electrophoresis

It was found that two DNA bands, including 490 ACE genes with ID genotype and 190 base pairs, make them suitable for endurance athletes, including boxers, But the II genotype has 490 base pairs, which can be found in general people but is likely to be a muscle endurance athlete (Woods et al., 2000; Ahmetov & Rogozkin, 2009). And DD genotype with 190 base pairs found

in muscle strength athletes such as short runners (Myerson et al., 1999) weightlifting athletes (Pimjan et al., 2018). There was difference in the detection of angiotensin, converting enzyme (ACE) by restriction fragment length polymorphism (RFLP). ACE genes can be identified by increasing the number of genes. With polymerase chain reaction, the RFLP process will be used for the ACTN3 and VDR genes, which requires expensive DdeI and FokI, which will be used in future studies.

The distribution of ACE polymorphisms in Muay Thai and control group and their association with muscle power. The main finding of this study were the present of ACE ID and II genotypes and allele frequency in both groups. Our findings were disagreement with Mikami at al., (2014) demonstrated and excess of DD genotype in Japanese wrestlers.

II genotype show the correlation with high CMJ in Thai boxers that relate with the study of Pitsiladis et al., (2013) found that 14 boxers and 28 combat athletes were used to analyze the ACE gene pattern. Knowing the number of genes found in this sample and some studies have shown that 19 athletes use muscle strength and endurance (Uchiyama et al., 2011). Found that most athletes are athletes using muscle strength when compared to the normal group. In this study, the researchers used 20 controls and one Thai boxer to study the variation of the ACE gene. It was found that ID genotype was the most in Thai boxers and was found to be the second genotype in the controls because the vapor allele showed a gene expression when trained for physical fitness training and the ACE I protein was modified to affect the mechanism of mitochondria is that ATP is the energy needed for muscle function. And has more volume of mitochondria and capillaries resulting in the muscles that are resistant to sports that use muscle endurance. However, the study of Pimjan et al., (2018) presented that the DD genotype affect the CMJ and SJ in Thailand weightlifters. However, the DD genotype was found to be 48.97% in the control group. DD genotype may be gene patterns that are suitable for sports that use muscle power. By giving the muscle power for maximum strength in a short period of time and requiring speed of exercise (Myerson et al., 1999, Nazarov et al., 2001, Pimjan L. et al., 2018) but found II genotype accounting for 50.70% in Thai boxers, which II genotype is a gene pattern that is likely to be an athlete using both muscle strength and endurance. By causing the muscles to have a rapid contraction (concentric contraction) to resist resistance in a long time (Myerson et al., 1999, Nazarov et al., 2001).

Collection of buccal cells by mouthwash method get higher yield than another method and it is the easiest and most convenient way to extract cells. It is much higher when compared to the DNA extraction using the TNE buffer. The variability of the ACE ID genotype was the highest percentage among Thai boxers.

5 SUGGESTIONS

The future studies may have to study of ACE genes among female Thai boxers and should be conducted with other genes and other sports that affect the physical performance.

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