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The relationship between ACTN3 gene polymorphism with VO2 max and flexibility

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Abstract. Maximum oxygen consumption (VO2 max) and flexibility are essential biomotoric components for football athletes. The influence of genetics on the biomotoric element has not been widely studied. The ACTN3 gene probably affects VO2 max and flexibility. This study aims to determine the relationship between ACTN3 gene polymorphism to VO2 max and flexibility in students of UKM Olahraga Jenderal Soedirman University. This was an observational analytic study with a cross-sectional approach. Seventy-eight subjects chosen by consecutive sampling underwent the protocol study. Venous blood samples were taken for ACTN3 gene polymorphism examination. The respondents were also tested for VO2 max with the multi-stage fitness test and flexibility tests using sit and reach method. Data were analyzed by a one way ANOVA test with a significance level of p=0.05.The results of this study showed that there was no significant relationship between ACTN3 gene polymorphism with VO2 max (p=0.763) and flexibility (p=0.912). The highest mean VO2 max was in the RR genotype (35.25±7.15 ml/kg.min), while the highest mean of flexibility was in the XX genotype (37.02±7.89 cm). There was no relationship between ACTN3 gene polymorphism with VO2 max and flexibility, in students of Jenderal Soedirman University.

1. Introduction

VO2 max is a dominant physical fitness parameter, as well as a marker of the cardiovascular and respiration systems. VO2 max is also used by athletes to measure endurance and athletic ability. Due to the importance of this component, athletes are always trained in regular and specialized exercises so that most of their VO2 max measurements are higher than non-athletes [1,2].

Many factors influence VO2 max such as the lungs, heart, muscles, body composition, age, gender, haemoglobine, physical activity and genetic factor [3]. Genetics have a significant role in the VO2 max capacity. The genes affect the cardiovascular, respiratory and muscular systems [4]. One of the genes that can influence athletic performance is the ACTN3 gene because it affects muscle components. The influence of the ACTN3 gene on VO2 max as the dominant determinant of body performance had not been widely studied. ACTN3 gene polymorphism probably affects VO2 max capacity due to changes in muscle metabolic processes [5]. Another bio motoric component that is equally important to athletes is flexibility. VO2 max can affect the performance of the athlete;

flexibility is essential in minimizing risk factors for injury. A person that has high flexibility will find it easier to perform manipulative movements to reduce the possibility of falling and injury.

The association of ACTN3 gene polymorphism with VO2 max had been studied in several countries, but there are not consistent results. Also, the VO2 max and flexibility related to the ACTN3 gene were also unclear and minimum in literature. Therefore, the study was interested in researching the relationship of ACTN3 gene polymorphism with VO2 max and flexibility in students of sports majoring at Jenderal Soedirman University.

2. Methods

The study was an analytic observational study with a cross-sectional approach. 78 people, selected from student underwent the physical examination, treatments, and genotyping analysis method. Subjects were male, enrolled in the football club and aged 18-24 years. Subjects with neuromuscular disorders, cardiovascular disorders, and active smokers were not allowed to participate. Each willing participant was given a full explanation and was asked to sign informed consent.

Venous blood samples were taken from the subjects for ACTN3 gene polymorphism examination. The identification process of ACTN3 gene polymorphism was done by DNA isolation, followed by PCR and RFLP protocol using the Dde1 enzyme. Primers used in this experiment were 5'CTGTTGCCTGTGGTAAGTGGG 3' and 5'TGGTCACAGTATGCAGGAGGG 3'. PCR coctail contains PCR mix (Invitrogen-USA), primers, DNA template, and nuclease free water up to 50ul were run in termocycler in condition as follows: 95°C for 5 minutes, followed by 35 cycles of 95°C for 30", annealing temperature 55°C for 45" and 70°C for 30". Digestion of 20 ul PCR product was using Dde1 enzymes to capture ACTN3 polymorphism. The ACTN3 gene visualisation was observed using a UV transilluminator following electrophoresis process (figure 1).

VO2max testing was using the multi-stage fitness test/MSFT method (figure 2). The respondents were also tested for the flexibility using the sit and reach test (figure 3). Data obtained from the examination were tested with ANOVA test in the significance level of $p<0.05$. Ethical approval was obtained from the Health Research Ethics Committee of Medical Faculty of Diponegoro University in Indonesia.

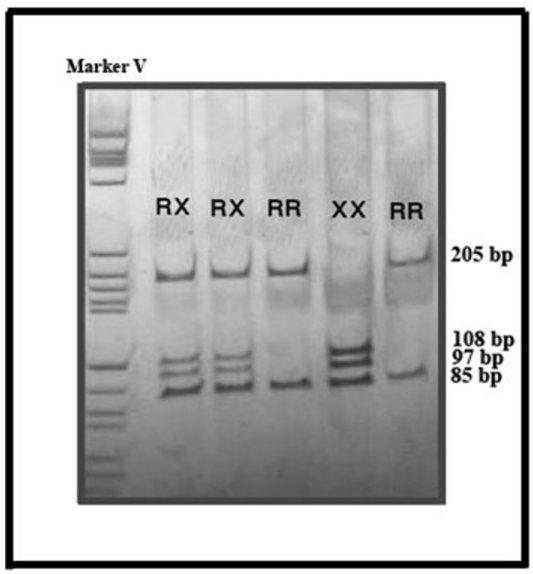

Figure 1. PCR-RFLP of ACTN3 Gene and band visualization of RR, RX, and XX Polymorphism [6].

3. Results

ACTN3 gene polymorphism revealed the highest percentage of genotypes to be RX 56,4%, followed by XX 26,9% and RR 16,7% (table 1). Subjects had a mean VO2 max of 34,18 ± 4,64 ml / kg.min and a mean flexibility of 36,70 ±7,26 cm (table 2). All of these variables were normally distributed by a Kolmogorov-Smirnov test $p>0,05$.

Figure 2. *Multi-stage fitness test* [7], the subject was asked to run back and forth on 20 meters track following the speed of the sound played in the sound player.

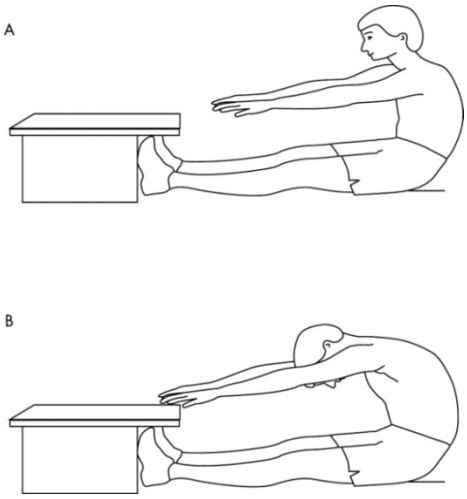

Figure 3. *Sit and reach test* [7], the subject slowly bowed with the position of the two arms straightened and the hands straight forward along the scale of the ruler.

Table 1. Genotyping of ACTN3 gene.

ACTN3 Gene Polymorphism	Number	Percentage (%)
RR	13	16,7
RX	44	56,4
XX	21	26,9
TOTAL	**78**	**100**

The VO2 max value and flexibility distribution among the ACTN3 gene polymorphism was depicted in Table 3. The highest mean VO2 max value was in the XX genotype (36.25 mL/kg.min). The genotype with the lowest mean VO2max was RX (33,42 mL/kg.min). Bivariate analysis showed no association between ACTN3 gene polymorphism with VO2 max (p=0,103).

Table 2. VO2 max and flexibility of subjects

Variable	Mean ± SD	Median (Min – Max)	95% CI
VO2 max	34,18 ± 4,64	33,70 (21,10 - 50,50)	33,22 – 35,63
Flexibility	36,70 ± 7,26	37,50 (17,50 – 51,50)	35,06 – 38,34

The highest mean value of flexibility was in the XX genotype (37.02 cm). The genotype with the lowest mean flexibility was RR (35.94 cm). Bivariate analysis showed no relation between ACTN3 gene polymorphism and flexibility (p=0,912).

Table 3. Relationship between ACTN3 gene polymorphism and VO2 max and flexibility.

ACTN3 Gene	VO2 Max (ml/kg.min)	Flexibility (cm)	One Way ANOVA Test (p)
RR	33,92 ± 6,17	35,94 ± 7,47	
RX	33,42 ± 3,77	37,02 ± 7,89	0,103[a] 0,912[b]
XX	36,25 ± 4,94	37,02 ± 7,89	

[a]ACTN3 vs VO2 max
[b]ACTN3 vs Flexibility

4. Discussions

The examination of ACTN3 gene polymorphism using Dde1 enzyme found three genotypes: RR, RX, and XX. The genotype differences in polymorphism could be seen from the length of each visible band (figure 1). The percentage of ACTN3 gene polymorphism showed that the RX genotype was the highest percentage (56.4%), followed by XX (26.9%) and RR (16.7%) (table 1). Research conducted by Nan-Xiang [9] in China showed similar proportions, where the RX genotype was higher than the other two genotypes. This result was also in accordance with Goel and Balraj [6] who stated that Asians have more RX genotypes than RR and XX.

Cardiorespiratory endurance or VO2 max was assessed using a multi-stage fitness test with the highest value was 50.50 mL/kg.min, the lowest value was 21.1 mL/kg.min, and the mean was 34.18±4.64 mL/kg.min (table 2). For males aged 20-29 years, normal VO2 max is 36.5-42.4 mL/kg.min, so the average maximum oxygen consumption capability of the subjects was lower than normal [7]. Flexibility was assessed using a sit and reach test, in which the highest score was 51.50 cm, the lowest value was 17.50 cm, and the mean was 36.70±7.26 cm (table 2). For men, the flexibility rate was normal (≥28 cm).

The results of this study showed that there was no significant relationship between ACTN3 gene polymorphism with VO2max (p=0,103) and flexibility (p=0,912). In this study, although there was no significant association between ACTN3 gene polymorphism and VO2 max, table 3 showed the highest mean of VO2 max in the XX genotypes (36,25±4,94 ml/kg.min). This result was similar to the previous research [10–12] which did not find a relationship between ACTN3 polymorphism and cardiorespiratory endurance but the highest mean of VO2max found in XX genotype. The fact that higher values of maximal oxygen uptake are recorded for individuals with the XX genotype, which is characterized by a lack of actinin 3 in muscle fibres, is consistent with the mechanism of development of physical fitness towards the aerobic type in case of a lack of predisposition to generate high power by muscles.

This study also showed that there was no significant relationship between ACTN3 gene polymorphism and flexibility (p=0.912). Although there was no significant association between ACTN3 gene polymorphism and flexibility, table 3 showed the highest flexibility rate in the XX genotype (37.02 ±7.89 cm). The skeletal muscle that has no α-actinin three protein (XX genotype) has better resistance to damage because the loss of this protein will be compensated by an increase in α-actinin protein 2, which then increases the number of ZASP proteins, titin, and vinculin in the z line. These three proteins have the function of maintaining a stronger Z-Z bond, adjusting the Z-line width and increasing its elasticity, so the muscles are more resistant to damage during activity[13]. It can assume that individuals with genotype XX have better flexibility than the others.

Quinlan [14] stated that the α-actinin 3 protein has functions as a binding enzyme to glycogen phosphorylase which is an important enzyme in anaerobic metabolism. The amount of glycogen phosphorylase is lower in XX genotypes than RR or RX. A decrease in the amount of glycogen phosphorylase can cause an increase in aerobic metabolism by increasing in the aerobic enzyme (NADH-TR and succinate dehydrogenase). Both of these enzymes increase only when the aerobic metabolism in the muscle also increases. Increased aerobic metabolism can cause the increasing of oxygen consumption (VO2) and maximum oxygen consumption (VO2max), because oxygen is needed as a source of energy [14–17].

The insignificant results of ACTN3 gene polymorphism with VO2max and flexibility in this study could be attributed to many things, one of which is the skeletal muscle system. Skeletal muscle is a complex component of the body because it involves a variety of metabolisms, proteins, and enzymes to work. In individuals that have an "Mc Ardle" disorder, the disease results from a failure of the glycogen breakdown of muscles which can cause muscle contraction disorders [18]. In this study, there was no in-depth examination of the muscular system.

The results of this study might be influenced by other things such as oxygen-carrying capacity, which is governed by hemoglobin, which can affect VO2 max because it acts as an oxygen carrier to all part of the human body, including muscles. Disorders of hemoglobin levels such as anemia will directly affect VO2 max and indirectly affect flexibility, as the oxygen supply required by muscles and joints for metabolism is reduced. In this study, there was no examination of hemoglobin levels, although it probably affected the results [19].

This study was different from the previous research by Pimenta [16], which used professional soccer athletes from top football clubs who were usually the best athletes in their respective countries. National athletes have undergone training since youth, and a controlled schedule of exercise each week. The level of physical activity or training and diet of professional athletes are closely guarded, as they are required to deliver optimal performance in every game. In this study, the subjects were students who played for a football club in the university and could be called an amateur athlete. There was no history taken on a diet and motivation, which probably affected the results of this study.

In summary, this study had limitations in its implementation. Measurement of VO2 max (using the MSFT) and flexibility (using sit reach test methods) require high motivation from the subject to produce results that can represent actual values. The effect of the muscle system and hemoglobin levels on VO2 max and flexibility was not observable in this study. The study also used students who were mostly amateur athletes and therefore had differences with professional athletes.

5. Conclusions

There was no relationship between ACTN 3 gene polymorphism with VO2max and flexibility in students of Jenderal Soedirman University.

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