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# Impact of angiotension I converting enzyme gene I/D polymorphism on running performance, lipid, and biochemical parameters in ultra-marathoners

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**Abstract**

The insertion (I) or deletion (D) polymorphism in the angiotension I converting enzyme gene, (ACE I/D, rs1799752) is associated with human exercise endurance and performance. However, most of the aforementioned studies focus on marathons, swimming, and triathlons, while the ACE polymorphism in ultra-marathoners has not yet been reported. We studied the impact of ACE I/D polymorphism in ultra-marathoners and investigated its relationship with lipid profiles, interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hs-CRP) levels in runners before and after ultra-marathon racing.

This observational study used data from a 100-km ultra-marathon in Taipei, Taiwan. Twenty-four male participants were analyzed for their ACE insertion/deletion polymorphism, lipid profiles, hs-CRP, IL-6 in serum immediately before and after ultra-marathon running.

In our 24 subjects analyzed, 7, 14, and 3 subjects were of I/I, I/D, and D/D genotypes, respectively. Runners with the D polymorphism (I/D and D/D) showed a trend of better performance in the 100-km ultra-marathon (measured by completion time in minutes, $P = .036$). In this group, the previous best marathon performance was also significantly better than the I/I group ($P = .047$). After adjusting for body mass index (BMI), the difference in performance was not significant. Ketone levels, IL-6, and hs-CRP levels were highly increased at immediately and 24-hour post-race. No correlation was found between different ACE polymorphisms and common biochemical parameters examined.

We report the first study in the impact of the ACE I/D (rs1799752) on ultra-marathoners. Presence of the D polymorphism in ACE gene is associated with better performance, although the BMI of the runners contribute as a major factor. There was no difference in the biochemical or lipid parameters measured among different ACE polymorphisms.

**Abbreviations:** ACE = angiotension I converting enzyme gene, ANOVA = analysis of variance, AST = aspartate aminotransferase, BMI = body mass index, CK = creatine kinase, CK-MB = creatine kinase-MB, CRP = C-reactive protein, DNA = deoxyribonucleic acid, ELISA = enzyme-linked immunosorbent assay, FFA = free fatty acids, HDL = high density lipoprotein cholesterol, hs-CRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, IMTG = intramyocellular triglyceride, LDH = lactate dehydrogenase, LDL = low density lipoprotein cholesterol, PCR = polymerase chain reaction, RAS = renin-angiotensin system, SNP = single nucleotide polymorphism, TC = total cholesterol, TG = triglyceride.

**Keywords:** angiotension I converting enzyme gene polymorphism, lipids, ultra-marathoner

*Editor: N/A.*

*Y-HC and J-IL are co-first authors and have equally contributed to the article.*

*This study was supported by Taipei Veterans General Hospital (V100C-202) and Mackay Memorial Hospital (MMH107129), Taiwan.*

*The authors have no conflicts of interest to disclose.*

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*Medicine (2019) 98:29(e16476)*

*Received: 19 November 2018 / Received in final form: 5 May 2019 / Accepted: 24 June 2019*

*http://dx.doi.org/10.1097/MD.0000000000016476*

## 1. Introduction

The insertion (I) or deletion (D) polymorphism in the angiotension I converting enzyme gene, or *ACE*, (*ACE* I/D, rs1799752) has been demonstrated in multiple studies to be associated with human exercise endurance and performance. Since the first study in 1998 which reported an association between higher allele frequency of I polymorphism in ACE and improved endurance performance in mountain climbers,[1] various studies have linked the polymorphism to performance. The I allele was associated with better performance in short distance runners,[2] triathlon runners,[3] long distance runners and cyclists,[4] and others. Conversely, the D allele was associated with better performance in swimmers.[2,5] Yet other studies report no difference in allele frequency in athletes versus non-athletic controls.[6,7] The studies above, and many others, have led to a conclusion that the I allele seems to be associated with endurance orientated events such as marathon running, while the D allele seems associated with strength and power-orientated sports, such as swimming,[8] although several studies are less conclusive. A meta-analysis in 2013 that pooled a total of 366 articles on ACE polymorphism came to a similar conclusion that the I allele was related to endurance sports.[9] However, another multi-cohort study in 2018 that enrolled a total of 698 Caucasian endurance athletes from 6 countries on ACE polymorphism came to a conclusion that the there was no association between ACE polymorphism and endurance running times.[10] Most of the aforementioned studies focused on marathons, swimming, and triathlons, while the ACE polymorphism in ultra-marathon runners have not yet been reported. Ultra-marathon, by definition, is a running distance longer than the standard marathon length (42.195 km). One hundred kilometer, a common ultra-marathon length, usually takes up to 10 hours or more to complete, and poses as a higher physiological challenge to the human body compared with a 42.195-km full marathon.

Ultra-marathons place a significant physical demand on runners, resulting in wide ranges of metabolic changes. Among the numerous biochemical parameters that are affected, important indices include interleukin-6 (IL-6), which is derived from muscle and plays an immunomodulatory role,[11] as well as C-reactive protein (CRP), an important acute phase protein reflecting changes post-race.[12] Besides IL-6 and CRP, which are mostly indicative of the inflammation status changes, recent studies have started to investigate the role of lipid profile changes during ultra-marathons.[13,14] There is a renewed interest in the role of intramyocellular triglyceride (IMTG) as an important energy provider during endurance sports.[15,16]

In light of the above background studies, we sought to examine whether the ACE I/D polymorphism would impact performance (race scores) in ultra-marathon runners. We also measured IL-6, high-sensitivity C-reactive protein (hs-CRP), and lipid profile parameters before and after the race to investigate the role of lipids and inflammation in endurance races, and correlated the measurements with ACE polymorphism genotypes. Interestingly, albeit our relatively small sample size, our results show that runners with the D polymorphism have a better running performance. This difference was offset by body mass index (BMI) differences in runners. Our measurement also showed an immediate increase of lipid parameters, IL-6, and hs-CRP immediately after the race, although we did not observe any specific differences between ACE polymorphism genotypes in IL-6 or hs-CRP levels. Our findings provide experimental data for deeper insight in physiological changes and the role of ACE polymorphisms in endurance sports such as ultra-marathons.

## 2. Materials and methods

### 2.1. Study design and population

Twenty-seven men experienced ultra-marathon runners participating in the 2011 Flexpower Cup National 100-Km Ultra-Marathon, in Taipei, Taiwan volunteered for this study. The competition began at 7 am and ended at 9 pm on October 10, 2011. Two runners were unable to finish the 100-km race and were excluded from the study. Technical details precluded complete serological sample collection in one runner, and the runner was also excluded from the study. The data of the remaining 24 male runners were included in the analysis. All runners ran around a 400 m oval track and were permitted to rest and to intake water and food freely throughout the race, as according to standard ultra-marathon regulations.

Before the competition, all runners were required to complete a questionnaire for demographic data and information on medical and training history. Body weights were measured for the 24 subjects 30 minutes before, immediately following, and 24 hours after the race.

### 2.2. Ethics statement

Institutional Review Board approval was obtained from the Ethics Committee of Taipei Veterans General Hospital, and all participants provided written consent to participate in the study.

### 2.3. Laboratory assessment

Blood (20 mL) was drawn from an antecubital vein, using sterile techniques, 1 week before, immediately following and 24 hours after the race. All specimens were refrigerated and transported to the laboratory within 4 hours of sampling.

Plasma samples were assayed on the Siemens Dimension RXL Max Integrated Chemistry System (Erlangen, Germany) using reagents supplied by the manufacturer. Aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase-MB (CK-MB), high density lipoprotein cholesterol (HDL), hs-CRP, lactate, lactate dehydrogenase (LDH), low density lipoprotein cholesterol (LDL), total cholesterol (TC), and triglyceride (TG) were determined with the bichromatic rate method. Free fatty acids (FFA) were measured using the Free Fatty Acid Quantitation Kit (Sigma-Aldrich, St. Louis, MO). Ketone bodies were measured by the representative beta-hydroxybutyrate levels using the colorimetric assay kit (Cayman Chemicals, Ann Arbor, MI). Plasma IL-6 was measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Europe) following standard ELISA protocols.

### 2.4. ACE polymorphism analysis

Genomic deoxyribonucleic acid (DNA) was extracted from sample leukocytes using the Qiagen mini blood kit (cat. No. 51104). Polymerase chain reaction (PCR) was performed using Herculase II Fusion DNA polyemerase (Agilent), with 250 nanomolar of each primer: forward primer: 5′-CTGGAGAC-CACTCCCATCC1TTCT-3′ and reverse primer: 5′-GATGTGG CCATCACATTCGTCAGAT-3′. The protocol used was 98 °C

for 2 minutes, followed by 30 cycles of 98 °C 20 seconds, 58 °C 60 seconds, and 72 °C 30 seconds, with a final extension step of 72 °C for 3 minutes. The product was run on a 2% TBE gel and imaged for further analysis.

PCR based genotying of the ACE I/D polymorphism is traditionally defined as following: (I) polymorphism would have an appearance of 490 base pairs using the aforementioned primers, and (D) polymorphism would have an appearance of 190 base pairs.[17] Assuming no karyotypic abnormalities, we could classify our samples into haplotype groups as I/I (one single 490 bp band), I/D (one band at 490 bp and 190 bp each), and D/D (one single 190 bp band). I/I would represent homogenous insertion polymorphism of ACE gene, I/D represent heterogenous (1 insertion and 1 deletion polymorphism), and D/D represent homogeneous deletion polymorphism.

### 2.5. Statistical analysis

Descriptive results were reported as median (range). The repeated measured analysis of variance (ANOVA) and Wilcoxon signed-ranks test were applied for evaluating the biochemical association between the 3 timepoints (pre-, immediately post-, and 24-hour post-race). The subjects' characteristics, and plasma biochemical association between the I/I group (I/I polymorphism) and the D group (including I/D and D/D polymorphism) groups over the pre-, immediately post-, and 24-hour post-race were using the Mann–Whitney *U* test. Spearman rank correlation coefficient was applied for evaluating the correlation between ACE I/D polymorphism and final scores of these runners. Quade test adjusting body mass index (BMI) was applied for evaluating the comparison between ACE I/D polymorphism and final scores of these runners. Commercially available statistical software (SPSS version 21.0, IBM Corp, Armonk, NY) was used for statistical analysis. Differences were considered to be statistically significant when 2 tailed *P* value was $<.05$.

## 3. Results

### 3.1. Race details and runner demographics

During the race, the temperature ranged from 24.9 to 28.7 °C, the relative humidity was 66% to 87%, and wind speed ranged from 0 to 6.5 m/s (data provided by the Central Weather Bureau, Taiwan). Twenty-four male runner participants completed the 100-km ultra-marathon race and were enrolled in our study. Their characteristics, including age, weight, height, BMI, years of running marathon, training distance, best marathon time, and running time for the current race are summarized in Table 1.

### 3.2. The D polymorphism is associated with better performance in the 100-km ultra-marathon

In our 24 subjects analyzed, 7, 14, and 3 subjects were of I/I, I/D, and D/D genotypes, respectively (Fig. 1). The relative genotype frequency was 71% and 29% for I/I and D polymorphism, respectively (Table 1). When comparing marathon scores between the 2 groups, the D group had a better personal best marathon score versus the I/I group (209 minutes vs 225 minutes, $P=.047$). For this race, the average performance of the D group was still better, although statistical significance was not reached (651 minutes vs 724 minutes, $P=.099$). With Spearman rank correlation analysis (*P*-value: .036), D/D genotype was associated with best performance ($607.7 \pm 45.5$ minutes), followed by I/D ($660.8 \pm 102.2$ minutes), and then I/I ($670.1 \pm 88.7$ minutes). It must be noted that this is the raw data of race performance (measured in minutes). To normalize the results, after normalizing each group to BMI, we discovered that there was no statistical difference between the 2 groups in comparing scores ($P=.680$).

We measured the hematological levels of AST, CK, CK-MB, lactate, and LDH immediately after the race. Then we compared the average levels of the aforementioned parameters by Mann–Whitney *U* test between the 2 groups. The results are shown in Table 2. In all the biochemical parameters measured, there was no difference across the average levels of each genotype group.

### 3.3. Analysis of lipid profiles and ACE polymorphism

A panel of lipid parameters (FFA, ketone, LDL, HDL, TC, TG) was measured at 3 different time points: before the race, immediately after the race, and 24 hours after the race. The exact measurement values are summarized in Table 3, and fold changes differences (comparing immediate post-race or 24-hour post-race to pre-race values, respectively) are illustrated in Fig. 2.

**Table 1**

**Background information of participants (n = 24).**

| | Median (range) | | | |
|---|---|---|---|---|
| | Total subject | D group (D/D + I/D) | I/I group | |
| | n = 24 | n = 17 (71%) | n = 7 (29%) | *P* |
| Parameter | | | | |
| Age, y | 48.5 (22–60) | 49 (22–57) | 44 (28–60) | .757 |
| Weight, kg | 63.6 (50.3–95.2) | 60.6 (50.3–72.7) | 72.7 (64.3–95.2) | **.001** |
| Height, m | 1.69 (1.55–1.85) | 1.66 (1.55–1.78) | 1.72 (1.66–1.85) | **.028** |
| Body mass index, kg/m$^2$ | 22.8 (18.9–27.8) | 22.5 (18.9–24.8) | 24.7 (22.2–27.8) | **.028** |
| Years of running marathon | 5 (1–12) | 5 (2–10) | 5 (1–12) | .455 |
| Training distance | | | | .575 |
| <40 km/wk | 4 | 2 | 2 | |
| 40–100 km/wk | 13 | 10 | 3 | |
| >100 km/wk | 7 | 5 | 2 | |
| Best marathon score, min | 212 (167–239) | 209 (167–237) | 225 (211–239) | **.047** |
| This ultra-marathon score, min | 683 (487–827) | 651 (487–827) | 724 (656–780) | .099 |

D = deletion polymorphism, I = insertion polymorphism.
Significant difference between D and I/I group, $P<.05$.

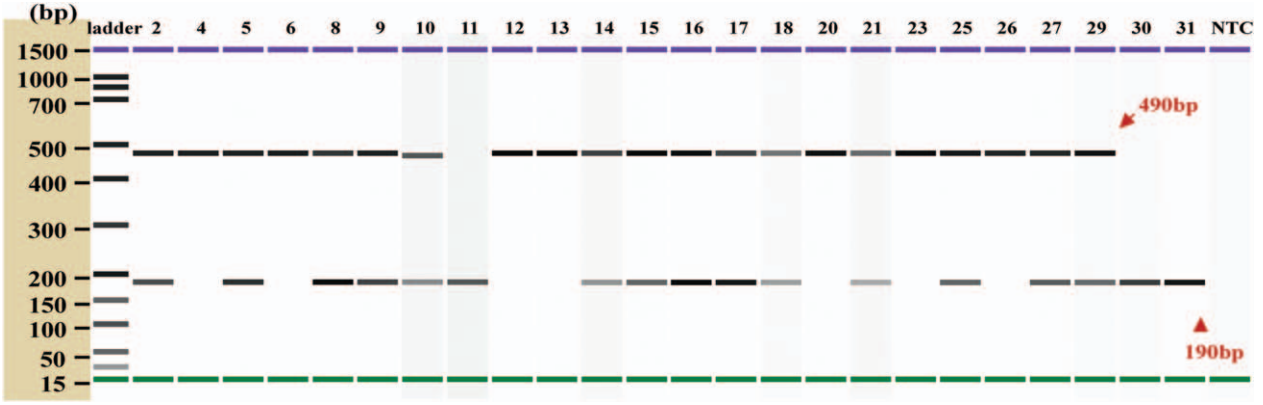


**Figure 1.** PCR analysis of I/D haplotype in 24 samples. NTC = no template control, PCR = polymerase chain reaction.

As presented in Table 3 and Fig. 2, at immediately post-race, there was a significant increase in ketone, TC, and TG levels. When measured at 24-hour post-race, there was a significant increase in HDL levels, and a significant decrease in FFA, TC, TG, and LDL. The most significant change was in ketone levels, showing a ~7-fold increase immediately post-race and 3-fold increase at 24-hour post-race. Also, there was a ~2-fold decrease of FFA at 24-hour post-race, although the immediate post-race levels showed no significant change. The changes in TC, LDL, and TG at 24 hours were modest despite statistically significant.

We compared average levels of lipid parameters between D and I/I groups using Mann–Whitney *U* test. In all the parameters measured, there was no significant difference between each group in either timepoint. This suggested that the ACE polymorphism is not associated with differences of lipid parameters.

### 3.4. Analysis of hs-CRP, IL6, and ACE polymorphism

Hs-CRP and IL-6 levels were also measured at 3 different time points: before the race, immediately after the race, and 24 hours after the race. The exact measurement values are summarized in Table 3, and fold changes differences (comparing immediate post-race or 24-hour post-race to pre-race values, respectively) are illustrated in Fig. 3.

Both IL-6 and hs-CRP were significantly elevated at immediate and 24-hour post-race when compared with pre-race. IL-6 had the highest increase at immediate post-race to >20-fold, and subsided to around 3-fold at 24 hours. Hs-CRP levels were around 5-fold at immediate post-race and further increased to >25-fold at 24 hours, possibly reflecting the longer half-life of hs-CRP.

**Table 2**

**Biochemical parameters measured at before, immediately after, and 24 hours after the ultra-marathon event, grouped according to ACE polymorphism (total subject, n = 24; D group [D/D + I/D], n = 17; I/I group, n = 7).**

| | Median (range) | | |
|---|---|---|---|
| | Pre-race | Post-race | |
| Parameter (reference range) | | Immediate | 24 h |
| AST (5–45 U/L) | | | |
| Total subject | 27.5 (12–41) | 96 (33–964)* | 243 (53–999)* |
| D group | 27 (12–38) | 95 (33–964) | 231 (78–999) |
| I/I group | 28 (25–41) | 150 (55–357) | 282 (53–592) |
| CK (15–210 U/L) | | | |
| Total subject | 148.5 (80–451) | 2432.5 (599–28,963)* | 4565 (816–11,588)* |
| D group | 142 (80–369) | 2172 (599–28,963) | 4062 (1124–10,873) |
| I/I group | 157 (128–451) | 3632 (1043–13,730) | 6230 (816–11,588) |
| CK-MB (<16 U/L) | | | |
| Total subject | 10 (4–16) | 46.5 (23–464)* | 61 (19–163)* |
| D group | 10 (4–16) | 47 (23–464) | 60 (21–163) |
| I/I group | 10 (4–15) | 46 (25–173) | 62 (19–126) |
| Lactate (4.5–19.8 mg/dL) | | | |
| Total subject | 22.8 (11.6–38.9) | 59.8 (40.1–94.6)* | 29.5 (9.3–56.9)* |
| D group | 24.9 (13.0–38.9) | 59.8 (40.1–94.6) | 30.2 (16.4–56.9) |
| I/I group | 19.8 (11.6–27.4) | 57.9 (43.9–78.1) | 20.6 (9.3–39.6) |
| LDH (131–250 U/L) | | | |
| Total subject | 213.5 (163–329) | 502 (351–1332)* | 501.5 (312–1413)* |
| D group | 218 (168–329) | 463 (373–1332) | 492 (366–1413) |
| I/I group | 207 (163–250) | 520 (351–1041) | 576 (312–844) |

AST = aspartate aminotransferase, CK = creatine kinase, CK-MB = creatine kinase-MB, D = deletion polymorphism, I = insertion polymorphism, LDH = lactate dehydrogenase.
* $P < .05$ versus pre-race value.

**Table 3**

**Lipid and inflammatory parameters measured at before, immediately after, and 24 hours after the ultra-marathon event (total subject, n = 24).**

| | Median (range) | | | |
|---|---|---|---|---|
| | | Post-race | | |
| Parameter | Pre-race | Immediate | 24 h | Normal range |
| Weight, kg | 63.6 (50.3–95.2) | 62.1 (50.0–92.1)* | 62.3 (48.5–93.2)* | – |
| Osmolarity, mOsmo/kg$H_2O$ | 289 (272–299) | 302 (288–311)* | 295 (282–315)* | 275–295 |
| Lipid parameters | | | | |
| FFA, mmol/L | 0.15 (0.09–0.43) | 0.21 (0.07–0.30) | 0.07 (0.04–0.15)* | 0.00–0.72 |
| Ketone, mmol/L | 0.0 (0.0–0.8) | 0.6 (0.2–1.4)* | 0.2 (0.0–0.8)* | – |
| TC, mg/dL | 181.5 (127–242) | 199.5 (130–251)* | 175.5 (116–233)* | 125–240 |
| HDL, mg/dL | 67.5 (37–109) | 69.5 (48–125) | 75.0 (52–121)* | 30–70 |
| LDL, mg/dL | 93 (32–185) | 102 (31–171) | 75.5 (23–130)* | <160 |
| TG, mg/dL | 56.5 (30–228) | 115.5 (76–409)* | 55.5 (26–141) | 20–200 |
| Immunological parameters | | | | |
| IL-6, pg/mL | 0.75 (0.35–2.01) | 18.17 (9.27–24.08)* | 1.40 (0.54–4.95)* | 0–0.01 |
| Hs-CRP, mg/dL | 0.05 (0.02–1.22) | 0.50 (0.04–9.70)* | 3.54 (0.39–11.40)* | 0–0.05 |

FFA = free fatty acids, HDL = high density lipoprotein cholesterol, Hs-CRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, LDL = low density lipoprotein cholesterol, TC = total cholesterol, TG = triglyceride.

* $P < .05$ (compared with the pre-race column).

When measured by Mann–Whitney *U* test, there was also no significant all the timepoints measured.

## 4. Discussions

In our study, we describe the following findings: there was a trend of better performance in the D group versus the I/I group in this ultra-marathon race. This observation is consistent that the D group had a significantly better previous best marathon score. This suggests that the D allele contributes to better performance. However, this difference in genotypes was not significant when adjusting for BMI. Ketone levels were highly increased immediately post-race and remained elevated at 24 hours. IL-6 and hs-CRP levels were highly increased at immediately and 24-hour post-race. None of the biochemical parameters were associated with either polymorphism genotype group at any measured timepoints, suggesting that the ACE polymorphism is not related to lipid, IL-6, or hs-CRP levels.

To our knowledge, this is the first study reporting the ACE polymorphism linked to performance in ultra-marathoners. An ultra-marathon is any footrace longer than the traditional marathon length of 42.195 km (26.219 mi). Following the original report of ACE I/D polymorphism having a role in physical performance,[1] a number of studies have examined the relationship of the polymorphism to performance in different sports.[2–8] Currently there is no clear consensus on the nature of sports that the insertion (I) polymorphism or the deletion (D) polymorphism would affect athlete performance.[10,18] An extensive review by Puthucheary et al[8] concluded that the (I) allele is related to better performance in "endurance" sports such as marathons or swimming, while the (D) allele is related to sports that typically require "bursts" of strength, such as short distance running or swimming. Many studies seem to be in agreement with this conclusion, such as a study showing that the frequency of I allele was linearly higher in runners who trained for longer running distances.[2,19] However, other studies have reported conflicting results which a clear association between (I) allele

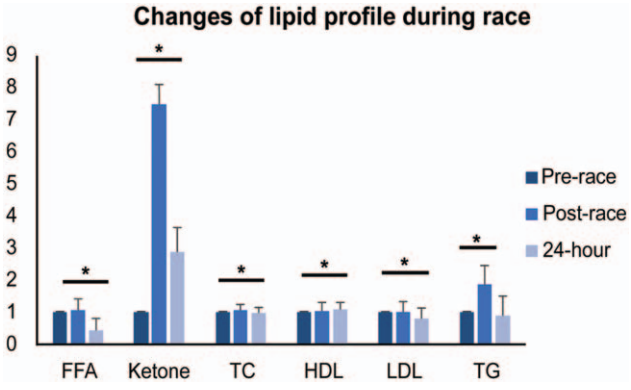


**Figure 2.** Changes of lipid profiles during the ultra-marathon. Lipid levels were measured at timepoints of before the race (Pre-race), immediately after (Post-race), and 24 hours after the race (24-hour). *Y*-axis denotes fold changes compared with pre-race levels.

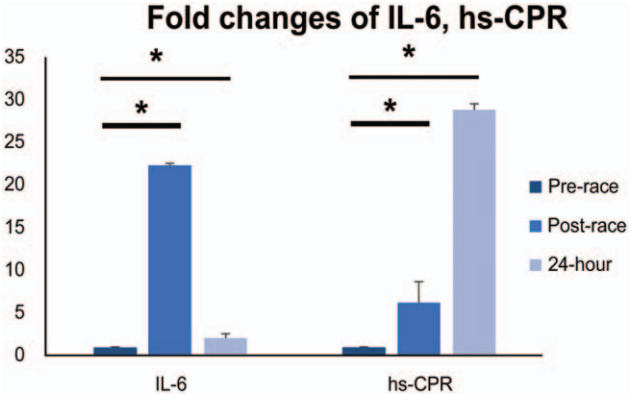


**Figure 3.** Changes of IL-6, hs-CRP during the ultra-marathon. Levels were measured at timepoints of before the race (Pre-race), immediately after (Post-race), and 24 hours after the race (24-hour). *Y*-axis denotes fold changes compared with pre-race levels. hs-CRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6.

frequency and better marathon performance could not be established.[10,20,21] Ethnicity differences,[20] sample size variations and individual physical differences have all been implied in confounding the interpretation of the data.[8]

It is an interesting point that the I allele is previously known to be linked to endurance and D allele is associated with burst exercise, but in our study D allele had better performance. One explanation would be that in ultramarathons, the ultra-long distance required a different physiological setting that the body have adapted after the start of the race. Therefore, results of our study might be suggestive of the distinctive differences physiologically between marathons and ultra-marathons. At this point it is still difficult to clearly pinpoint the discrepancies between results in previous marathon studies and our study. We speculate that a multifactorial etiology, or possibly a multi-gene etiology, may be involved in performance in endurance sports. We anticipate that further data on ultra-marathons could provide additional insight on this issue.

There has been extensive research on explaining how ACE polymorphisms affect exercise performance. Physiologically, the ACE polymorphism is linked to difference in contraction profiles, muscle strength, and other muscle parameters in human studies.[8,22] Left ventricular mass is also reported to be correlated with the ACE polymorphism, although there are many conflicting reports in subjects and patients under different situations.[23–25] Whether in cardiac or skeletal muscle, most studies point to the renin-angiotensin system (RAS) as a primary involved pathway since ACE is a crucial enzyme in this system. This implies that the ACE (I) or (D) polymorphism would have an effect on either the ACE protein function or levels for it to impact the RAS. Indeed, early studies reported higher cardiac tissue ACE protein activity in patients with D/D genotype,[26] as well as higher serum ACE levels in D/D genotype.[27] In agreement to the above, a study in Japanese patients show that I/I genotypes lead to lower serum ACE levels compared with I/D and D/D genotype in kidney biopsy samples,[28] and a similar finding was reported in patient lymphocyte samples.[29] The fact that the (I) polymorphism is an insertion comprising an *Alu* sequence in the 16th intron of the ACE gene makes it interesting to consider that a small 287 bp insertion in an intron would somehow affect protein half-life and/or activity. These suggest epigenetic controls of transcription at the mRNA level associated with the polymorphism, and further research is needed to clarify this study. An alternative explanation to reconcile our findings with the current state of genomics in ultramarathon running would be a multifactorial role, including other genes influencing running performance. COL5A1,[30] TTN,[31] as well as NOS3, BDKRB2, UCP2, AMPD1[32] have also been proposed and could potentially be pursued further for genomic influence studies. Most of these aforementioned genes are related to genes/proteins involved in muscle function, suggesting that genomic alterations in muscle cells may contribute to performance in endurance sports.

Several recent studies reported changes in lipid profile of ultra-marathoners.[13,14] These studies did not observe a substantial significant change of lipid levels immediately after ultra-marathon, but it must be noted that the sample sizes were relatively small. In our study, we observed a modest but significant increase in most of the lipid parameters measured, with the exception of ketone levels, which had a significant 7-fold increase immediately post-race and partially subsided to 3-fold increase at 24 hours post-race. This is compatible with the observation of post-exercise ketosis,[33] which has recently gathered increased interest due to the finding of ketones as an epigenetic modifier.[34] We also queried whether an association between ACE I/D genotype and lipids, hs-CRP, or IL-6 levels was present, but did not observe significant association.

This is the first study reporting the effects of a gene polymorphism on performance in ultra-marathoners. A recent study reported the C > T single nucleotide polymorphism (SNP, rs172722) in the COL5A1 gene, which encodes type V collagen, to be linked to performance in ultra-marathon.[35] Another previous study investigating the ACE I/D polymorphism reported a difference in left ventricular mass, but did not report performance results.[36] Other than these 2 studies, genotypic studies on ultra-marathoners are scarce and should be encouraged to provide more insight on genetic predisposition on athletic endurance and performances.

Our study has several limitations. Due to the lower number of participants in ultra-marathons compared with regular marathons, it was difficult to enroll a larger number of subjects. Our study did not include a control group, which brings into the questions whether our small sample size would be reflective of the allele frequency in the general population. The fact that our D/D group had only 3 patients supports the above criticism. Since ultra-marathons are extremely physically demanding, it is difficult to enroll a high number of participants. We believe that our observed trend of better performance in D group in this ultra-marathon is consistent with the same group having a better previous marathon performance ($P = .047$). It is our opinion that despite the small sample size, our study provides valuable data supporting the hypothesis that the D allele is correlated with better performance in endurance sports, as well as providing the first study on ACE polymorphism in ultra-marathoners.

## 5. Practical applications

In our study, we highlight the findings of the contribution of the *ACE* gene D allele polymorphism frequency to endurance sports performance. We also examine the impact of the D allele on selected biochemical parameters, although we did not observe a significant correlation. Our study provided another data point in sports counseling for coaches and exercise professionals. As "Precision medicine" and genomics have greatly impacted clinical medicine, we believe sports medicine will also benefit from exercise associated genomics to provide more sophisticated sports counseling, risk stratification, goal setting and planning for suitable sports. Our study follows this perspective as described in the manuscript.

## 6. Conclusions

In conclusion, we report that in ultra-marathoners, the ACE polymorphism (D) allele is correlated with better performance measured by overall race time, although this difference was not significant when normalized to BMI. The polymorphism does not significantly affect AST, CK, CK-MB, lactate, and LDH, and is not related to lipid, IL-6, or hs-CRP changes before and after the race. Our findings provide valuable data in the sports physiology field to understand the impact of genomics on exercise performance and metabolism.

## Acknowledgments

The authors thank all the ultra-marathoners who participated in this study and all the doctors, nurses, and emergency medical

technicians who provided professional care at this ultra-marathon race. They also express gratitude to our colleagues at Soochow University and the Chinese Taipei Association of Ultrarunners, all of which assisted at the ultra-marathon event. They further feel an immense gratitude to Yen-Kuang Lin, Research Fellow, Biostatistics Center, Taipei Medical University, Taipei, Taiwan to help their statistical analysis.

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