Alpha-Actinin-3 (ACTN3) R/X Gene Polymorphism and Physical Performance of Multi-Ethnic Malaysian Population

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ABSTRACT:

A disparity population data set in the current literature with limited reports among Asian samples and the inconsistent findings among different ethnic groups warrant further investigation on the association between alpha-actinin-3 (ACTN3) R/X gene polymorphism and human physical performance in Asian population. This study was designed to examine the association between ACTN3 R/X gene polymorphism and physical performance of multi-ethnic Malaysian population. One hundred eighty well-trained athletes (34 endurance, 41 strength, and 105 intermittent) and 180 controls were drawn from four ethnic groups in Malaysia (Malay, Chinese, Indian, and Other Bumiputra). A sample of deoxyribonucleic acid (DNA) was retrieved from a buccal swab from each participant and the ACTN3 R/X genotype was identified through polymerase chain reaction and restriction fragment length polymorphism analysis. The strength and endurance performances of the athletes were evaluated with maximal voluntary contraction and Yo-Yo intermittent recovery level 2 tests, respectively. The independent t-test, chi-square, multivariate, and one-way analysis of variance were used for data analysis. ACTN3 R/X alleles (p = 0.672) and genotype (p = 0.355) frequencies did not vary much between the multi-ethnic groups of Malaysian athletes. These small variations did not have any influence on handgrip strength (p = 0.334), leg strength (p = 0.256), and Yo-Yo intermittent recovery level 2 performance (p = 0.425) between these ethnic groups. The RR and XX genotypes were more frequent among strength and intermittent athletes, respectively. Athletes with the RR genotype had greater handgrip than those with the RX genotype (p = 0.031), but not different from athletes with the XX genotype (p = 0.228). Athletes with RR
genotype also have a higher leg strength than those with the RX (p = 0.001) and XX genotype (p = 0.010). However, the endurance performance was similar among genotype groups (p = 0.385). The ACTN3 R/X gene polymorphism may confer similar effect on physical performance across different ethnic groups in Malaysia with the R allele was associated with greater strength performance whilst the X allele was not associated with endurance performance of multi-ethnic Malaysian population.

**KEY WORDS** Strength, endurance, athletic status, Malaysian population

**INTRODUCTION**

The alpha-actinin-3 (ACTN3) R/X gene polymorphism (rs1815739) is one of the genetic variants that has been most widely studied for human physical performance [1]. R allele of the ACTN3 R/X gene polymorphism is responsible for the production of ACTN3 protein, which is one of the elements for the Z disk of fast twitch muscle fibers [2]. The presence of ACTN3 protein in skeletal muscle stabilizes and integrates the muscle contractile apparatus, thereby increasing the muscle's ability to generate high muscle power energy and velocity during movements [3]. In contrast, the X allele of this polymorphism was reported to prevent the formation of ACTN3 protein [2]. MacArthur et al. [4] suggested that the loss of ACTN3 protein leads to alterations in skeletal muscle metabolism towards more efficient aerobic metabolism. Given these different physiological functions, possession of the ACTN3 R and X alleles may grant beneficial effects for strength/power and endurance activities, respectively.

A study by Yang et al. [5] had been the first to link the ACTN3 gene to human physical performance. This study suggested that the R allele of ACTN3 R/X gene polymorphism could enhance sprint ability, as at least one copy of R allele was found in all male Olympian power and female elite sprint athletes [5]. Moreover, Niemi and Majamaa [6] reported similar findings with a higher frequency of the R allele in Finnish sprinters and a lower frequency of R allele in endurance athletes. They also reported that none of the top Finnish sprinters were predisposed for two copies of X allele [6]. Besides the positive finding on the effect of possession of the R allele on strength/power athletic status, individuals with R allele have also been reported to have greater strength/power capacity [7-10].

The association between the ACTN3 R/X gene polymorphism and human physical performance, however, remain ambiguous as some studies failed to replicate the results of others [6, 11-16]. Studies involving Indian [16], Finnish [6], Israeli [12], and Russian [11] athletes demonstrated that the R allele may confer an advantage for strength performance. In contrast, Scott et al. [13] reported that the R allele failed to confer any advantage to strength performance among Jamaican and African American athletes. In a study among an Asian population, individuals with R allele displayed significantly higher muscle strength than individuals with X allele [14]. Nevertheless, a study by Gineviciene et al. [15] among Caucasian samples reported that individuals with X allele had greater muscle strength than those individuals with R allele.

These contradictory results reported across different ethnicities has been speculated may be due to the different pattern in the distributions of ACTN3 R/X gene polymorphisms across different ethnicities [17]. It has been reported that the highest R allele frequency was found in Nigerian [18] and the lowest in Indian populations [16]. The prevalence of R and X alleles in the Black population ranged from
0.92 to 0.42 and 0.58 to 0.08, respectively [7, 18]. In the Caucasian population, the frequencies of R and X alleles range from 0.61 to 0.50 and 0.50 to 0.39, respectively [7, 11]. The R allele frequency in the Asian population ranges from 0.53 to 0.39 [7, 16], and the X allele frequency varies between 0.61 and 0.47 [7, 16]. Ethnic variation in the distribution of the ACTN3 R/X gene polymorphism thus raises the possibility that the association of this polymorphism on athletic performance may also varies across ethnic groups.

The association between the ACTN3 R/X gene polymorphism and human physical performance across different ethnic groups has yet to be largely examined. To date, the study by Ma et al. [1] reported that the association between this gene polymorphism and human physical performance has been well documented in Caucasian populations, but limited information is available for Asian populations. Hence, the objective of this research was to examine the association of ACTN3 R/X gene polymorphism with physical performance in a multi-ethnic Malaysian population. This research also determined if the association between the ACTN3 R/X gene polymorphism and human physical performance differ by ethnicity.

METHODS

Participants
This study comprised of 180 university athletes aged 20.5 ± 1.9 (mean ± SD) years (148 male, 32 female) and 180 sedentary controls aged 20.4 ± 1.6 years (70 male, 110 female). Based on the current distribution ratio in Malaysia [19], both athlete and control groups have same number of samples for each ethnic group (55% Malays (n=99), 24.7% Chinese (n=45), 12.9% Other Bumiputras (n=23), and 7.4% Indians (n=13)). All sedentary individuals reported of having sedentary lifestyle defined by performing two or fewer days a week of recreational exercise for less than 30 minutes a day for the past three months prior to data collection [20]). The athletes were trained for at least one year and represent the university in national sporting competition. Within the cohort of athletes, 34 participants (25 male, 9 female) aged 19.8 ± 1.9 years were classified as endurance athletes, 41 participants (25 male, 16 female) aged 19.7 ± 1.8 years as strength athletes, and 105 participants (99 male, 6 female) aged 21.0 ± 1.8 years as intermittent athletes according to the classification described by Maciejewskakarłowska et al. [21]. Participants were excluded from this research if they had mixed ancestry within three generations. The research protocol on Malaysian samples were permitted by the Human Research Ethics Committee in Universiti Sains Malaysia.

Genotyping
Prior to DNA sampling, the written consent was obtained from each participant. By using a sterile swab applicator (Classic Swabs by Copan Flock Technologies, Brescia, Italy), DNA sample from each participant was obtained via buccal swab. Isolation of genomic DNA from the swab samples was performed by using the GeneAll® ExgeneTM Cell sv kit (GeneAll Biotechnology Co. Ltd., Seoul, South Korea). Then, the DNA samples were analysed using polymerase chain reaction (PCR) with a set of primer reported by Mills et al. [3] (F: 5’CTGTTTCGACTGAAGGAGGG and R: 5’TGGTCAGATGAGGAGGAGG3’). The target fragment bearing the ACTN3 R/X gene polymorphism was performed under the following conditions: 95°C/ 2 minutes x 1 cycle; 95°C/ 30 seconds, 61.6°C/ 30 seconds, 72°C/ 30 seconds x 25 cycles; 72°C/ 5 minutes x 1 cycle. The presence of 291 bp band indicated the successful amplification of ACTN3 gene. The PCR product was then confirmed by DNA sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia). In order to obtain the genotype of the ACTN3 R/X gene polymorphism, the amplified PCR product was digested with DdeI restriction
enzyme (New England Biolabs, Beverly, MA, USA). The presence of 205 bp and 86 bp bands is an indication for R allele while the presence of 108 bp, 97 bp, and 86 bp bands is an indication for X allele.

**Anthropometric Measurements**

Subsequent to DNA sampling, a portable stadiometer (Seca 213, Seca Corporation, USA) was used to measure participant’s body height. Meanwhile, an Omron KARADA Scan Body Composition & Scale (HBF-362, Omron Corporation, Japan) was used to measure participant’s body weight, body mass index, and body fat.

**Physical tests**

Twenty meter Yo-Yo intermittent recovery level 2 was administered to the athletes in order to determine their endurance performance. Meanwhile, isometric handgrip and leg strength tests were performed by the athletes to determine their handgrip and leg strength performance. Detailed descriptions of the validity and reliability of these tests are available elsewhere [22-24].

**Isometric handgrip strength test**

The handgrip test was performed on a hand dynamometer (Takei A5401, Takei Scientific Instruments Co. Ltd., Japan) to determine an athlete’s isometric handgrip strength. A standard procedure of measuring handgrip strength (kg) using a hand dynamometer followed the procedure defined by Tomchuk [16]. The dynamometer was calibrated according to manufacturer’s instruction. Prior to the start of the test, the athletes held the dynamometer with the arm hanging straight down and the elbow by the side of the body. When all set, the athletes squeezed the dynamometer maximally for about 5 seconds, with the athletes were verbally encouraged during the test. The athletes performed the test three times with 10 to 20 seconds rest interval between each trial, and the average score was used for data analysis.

**Isometric leg strength test**

A back and leg dynamometer (Takei A5402, Takei Scientific Instruments Co. Ltd., Japan) was used to determine isometric leg strength (kg) of the athletes. The procedure for this test followed the procedure described by Ashok [25]. The dynamometer was calibrated according to manufacturer’s instruction. Prior to the start of the test, the athletes stood upright with both feet on the base of the dynamometer and the chain length of the dynamometer was adjusted until the athlete’s knees were bent around 110 degrees. When ready, the athletes pulled the handle bar maximally for 5 seconds, with the athletes were verbally encouraged during the test. The athletes performed the test three times with 10 to 20 seconds rest interval between each trial. The average score obtained by the athletes was used for data analysis.

**Yo-Yo intermittent recovery level 2 test**

Before the test began, participants were fitted with a Polar transmitter belt (T61, Polar Electro Oy, Finland) onto their chest. This test required the athletes to shuttle from one end of the marked course to the other at a relatively slow pace and then quickly ramped up their speed according to the pace set by the recorded beeps, with 10 seconds of active recovery in each bout of intense running. Athletes were verbally encouraged during the test. If the athletes did not complete a successful out and back shuttle within the allocated time, a warning was given to them. The score for the test was determined from the last speed level and number of shuttles reached before the athletes received a second warning or voluntarily withdrew from the test. By using a standard norm for Yo-Yo intermittent recovery level 2 test, the score for the test was converted to the total distance covered to compute the endurance capacity of the athlete. Their maximum heart rate achieved immediately after running was also recorded. The procedure of the Yo-Yo intermittent recovery level 2 was consistent to the earlier method published by Bangsbo et al. [26].
Mean ± standard deviation (SD) was used to present the descriptive data. An independent t-test was used for comparing the means of two independent samples. Allele frequency of ACTN3 R/X gene polymorphism was determined by direct counting. The Hardy-Weinberg equilibrium (HWE) test was used for genotyping quality control [27]. As HWE may be violated in athletes without being caused by genotyping errors [28, 29], the HWE test was only tested for the control group. The multivariate analysis was used to examine the association between ethnicity and ACTN3 R/X gene polymorphism factors on performance measured in this study. The difference in the ACTN3 R/X allele and genotype frequencies between the groups (i) the whole cohort of athletes and controls, and (ii) endurance athletes, strength athletes, intermittent athletes, and controls) was analysed by the chi-square (X²) test.

### STATISTICAL RESULTS

Table 1 shows the descriptive statistics for the physical characteristics of the whole cohort of athletes and controls. The controls having lower mean values for height (t(358) = 8.649, p < 0.001), body weight (t(358) = 7.882, p < 0.001), and body mass index (t(358) = 4.098, p < 0.001) compared to athletes. On the other hand, the mean value of body fat was observed to be higher in controls than in athletes (t(358) = -4.244, p < 0.001).

Physical characteristics of endurance, strength and intermittent athletes were presented in Table 2. All athletes were similar in body mass index (F(2, 177) = 2.894, p = 0.058) and body fat (F(2, 177) = 0.635, p = 0.531). There were significant differences in height (F(2, 177) = 9.492, p < 0.001) and body weight (F(2, 177) = 6.027, p = 0.003) between the athlete groups. Bonferroni’s post-hoc test revealed that the mean values of height and body weight in the strength group were significantly lower compared with the intermittent group (Height: p < 0.001, Body weight: p = 0.001), though it was not significantly different with the values among endurance group (Height: p = 0.056, Body weight: p = 0.940).

Table 3 shows the scores obtained by the athletes for the Yo-Yo intermittent recovery level 2 performance, handgrip and leg strength tests. Yo-Yo intermittent recovery level 2 performance differed significantly between athletes from different sporting disciplines (F(2, 177) = 4.340, p = 0.014), with the performance score was higher in the endurance athletes than the strength (p = 0.021) and intermittent athletes (p = 0.029). Handgrip strength differed significantly among athletes from the three different sporting disciplines (F(2, 177) = 3.994, p = 0.020), with the intermittent athletes had significantly higher values of handgrip strength than the strength athletes (p = 0.025), but not significantly different compared to endurance athletes (p = 0.336). Nevertheless, the leg strength value did not differ significantly between athletes from different sporting disciplines (F(2, 177) = 0.538, p = 0.506).
### Table 1  
Physical characteristics of athletes and controls in the Malaysian population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Athletes (n=180)</th>
<th>Controls (n=180)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>168.8 ± 9.1*</td>
<td>160.7 ± 8.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>67.5 ± 13.6*</td>
<td>56.3 ± 12.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>23.6 ± 4.0*</td>
<td>21.8 ± 3.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>18.6 ± 6.3*</td>
<td>21.5 ± 7.7</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Note.*

Data shown as mean ± SD

*Significantly different compared to controls (p < 0.001)

### Table 2  
Physical characteristics of endurance, strength and intermittent athletes in the Malaysian population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Endurance (n=34)</th>
<th>Strength (n=41)</th>
<th>Intermittent (n=105)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>168.5 ± 9.8</td>
<td>163.7 ± 10.4*</td>
<td>170.8 ± 7.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>63.6 ± 17.0</td>
<td>62.5 ± 9.2#</td>
<td>70.0 ± 13.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>22.1 ± 4.1</td>
<td>23.2 ± 3.0</td>
<td>24.0 ± 4.3</td>
<td>0.058</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>18.6 ± 6.1</td>
<td>19.6 ± 7.3</td>
<td>18.7 ± 6.4</td>
<td>0.531</td>
</tr>
</tbody>
</table>

*Note.*

Data shown as mean ± SD

*Significantly different compared to intermittent athletes (p < 0.001)

# Significantly different compared to intermittent athletes (p = 0.001)
Allele and genotype frequencies of *ACTN3 R/X* in the athletes and controls groups were presented in Fig. 1. *ACTN3 R/X* genotype distribution for the control group was in agreement with HWE (p > 0.050). The frequency of *ACTN3 R/X* allele frequency in the whole cohort of athletes was not significantly different from that the whole cohort of controls ($X^2 = 2.1998$, df = 1, p = 0.138). However, there is a significant difference in *ACTN3 R/X* genotype frequency between athletes and controls ($X^2 = 11.111$, df = 2, p = 0.004) with the athletes had a higher frequency of *RR* genotype than controls.

*Fig. 1*  
*ACTN3 R/X* allele and genotype frequencies in the whole cohort of athletes and controls in the Malaysian population.

*Note.*  
*p = 0.004* for genotype frequency in athletes vs. controls.
The ACTN3 R/X allele and genotype frequencies were compared between endurance athletes, strength athletes, intermittent athletes, and controls as shown in Fig. 2. The ACTN3 R/X allele frequency was similar between the four groups ($X^2 = 5.291$, df = 3, $p = 0.152$). However, the genotype frequencies differed significantly across these groups ($X^2 = 15.004$, df = 6, $p = 0.020$). ACTN3 R/X genotype frequency in the endurance athletes did not significantly differ from those frequencies in the controls ($X^2 = 5.504$, df = 2, $p = 0.064$), the strength ($X^2 = 0.490$, df = 2, $p = 0.783$), and intermittent ($X^2 = 0.903$, df = 2, $p = 0.637$) athletes. Nevertheless, ACTN3 R/X genotype frequency of the strength athletes differed from those of the controls ($X^2 = 10.074$, df = 2, $p = 0.006$), but not to those in intermittent athletes ($X^2 = 3.298$, df = 2, $p = 0.192$). Meanwhile, ACTN3 R/X genotype frequency in the intermittent athletes did not significantly differ from those of controls ($X^2 = 5.781$, df = 2, $p = 0.056$). Among these four groups, the strength and intermittent athletes had the highest frequencies of RR and XX genotypes, respectively.

There was no significant interaction between ethnicity and ACTN3 R/X gene polymorphism on handgrip strength ($F(2, 174) = 1.153$, $p = 0.334$), leg strength ($F(2, 174) = 1.308$, $p = 0.256$), and Yo-Yo intermittent recovery level 2 performance ($F(2, 174) = 1.003$, $p = 0.425$). Nevertheless, handgrip strength differed significantly among athletes with different genotype groups as shown in Fig. 3 ($F(2, 177) = 3.647$, $p = 0.028$). Using a Bonferroni’s post-hoc test, the handgrip strength recorded by the athletes with RR genotype is significantly higher than those with the RX genotype ($p = 0.031$), but not significantly different from athletes with the XX genotype ($p = 0.228$). There is also significant difference in leg strength across the genotype groups as presented in Fig. 4 ($F(2, 177) = 7.979$, $p < 0.001$), with leg strength was significantly higher in the athletes with RR genotype compared to those athletes with the RX genotype ($p = 0.001$) and XX genotype ($p = 0.010$). However, there was no significant difference for the performance of Yo-Yo intermittent recovery level 2 among athletes with the RR genotype ($377.7 \pm 208.1$), RX genotype ($342.4 \pm 138.9$), and XX genotype ($328.7 \pm 174.8$) ($F(2, 177) = 1.663$, $p = 0.385$).
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**Fig. 2** ACTN3 R/X allele and genotype frequencies among endurance athletes, strength athletes, intermittent athletes, and controls in the Malaysian population

*Note.*

\*p = 0.006 for genotype frequency in strength athletes vs. controls

**Fig. 3** The mean value of handgrip strength in athletes with different ACTN3 R/X genotypes in the Malaysian population

*Note.*

Data shown as mean ± SD

*Significantly different compared to RX genotype (p = 0.031)

**Fig. 4** The mean value of leg strength in athletes with different ACTN3 R/X genotypes in the Malaysian population

*Note.*

Data shown as mean ± SD

*Significantly different compared to RX genotype (p = 0.001)

**Significantly different compared to XX genotype (p = 0.010)
DISCUSSION

The main finding of this study is that ACTN3 R/X alleles and genotype frequencies and its association with physical performance did not vary much between the multi-ethnic groups of Malaysian athletes. Also, the present study identified that the presence of R allele was associated with greater strength performance whilst the presence of X allele was not associated with endurance performance of multi-ethnic Malaysian population.

The athletes in the present study were drawn from different ethnic groups in Malaysia, thus it is probable that different composition of ACTN3 R/X gene polymorphism in different ethnic groups could be a factor that influence the performance of the athletes, which has been previously suggested by Zilberman-Schapira et al. [17]. The present study showed that the ACTN3 R/X gene polymorphism did not interact with ethnicity to impact endurance and strength performances in the Malaysian population. Thus, the finding of the current study suggested that ACTN3 R/X gene polymorphism may has universal effect on human physical performance across different ethnic groups in Malaysia.

As expected, the present study found a higher frequency RR genotype in the strength athletes, which is in line with the previous finding among Asian [16] and Caucasian [5, 12] athletes. The present study also found that athletes with RR genotype had greater hand and leg strength than athletes with other genotypes. This finding is consistent with the findings of earlier studies involving Caucasian [8, 10] and Asian [9] populations, which demonstrated that the RR genotype carriers have greater strength capacity than other genotype carriers, thereby reaffirm the notion that the R allele was associated with greater strength performance.

The present data demonstrate a higher frequency of XX genotype among intermittent athletes than endurance and strength athletes. This result is not in agreement with the previous reports in the Caucasian population, which reported a higher frequency of XX genotype among endurance athletes compared to those frequencies in the strength and intermittent athletes [12, 30]. This finding is contrary to those observed in the Chinese population [14]. The reliability of a higher frequency of X allele in intermittent athletes could be explained by the fact that intermittent sport involves aerobic-anaerobic demand. Hence, an intermittent athlete requires both aerobic and anaerobic capacity. Therefore, the presence of X allele in intermittent athletes may confer a beneficial effect for aerobic activity.

In addition, this study found that the endurance performance as measured by Yo-Yo intermittent recovery level 2 test was similar between the ACTN3 R/X genotype. Nevertheless, this finding was not comparable to the findings obtained by Lucia et al. [31]. According to the study by Lucia et al. [31], the carriers of the XX genotype had a higher endurance performance compared to carriers of RR and RX genotype. The contradictory finding between the present study and the study by Lucia et al. [31] may be attributed to the difference in the study sample criteria. Lucia et al. [31] had studied the effect of ACTN3 R/X gene polymorphism on endurance performance among untrained people, whilst the present study focused on a group of athletes.

The present study is presented with several limitations. The first limitation was related to the number of athletes used in this study as when the athletes were divided into three groups based on their sporting disciplines, the sample size decreased considerably. As in all such studies, extension to, and replication with a larger sample size is warranted. The studied groups were comprised of both females and males. Thus, it remains unknown if the effect of ACTN3 R/X gene polymorphism may differ by gender. As the aims of the present study was not related to training effect, there is no data is available on the training status of the athletes. Therefore, it is
likely that performance measured in the present study due to the training effect which could mask gene-related effects on physical performance. The present study did not measure the physical performance of controls to reduce variability of the results. However, replicate study should be performed in future studies to validate the present findings. Despite the above-mentioned limitation, confidence in the validity of the present findings is increased by the fact athlete and control groups were ethnically-matched, and genotype distribution of the controls was in HWE.

In summary, this study demonstrates that the distribution of \( ACTN3 \ R/X \) gene polymorphism and its association with physical performance did not vary much between different ethnic groups of Malaysian athletes. This study confirmed the association between the \( R \) allele and strength performance within Asian ethnicity as has been widely investigated among the Caucasian population. It is further concluded that \( X \) allele was not associated with endurance performance within the Malaysian population. This research provide better comprehension on the involvement of the genetic factor on human physical performance among different ethnic groups in Malaysian population.

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REFERENCE

