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ASSOCIATION BETWEEN SPORT RELATED POLYGENIC PROFILE AND MAGNITUDE OF PHYSICAL PERFORMANCES CHANGES FOLLOWING RESISTANCE TRAINING AMONG NOVICE FIELD HOCKEY ATHLETES

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ABSTRACT

Background: Studies have shown that inter-individual variation in response to resistance training is attributed to genetic variation. Aim: To correlate total genotype scores (TGS) with the magnitude of physical performances (skeletal muscle mass, muscular strength, muscular power, performance decrement, and VO_2max) change, following 8 weeks of resistance training. Methods: We included forty-five (N=45) participants (age = 16.53 ± .5 years old, body mass = 61.03 ± 6.67 kg, height = 1.67±.05 m) and randomly assigned into; high intensity resistance training (n=15), moderate intensity resistance training (n=15) and a control group (n=15). They were prescribed with the same upper and lower body exercise routines. Pre- and post-test physical performances were measured. Genotyping was conducted using in-house designed allele-specific polymerase chain reaction assays. TGS for nine SNPs: Angiotensin Converting Enzyme, ACE rs1799752; Alpha-acitin-3, ACTN3 rs1815739; Adrenergic Receptor B3, ADRB3 rs4994; Angiotensinogen, AGT rs699; Bradykinin Receptor B2, BDKRB2 rs1799722; Peroxisome Proliferator-Activated Receptor Alpha, PPARA rs4253778; Peroxisome Proliferator-Activated Receptor Gamma Co-Activator 1-Alpha, PPARGC1A rs8192678; Thyrotropin Releasing Hormone Receptor, TRHR rs7832552 and Vascular Endothelial Growth Factor, VEGF rs1870377,were calculated and assigned with strength-power and endurance quality. Pearson correlation analysis was employed to investigate the correlation between TGS, and magnitude of physical performances change following intervention. Result: There is a significant correlation between strength-power TGS with lower body muscular strength (r= .65, p<.01) and power (r= -.69, p<.01) following moderate intensity resistance training. Conclusion: In this study, it is demonstrated that participants with higher strength-power TGS, gained greater lower body strength improvement and lesser muscular power decrement even with moderate intensity as compared to high intensity resistance training. Therefore, personalising training based on athlete's genetic profile may optimise performance.

Keywords: *Field hockey; Single nucleotide polymorphism; Resistance; Training.*

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INTRODUCTION

Field hockey is a competitive, physically demanding, high intensity intermittent sport (Sharma & Kailashiya, 2018; Razali et al., 2017). This sport requires the action of walking, jogging, jumping, accelerating, and decelerating while changing the movement direction (Sharma & Kailashiya, 2018; Razali et al., 2017; Bazyler et al., 2015; Pimenta et al., 2012). Critical to success and excellence in most intermittent sports are the strength-power development aside from an excellent aerobic capacity (Bazyler et al., 2015; Bishop et al., 2015; Bishop & Girard, 2015; Elferink-Gemser et al., 2004). Muscle strength-power development enabled the athletes to maintain a good, adapted posture, pass over the ball at a greater distance, achieve optimal sprinting speed and recover at a faster rate in between sprint during tournament and training (Bazyler et al., 2015; Elferink-Gemser et al., 2004; Lemos et al., 2017).

It is generally accepted that the interaction between intrinsic and extrinsic factors deter individual's athletic abilities in term of their physical capacities (Ginevičienė et al., 2011; Vancini et al., 2014). Extrinsic factors (e.g., training) influenced athlete development, whilst intrinsic factor (e.g., genetic) is equally important to the inter-individual differences in the development of sport performance phenotypes (Guilherme & Lancha, 2020). The achievement of athletes is determined by the combination of skills acquired through training and their phenotypic traits, which are genetically influenced (Ginevičienė et al., 2011; Tucker & Collins, 2012). It was suggested that heritability explains 66% of athletic status (Tucker & Collins, 2012). Two recent meta-analyses estimated that heritability contribute 49 to 56% variability of strength-power and 44 to 68% of endurance phenotype (Miyamoto-Mikami et al., 2017; Zempo et al., 2017).

Individual physical capacity reflects the combination of complex phenotype influenced by genetic and environmental factors (Atabas et al., 2020). To meet the physical demand for optimal performance, the prescription of appropriate training is essential (Sharma & Kailashiya, 2018). Generally, resistance training (RT) had been well known to improve the strength-power performance of athletes (Baechle & Earle, 2008). Effective RT prescription involves manipulating several training variables (e.g., intensity, volume, frequency, rest interval, exercise selection and order) (Mangine et al., 2015). High intensity and moderate intensity RT had significantly improved several performance components, especially in intermittent sport (Mangine et al., 2015; Assuncao et al., 2016; Christou et al., 2006).

Association studies in twin have shown that gene polymorphism plays its role in younger age groups in both health and performance related phenotypes (Ahmetov et al., 2013; Pickering & Kiely, 2019). In this is younger age groups, there was expected to be a higher genetic contribution to phenotypic variation and more readily recognisable interaction between genetic and environmental components (Ahmetov et al., 2013). However, there are only a few studies have reported the association between sport-related polygenic profiles and physical performances following different RT intensities, especially among novice athletes. Therefore, this study aimed to investigate the correlation of TGS with the magnitude of changes in physical performances following different RT intensities among the novice male field hockey athletes.

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METHODS

Participants

A true experimental design was used in this study. Purposive sampling was employed to recruit forty-five (N=45) male field hockey athletes from several local high school clubs. The sample size was calculated using G-power software with consideration of 20% drop-out rate according to Cohen (1988). The participants were randomly assigned to three groups: i) high intensity RT (HI RT, n=15), ii) moderate intensity RT (MI RT, n=15), and iii) Control group (C, n=15). The inclusion criteria were male, youth field hockey athletes (age = 16.53 ± .08 years old), healthy, free from any injuries for the past six months. They also must be active players for the past one year, participating in any competitive field hockey tournaments without prior experiences to RT. The study protocols and objectives were explained to the participants before obtaining their written informed consent. A questionnaire and PAR-Q+ was distributed to obtain their demographic data and to determine their readiness for RT. Electrocardiogram (ECG) test was also conducted to detect cardiac abnormalities. This study was approved by the university's Research Ethics Committee [ref no.:600-IRMI (5/1/6)].

Physical performance assessments

Anthropometry and body composition

Participants dressed in light clothing. Their standing height was measured by a digital scale, BSM170 (Biospace, Seoul, South Korea) to the nearest 0.1 cm, whilst their body weight was measured using Bio-Impedance Analyser (BIA) machine, InBody270 (Biospace, Seoul, South Korea) to the nearest 0.1 kg. Then, skeletal muscle mass (SMM) was also assessed with BIA according to the standardized procedures described elsewhere (Padkao & Prasertsri, 2019).

Muscular strength

The upper body (UB) and lower body (LB) muscular strength were measured by the 1-RM bench press (BP) and leg press (LP) procedures based on the indirect one-repetitive maximum (1-RM) measurement protocol described in Baechle and Earle (2008). The proper lifting procedures were explained to ensure correct lifting technique to prevent injury. The participants were instructed to lift the weight at ~50% BW to allow 8 to 10 repetitions followed by 1 minute rest. Then, 4 to 9 kg and 14 to 18 kg of weight were added, respectively to allow 7 to 10 repetitions. They were instructed to lift maximally. Familiarization session was conducted before the actual measurement. Measurement was assisted by an experienced instructor. Proper stretching, warm up and cooling down sessions were conducted before and after measurement. The calculation 1RM was based on an equation published in Brzycki (1993).

Muscular power

LB muscular power was conducted through vertical jump (VJ) procedures (Sayers et al., 1999). A commercial vertec device was used, and the participant's standing reach height was measured. They were instructed to squat >90º and pause before jumping as high as possible with dominant hand reaching the swivel vane. Three jump attempts were carried out with 1-minute rest between each jump. The best jump was equated into an equation by Sayers et al.

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(1999). The jumping procedure was explained and demonstrated to the participants to ensure the correct technique was used to prevent injury. Proper stretching, warm up and cooling down sessions were conducted before and after measurement to prevent injury. Familiarization session was provided before the actual measurement.

Repeated sprint ability

was measured by 6 × 30 m repeated sprint ability (RSA) test. The test procedure was explained and demonstrated to the participants to ensure the correct techniques were used to prevent injury. Participants were instructed to stand 0.5 m behind the starting line and accelerated for 30 m for 6 repetitions with 25 seconds of active recovery for about 40 m. They were advised to sprint maximally as pacing was discouraged. Proper stretching, warm up and cooling down sessions were conducted before and after measurement to prevent injury. The indices of a single sprint time, fastest sprint time, average sprint time, and total sprint time were measured and incorporated into the equation to calculate PD% as described in Spencer et al. (2006).

VO_2max

Bruce treadmill protocol was used to measure VO_2max. The participants were instructed to run on the treadmill until exhaustion. The use of handrails was discouraged. The heart rate (HR) was monitored continuously. Time of exercise cessation was taken and incorporated into the equation as described in Foster et al. (1984).

Resistance training protocol

Both RT groups trained three times per week on non-consecutive days. The training volume was pre-determined and equated as described in Klemp et al. (2016). One week of familiarization was designed to ensure they performed the correct lifting techniques. The same upper body (biceps curl, triceps extension, shoulder press) and lower body (leg press, hamstring curl, calf raise) exercise routines were performed by the participants in a gym facility nearby. The HI RT was performed at 3 sets of 1 to 6 repetitions at 80 to 90% 1-RM, whilst the MI RT was performed at 3 sets of 8 to 12 repetitions at 60 to 75% 1-RM. Proper stretching, warm-up and cooling-down sessions were conducted before and after each of the training sessions to prevent injury. During the intervention period, all participants were advised to refrain from any additional RT sessions and maintain their habitual physical activity and dietary intake.

Genotyping

DNA was extracted using an in-house modified conventional DNA extraction method. Five (5 ml) of 1 × lysis buffer (0.64 M sucrose, 0.02 M Tris hydrochloric acid, 2% Triton X-100, autoclaved Mili-Q water) was added to 5 ml of blood sample and inverted (10 times). Later, another 5 ml of 0.5 × lysis buffer, was added to the blood sample and inverted (10 times), left on ice (10 minutes) and later centrifuged (Eppendorf, model 5810R, Hamburg, Germany) at 2,700 × g, 20℃ for 15 minutes. The pellet was rinsed with 25 ml of Tris Ethylenediaminetetraacetic acid (EDTA) (pH 8.0) buffer followed by centrifugation at 2,700 × g, 20℃ for 15 minutes. The supernatant was discarded whilst the pellet was kept. The steps above were repeated three times until a clear supernatant and pellet were formed. The pellet was re-suspended with 2 ml saline EDTA (pH 8.0) and incubated overnight in a water bath at 37℃ with 100 µl of 20 % Sodium Dodecyl Sulfate (SDS) and 10 µl proteinase-K solution (20

mg/ml). Then, 100 µl of 2.0 M Potassium Chloride (KCl) was added followed by 4 ml of cold 100% ethanol. DNA became visible as a floating strand in the solution. Then, 700 µl of cold 70% ethanol was used to rinse the DNA and left to dry. The DNA was reconstituted with Tris EDTA buffer and stored at -20℃. The DNA's concentration and purity were measured using NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, USA).

Genotyping was conducted using ASPCR assays. The primers were designed to target specific single nucleotide polymorphism (SNPs). The most prominent SNPs that have been associated with sport performances had been chosen for this study (Atabas et al., 2020; Hughes et al., 2011). ASPCR was conducted using a thermal cycler (Takara Bio, CA, USA) with the final mixture volume of 20 µl, containing participant's DNA (100 ng/µl), 0.5 U/µl of *taq* DNA polymerase (NEB, MA, USA), various concentrations of primers (0.1-2.0 µM), 0.16 m M deoxyribonucleotide triphosphate (dNTPs) and autoclaved Mili-Q water. A touchdown thermal cycle condition was composed of; pre-denaturation at 95℃ (2 minutes), denaturation at 95℃ (30 seconds), and extension at 68℃ (30 seconds). In the initial 10 cycles of annealing, the temperature was gradually decreased (-1℃) from 65℃ to 55℃ (30 seconds). The latter half (30 cycles) temperature was maintained at 55℃ (30 seconds). Finally, post-extension was set at 68℃ (5 minutes). PCR products were electrophoresed at 200 V for 60 minutes and examined on 3.5 % agarose gel stained with Ethidium Bromide (EtBr). The gel was visualized under ultraviolet light to detect the presence of specific bands sizes which indicates the alleles.

Calculation of TGS

The TGS was calculated using an algorithm that incorporated all genotype score in a simple additive model (Ruiz et al., 2009; Ruiz et al., 2010). 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 presented in table 1

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Table 1: Genotype scoring

Variables	Genotype scoring
Genes	
ACE	
S-P	DD=2, ID=1, II=0
E	DD=0, ID=1, II=2
ACTN3	
S-P	CC=2, CT=1, TT=0
E	CC=0, CT=1, TT=2
PPARA	
S-P	CC=2, CG=1, GG=0
E	GG=2, CG=1, CC=0
ADRB3	
E	AA=2, AG=1, GG=0
BDKRB2	
E	TT=2, TC=1, CC=0
VEGF	
E	CC=2, CG=1, GG=0
PPARGC1A	
E	GG=2, AG=1, AA=0
AGT	
S-P	TT=2, TC=1, CC=0
TRHR	
S-P	CC=2, AC=1, AA=0

Abbreviation: E=endurance, S-P=strength-power

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 26.0 (SPSS, Illinois, USA). All measurement was found to be normally distributed (Shapiro-Wilk test, $p>.05$) except for LB muscular power variables. Descriptive analysis of the TGS was performed. Pearson correlation analysis was used to investigate the association between TGS and magnitude of physical performances improvement following different RT intensities. Statistical significance was set at ($p<.05$), and the data was presented in (M±S.D) unless otherwise stated.

RESULTS

Participant's description

Majority of the participants presented with heterozygous genotypes except for PPARA GG=100.0% and ADRB3 AA=100.0% which are homozygous genotypes. The genotype frequencies are presented in table 2.

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Table 2: Genotype frequencies of the sport-related genetic variants

Variables	(%)
Genes	
ACE	
II	31.10
ID	55.60
DD	13.30
ACTN3	
CC	24.40
CT	53.30
TT	22.20
PPARA	
CC	.00
CG	.00
GG	100.00
ADRB3	
AA	100.00
AG	.00
GG	.00
BDKRB2	
TT	17.80
TC	44.40
CC	37.80
VEGF	
CC	15.60
CG	48.90
GG	35.60
PPARGC1A	
GG	22.20
AG	93.30
AA	4.40
AGT	
TT	.00
TC	33.30
CC	66.70
TRHR	
CC	20.00
AC	55.60
AA	24.40

Baseline characteristics and TGS

There was no significant different (p>.05) across groups at baseline except for LB muscular power (p=.01) whereby HI RT (M=4.27) is higher as compared to MI RT (M=3.58 kW) and C (M=3.79 kW). The overall mean TGS for strength and endurance are 17.67 and 48.93, respectively. There was no significant different (p>.05) in TGS across groups. The baseline physical performances and TGS across groups are presented in table 3.

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Table 3: Baseline characteristics and TGS across training group

Variables	HI (Mean ± S.D)	MI (Mean ± S.D)	C (Mean ± S.D)	*p*-value
TGS				
Strength-power	17.42 ± 1.07	18.53 ± 1.29	17.05 ± 1.58	.72
Endurance	48.56 ± 1.92	49.67 ± 1.99	48.56 ± 2.39	.91
Physical performances				
SMM (kg)	28.80 ± .89	27.80 ± 3.75	27.56 ± 2.27	.48
UB muscular strength (kg)	44.93 ± 3.84	44.13 ± 3.81	44.13 ± 3.81	.80
LB muscular strength (kg)	123.00 ± 11.62	122.33 ±13.21	118.47 ± 9.08	.51
LB muscular power (kW)	4.27 ± .61	3.58 ± .72	3.79 ± .51	.01*
PD (%)	8.75 ± 1.92	9.56 ± 2.35	9.25 ± 2.38	.61
VO2max (kg.ml/min)	50.56 ± 4.45	48.79 ± 3.89	49.56 ± 4.15	.52

*significant different (p<.05)

Correlation between TGS and physical performance changes

Significant correlation was found between strength TGS with LB muscular strength (r=.65, p<.01) and LB muscular power (r=-.69, p<.01) following MI RT. Participants with higher strength TGS, gained greater increment in LB muscular strength, whilst LB muscular power experienced lesser decrement following MI RT. The correlation coefficients (r) from the association between magnitude of physical performance changes and TGS are presented in table 4.

Table 4: Correlation between TGS and magnitude of physical performance changes

Variables	Mean difference	TGS Strength-power r	*p*-value	Endurance r	*p*-value
Training groups					
HI					
SMM (kg)	0.90	.37	.18	.27	.34
UB muscular strength (kg)	21.87	.22	.43	.52*	.05
LB muscular strength (kg)	42.60	.02	.94	.11	.69
LB muscular power (kW)	0.78	-.07	.81	-.50	.06
PD (%)	-1.71	.23	.41	.12	.66
VO2max (kg.ml/min)	-0.25	-.44	.10	.45	.10
MI					
SMM (kg)	0.38	-.14	.62	-.32	.24
UB muscular strength (kg)	16.27	.02	.95	-.54*	.04
LB muscular strength (kg)	18.53	.65**	.008	-.24	.40
LB muscular power (kW)	0.45	-.69**	.005	-.05	.87
PD (%)	-0.33	.44	.10	.05	.86
VO2max (kg.ml/min)	-0.15	.33	.24	-.01	.96
C					
SMM (kg)	0.04	.12	.66	-.20	.47
UB muscular strength (kg)	-0.27	.07	.80	.12	.68
LB muscular strength (kg)	-4.47	.14	.61	.12	.67
LB muscular power (kW)	-0.10	-.44	.10	.16	.57
PD (%)	0.55	.30	.28	-.20	.47
VO2max (kg.ml/min)	-0.47	-.47	.08	.45	.09

*significant different (p<.05), **significant different (p<.01)

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DISCUSSIONS

Human physical capacity is influenced by many environmental and genetic factor (Murtagh et al., 2020; Hughes et al., 2011). Numerous single SNPs had been associated with physical performances and had been replicated in number of investigations (Thomis et al., 2004; Pescatello et al., 2006; Charbonneau et al., 2008; Colakoglu et al., 2005; Giaccaglia et al., 2008; Pereira et al., 2013; Gentil et al., 2011; Moraes et al., 2018; Erskine et al., 2014). However, physical performances phenotype had been accepted to be polygenic (multiple SNPs) in nature (Hughes et al., 2011). Combinations of multiple SNPs rather than single SNPs likely deter human physical capacity (Murtagh et al., 2020). Most of the participants harboured endurance related genotypes compared to strength genotypes. Amato et al. (2018) demonstrated similar findings whereby their professional Italian soccer athletes were more endurance oriented (TGS = 56.44 %) as compared to strength (TGS = 43.52 %). However, the Russian professional soccer players presented with higher strength TGS (M = 52.00) as compared to healthy control (M = 41.30) (Egorova et al., 2014).

The features of field hockey itself required both UB and LB to work simultaneously to hold the stick and hit the ball whilst the LB need to maintain semi crouched posture and the capacity to sprint over a ball or even goal shooting (Razali et al., 2017; Bishop et al., 2015). Specifically, the aerobic component dominates energy delivery, however, most decisive actions such as winning ball possession, scoring, and conceding goals were mostly covered by anaerobic metabolism (Bazyler et al., 2015; Lemmink et al., 2004). Both energy system plays their role and understanding athlete's genetic background might aid in the planning for training or placement of the athlete during games appropriately.

The present result indicates that participants with higher strength-power TGS, shown greater improvement in their LB muscular strength although trained in MI RT. In addition, the HI RT group revealed no significant correlation between strength-power TGS with the physical performance variables. There might be a possibility of non-responder individuals in the HI RT group which resulted in non-significance correlation with physical performances (Pickering & Kiely, 2019). The individual variation in response to RT must be taken into consideration, with some participants experiencing greater or lesser improvement as compared to others with the same training intensity prescribed (Pickering & Kiely, 2019). Accordingly, the level of adaptation experienced by an individual will be dependent on the interaction between specific training performed and genotype (Tucker & Collins, 2012).

Christou et al. (2006) reported that additional 16 weeks of MI RT (55 to 80% 1RM) to soccer training in adolescent novice soccer athletes led to improvement of 1RM UB, 1RM LB, VJ, and sprint ability. Another investigation revealed, 12 weeks of RT, increased both muscle strength and power performance in the population of adolescent football players (Gavanda et al., 2019). However, another study among healthy untrained adolescents reported no significant 1RM UB and LB strength improvement following 9 weeks of either HI or MI RT (Assuncao et al., 2016). Lesinski et al. (2016) stated that beneficial effect of short-term (<24 weeks) RT is almost similar with 9 to 12 weeks of RT, but longer period of training (>24 weeks) was more effective in youth or novice athletes.

Increased muscular power is a priority in team sports as it is required in most of the decisive action during games (Moore et al., 2005). Presently, participants experienced lesser

LB muscular power improvement following MI RT. RT alone is insufficient to improve power performance (Moore et al., 2005; Vissing et al., 2008). Additional power training is essential as Moore et al. (2005) revealed that combination of RT with power training resulted in significant muscular power improvement among novice soccer athletes. Combination of power training with RT will provide more substantial power improvement as compared to RT alone in youth athletes.

CONCLUSIONS

In conclusion, this study revealed that there is a relationship between strength-power TGS and magnitude of LB muscle strength and power changes following MI RT intensities among novice field hockey athletes. Practical application from this study may include genotyping for performance SNPs at an early age before the development of performance phenotype to help predict response to training. The potential to predict RT response may be useful to ensure an individual with higher strength-power or endurance TGS could be given appropriate training to maximize adaptation towards training. Young talented adolescent with favourable strength-power or endurance associated genetic profile could be selected and given appropriate training with the aim to compete in higher level later in their sporting careers.

Authors' contributions:

Elin Elisa Khairul (elinelisa89@gmail.com) (ORCID ID: 0000-0003-3433-5135) data collection and draft preparation.

Teh Lay Kek (tehlaykek@uitm.edu.my) (ORCID ID: 0000-0002-2830-4263) verify genotypes data and edit the paper for accuracy of data presented.

Mohd Zaki Salleh (zakisalleh@uitm.edu.my) (ORCID ID: 0000-0001-9882-0661) verify the data for publication, approval of the final draft.

Raja Mohammed Firhad Raja Azidin (firhad@uitm.edu.my) (ORCID ID: 0000-0003-4770-2870) verify resistance training programme.

Sarina Md. Yusof (sarin864@uitm.edu.my) (ORCID ID: 0000-0002-3764-7815) conceive and design the study, verify physical performance data.

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