Gene Polymorphism in Angiotensin-I-Converting Enzyme and Physical Activity Among Normotensive Chinese

Wai Pong Wong, Yi Zhao, and Woon-Puay Koh

The angiotensin-I-converting enzyme (ACE) I/D gene polymorphism has been studied for its role in determining habitual physical activity level, but there is no information from Asian populations. The objective of this study was to determine whether this ACE gene polymorphism was associated with physical activity level among Chinese in Singapore. In this cross-sectional study, 110 normotensive Chinese in Singapore, age 21–61 yr, completed the short-form version of the International Physical Activity Questionnaire and contributed buccal cell samples for genotyping of the ACE I/D gene polymorphism using polymerase chain-reaction amplification. They also provided demographic information and underwent anthropometric measurements. Physical activity level was expressed as continuous (in kcal/wk) and categorical (low, moderate, or high) data. The 3 genotypes of ACE were DD (homozygous for the deletion allele), II (homozygous for the insertion allele), and ID. Among the participants, 28.2% reported low, 49.1% moderate, and 22.7% high physical activity level. Frequencies of the genotypes were 11.8% for DD, 42.7% for ID, and 45.5% for II. ACE genotype was independently associated with physical activity level. After age, gender, and body-mass index were adjusted for, individuals with DD or ID genotypes were more likely to report insufficient or low physical activity level than those with II genotypes (odds ratio = 6.88; 95% confidence interval: 2.26, 20.94). In conclusion, the I/D polymorphism of the ACE gene is significantly associated with self-reported physical activity level in normotensive Chinese Singaporeans.

**Keywords**: health, metabolism, exercise physiology, Asian

Physical inactivity, a common risk factor for many chronic noncommunicable diseases such as cardiovascular disease (Sesso, Paffenbarger, Ha, & Lee, 1999), is responsible for 1.9 million preventable deaths worldwide (World Health Organization, 2002). The World Health Organization has reported that two in five adults participated in less than the 150 min/week of moderate-intensity physical activity recommended to improve or maintain health (Haskell et al., 2007; World Health Organization, 2002). Habitual physical activity level may be explained by environmental and psychosocial factors (Chinn, White, Howel, Harland, & Drinkwater, 2006; Humpel, Owen, & Leslie, 2002), but heritability and genetic influence may also account for some of the propensity to be physically active (Lauderdale et al., 1997; Lightfoot, Turner, Daves, Vordermark, & Kleeberger, 2004; Perusse, Tremblay, Leblanc, & Bouchard, 1989; Simonen et al., 2003).

The angiotensin-converting enzyme (ACE) gene polymorphism, in particular, has been studied for its role in determining physical activity preference (De Moor et al., 2009; Fuentes, Perola, Nissinen, & Tuomilehto, 2002; Hagberg, Ferrell, McCole, Wilund, & Moore, 1998; Winnicki et al., 2004).

The ACE is found in circulation and on the surface of many cell types, including skeletal muscle, as a membrane-bound protein (Gordon, Davis, Carlson, & Booth, 2001). Its cardiovascular role is to regulate systemic blood pressure and vascular tone by converting angiotensin I to angiotensin II (a vasoconstrictor) and inactivating bradykinin (a vasodilator). Angiotensin II also augments overload-induced hypertrophy of skeletal muscle (Gordon et al., 2001). About half of the interindividual variations in plasma ACE levels are due to the presence of an insertion/deletion (I/D) polymorphism, determined by the presence or absence, respectively, of a 287-base-pair (bp) element on intron 16 of the ACE gene, which is located on the chromosomal region 17q23 (Cambien et al., 1988). Individuals who are homozygous for the deletion allele (DD) have serum ACE levels higher than those who are homozygous for the insertion allele (II). Furthermore, the D allele is associated with a higher proportion of fast-twitch or Type 2 muscle fibers (associated with strength development), while the I allele is associated with slow-twitch or Type 1 muscle fibers (associated with endurance development; Zhang et al., 2003), thus fueling speculation that individuals with the D allele tend to perform better in resistance (muscle strengthening) training but poorer in aerobic physical...
activity than those with the I allele (Jones, Montgomery, & Woods, 2002; Williams et al., 2000). Indeed, studies in White athletes have shown that the I allele is associated with performance in aerobic- or endurance-type sports (Alvarez et al., 2000; Dekany et al., 2006; Hagberg et al., 1998; Hagberg et al., 2002; Hruskovcová et al., 2006; Lucia et al., 2005; Myerson et al., 1999; Tanriverdi et al., 2005; Tsianos et al., 2004). The ACE I/D genotypes have also been linked to physical performance in different Asian populations (Kim et al., 2010; Moon, Park, & Kwon, 2010; Yoshihara et al., 2009; Zhao et al., 2003). These studies collectively suggest that physical activity could be genetically influenced, and the ACE gene is a strong candidate for this role. Several studies involving American and European participants have examined the association between ACE genotypes and habitual physical activity level (De Moor et al., 2009; Fuentes et al., 2002; Hagberg et al., 1998; Winnicki et al., 2004). However, findings on the association of ACE polymorphisms and physical activity remain inconsistent, which may in part be due to differences in study population. The current study aimed to determine whether the I/D polymorphism of the ACE gene could be associated with physical activity level in normotensive Chinese Singaporeans.

**Methods**

**Participants**

This study recruited 110 Chinese Singaporeans age 21–61 years who were employees working in a single hospital. Exclusion criteria were hypertension (systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg), inability to perform activities of daily living, cognitive impairment, and inability to communicate. The Singapore General Hospital institutional review board approved the study, and informed consent was obtained from the participants.

Demographic information included age, gender, smoking history, self-reported medical history, and medications. The participants underwent measurements of body weight, height, body composition, and resting blood pressure. Body-fat composition was determined by bioimpedance (InBody 4.0, Biospace Inc., Beverly Hills, CA). All tests were performed by the same assessor during daytime. The participants were asked to refrain from exercise and meals for at least 1 hr before the measurements were taken.

The resting blood pressure of the left upper limb was measured using a validated manual blood-pressure set (model: Welch Allyn 420 series, Skaneateles Falls, NY). A standardized blood-pressure measurement protocol was implemented to minimize measurement error (Pickering et al., 2005). The average of two readings taken a minute apart was computed for each participant. Height and weight of each participant were measured using standardized measuring tapes and weighing scales, and training was conducted to ensure standardization of measurement protocols.

**Self-Reported Physical Activity**

Participants completed the self-administered short-form version of the International Physical Activity Questionnaire (IPAQ). The questionnaire ascertains the frequency and duration of vigorous-intensity (such as heavy lifting), moderate-intensity (such as jogging), and walking activities undertaken for leisure, at home, at work, and during traveling in the previous 7 days. Scores for each of three types of physical activity were summed and converted to kilocalories per week according to the recommended formulas (“Guidelines for Data Processing,” 2005). Physical activity level was also categorized as low, moderate or high, based on the IPAQ criteria (“Guidelines for Data Processing,” 2005).

**ACE Genotyping**

Buccal epithelial cells were collected by the mouthwash method (Koh et al., 2003), and genotyping of the ACE gene was determined by polymerase chain-reaction (PCR) amplification. Participants swished 10 ml of antiseptic mouthwash (Listerine, Johnson and Johnson) vigorously in their mouths for 1 min and then returned it into a specimen bottle. The specimen was stored at –20 °C. Fifty microliters of reaction mixture was prepared, consisting of about 50 ng DNA, PCR buffer (Fermentas Inc., Burlington, ON, Canada), primers concentration 0.4 μM (the forward primer 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and the reverse primer 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′) flanked the insertion/deletion region and were used for PCR analysis of chromosomal DNA), 0.2 mM dNTPs, and 1 unit Taq polymerase (Fermentas Inc.; Lau et al., 2002). The amplification was conducted in an automated thermocycler (GeneAmp 9700, Applied Biosystems, Foster City, CA) for 35 cycles (94 °C, 30 s; 60 °C, 45 s; 72 °C, 60 s). The products were separated in 2% agarose gel and visualized by ethidium bromide staining. Amplification of the I allele produces one band at 490 bp for homozygote II. Amplification of the D allele produces one band at 190 bp for homozygote DD. Both bands at 490 and 190 bp are produced by heterozygote. All DD cases were subject to confirmation with a second PCR, performed using the insert-specific forward primer 5′-TTCGAGACG GAG TCT CGC TC-3′ together with the same reverse primer as earlier, since mistyping of ACE heterozygotes as DD homozygotes could occur. Of 114 participants recruited for this study, 4 (3.5%) were excluded from analyses due to noninformative genotypes. Hence, 110 participants were included in this study.

**Statistical Analysis**

Pearson chi-square test was used to examine whether the ACE genotype distributions were consistent with expected frequencies assuming Hardy-Weinberg equilibrium. All variables were presented in descriptive statistics grouped by the genotype. Since the data for physical activity level in kilocalories per week were positively
skewed, we carried out logarithmic transformation of the data and used the natural logs of the values in the analyses. Significant differences among the genotypes in terms of physical activity level were tested by one-way analysis of variance, and pairwise comparisons were tested using post hoc Bonferroni tests. Logistic-regression analysis was used to examine the association between ACE gene polymorphism and physical activity categories, with further adjustment for potential confounders such as age, gender, and body-mass index (BMI). The strength of the association was indicated by odds ratios (ORs) and their 95% confidence intervals (CIs) in the logistic-regression analyses. Level of significance was set at $p < .05$. Stata/IC version 10.0 for Windows (StataCorp LP, TX) was used for all analyses.

**Results**

The genotype distributions (DD 11.8%, ID 42.7%, II 45.5%) were in Hardy-Weinberg equilibrium ($\chi^2 = .702, p = .146$). Frequencies for the I and D alleles were .67 and .33, respectively. The ages of the participants ranged from 21 to 61 years, with a mean of 32.7 (SD 11.2). Table 1 shows the characteristics of individuals with different genotypes. None of the participants were smokers. In this sample, the overall proportions of individuals who reported low, moderate, and high physical activity levels were 28.2%, 49.1%, and 22.7%, respectively. The mean BMI for those with sufficient physical activity level was 21.7 (SD 3.8) kg/m$^2$, whereas that for low physical activity level was 23.0 (SD 4.2) kg/m$^2$. Although participants with low physical activity level appeared to have higher BMI than those with sufficient physical activity level, this difference was not statistically significant ($p = .15$).

The geometric means of physical activity levels in the three genotypes were 566 kcal/week for the DD genotype, 1,160 kcal/week for the ID genotype, and 1,798 kcal/week for the II genotype, one-way analysis of variance: $F(2,104) = 6.97, p = .0014, R^2 = .118$. These means were significantly different between DD and II genotypes (post hoc Bonferroni test: $p = .002$), although they were not significantly different for the other pairwise comparisons, namely, between ID and II genotypes (post hoc Bonferroni test: $p = .110$) and between DD and ID genotypes (post hoc Bonferroni test: $p = .091$). After adjustment for age, gender, and BMI, individuals who carried the D alleles were almost 7 times as likely as those with the II genotype to report having low physical activity level (Table 2).

**Discussion**

We found a significant association between physical activity level and ACE gene polymorphism in normotensive Chinese Singaporeans, which supported the hypothesis that this gene may play a role in influencing physical activity preference.

Findings of the current study involving normotensive individuals agreed with those from a report that examined 355 Italians from the HARVEST trial (Winnicki et al., 2004). The participants (mean age 33 years) from the three different ACE genotypes differed significantly in frequency of physical activity participation, which was ascertained using a validated multi-item questionnaire (Reaven, Barrett-Connor, & Edelstein, 1991). Of note,
11.8% of the variance (estimated $R^2$ from ANOVA) in physical activity level.

Three other reports appear to contradict the current finding. An early study described the lack of significant difference in the ACE genotypic distributions among three groups of postmenopausal older women (mean age for the three genotypes were 62, 63, and 65 years), who were further divided into three groups: athletic (competitive sports participation, $n = 20$), physically active (low- to moderate-intensity activity at least 30 min/day for most days of the week, $n = 19$), or sedentary (no aerobic physical activity, $n = 19$; Hagberg et al., 2002). In another study, Finnish researchers analyzed data from 454 participants (mean age 44 years) who took part in the MONICA study and concluded that the ACE genotypic distributions were not significantly different between physically active (those who said “yes” to a single question on whether they participated in at least 20–30 min of leisure-time exercise that caused them to be a little out of breath and sweating) and sedentary (those who said “no” to the same question; Fuentes et al., 2002). Finally, in a recent genome-wide association study involving 1,644 Dutch and 978 Americans whose mean age was also 44 years, no significant difference in ACE genotypic distributions was demonstrated between exercisers and nonexercisers (De Moor et al., 2009). It should be noted that participants were divided into these two groups of exercise behaviors based on yes or no response to a single question: “Do you participate in exercise regularly?” for the Dutch and “Do you exercise for 60 minutes per week?” for the Americans (De Moor et al., 2009).

Several factors could account for the difference in findings. First, all these reports employed single-item questions to quantify or define physical activity (De Moor et al., 2009; Fuentes et al., 2002; Hagberg et al., 1998), which could potentially lead to inadequate assessment of physical activity level (Perusse et al., 1989; Slater, Green, Vernon, & Keith, 1987), resulting in nondifferential misclassification bias and null effect measures. Physical activity is defined as “bodily movement that is produced by the contraction of skeletal muscle and that substantially increases energy expenditure” (American College of Sports Medicine, 2006, p. 3). Given that energy expenditure is estimated from several variables, namely, the type of physical activity and its intensity, frequency, and duration, a single-item question on physical activity will lack construct validity compared with a multi-item questionnaire (Slater et al., 1987). Moreover, “moderate intensity” in one study was inconsistent with contemporary definition (Fuentes et al., 2002). In our study, the questionnaire used has been shown to have acceptable test–retest reliability (.76; 95% confidence interval, CI: 0.73–0.77; tested 3 or 7 days apart), as well as fair concurrent (.65; 95% CI: 0.64–0.70; short- vs. long-form) and criterion validity (.30; 95% CI: 0.23–0.36; vs. accelerometry; Craig et al., 2003). In our analysis, subjects were categorized in the two physical activity categories of either low or moderate to high level, since at least moderate-intensity physical activity is necessary for health gains (Haskell et al., 2007). Second, ethnic differences could also account for the conflicting findings. For example, although studies involving Whites have demonstrated greater endurance-training effects in athletes with the $I$ allele than in those with the $D$ allele, the effects were reversed in Kenyan national athletes (Scott et al., 2005), Korean obese men (Moon et al., 2010), and Chinese male military personnel (Zhao et al., 2003). Finally, age may also play a role in preferred physical activity pattern. Participants in studies reporting a lack of association between ACE genotypes and physical activity level tended to be older, with average age in the mid-40s (De Moor et al., 2009; Fuentes et al., 2002) or 60s (Hagberg et al., 1998). Activity level in older adults did not seem to be affected by their ACE genotypes, as limitations in mobility and activities of daily living are likely to become more dominant factors influencing physical activity level (Kritchevsky et al., 2005).

Although precisely how ACE genotypes influence voluntary physical activity participation remains to be elucidated, two postulates could explain this relationship. First, cellular differences between individuals carrying the $D$ alleles render participation in aerobic physical activity less favorable and preferred. Earlier investigators have speculated that a higher level of circulating ACE in $DD$ individuals could lead to greater conversion of angiotensin I to angiotensin II and degradation of kinins, the latter having the effect of lowering the muscle blood flow and glucose utilization necessary for aerobic work.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Level of Physical Activity</th>
<th>Odds Ratio for Low Physical Activity Level (95% Confidence Interval)</th>
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<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Moderate or high</td>
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<td>6</td>
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<tr>
<td>$ID$</td>
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<tr>
<td>$ID$ or $DD$</td>
<td>25</td>
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$^a$Further adjusted for age, gender, and body-mass index.
were not determined or included in the analyses. This activity behavior (Chinn et al., 2006; Humpel et al., 2002) chose social variables known to influence habitual physical validity. Other environmental, socioeconomic, or psychosocial variables could potentially confound the association between ACE gene polymorphisms and physical activity level. The wide confidence interval in the current study underscores the need for a larger sample size. Although the frequencies of genotypes and physical activity levels were similar to those previously reported for Singaporeans, participants in the current study were Chinese Singaporeans, predominantly female, fairly young, and nonsmoking, with a health care background. If this gene–physical activity association is modified by gender, age, smoking status, or other medical conditions, the risk estimate of this association may only be generalized to similar populations. In terms of the ACE genotyping method, both primers used in the first PCR had one nucleotide mismatch when compared with NCBI gene reference sequence. However, we believe that the PCR products were unlikely to be affected by this error, given that the same method had been used in previous studies (Lau et al., 2002). Finally, the cross-sectional nature of the study limits causation inference, although an individual’s ACE genotype should generally be accepted as preceding the predilection for physical activity.

**Conclusions**

In conclusion, normotensive Chinese Singaporeans who had the D allele in their ACE genotype were more likely to report low, or insufficient, physical activity level, after adjusting for age, gender, and BMI. Unlike previous studies that have reported a lack of association between genotypes and level of physical activity participation, the current study used an objective multi-item questionnaire instrument to determine physical activity level. Further studies, however, are needed to understand the relationships among genotypes, physical inactivity, and development of diseases known to be associated with physical inactivity.

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W.P.W. recruited participants, undertook all assessments, carried out the genotyping studies, and drafted the manuscript. Y.Z. provided the laboratory facilities for the work and supervised and checked the results of the genotyping studies. W.P.K. supervised the statistical analyses and their interpretations and provided input to the manuscript draft. All authors read and approved the final manuscript.

**References**


