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CORRELATION OF TOTAL GENOTYPE SCORES AND AEROBIC CAPACITY AMONG FIELD HOCKEY PLAYERS

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ABSTRACT

A balanced aerobic and anaerobic capacity is an important determinant of performance among field hockey players during the competition. Training and heritable talent play important roles in determining the performance of the athletes. Therefore, this study aimed to investigate the influence of sport-related genetic variants on aerobic capacity and repeated sprint ability performance among field hockey players. A total of 45 participants (age = 16.42 ± 0.50 years old, height = 1.67 ± 0.06 m) were selected based on the inclusion criteria of this study. Participants were divided into three groups based on training intensity for eight weeks (high intensity, moderate intensity, and control). Three genetic variants associated with endurance and power (ACE rs1799752, ACTN3 rs1815739, and PPARA rs4253778) were used to calculate the total genotype score (TGS). Pearson's correlation was used to analyse the correlations between the performance and TGS. There was a significant correlation between the aerobic capacity with endurance TGS (r=0.55, p=0.03) and power TGS (r=-0.55, p=0.03) following the moderate intensity group. It is concluded that the higher the endurance TGS, the better the aerobic capacity in the moderate intensity endurance exercise. Therefore, the short-term performance of the athletes was significantly influenced by the genotypes and training intensity.

Keywords: *Total genotype Score, aerobic capacity, field hockey players*

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INTRODUCTION

Field hockey is a popular sport that has received much interest from Malaysians. Among the demand of field hockey players is to perform high intensity activities such as running and sprinting and quickly recover during low-intensity activity intervals like walking and jogging (Lemmink et al., 2004). Due to the activity profiles of field hockey, sprinting capability, endurance, and technical performance execution may prove to be underwhelming if the training period is insufficient for the athletes (Elferink-Gemser et al., 2004). According to Gronek et al. (2013), field hockey involves a mixture of energy systems (60% aerobic and 40% anaerobic).

Maximal oxygen uptake (VO_{2max}) is one of the direct measurement tests to assess aerobic capacity, which may be affected by one's predisposition of slow-twitch muscle fibers that may be influenced by genetic variations (Ahmetov & Fedotovskaya, 2015). VO_{2max} refers to the intensity of the aerobic process and denotes the maximum capacity to transport and utilize oxygen during exercise (Shete et al., 2014). Infield hockey, short, low intensity bouts intercede high intensity activities, thus demanding high aerobic capacities to allow better recoveries between the high intensity bouts (Watson et al., 2017).

The athletes' ability to sustain power outputs over repeated bouts throughout the match-play is vital as physical performances may deteriorate as fatigue develops from prolonged exertions. Like soccer players, field hockey players must repeatedly produce speed during training or competition (Bradley et al., 2010). Repeated sprint ability (RSA) is a well-known test battery for field-based sports (Barbero-Álvarez et al., 2010) to assess a player's fulfillment of such demand.

Besides training, genetic factors with related phenotypes play a crucial role in determining athletic performance, namely strength and endurance (Ahmetov et al., 2016). When inherited, performance-enhancing polymorphisms may lead to improved athletic performance (Ostrander et al., 2009). Over 150 DNA polymorphisms have been linked to human physical performance (Rankinen et al., 2006). The optimum polygenic profile probably differs between endurance and power sports because the phenotype traits that determine performance in both types of events are likely to be different (Buxens et al., 2011).

Sports genetics is a new scientific discipline focusing on the functioning of an athlete’s genome. It is considered a key role in athletic performance and related phenotypes such as endurance and strength (Ahmetov et al., 2016). Endurance and strength show different genetic sequence variants. There are many endurance and strength-related markers for the endurance athletes, such as the ACE gene, PPARA gene, PPARGCIA gene, ACTN3 gene, and NOS3 gene, and over 200 polymorphisms of genome influencing exercise performance traits (Ahmetov & Fedotovskaya, 2015).

Total genotype score (TGS) is a well-known method to predict the potential of a genome for athletic performance (Williams & Folland, 2008). It calculates the abundance of various genetic polymorphisms and associates an athlete with performance characteristics related to the polymorph. *ACE* (angiotensin I-converting enzyme), *ACTN3* (actinin- α3) (Dionísio et al., 2017; Sessa et al., 2011; Williams & Folland, 2008), and *PPARA* (peroxisome proliferator-activated receptors) (Lopez-Leon et al., 2016) are among the genes associated with athlete's strength and endurance developments. Hence *ACE*, *ACTN3*, and *PPARA* were included in this study to investigate their association with endurance performance. Several studies investigated the influence of TGS on different sports, such as soccer players (Fazli et al., 2021; Suraci et al., 2021). However, to our knowledge, no such study has been conducted for a field hockey setting. A different type requires different intensity and energy of sports; thus, the effects of the three variants may have been different in field hockey players. Therefore, this study aimed to investigate the correlation between endurance and power genotypic scores with aerobic capacity and repeated sprint ability performance among field hockey players.

METHODOLOGY

Participants

A true experimental design was used in this present study. Purposive sampling was employed to recruit the participants in this study. The study was conducted during the off-season, so no official training was conducted. A total of 45 male field hockey players (age = 16.42 ± 0.50 years old, height = 1.67 ± 0.06 m, and body mass = 60.60 ± 7.12 kg) were recruited for this study. The criteria for the selection of the participants were as follows; (1) the participants must play at least at the district level, (2) the participants must be active players and involved in the games for the past year, (3) the participants must be physically healthy and free from injuries, (4) the participants must be free from any drug or supplement consumption. The sample size was calculated using the statistical software G*Power (G*Power, Universitat Kiel, Germany) by Faul et al. (2007). To achieve a moderate effect size, f, of 0.25, and 80% statistical power, a total sample size of 42 was required for three groups. A total of 45 participants were recruited for this study to account for the 20% dropout rate. Participants were divided into three groups: (1) high intensity group (HI), (2) moderate intensity group (MI), and (3) control group (C). The study was approved by the Human Research Ethics Committee, Universiti Teknologi MARA (600-IRMI (5/1/6) REC/35/19). A flow chart of the study design is presented in figure 1.

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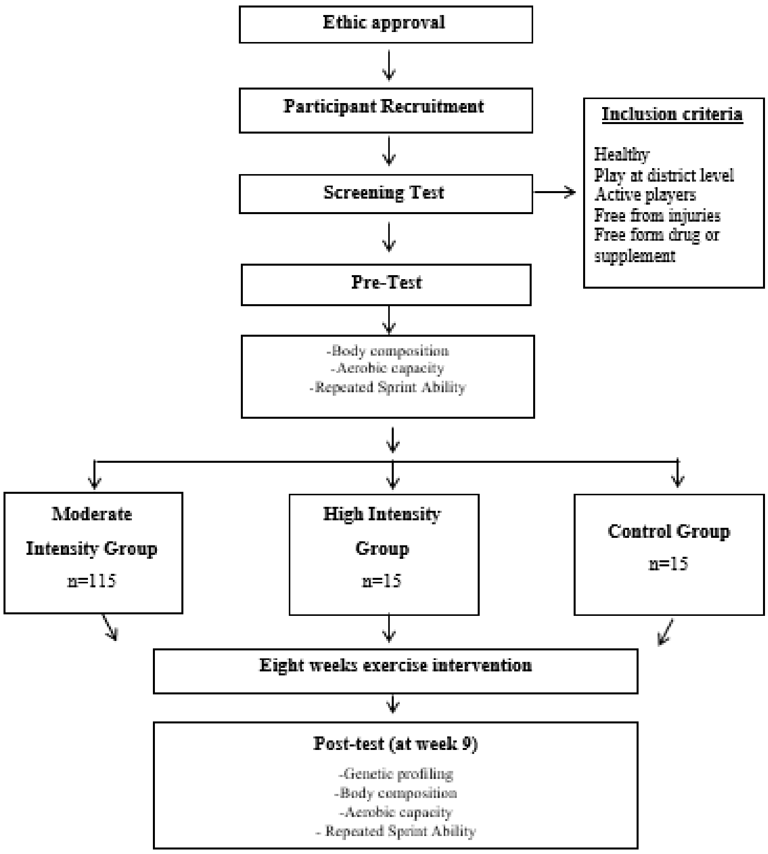

Figure 1: Study design

Procedure

All participants were required to fill in the Physical Activity Readiness Questionnaire (PAR-Q) and pass the resting electrocardiogram (ECG) with no abnormality. In addition, consent was signed by each of the participant's parents or legal guardian before start of the study. All groups did the sport performance components test, which is body composition test, aerobic capacity (VO2max test), and sprint performance decrement (RSA test) for pre and post- test. Table 1 describes the different types of training prescribed to the participants.

Table 1: Endurance training intensity for the field hockey players

Weeks	High intensity group (HI)	Moderate intensity group (MI)
1-2	Intensity: 90% Exercise: 4 bouts x 4 mins running Frequency: Three times a week	Intensity: 70% Exercise: 50 mins running Frequency: Three times a week
3-4	Intensity: 90% Exercise: 4 bouts x 4 mins running Frequency: Three times a week	Intensity: 70% Exercise: 50 mins running Frequency: Three times a week
5-6	Intensity: 95% Exercise: 4 bouts x 4 mins running Frequency: Three times a week	Intensity: 75% Exercise: 50 mins running Frequency: Three times a week

7-8	Intensity: 95% Exercise: 4 bouts x 4 mins running Frequency: Three times a week	Intensity: 75% Exercise: 50 mins running Frequency: Three times a week

Selected participants for the control group (C) were not prescribed any exercise for the eight-week study duration. Performance tests to measure aerobic capacity (AC) and repeated sprint ability (RSA) were conducted on the first and 9th week. All the post-tests followed the same testing sequence and procedures as the pre-test.

Genotyping

Deoxyribonucleic Acid (DNA) was extracted from the blood using the standard salting-out method for genotyping as described by MWer et al. (1988). Allele-specific polymerase chain reaction assays were used to identify the genotypes of *ACE*, *ACTN3*, and *PPARA*. Five (5) ml of 1x red cell lysis buffer was added into the blood sample (5 ml) in a 50 ml Falcon tube, and the tube was mixed gently. Subsequently, 5 ml of 0.5x red cell lysis buffer was added to make the final total volume of 15 ml, and the tube was mixed gently. Then, the sample was incubated on ice for 10 minutes before centrifuged 3500 x g (Eppendorf, Hamburg, Germany) for 15 minutes. After centrifuged, the supernatant was discarded, leaving the pellet, which was rinsed with 25 ml of 1x tris EDTA (pH 8.0) two times. Next, saline EDTA (pH 8.0) was added, and the pellet was broken up using the wide-bore pipette tips.

Subsequently, 100 µl of 20% sodium dodecyl sulfate (SDS) and 12.5 µl of RNAase A and 50 µl of proteinase-K was added to the samples for overnight incubation at 37°C. The next day, 100 µl of 2 M potassium chloride (KCl) was added to the sample, and the tube was swirled gently to dissolve the salt. DNA was visible as strands floating in the solution after 4 ml of cold 100% ethanol was added into the tube. The DNA was removed using a pipette and transferred into a new microcentrifuge tube. 70% ethanol was used to clean up the DNA. The DNA was left to air dry and reconstituted with Tris EDTA (TE) buffer (pH 8.0) before storing at -20°C.

Allele-specific polymerase chain reaction (ASPCR) assays were used to identify the alleles for *ACE* (rs1799752), *ACTN3* (rs1815739), and *PPARA* (rs4253778). ASPCR was performed using a thermal cycler (Takara Bio, CA, USA). The cycling condition was: initial denaturation at 95°C (20 seconds), denaturation 95°C (30 seconds), annealing temperatures (Touch-down from 63°C to 55°C (20 seconds), extension at 68°C (30 seconds), and final extension at 68°C (5 minutes) with 20 µl of final mixture volume that contained DNA (100 ng/µl), 0.5 U Taq DNA polymerase (NEB, MA, USA), primers (0.1-2.0 µM), 0.16 mM deoxyribonucleotide triphosphate (dNTPs) and autoclaved double-distilled water (ddH2O). PCR product was mixed with 2µl loading dye and loaded into agarose gel (3.5%) for further analysis.

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Total Genotype Score (TGS)

The 'optimal' homozygous, intermediate 'heterozygous' and 'less optimal' homozygous were assigned with the scoring of 2,1, and 0, respectively and associated with strength-power and endurance qualities. The total score was transformed in the range from 0 to 100 and labelled as TGS. The genotype scoring is presnted in Table 2. The TGS was calculated and describe by Miyamoto et al. (2017) using the equation (Equation 1).

TGS = (100/42) × (GS*ACE* + GS*ACTN3* + GS*PPARA*) [Equation 1]

Table 2: Genotype scoring

Genes	Genotype scoring
ACE	
Endurance	II=2, ID=1, DD=0
Power	DD=2, ID=1, II=0
ACTN3	
Endurance	TT=2, CT=1, CC=0
Power	CC=2, CT=1, TT=0
PPARA	
Endurance	GG=2, CG=1, CC=0
Power	CC=2, CG=1, GG=0

Performance Test

Aerobic capacity

An indirect method was used to measure the VO2max of the participants. Bruce Protocol was used in this study, and participants were required to wear a heart rate monitor (Polar USA, Lake Success, NY, USA). A motorized treadmill (K4B2 COSMED Inc. Italy) was used in this procedure. A 10 stage VO2max test on the treadmill started at 2.74km/h with a gradient of 10%. Usually, the participant will begin by walking on the treadmill in the first stage. 2% of gradient was added for every stage, and the speed was also increased according to the Bruce Protocol. Hall-López et al. (2015) reported high reliability, r=0.907 of this test. The equation for indirect VO2max calculation was described by Foster et al. (1984):

VO_2max (kg/ml/min) = 14.8 – [(1.39 x T) + (0.452 x T2) – (0.012 x T3)] [Equation 2]

Repeated Sprint Ability (RSA)

A 6 x 30 m shuttle sprint test was used to measure their performance (Bishop et al., 2001). The participants were required to sprint six times along the 30 m shuttles, and the time taken for each sprint was recorded. Cones were numbered to avoid confusion among the participants during the run. The equation for performance decrement was calculated as described by Spencer et al. (2006):

Performance decrement (%) = [total sprint time / fastest sprint time) x 100] – 100 [Equation 3]

Statistical Analysis

Statistical Package for Social Science (SPSS) software for Windows application version 26.0 (SPSS, Illinois, USA) was used to analyze the data. Alpha was set at .05 for all analyses, and data are presented as mean ± standard deviation. Descriptive statistics were used for TGS. Mixed between- and within- ANOVA was employed to determine the effect of performance on different endurance exercise intensities changes. The correlations between the performance and TGS were determined using Pearson Correlation analysis.

RESULTS

The demographic characteristics of participants were summarized in table 3. Based on the table below, all groups' characteristics were similar.

Table 3: Demographic Data

Participant	*Age (y)*	*Height (m)*	*Weight (kg)*	*BMI*
High Intensity Group (n=15)	*16.20 ± 0.41*	*1.64 ± 0.70*	*57.52 ± 8.21*	*21.41±2.55*
Moderate Intensity Group (n=15)	*16.40 ± 0.51*	*1.69 ± 0.53*	*60.84 ± 6.32*	*21.31±1.49*
Control Group (C) (n=15)	*16.67 ± 0.49*	*1.69 ± 0.05*	*63.45 ± 5.75*	*22.18±1.74*

The most common genotype was *PPARA* GG (100%), followed by *ACTN3* CT (57.75%) *ACE* II (55.56%). The frequencies of sport-related genetic variants for all the participants are presented in Table 4.

Table 4: Genotype frequency of the sport- related genetic variants

Genes	Polymorphism	N of frequency of the genotype	Genotype frequency (%)
ACE752	287-bp Ins(I)/Del(D) (rs1799752)	II=25, ID=20, DD=0	II= 55.56, ID= 44.44, DD= 0
ACTN3739	Arg(C)577Ter(T) (rs1815739)	TT=11, CT=26, CC=8	TT= 24.44, CT= 57.75, CC= 17.78
PPARA778	Intron 7 G/C (rs4253778)	GG=45, CG=0, CC=0	GG= 100, CG= 0, CC= 0

Most participants carry endurance-related genotypes, with the mean TGS for endurance-related genotypes higher than the mean TGS for power-related genotypes (78.81 and 25.19, respectively). Table 5 describes the means TGS for power and endurance of the field hockey players.

Table 5: The Means TGS for power and endurance of the field hockey players

Total genotype score (TGS)	***Mean (SD)***	***Range (minimum-maximum)***
Endurance TG	*74.81 (14.92)*	*50.00-100.00*
Power TGS	*25.19 (14.92)*	*0.00-50.00*

The performance tests of the field hockey players are presented in Table 6. There was a significant effect of time for all performance measured ($p < 0.05$). The difference in group interventions appears to significantly affect the measured parameters after the eight weeks of training (AC: $p < 0.001$; RSA: $p = 0.002$). Notable changes can be observed in all groups for AC performance after eight weeks of intervention. The pairwise comparison revealed a significant difference for all groups over time for AC ($p = 0.000$, $p = 0.011$, and $p = 0.023$ respectively). For RSA, a significant difference was found in the HI group only with $p = 0.000$. There also was a significant difference between HI and MI, and MI and C at the post-test ($p < 0.05$) in AC and RSA performance. However, there was no significant difference between the M and C groups for AC and RSA performance tests.

Table 6: Performance of the field hockey players

Variables	***HI (n=15)***		***p value***	***MI (n=15)***		***p value***	***C (n=15)***		***p value***
	Pre-test	***Post-test***		***Pre-test***	***Post-test***		***Pre-test***	***Post-test***	
Aerobic Capacity (AC)									
VO_{2max} (kg/ml/min)	*46.65 ± 4.85*	*54.92 ± 2.71*	*0.000**	*42.85 ± 5.87*	*44.95 ± 5.68*	*0.011**	*45.81 ± 8.00*	*43.94 ± 6.60*	*0.023**
Repeated Sprint Ability (RSA)									
Performance decrement (%)	*9.42 ± 6.59*	*4.76 ± 2.68*	*0.000**	*9.88 ± 3.68*	*8.15 ± 3.21*	*0.153*	*8.97 ± 4.06*	*10.78 ± 3.55*	*0.135*

*Significant different between groups ($p<0.05$)

The correlation coefficients (r) of performance changes and TGS are presented in table 7. There was a significant correlation between the endurance TGS and AC (r=0.55, p=0.03) for the MI group. MI group also found a significant correlation between power TGS and AC with r=-0.55, p=0.03. There was negative correlation between power TGS and AC because the study only focuses on aerobic training and there was no strength training that was given throughout the study. Participants with higher endurance TGS improved in AC while higher power TGS reduced in AC. However, there was no significant correlation between TGS and performance changes in HI and C groups.

Table 7: Correlation between TGS for endurance and power with aerobic capacity and repeated sprint ability of the field hockey players

Group	Variables	Mean difference (Post-test – Pre-test)	Endurance TGS		Power TGS	
			Correlation (r)	*p* value	Correlation (r)	*p* value
High Intensity Group	Aerobic Capacity VO_{2max} (kg/ml/min)	9.81	-0.14	0.61	0.14	0.61
	Repeated Sprint Ability					

Malaysian Journal of Sport Science and Recreation
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	Performance Decrement (%)	-4.66	0.01	0.97	-0.01	0.97
Moderate Intensity group	Aerobic Capacity VO_{2max} (kg/ml/min)	3.21	0.55	0.03*	-0.55	0.03*
	Repeated Sprint Ability Performance Decrement (%)	-1.70	-0.23	0.41	0.23	0.41
Control group	Aerobic Capacity VO_{2max} (kg/ml/min)	-3.60	0.18	0.52	-0.18	0.52
	Repeated Sprint Ability Performance Decrement (%)	1.81	-0.09	0.74	0.09	0.74

**Significant difference (p<0.05)*

DISCUSSION

There was a significant correlation between the endurance TGS and AC in the MI group in this study. This study revealed that moderate intensity improves VO_{2max} of the participants with high endurance TGS. This study supported an earlier finding by Bae et al. (2007), where a significant association was found between the genetic polymorphism for *ACE* T-3892C and VO_{2max} after 12 weeks of endurance training among the Korean women. A previous study from Manna et al. (2009) found that maximal aerobic capacity (VO_{2max}) increased in young Indian hockey players aged 14–16 years old. Increased aerobic activity leads to increased myoglobin in the muscle that stores and transports oxygen into cells, allowing more ATP molecules to be produced. This will improve the blood supply to the whole body and enhance the capacity of the muscle to work for a longer duration (Manna et al., 2009). Genetic heritage influences the mass and function of cardiovascular and muscle (Costa et al., 2012), leading to improved performance of the athletes.

Our study also showed no correlation between the TGS and performance test for HI and C groups (p>0.05). Miyamoto et al. (2017) found that *ACE* and *ACTN3* were not associated with the sprint/power performance in the Japanese population. Similarly, Collins et al. (2004) reported no association between *ACE* and *ACTN3* and sprint/power performance among South African athletes. Recently, Drozdovska et al. (2013) further demonstrated the finding that there was no significant difference for the genotype and allele frequencies between Ukrainian athletes and control.

However, some studies determined that the *ACE* II representative the speed or power performance (Amir et al., 2007; Rankinen, Wolfarth, et al., 2000; Scott et al., 2005). Another study involving the Chinese males found that the *ACE* DD genotype was associated and improved with VO_{2max}, maximal work rate, and most significant decreases in heart rate (Rankinen, Pérusse, et al., 2000; Zhao et al., 2003). Individual who carries the DD genotype was reported to have higher power performance (Ahmetov et al., 2016). DD genotype was found higher in Russian athletes (Nazarov et al., 2001) and European and Commonwealth championship swimmers (Woods et al., 2001). Lucia et al. (2006) found no significant difference in VO_{2max} between three genotypes of *ACTN3* among the Caucasian cyclists and runners. And X allele was associated with endurance phenotype (Pimenta et al., 2013).

In the previous study, the *ACE* I allele was associated with endurance (Ahmetov & Fedotovskaya, 2015). This coincides with Ahmetov and Fedotovskaya (2015). Lower *ACE* enzyme activity related to the II genotypes may enhance local nitric oxide concentrations, improving mitochondrial respiration efficiency, thus improving contractile function in cardiac and skeletal muscle (Williams et al., 2000). *ACE* D allele in the current study is associated with power. This supports by Collins et al. (2004). The DD genotype was associated with a higher level of angiotensin II enzyme activity that can directly affect skeletal muscle (hypertrophy) through vasoconstriction and indirectly affects renal salt and water retention via aldosterone (Jones et al., 2002; Puthucheary et al., 2011). These physiological changes due to genetic polymorphism of *ACE* could benefit athletes for the power or sprint-oriented sports.

Next, for the *PPARA* gene, GG genotypes are associated with endurance, while the CC genotypes are associated with power. G allele increased the fatty acid oxidation in skeletal muscle and increased the proportion of slow-twitch muscle fibers (Lopez-Leon et al., 2016). In response to anaerobic exercise, the CC genotypes were associated with a tendency of skeletal muscle hypertrophy and ease of glucose utilization (Ahmetov et al., 2013).

The current study shows different gene role results could be because of the bias in allele disposition. The present study showed that more participants carried the endurance-related genotype than the power-related genotype. The I allele (55.56%) and the D allele (44.44%) in the *ACE* gene while the TT genotype (24.44%), CT genotypes (57.78%), and CC genotypes (17.78%) in the *ACTN3* gene. For *PPARA*, all players carry the GG genotype (100%). Eynon et al. (2011) supported by showing the results, mean for endurance TGS was significantly higher for endurance athletes instead of power athletes and controls. A previous study from Dionísio et al. (2017) showed for the *ACTN3* genotype, 53% were heterozygous, while for the *ACE* genotype were 19% homozygous (II) from 220 Brazilian soccer players. *ACE* DD genotypes and *ACTN3* CT genotypes were found higher among the Serbian national soccer team, and the total participant was 27 females aged 16-18 years old. However, *PPARA* homozygous (GG) was found higher in athletes than in GC and CC genotype in the study (Ahmetov et al., 2013). The study focused on a team sport, and 439 participants carried endurance-related genotype. Tural et al. (2014) supported the study with a total of GG was higher in endurance athletes (63.3%) compared to control (38.2%). In the future, the present study suggests a larger sample size (with a more even distribution of allele in genotypes (i.e., *PPARA*: GG=CG=CC). This could show a different result or a significant difference between the endurance/power TGS and the performance test.

In this present study, the training program was eight weeks. It may not be enough to induce changes in the aerobic capacity and repeated sprint ability. Past research has shown that 16 weeks of training could increase aerobic capabilities and trainability in adolescent field hockey players (Bonova, 2020). Moreover, the study by Subramaniam et al. (2015) showed that 12 weeks of circuit resistance training significantly improved the performance of the 13-

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15 years old novice athletes. Besides that, the fact that alleles cannot be trained may be the cause of the disparity in performance development between each individual (Woods et al., 2002). Future research should increase the endurance training volume from eight weeks to 12-16 weeks. Moreover, future studies should also include other sports so that researchers can distinguish the relationship between the TGS and sports performance.

CONCLUSIONS

In conclusion, current studies prove a significant correlation between the total genotype scores and running performance for the MI endurance training group. A correlation was shown between the endurance total genotype scores and VO_{2max} following the moderate intensity of endurance exercise. This study suggests that moderate intensity exercise can help athletes with high endurance total genotypes scores enhance their performance in aerobic capacity or running capabilities. Therefore, the short-term performance of the athletes can be significantly influenced by the genotypes and training intensity.

The limitation of this study was the adherence of the participants. Absence during the exercise that has been scheduled could affect the data of this study. The researcher encouraged all the participants to overcome this limitation by rewarding them after the eight weeks of intervention. Next, all the participants were required to draw 5ml of their blood for the genotype. It could intimidate some participants to not turn up during the extraction procedure. To overcome this, the researcher had to encourage them by showing the extraction process first and trying to motivate all the participants during the extraction procedure.

Contribution of Main author and Co - authors

Wan Atiyyah Ab Wahab - collect data, lab work, prepare the draft, and revise the manuscript.

Sarina Md Yusof - verify data on sports performance and given final approval

Teh Lay Kek - verify the genotypes data

Suhana Aiman - verify data on sports performance

Elin Elisa Khairul - collect data and lab work

Nur Amirah Asyiqin Zaihuri - verify the genotypes data

Mohd Zaki Salleh - verify the genotypes data

All authors participate and agree to be accountable for all aspect and no self- interest

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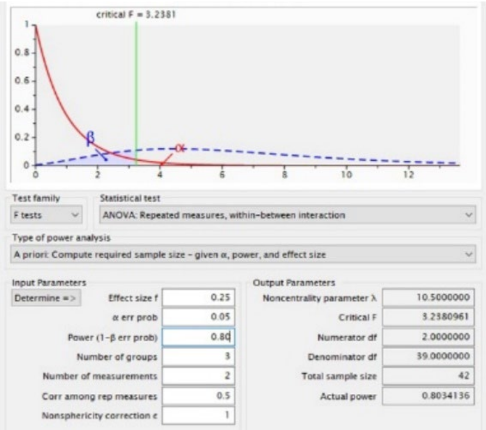
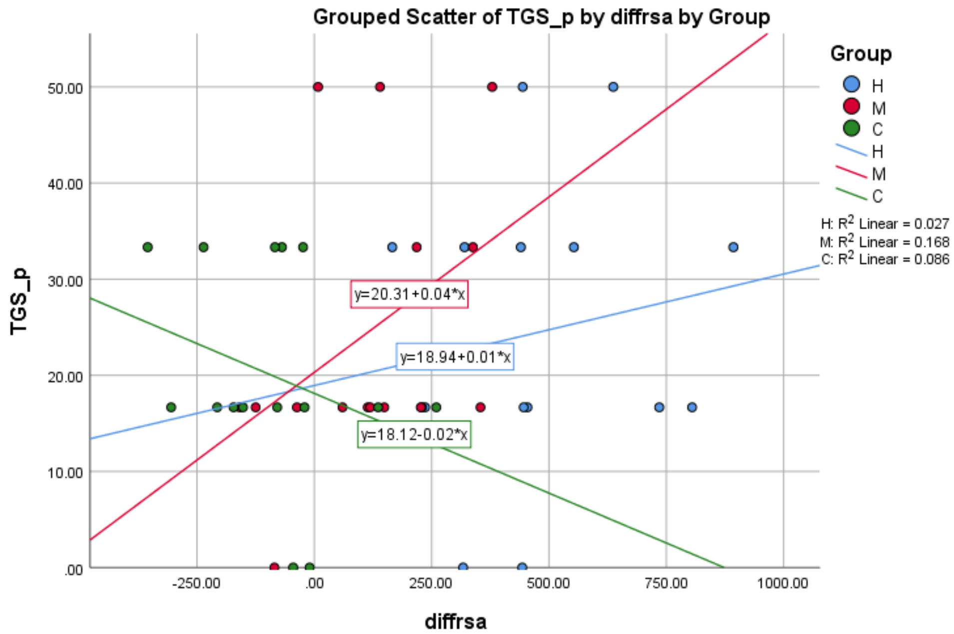
APPENDIX

1.



G-power

2. Scatterplots



Scatterplot relationship between the TGS power and repeated sprint ability

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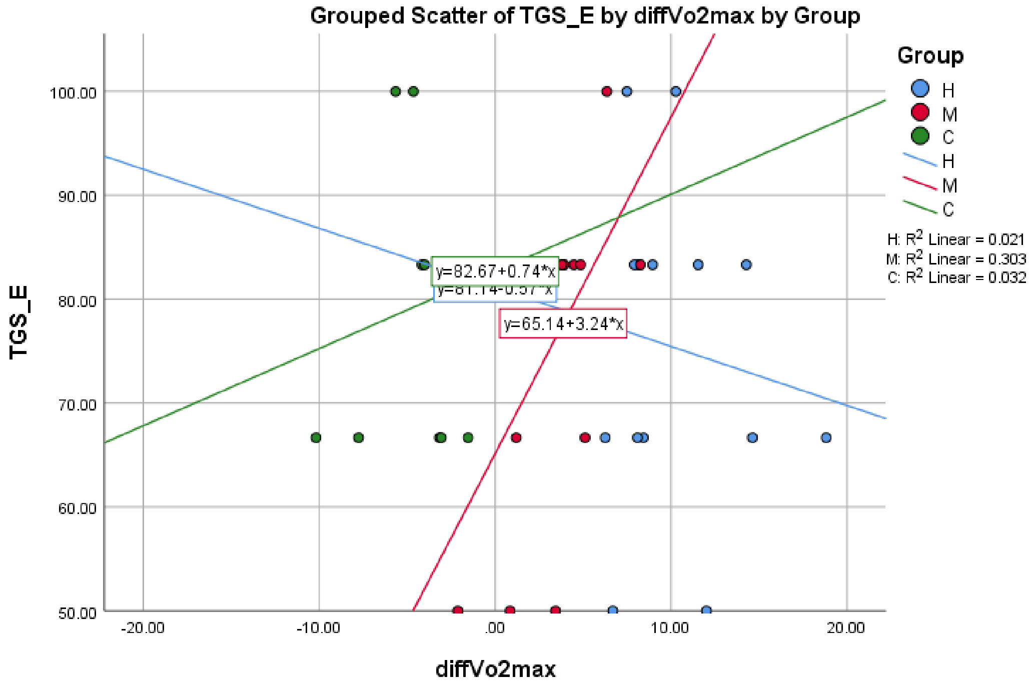

Scatterplot relationship between the TGS endurance and aerobic capacity

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