

Association and Predictive Ability of *ACTN3 C/T*, *ACE I/D* and *PPARGC1A A/G* Polymorphisms on Jump Height of Elite Malaysian Footballers

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Submission date: 31 July, 2024

Accepted date: 22 August, 2024

Published date: 15 September, 2024

ABSTRACT

Footballing performance is a complicated phenotype influenced by various factors that include individual genetic makeup and environmental variables. Genetic association research in football is gaining significant interest but the majority of the studies involve Caucasian and Brazilians footballers. Therefore, this study aims to investigate the association and predictive ability of a total genotype score derived from *ACTN3 C/T*, *ACE I/D* and *PPARGC1A A/G* polymorphisms on jump height of elite Malaysian footballers. A total of sixty-seven elite Malaysian Malay male footballers (19.8 ± 1.4 years) participated in this study and genomic DNA was extracted from saliva samples for genotyping and calculation of the total genotype score. The jump height of each participant was assessed using the Yardstick vertical jump device. The mean jump height and total genotype score of the participant were 61.9 ± 6.5 cm and 38.8 ± 18.0 , respectively. Spearman rank-order correlation indicated that a significant positive correlation was found between the total genotype score and jump height that remained significant ($r_s = 0.276$; $p = 0.027$) after adjusting for height and body mass of the participants. A significant regression was found $F(1, 65) = 6.554$, $p = 0.013$ with the r^2 value of 0.092 indicating that the total genotype score explained 9% of the variance in jump height. The positive association between total genotype score and jump height in elite Malaysian footballers indicated that footballers with a greater total genotype score tend to attain a higher jump height and may have further implications in football as literature has indicated that jump performance could be used to infer speed and maximal strength.

Keywords: Football, Sport Genomic, ACE, ACTN3, PPARGC1A

INTRODUCTION

Elite performance in athletics is a complicated phenotype influenced by various factors that include individual genetic makeup and environmental variables such as diet and training (Ahmetov et al., 2022). Eventhough the abilities exhibited at the highest competitive level in different sports required sport-specific and individualized training schemes, it is not possible to ignore the growing results of genetic contributions to individual physical performance variations. In the past two decades, there has been a significant surge in interest surrounding genetic factors influencing every aspect of athletic performance (Sarmiento et al., 2020). As of May 2023, there are 251 genetic markers reported to be associated with athletic performance and these include endurance- and power/strength-related genetic markers (Semenova et al., 2023). Although sports at the opposing ends of the endurance-strength continuum continued to be the main focus in genetic association research, an increase in interest on team-sports, such as football, is seen in the last ten years (McAuley et al., 2021b). Football is an intermittent sport with high intensity that relies on a number or interconnected parameters, such as physiological, psychological and technical components. Several studies have highlighted the influence of specific sport-related genetic polymorphisms on footballers' performance, status and/or training responses such as that of Jones et al. (2016) who reported that the matching footballers' genotype with the appropriate training modality led to a more effective resistance training and Petr et al. (2022) who reported speed and power-related gene polymorphisms were associated with playing position in elite soccer players.

A recent systematic review reported that the *angiotensin I converting enzyme (ACE)* and *actinin alpha 3 (ACTN3)* genes were the most commonly investigated genetic markers in football genetic association research (McAuley et al., 2021b). The insertion (*I*) allele of the *ACE* gene involves an Alu repeat sequence of 287 base pairs within intron 16 is associated with reduced serum and tissue ACE activity in comparison to the deletion (*D*) allele which lacks the 287-bp Alu repeat sequence (Puthuchery et al., 2011). On the other hand, the substitution of *C* allele with *T* allele in the *ACTN3* gene that causes arginine at position 577 to be replaced with a stop codon leads to the absence of ACTN3 production in fast twitch muscle fibers that are involved in explosive movements (El Ouali et al., 2024). The *D* allele of *ACE* and *C* allele of *ACTN3* tend to be associated with power athlete status while endurance athlete status has been associated with the *I* allele of *ACE* and *T* allele of *ACTN3*. Several case-control studies have indicated that the *ACE DD* and *ACTN3 CC* genotype is often linked to success in speed-strength disciplines like short-distance running, long jump, high jump, and short-distance swimming (Ahmetov et al., 2013; Cieszczyk et al., 2016; Dionísio et al., 2017; Papadimitriou et al., 2016). Conversely, individuals with the *ACE II* genotype tend to excel in endurance-related disciplines such as medium- and long-distance running, race walking, hockey, and rowing (Ulucan et al., 2015). However, it should be noted that conflicting findings exist in some studies regarding these associations as described by McAuley et al. (2021a).

Other than *ACE* and *ACTN3* genes, the *PPARGCIA* gene also appears to be genetic marker of interest as it encodes for is involved in mitochondrial biogenesis, fatty acid oxidation, glucose utilization, thermogenesis and angiogenesis also represents a genetic marker of interest (Hall et al., 2023). Specifically, the *A* allele which results in glycine (*G* allele) being substituted by serine at position 482 of the encoded protein, has been found to be a power-associated marker (Gineviciene et al., 2016). It has been observed that in Lithuanian footballers, specifically attackers, the distribution of *PPARGCIA* genotypes differ significantly from sedentary controls (Gineviciene et al., 2014). However, these genetic associations were mainly discovered among athletes of other nationalities and hence, highlights the importance of replication studies to be conducted among Malaysian athletes given the contrasting allelic associations reported by McAuley et al. (2021a). The present study aims to investigate the association and predictive ability of a total genotype score derived from the *ACE I/D*, *ACTN3 C/T* and *PPARGCIA A/G* polymorphisms on jump height of Malaysian elite footballers. The present study also focused on jump height as it is commonly performed in footballers to assess their lower limb explosive power and prior studies have reported of its correlation with sprinting and maximal strength.

METHODOLOGY

Participants

A total of sixty-seven ($n = 67$) Malaysian Malay male footballers who competed at either Malaysia Youth Cup, President Cup or Premier League provided informed consent and participated in this study. Only participants who were of Malay ethnicity with no family history of admixture or inter-marriage for at least three generations and no contraindications to physical activity as indicated by the Physical Activity Readiness Questionnaire plus questionnaire were eligible to participate in the study. Post hoc power analysis using G*Power indicated that a sample size of 67 was sufficient to detect correlation of a small effect size. All players were informed about the aim and methodology of this study prior to the testing. The study protocol was approved by the Research Ethics Committee at Universiti Teknologi MARA (Approval code: REC/07/2022 (ST/MR/151)).

Allele-specific PCR

Saliva sample from each participant was collected and genomic DNA was extracted using the PSP SalivaGene DNA Kit (Invitex Molecular, Germany) according to manufacturer's recommendations. Genotyping of *ACTN3* C/T, *ACE* I/D, and *PPARGC1A* A/G polymorphisms were performed using allele-specific PCR as described previously (Ang et al., 2023). Genotyping by PCR was carried out in a final volume of 20 μ l consisting of 1 \times PCR reaction buffer, 2 mM magnesium chloride, 0.16 mM deoxyribonucleotide triphosphates, 0.5% (v/v) of dimethyl sulfoxide, varying concentrations of primers, 0.5 U of Taq DNA polymerase (Biotools B&M Labs., Madrid, Spain), 100 ng of genomic DNA and sterile deionised water. The primer sequences used are listed in Table 1. Amplification of the targeted DNA fragments was carried out in a T100 thermal cycler (Bio-Rad Laboratories, California, USA) under the following conditions: initial denaturation at 95°C for two minutes, followed by 30 cycles of denaturation at 95°C for 20 seconds, annealing at the optimised temperature for 20 seconds, and extension at 68°C for 30 seconds. A final extension step at 68°C was carried out for five minutes before the amplification products were electrophoresed in a pre-stained agarose gel (iNtRON Biotechnology, Seoul, Korea) and visualized under ultraviolet light with a gel documentation system (Uvitec, Cambridge, UK).

Table 1: List of primer sequences used in this study.

Gene (rsID)	Sequence (5'-3')	Amplicon size	
<i>ACE</i> (rs4343)	Forward outer primer:	TAAGCCACTGCTGGAGAGCCACTC	Common: 373 bp
	Reverse outer primer:	TGAGGAAAATGAAGGGACCCAAGTG	D allele: 228 bp
	Inner primer-D allele:	CCCATAACAGGTCTTCATATTTCCGGTAC	I allele: 197 bp
	Inner primer-I allele:	ATCTGACGAATGTGATGGCCCCA	
<i>ACTN3</i> (rs1815739)	Forward outer primer:	ACAGGAGGCCGGGTTCTTGT	Common: 292 bp
	Reverse outer primer:	TGTCCTGCGGGCTGAGGG	C allele: 201 bp
	Inner primer-C allele:	CATGATGGCACCTCGCTCGCG	T allele: 132 bp
	Inner primer-T allele:	AACACTGCCCGAGGCTGCCT	
<i>PPARGC1A</i> (rs8192678)	Forward outer primer:	TGAGAGAGACTTTGGAGGCAAGCAAG	Common: 306 bp
	Reverse outer primer:	CATTGAACAATGAATAGGATTGCGTGC	A allele: 187 bp
	Inner primer-A allele:	TTGACGACGAAGCAGACAAGAACA	G allele: 170 bp
	Inner primer-G allele:	CTGAAATCACTGTCCTCAGTCCCC	

Measurements

The standing height and body mass of each footballer was recorded using a stadiometer and a scale. Following anthropometric measurement, participants completed the FIFA 11+ warm-up before vertical jump height was assessed using the Yardstick vertical jump device (Swift Performance Equipment, Australia; ICC = 0.98) (Gabbett et al., 2008). The Yardstick was adjusted to each of the participant's

standing reach height before a countermovement jump was executed by performing a rapid downward movement to a self-selected depth followed immediately by a maximal intensity vertical jump. At the peak of the jump, participants used the fingers of their dominant arm to displace horizontal vanes of the Yardstick vertical jump device. Each participant completed two trials separated by a one-min rest and the highest jump height based on the number of vanes that were displaced was used for analysis.

Data analysis

All statistical analyses were performed with SPSS 29 (SPSS, IBM, USA). In order to generate the total genotype score, a genotype score of 2 was assigned to the ‘optimal’ power genotype whereas the ‘less optimal’ was assigned a score of 0. The heterozygous genotype was assigned a score of 1. The genotype score for *ACTN3* C/T, *ACE* I/D, and *PPARGCIA* A/G polymorphisms were summed up for each participant and transformed to the scale of 0-100 to generate the total genotype score. Spearman rank-order correlation was conducted to determine the association between total genotype score and jump height. Spearman rank-order correlation is denoted as r_s . Simple linear regression was also performed to study the predictive ability of the total genotype score on jump height.

RESULTS AND DISCUSSION

The mean age, height and body mass of sixty-seven Malaysian Malay male footballers were 19.9 ± 1.4 years, 170.7 ± 6.4 cm and 65.8 ± 7.5 kg, respectively. The distribution of all genotypes conformed with Hardy-Weinberg equilibrium ($p > 0.05$). The most prevalent genotype for *ACTN3*, *ACE* and *PPARGCIA* polymorphisms were CT genotype (56.7%), II genotype (58.2%) and AG genotype (52.2%), respectively (Table 1). The mean jump height of the participants was 61.9 ± 6.5 cm. The lowest jump height recorded was 48 cm and the highest jump height was 77 cm. The total genotype score of the participants ranged from 0 to 83. The mean of the total genotype score was found to be 38.8 ± 18.0 , indicating that most of the players carried less favourable power-associated genotypes. Spearman rank-order correlation was performed to determine the association between total genotype score and jump height as the total genotype score was found to be not normally distributed. Preliminary scatterplot analysis indicated a monotonic relationship between total genotype score and jump height. A significant positive correlation ($r_s = 0.274$; $p = 0.025$) was found between the total genotype score and jump height. The positive correlation remained significant and unchanged ($r_s = 0.276$; $p = 0.027$) after adjusting for height and body mass of the participants. Simple linear regression analysis was conducted to assess the ability of the total genotype score to predict jump height. A significant regression was found $F(1, 65) = 6.554$, $p = 0.013$. The r^2 value was 0.092, indicating that the total genotype score explained 9% of the variance in jump height. The regression equation was: jump height = $57.715 + 0.109$ (total genotype score).

Table 1: Genotype distribution of the Participants

Gene	Genotype	Frequency	Percentage (%)	Jump height (mean \pm SD)
<i>ACTN3</i>	CC	13	19.4	65.0 ± 6.5 cm
	CT	38	56.7	60.7 ± 6.1 cm
	TT	16	23.9	62.3 ± 6.7 cm
	DD	3	4.5	71.0 ± 6.0 cm
<i>ACE</i>	ID	25	37.3	63.0 ± 5.1 cm
	II	39	58.2	60.6 ± 6.7 cm
<i>PPARGCIA</i>	AA	13	19.4	63.6 ± 5.1 cm
	AG	35	52.2	61.4 ± 7.0 cm
	GG	19	28.4	61.8 ± 6.4 cm

Recent studies have indicated that several genes are involved in determining both the physiological and psychological performance of players, supporting the widely accepted concept that genetics are strongly associated with human physical performance (Atabaş et al., 2020; Jeremic et al., 2019). Previous studies have primarily focused on athletes from either strength/power-oriented sports (such as swimmers and sprinters) or endurance-based sports (like marathon runners and rowers) and these genetic association findings were summarised in a recent systematic review in which 41 markers were reported to be endurance-related while 45 were power-related and 42 were strength-related (Semenova et al., 2023). Considering that football is an intermittent high-intensity sport, sprinting, jumping, kicking, turning, changing pace, and tackling take place at a higher frequency during the 90-minute game (Clos et al., 2021), it would be interesting to find out if there is any significant relationship between genetic with power performance in football. Therefore, we seek to investigate the association and predictive ability of a total genotype score derived from the *ACTN3* C/T, *ACE* I/D, and *PPARGC1A* A/G polymorphisms on jump height, particularly in football.

In a cohort of 67 Malaysian elite male footballers, we identified that the most common genotypes observed were *CT* for *ACTN3* (56.7%), *II* for *ACE* (58.2%), and *AG* for *PPARGC1A* (52.2%). Similar findings were reported by Clos *et al.* (2021) whereby the heterozygous genotype *CT* for *ACTN3* genotype was the most prevalent in elite professional football players. Systematic reviews of genetic association studies in sports have indicated that the *C* allele of *ACTN3* is more common among individuals involved in explosive activities, whereas the *T* allele is more prevalent in those participating in endurance activities (Ahmetov et al., 2022; Semenova et al. 2023). Given that muscle performance related to the *ACTN3* gene differs by genotype, individuals with the *CT* genotype are expected to adapt most effectively to the physical demands of football as the it is an endurance sport characterized by intermittent bursts of high-intensity effort (Clos et al., 2021). Similar results were also reported by previous studies involving footballers on *ACTN3* gene (Clos et al., 2019; Coelho et al., 2018; Dionísio et al., 2017). The high frequency of the *PPARGC1A* *AG* genotype in the present cohort of footballers also supports the notion that the *G* allele is important for athletes that rely on the aerobic energy system for sports with prolonged exertion of moderate to high intensity as the *G* allele has been postulated to increase the expression of *PPARGC1A* and hence, enhancing the efficiency of aerobic metabolism (Gineviciene et al., 2014).

One of the main findings in relation to the average total genotype score in the present cohort of footballers showcased that most of studied players possessed less advantageous power-related genotypes. Similar to the study by Mohd Fazli et al. (2022), professional Malaysian footballers tend to carry endurance-related alleles rather than power-related ones. Specifically, most of them had the endurance-associated *ACE* *II* genotype that do not align with previous studies that reported the higher frequencies of *ACE* *ID* genotype in the footballers (Ciężczyk et al., 2016; Gineviciene et al., 2014; Juffer et al., 2009). Although success in football is affected by many factors, including genetics, it is not determined by a single specific "power and/or sprint gene". However, there may be specific genes and genetic profiles that are associated with physical traits relevant to explosive strength in football, offering a more precise understanding of how genetics influence performance, injury risk, and explosive strength (González-García & Varillas-Delgado, 2024).

The current study also revealed that a significant positive correlation was observed between the total genotype score and jump height. This is likely due to the greater muscle strength, power, and volume seen in *ACTN3* *C*-allele and *ACE* *D*-allele carriers compared to *ACTN3* *TT* and *ACE* *II* genotypes (Erskine et al., 2014). Since the *ACTN3* *C* allele has been linked to more extensible knee extensor tendons, it has been postulated that *CC* homozygotes' tendons can store and release more energy, potentially enhancing power during sprinting and acceleration (Murtagh et al., 2020). The study by Dionísio et al. (2017) also reported that footballers who carried *ACTN3* *CC* and *ACE* *DD* genotypes presented better performance during jump and sprint tests. While genetics can predispose an individual toward certain sports, it is the interaction between phenotype and genotype that determines the final outcome. Athletic performance results from a combination of factors, including training, anthropometric

and morphometric characteristics, and more. These phenotypes are shaped by various processes and cellular pathways, which are ultimately influenced by numerous relevant genes. As genetic analyses become more accessible to athletes, coaches, medical staff, and sports federations, they enable the identification of potential training responders. This, in turn, facilitates the individualization and personalization of sports training programs (González-García & Varillas-Delgado, 2024).

CONCLUSION

The present study revealed the positive association between total genotype score and jump height in elite Malaysian footballers whereby footballer with a greater power-related total genotype score tend to attain a higher jump height. Findings from the present study may have further implications in football as literature has indicated that jump performance could be used to infer speed and maximal strength. However, other variables should be explored to improve the predictive ability of the regression model on jump height as the current model only accounted for 9% of the variance.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Higher Education Malaysia through the Fundamental Research Grant Scheme (FRGS/1/2018/SS06/UITM/02/1).

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR'S CONTRIBUTIONS

GYA, TCH, RMFRA conceptualize the study. All authors contributed in the data collection and data analysis. NFS and GYA wrote the manuscript while all authors participated in the final approval of the manuscript.

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