DISTRIBUTIONS OF ACE I/D AND ACTN3 R/X GENE POLYMORPHISMS IN MULTI-ETHNIC MALAYSIAN AND AUSTRALIAN POPULATIONS

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ABSTRACT

Background: The distribution patterns of the most studied variants related to sports performance which are the angiotensin I-converting enzyme (ACE) I/D and alpha-actinin-3 (ACTN3) R/X polymorphisms in the multi-ethnic Malaysian population have not been well documented. Thus, this study aims to examine the distribution patterns of ACE I/D and ACTN3 R/X polymorphisms in four ethnic groups in Malaysia, and ethnic variation of these polymorphisms across different ethnicity.

Materials and Methods: DNA samples were retrieved via buccal cell from 180 Asians from Malaysia (99 Malays, 45 Chinese, 23 Other Bumiputras, and 13 Indians) and 180 Caucasians from Australia. The polymorphisms were identified through Polymerase Chain Reaction and Restriction Fragment Length Polymorphism analysis. The chi-square test was used to analyse the data.

Result: This study found that the distribution of ACE I/D polymorphism varied among different ethnic groups within Malaysia as well as between Malaysian and Australian populations, but not for the ACTN3 R/X polymorphism. Among the four ethnic groups in Malaysia, the Malay and Other Bumiputra groups had the highest frequencies of II (0.40) and DD (0.26) genotypes, respectively. Meanwhile, the Malaysians had the higher frequencies of the II (0.31 vs. 0.25) and ID (0.54 vs. 0.47) genotypes compared to the Australians.

Conclusion: In conclusion, the different pattern in the distribution of ACE I/D polymorphism observed in this study increases the possibility that the effect of ACE I/D polymorphism on sports performance may also differ by ethnicity. Conversely, the present finding on ACTN3 R/X polymorphism suggests that its effect on sports performance may not vary by ethnicity.

Keywords: ACE I/D polymorphism, ACTN3 R/X polymorphism, multi-ethnic Malaysian population, Australian population, ethnicity
1.0 Introduction

The ACE I/D gene polymorphism in the ACE gene has been thought to confer a greater advantage in endurance activity due to its role in determining the level of ACE circulation in tissues (Rigat et al., 1990); a main component in the renin-angiotensin system (RAS) (Sayed-Tabatabaei, Oostra, Isaacs, van Duijn, & Witteman, 2006). Meanwhile, the ACTN3 R/X gene polymorphism in the ACTN3 gene has been thought to present an advantage to activities that require short bursts of intense strength and power because it codes for ACTN3 protein (North et al., 1999); a protein found only in the fast-twitch skeletal muscle fibre. Most studies involving the Caucasian population reported that possession of the I allele of ACE I/D gene polymorphism influences endurance performance (S. Cam, Colakoglu, Colakoglu, Sekuri, & Berdeli, 2007; Hagberg, Ferrell, Dengel, & Wilund, 1999; Kasikcioglu et al., 2004). Conversely, some studies in the Asian population revealed that possession of the ACE I allele did not appear to influence endurance performance, as the endurance performance was significantly higher in those with ACE D allele than those with ACE I allele (Tobina et al., 2010; Zhaoa et al., 2003). With regard to ACTN3 R/X gene polymorphism, studies involving Indian (Kothari et al., 2011), Finnish (Niemi & Majamaa, 2005), Israeli (Eynon et al., 2009), and Russian (Druzhevskaya, Ahmetov, Astratenkova, & Rogozkin, 2008) athletes demonstrated that possession of the ACTN3 R allele may confer an advantage for strength/power performance. In contrast, Gineviciene et al. (2011) reported that possession of the ACTN3 R allele failed to confer any advantage to strength/power performance among Lithuanian athletes.

The reason for the existence of ethnic variation in the effects of these polymorphisms on physical performance has remained unknown. The ethnic variation in the distributions of ACE I/D and ACTN3 R/X gene polymorphisms globally maybe partly responsible for these differences as well (Zilberman-Schapira, Chen, & Gerstein, 2012). Studies showed that I allele frequency of the ACE I/D gene polymorphism was more frequent in the healthy Australian Aboriginal population (Lester et al., 1999) and least in European (Tiret et al., 1992). As for the ACTN3 R/X gene polymorphism, the highest R allele frequency was found in Nigerian (Yang et al., 2007) and the lowest in Indian populations (Kothari, et al., 2011).

To date, the distribution patterns of ACE I/D and ACTN3 R/X gene polymorphisms in the Malaysian population have not been well documented. The distribution of ACE I/D gene polymorphism in Malaysia was reported in 2008 (Jayapalan, Muniandy, & Chan, 2008). However, the data retrieved from this previous study (Jayapalan, et al., 2008) still need to be validated. In contrast, there has been no report thus far for ACTN3 R/X gene polymorphism distribution in the Malaysian population. Besides, it had been necessary to obtain information concerning distributions of ACE I/D and ACTN3 R/X gene polymorphisms in the general population of Malaysia before establishing their effects on physical performance in this population. Since there was ethnic variation in the distributions of ACE I/D and ACTN3 R/X gene polymorphisms, it would also be interesting to compare the distribution patterns of ACE I/D and ACTN3 R/X gene polymorphisms in the Malaysian population to other population for a comprehensive description concerning the ethnic variation on these polymorphisms.

Therefore, the aims of the present study were to examine (i) the distribution patterns of ACE I/D and ACTN3 R/X gene polymorphisms in the multi-ethnic Malaysian population, and (ii) ethnic variation on these polymorphisms by comparing the distribution of the data between
Malaysian and Australian populations, as well as between four ethnic groups (Malay, Chinese, Indian, and Other Bumiputra) in Malaysia. The distributions of \textit{ACE I/D} and \textit{ACTN3 R/X} gene polymorphisms were hypothesized to vary between ethnic groups.

### 2.0 Materials and Methods

#### 2.1 Participants

The study group consisted of 180 Asians from Malaysia, and 180 Caucasians from Australia, who had provided informed consent to participate in this study. All participants were sedentary healthy individuals. To limit variation due to genetic admixture, participants reported with mixed ancestry within three generations were excluded from this study. The number of participants for this study was based on the sample size calculation using the Power and Sample Size Calculation version 3.1.2 software (Dupont & Plummer, 1990) [Calculated sample size in each group = 164 participants; Research sample size = 164 participants + (164*10% (expected drop out)) = 180 participants]. The power of the study was set at 0.80 with the alpha level of 0.05 and the effect size of 0.25. The participants from the Malaysian population (70 males, 110 females) aged 20.4 ± 1.6 years were drawn from four ethnic groups in Malaysia, who were also students from several universities in Malaysia. In order to obtain comprehensive results for the distributions of \textit{ACE I/D} and \textit{ACTN3 R/X} gene polymorphisms in the Malaysian population, the proportion of participants with different ethnic backgrounds had been set according to the current distribution ratio in Malaysia (Statistic, 2011). Based on the respective ratio, 99 Malays (55%), 45 Chinese (24.7%), 23 Other Bumiputras (12.9%), and 13 Indians (7.4%) were selected as participants for this study. In comparison to the Malaysian population, 180 Caucasian origin (62 males, 118 females) aged 23.3 ± 3.6 years had been recruited from the Australian population. All the participants for Australian population were students at the University of Sydney, Sydney, Australia. The study protocols on Malaysian and Australian samples were approved by the Human Research Ethics Committee in Universiti Sains Malaysia and the Human Research Ethics Committee in University of Sydney, respectively.

#### 2.2 Genotype Determination

Deoxyribonucleic acid (DNA) samples from each participant were obtained via buccal swab using a sterile swab applicator (Classic Swabs by Copan Flock Technologies, Brescia, Italy). Genomic DNA was isolated from the swab samples using the GeneAll® ExgeneTM Cell SV kit following the manufacturer’s protocol (GeneAll Biotechnology Co. Ltd, Seoul, South Korea). Polymerase chain reaction (PCR) for the \textit{ACE I/D} gene polymorphism was carried out in a final volume of 25 μl consisting of 2.5 μl of 10X standard reaction buffer (GeneAll Biotechnology Co. Ltd., Seoul, South Korea) (25 mm Mg2+, 50 mm Tris-HCl, 50 mm KCl, 0.1 mm EDTA, 1 mm DTT, 0.5 mm PMSF, and 50% glycerol), 2.0 μl of dNTP mix (200 μm from each dNTP (dATP, dCTP, dGTP, and dTTP)), 0.8 μm of each primer (forward primer: 5'-CTGGAGACCACCTCCCATCCTTTCT-3'; reverse primer: 5'-CTGGAGACCACCTCCCATCCTTTCT-3'), 0.5 units of Taq DNA polymerase, 2.5 μl of dimethylsulfoxide, 10.8 μl of sterilize distilled water, and 5 μl of genomic DNA (2-8 ng/μl). The target fragment bearing the \textit{ACE I/D} gene polymorphism was amplified under the following conditions; 7 minutes at 95°C, followed by 25 cycles of 30 seconds at 95°C, 30
seconds at 62°C, and 1 minute at 72°C, with a final step of 7 minutes at 72°C. The amplified products were electrophoresed on 1.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 490 base pair (bp) and 190 bp bands indicated the ACE insertion (I) and deletion (D) alleles, respectively. The PCR products for ACE I/D gene polymorphism were confirmed by sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia). Meanwhile, PCR for the ACTN3 R/X gene polymorphism was carried out in a final volume of 25 µl consisting of 2.5 µl of 10X standard reaction buffer (GeneAll Biotechnology Co. Ltd., Seoul, South Korea) (25 mm Mg2+, 50 mm Tris-HCl, 50 mm KCl, 0.1 mm EDTA, 1 mm DTT, 0.5 mm PMSF, and 50% glycerol), 2.0 µl of dNTP mix (200 µm from each dNTP (dATP, dCTP, dGTP, and dTTP)), 0.2 µm of each primer (forward primer: 5’-CTGTTGCCCCTGGTGTAAGTG-3’; reverse primer: 5’- TGGTCACAGTATGCAGGAGG-3’), 0.5 units of Taq DNA polymerase, 2.5 µl of dimethylsulfoxide, 12.3 µl of sterilize distilled water, and 5 µl of genomic DNA (2-8 ng/µl). The target fragment bearing the ACTN3 R/X gene polymorphism was amplified under the following conditions: 2 minutes at 95°C, followed by 26 cycles of 30 seconds at 95°C, 30 seconds at 61.6°C, and 30 seconds at 72°C, with a final step of 5 minutes at 72°C. The amplified products were electrophoresed on 1.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. 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) in a final volume of 10 µl consisting of 1.0 µl of 10X NEBuffer3 (New England Biolabs, Beverly, MA, USA), 1 U of DdeI restriction enzyme (New England Biolabs, Beverly, MA, USA), and 8.5 µl of amplified PCR product. The reaction mix was incubated at 37°C for 45 minutes and the digestion product was electrophoresed on 2.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 205 bp and 86 bp bands indicated R allele, while the presence of 108 bp, 97 bp, and 86 bp bands indicated X allele.

2.3 Statistical Analysis

Allele frequencies of ACE I/D and ACTN3 R/X gene polymorphisms were determined by direct counting. Simple HWE calculator (http://www.koonec.com/wp-content/uploads/k-blog/HWE.xls.) was used to confirm that the observed ACE I/D and ACTN3 R/X genotype frequencies were in HWE for all groups ((i) Malaysian population (Malay, Chinese, Indian, and Other Bumiputra) and (ii) Australian population (Caucasian)). The chi-square (X^2) test was used to examine the difference in allele and genotype frequencies of the ACE I/D and ACTN3 R/X gene polymorphisms between the ethnic groups ((i) Malay vs. Chinese vs. Indian vs. Other Bumiputra, and (ii) Malaysian vs. Australian). All statistical evaluations were performed by using the IBM SPSS statistical version 20.0 (Armonk, New York, USA), with the level of significance set at p < 0.050.
3.0 Result

3.1 ACE I/D gene polymorphism

Table 1 ACE I/D allele and genotype frequencies in the multi-ethnic groups in Malaysia

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>ACE I/D Genotype</th>
<th>ACE I/D Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II</td>
<td>ID</td>
</tr>
<tr>
<td>Malay (n=99)</td>
<td>40 (0.40)</td>
<td>50 (0.51)</td>
</tr>
<tr>
<td>Chinese (n=45)</td>
<td>13 (0.29)</td>
<td>22 (0.49)</td>
</tr>
<tr>
<td>Indian (n=13)</td>
<td>1 (0.08)</td>
<td>10 (0.77)</td>
</tr>
<tr>
<td>Other Bumiputra (n=23)</td>
<td>2 (0.09)</td>
<td>15 (0.65)</td>
</tr>
</tbody>
</table>

Note.
Data shown as number (relative frequency)
*p = 0.005 for genotype frequency in Malay group vs. Other Bumiputra group

The distribution of ACE I/D genotype for all groups had been in agreement with Hardy-Weinberg equilibrium (p > 0.05). The distribution of ACE I/D allele and genotype in the multi-ethnic population in Malaysia is presented in Table 1. The Malay group had ACE I/D genotype frequencies of 0.40, 0.51, and 0.09, for II, ID, and DD genotypes respectively. Meanwhile, in the Chinese group, the frequencies of II, ID, and DD genotypes were 0.29, 0.49, and 0.22, respectively. The frequencies of II, ID, and DD genotypes for Indian and Other Bumiputra groups were 0.08, 0.77, and 0.15, as well as 0.09, 0.65, and 0.26, respectively. Among the four ethnic groups, the Malay and Other Bumiputra groups had the highest frequencies of II and DD genotypes, respectively.

Figure 1 Distribution of ACE I/D genotype in Malaysian and Australian populations

Note.
Data shown as number (relative frequency)
*p = 0.009 for genotype frequency in Malaysian population vs. Australian population
The distributions of ACE I/D genotype in Malaysian and Australian populations are presented in Figure 1. In the Malaysian population, the frequencies of II, ID, and DD genotypes were 0.31, 0.54, and 0.15, respectively. Meanwhile, the frequencies of II, ID, and DD genotypes in the Australian population were 0.25, 0.47, and 0.28, respectively.

3.2 ACTN3 R/X gene polymorphism

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>ACTN3 R/X Genotype</th>
<th>ACTN3 R/X Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR</td>
<td>RX</td>
</tr>
<tr>
<td>Malay (n=99)</td>
<td>23 (0.23)</td>
<td>55 (0.56)</td>
</tr>
<tr>
<td>Chinese (n=45)</td>
<td>13 (0.29)</td>
<td>22 (0.49)</td>
</tr>
<tr>
<td>Indian (n=13)</td>
<td>0 (0.00)</td>
<td>9 (0.69)</td>
</tr>
<tr>
<td>Other Bumiputra (n=23)</td>
<td>4 (0.17)</td>
<td>16 (0.70)</td>
</tr>
</tbody>
</table>

Note. Data shown as number (relative frequency)

The distribution of ACTN3 R/X genotype for all groups had been in agreement with Hardy-Weinberg equilibrium (p > 0.05). The ACTN3 R/X allele and genotype distributions of four ethnic groups in Malaysia are presented in Table 2. The Malay group had ACTN3 R/X genotype frequencies of 0.23, 0.56, and 0.21, for RR, RX, and XX genotypes respectively. Meanwhile, in the Chinese group, the frequencies of RR, RX, and XX genotypes were 0.29, 0.49, and 0.22, respectively. The frequencies of RR, RX, and XX genotypes for Indian and Other Bumiputra groups were 0.00, 0.69, and 0.31, as well as 0.17, 0.70, and 0.13, respectively.

Figure 2 ACTN3 R/X genotype frequencies in Malaysian and Australian populations

Note. Data shown as number (relative frequency)
The distributions of ACTN3 R/X genotype in Malaysian and Australian populations are shown in Figure 2. In the Malaysian population, the frequencies of RR, RX, and XX genotypes were 0.22, 0.57, and 0.21, respectively. Meanwhile, the frequencies of RR, RX, and XX genotypes in the Australian population were 0.24, 0.57, and 0.19, respectively. In the Malaysian population, the frequencies of R and X alleles were 0.51 and 0.49, respectively. Meanwhile, the frequencies of R and X alleles in the Australian population were 0.53 and 0.47, respectively.

3.3 Association of ACE I/D and ACTN3 R/X gene polymorphisms with ethnicity

<table>
<thead>
<tr>
<th>Comparison of groups</th>
<th>ACE I/D Genotype frequency</th>
<th>ACE I/D Allele frequency</th>
<th>ACTN3 R/X Genotype frequency</th>
<th>ACTN3 R/X Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malay vs. Chinese</td>
<td>X² 0.75p</td>
<td>X² 0.15p</td>
<td>X² 0.71p</td>
<td>X² 0.75p</td>
</tr>
<tr>
<td>Malay vs. Indian</td>
<td>5.174</td>
<td>0.075</td>
<td>1.990</td>
<td>0.158</td>
</tr>
<tr>
<td>Malay vs. Other Bumiputra</td>
<td>5.319</td>
<td>0.070</td>
<td>1.883</td>
<td>0.170</td>
</tr>
<tr>
<td>Chinese vs. Indian</td>
<td>10.594</td>
<td>0.005*</td>
<td>5.503</td>
<td>0.019*</td>
</tr>
<tr>
<td>Chinese vs. Other Bumiputra</td>
<td>3.542</td>
<td>0.170</td>
<td>0.208</td>
<td>0.648</td>
</tr>
<tr>
<td>Indian vs. Other Bumiputra</td>
<td>3.656</td>
<td>0.161</td>
<td>1.229</td>
<td>0.268</td>
</tr>
<tr>
<td>Malaysian vs. Australian</td>
<td>9.516</td>
<td>0.009*</td>
<td>3.611</td>
<td>0.057</td>
</tr>
</tbody>
</table>

*p < 0.005

Table 3 shows the association analysis of ACE I/D and ACTN3 R/X gene polymorphisms with ethnicity. The statistical analysis showed that there was a significant difference in the distribution of ACE I/D genotype polymorphism between the ethnic groups (X² = 16.828, df = 6, p = 0.010). The Malay group differed significantly in ACE I/D genotype frequency when compared with the Other Bumiputra group (X² = 10.594, df = 2, p = 0.005), but not to those in the Chinese (X² = 5.174, df = 2, p = 0.075) and Indian (X² = 5.319, df = 2, p = 0.070) groups. The Chinese group did not differ significantly in ACE I/D genotype frequency when compared with the Indian (X² = 3.542, df = 2, p = 0.170) and Other Bumiputra (X² = 3.656, df = 2, p = 0.161) groups. In addition, the Indian group did not differ significantly in ACE I/D genotype frequency when compared with the Other Bumiputra group (X² = 0.602, df = 2, p = 0.740). In contrast, the distribution of ACE I/D allele was not significantly different between Malay (I = 0.66; D = 0.34), Chinese (I = 0.53; D = 0.47), Indian (I = 0.46; D = 0.54), and Others Bumiputra (I = 0.41; D = 0.59) groups (X² = 6.882, df = 3, p = 0.076). Moreover, the statistical analysis showed that there was a significant difference in the distributions of ACE I/D genotype between Malaysian and Australian populations (X² = 9.516, df = 2, p = 0.009) with the frequencies of the II and ID genotypes higher among Malaysians compared to Australians. However, the distribution of ACE I/D allele were not significantly different between Malaysian (I = 0.58; D = 0.42) and Australian (I = 0.48; D = 0.52) populations (X² = 3.611, df = 1, p = 0.057). The statistical analysis, however, showed insignificant difference between the ethnic groups with regard to ACTN3 R/X genotype frequency (X² = 6.926, df = 6, p = 0.328). The distribution of ACTN3 R/X allele was also not significantly different between Malay (R = 0.51; X = 0.49), Chinese (R = 0.53; X = 0.47), Indian (R = 0.35; X = 0.65), and Other Bumiputra (R = 0.52; X = 0.48) groups (X² = 0.9383, df = 3, p = 0.816). Nevertheless, there was no significant difference in ACTN3 R/X genotype frequencies between Malaysian and Australian populations (X² = 0.413, df = 2, p = 0.814). The statistical analysis also

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showed that the difference in \textit{ACTN3 R/X} allele frequency was insignificant between these two populations ($X^2 = 0.1002$, df = 1, $p = 0.752$).

\section*{4.0 Discussion}

The main finding of this study was that the distribution of \textit{ACE I/D} gene polymorphism varied by ethnicity, as defined by a significant difference in the distribution of this polymorphism between Malaysian and Australian populations, as well as among the four ethnic groups in Malaysia. In contrast, the present study found that distribution of \textit{ACTN3 R/X} gene polymorphism did not vary as much by ethnicity as no significant difference was observed in the distribution of this polymorphism among the ethnic groups.

As expected, a significant ethnic difference was observed in the distribution of the \textit{ACE I/D} gene polymorphism between Malaysian and Australian populations. The results demonstrated that the $DD$ genotype was significantly more frequent in the Australian population than in the Malaysian population. The frequency of the $DD$ genotype in the Australian group was similar to a previous report for the Australian population (Lea et al., 2005; Lester, et al., 1999). Meanwhile, the lower frequency of the $DD$ genotype observed in the Malaysian population had been consistent with the findings obtained from previous studies conducted among other Asian populations (Huang, Xie, Zhou, Yang, & Sun, 2004; Movva et al., 2007; Saha, Talmud, Tay, Humphries, & Basair, 1996).

The frequencies of \textit{ACE II}, \textit{ID}, and \textit{DD} genotypes in Malaysian population observed in this study were differed slightly from the previous report for the Malaysian population (Jayapalan, et al., 2008). The reason for this inconsistent finding may be due to the different sample sizes involved in the study. The sample size for each ethnic group in the present study was based on the current distribution of these groups in Malaysia (Statistic, 2011), which had not been controlled for in the previous study (Jayapalan, et al., 2008), thereby providing a more representative distribution of the \textit{ACE I/D} gene polymorphism in the Malaysian population.

Within the Malaysian population, there was also a difference in the distribution of the \textit{ACE I/D} gene polymorphism. Both Malay and Chinese groups showed to have higher frequency of $I$ allele over the $D$ allele, whereas the $D$ allele appeared to be more prevalent than $I$ allele in Indian and Other Bumiputra groups. The present result differed from the previous Malaysian study carried out by Jayapalan et al. (2008) in terms of allele and genotype frequencies though the similar trend of ethnic variation was observed. $I$ allele frequency was higher in the Malay group, followed by the Chinese, Indian, and the lowest in the Other Bumiputra group. The distribution pattern of \textit{ACE I/D} gene polymorphism for the Malay group in the present study was remarkably similar with those in Japanese (Matsubara et al., 2002) and Taiwanese (Chuang et al., 1997) populations. The similar pattern between the Malay group and these populations matched with the Taiwan model, which hypothesised that the Malay group in the

Malaysian population originated from the Austronesian group from Taiwan that have been thought to have migrated to the Malaysia Peninsular roughly 3,000 years ago (Comas et al., 1998). On the other hand, the distribution patterns of \textit{ACE I/D} gene polymorphism for Chinese and Indian groups were slightly similar with the previous reports from populations in China (Saha, et al., 1996) and India (Movva, et al., 2007), respectively. This is of no surprise.
as Chinese in Malaysia mostly originated from Southern China while the Indians in Malaysia were mostly immigrants from Southern India (Gan, Subramaniam, Lian, & Nadarajan, 2013). Nevertheless, there has been no any report on the frequency of $ACE\ I/D$ gene polymorphism in the Other Bumiputra group, hence, this study had provided the first data set of this ethnic group. The frequency of the $I$ allele in the Other Bumiputra group seemed to be among the lowest reported for Asian population, and was similar to that in Caucasian population (F. S. Cam et al., 2005; Ferrieres et al., 1999).

Apart from that, Batzer et al. (1994) suggested that the $ACE\ I/D$ gene polymorphism is of African origin and the current ethnic variation on this polymorphism was due to the migration of modern humans out of Africa. During the migration of human, the frequencies of $ACE\ I/D$ allele and genotype changed due to evolutionary factors, such as natural selection and gene flow (Batzер, et al., 1994). The $ACE\ I/D$ gene polymorphism was used as markers in numerous population structure analyses as it has been considered to be a highly stable polymorphism, where there is no mechanism for deletion of this newly inserted element (Stoneking et al., 1997). The $D$ allele is known as the ancestral form of this polymorphism, while the $I$ allele is the most recent version of this polymorphism (Stoneking, et al., 1997). The higher frequency of the $D$ allele in a certain ethnic group is, therefore, indicative of the ancestral ethnic origin for this polymorphism (Stoneking, et al., 1997). The frequencies of $D$ allele observed in the Malaysian and Australian populations in the present study was relatively lower compared to previously reported of the black population by Batzer et al. (1994), which appear to be consistent with this theory. Therefore, the finding of the present study supported the theory that the $ACE\ I/D$ gene polymorphism is of African origin and the current ethnic variation on this polymorphism was due to the migration of modern humans out of Africa. With regard to the present data obtained for $ACE\ I/D$ gene polymorphism, ethnicity factor plays a significant role for the distribution of $ACE\ I/D$ gene polymorphism, as previously suggested by Barley et al. (1994). These data indicate that the effect of $ACE\ I/D$ gene polymorphism on human physical performance, as previously reported for the Caucasian population could be different in the Malaysian population.

In addition, with regard to $ACTN3\ R/X$ gene polymorphism, this is the first study reporting its distribution in the Malaysian population. The allele frequency of $R$ allele in the Malaysian population was closely similar to those reported for Indian population (Goel & Mittal, 2007). Moreover, demarcation data analysis of the Malaysian population based on ethnicity showed that the frequency of $R$ allele in the Malay was 0.51, which was comparable with the findings obtained from Indian population (Goel & Mittal, 2007). The similar pattern between Malay group and Indian population had been concurrent with the finding retrieved from Comas et al. (1998), who reported that the Malay group in Malaysia are descendants of the Proto-Malays, who had admixed with Siamese, Javanese, Sumatran, Indian, Thai, Arab, and Chinese traders. On the other hand, the present results obtained for the Chinese and the Other Bumiputra groups were markedly similar to the previous report for the Asian population (Clarkson et al., 2005). Meanwhile, the findings for the Indian group matched with the report by Kothari et al. (2011) for the Indian population.

When the data for the Malaysian population were compared to the Australian population, insignificant difference was observed for the genotype and the allele distributions of $ACTN3\ R/X$ gene polymorphism between these two populations. These findings are consistent with a previous study carried out by Goel and Mittal (2007), who demonstrated that the frequencies of both alleles and genotypes of $ACTN3\ R/X$ gene polymorphism in the Asian population had
been similar to those of the Caucasian population. Within the Malaysian population, the statistical analysis also indicated insignificant difference in the distribution of this polymorphism between the four ethnic groups in Malaysia. A similar distribution between the Malaysian and the Australian populations, as well as between the four ethnic groups in Malaysia, indicated that these studied populations may share similar positive selection of ACTN3 R/X gene polymorphism, as opposed in the previous study (MacArthur et al., 2007). In fact, it had been revealed that the effect of ACTN3 R/X gene polymorphism on human physical performance previously reported for the Caucasian population may also appear in the Malaysian population.

**Conclusion and recommendation**

In conclusion, this study demonstrated that the distribution of ACE I/D gene polymorphism varies by ethnicity, as defined by a significant difference in the distribution of this polymorphism between Malaysian and Australian populations, as well as between Malay and Other Bumiputra ethnic groups in Malaysia. Conversely, the distribution of ACTN3 R/X gene polymorphism did not vary by ethnicity. This study suggests that the effect of ACE I/D gene polymorphism on human physical performance may also differ by ethnicity, whilst the effects of ACTN3 R/X gene polymorphism on human physical performance may be similar across different human populations. The data from this study should, therefore, serve as a basis for the assessment of the effects of ACE I/D and ACTN3 R/X gene polymorphisms on human physical performance in the Malaysian population. Therefore, further study is warranted for the assessment of the effects ACE I/D and ACTN3 R/X gene polymorphisms on human physical performance within the Malaysian population.

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**Declaration**

Author(s) declare that they have no conflict of interest regarding publication of this manuscript.
Authors contribution

Author 1: carried out the experimental studies, drafted the manuscript and performed the statistical analysis
Author 2: participated in the design of the study and editing the manuscript
Author 3: participated in the design of the study and editing the manuscript
Author 4: participated in the design of the study and editing the manuscript

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