Human angiotensin I-converting enzyme gene and endurance performance

SAUL MYERSON,1 HARRY HEMINGWAY,2 RICHARD BUDGET,3 JOHN MARTIN,1 STEVE HUMPHRIES,1 AND HUGH MONTGOMERY1
(With the Technical Assistance of Maj Mutch and Helen McGloin)
1Centre for Cardiovascular Genetics, University College London and Royal Free Hospital Medical School, Rayne Institute, London WC1E 6Jj; 2Department of Epidemiology and Public Health Medicine, University College London, London WC1E 6BT2; and 3British Olympic Medical Centre, Northwick Park Hospital, Middlesex HA1 3UJ, United Kingdom

Myerson, Saul, Harry Hemingway, Richard Budget, John Martin, Steve Humphries, and Hugh Montgomery. Human angiotensin I-converting enzyme gene and endurance performance. J. Appl. Physiol. 87(4): 1313–1316, 1999.—Human physical performance is strongly influenced by genetic factors. A variation in the structure of the human angiotensin I-converting enzyme (ACE) gene has been reported in which the insertion (I) variant is associated with lower ACE levels than the deletion (D) gene. We have previously reported that the I variant was associated with improved endurance performance in high-altitude mountaineers and British Army recruits. We now examine this genotype distribution in 91 British Olympic-standard runners (79 Caucasians). DNA was extracted from the buccal cells contained in 10 ml of saline mouthwash donated by the subjects, and the I and D variants of the ACE gene were identified by PCR amplification of the polymorphic region. There was an increasing frequency of the I allele with distance run [0.35, 0.53, and 0.62 for ≤200 m (n = 20), 400–3,000 m (n = 37), and ≥5,000 m (n = 34), respectively; P = 0.009 for linear trend]. Among 404 Olympic-standard athletes from 19 other mixed sporting disciplines (in which endurance performance was not necessarily a key factor), the I allele did not differ significantly from that found in control subjects: 0.50 vs. 0.49 (P = 0.526). These results support a positive association of the I allele with elite endurance performance.

exercise; angiotensin I-converting enzyme genotype; polymorphism

THE CIRCULATING OR ENDOCRINE renin-angiotensin system (RAS) (16) plays a homeostatic role in the human circulation. Angiotensin I-converting enzyme (ACE) is responsible for the breakdown of vasodilator kinins while promoting formation of the vasoconstrictor angiotensin II (ANG II). ANG II in turn stimulates adrenal aldosterone release, leading to salt and water retention. In this way, blood volume and pressure are influenced. A variant of the human ACE gene has been identified and consists of the presence of an extra 287-bp fragment. The presence of the extra fragment is associated with lower circulating and tissue ACE activity, and this variant of the ACE gene is called the I (or insertion) allele. The absence of this fragment (the deletion or D allele) is associated with relatively higher ACE activity (28).

Increasing evidence supports the existence of local RASs in human tissues, including skeletal muscle, which may play a metabolic role (10). Tissue ACE activity is also influenced by the I/D polymorphism of the ACE gene (6, 7). Our laboratory has recently reported that the I polymorphism of the human ACE gene was associated with increased duration of repetitive biceps flexion after a program of general physical training (23). The I allele was also overrepresented among elite mountaineers (23). Together, these findings suggest that the I allele (and thus lower tissue ACE activity) favors endurance performance. Among elite runners, we might thus predict an allele frequency to rise with increasing distance run. We have tested this hypothesis and, in addition, have examined I allele frequency in other elite athletes.

METHODS

Subjects and controls. All 1,086 elite athletes selected by the British Olympic Association as potential Olympic competitors were contacted. Of the 495 respondents, 91 were runners (48 men, 43 women; 79 Caucasian) who competed over 12 distances ranging from 100 m to 100 km (sprinters to ultramarathon runners). There were 404 respondents from other sports (219 men; 185 women) participating in 19 disciplines. These individuals provided a mouthwash sample using 10 ml of 0.9% sodium chloride solution, and DNA was extracted from the buccal cells contained in this sample. ACE genotype was then determined by using a three-primer PCR amplification by two independent staff blind to all subject data, as previously described (25). Primer ratios corresponded to 50 pmol of ACE1 (D-specific oligonucleotide) and ACE3 (common oligonucleotide) and 10 pmol of ACE2 (I-specific oligonucleotide) in a 20-µl reaction. This yielded amplification products of 84 bp for the D allele and 65 bp for the I allele. Amplification products were visualized by using ethidium bromide staining on 7.5% polyacrylamide gels. Genotyping accuracy was ensured by using replica PCRs set up without the I-specific primer (ACE2) to confirm the presence of the D allele.

The frequencies of the I allele for the runners as a group were compared with those of 1,906 British control subjects free of cardiovascular disease (relative frequency II, ID, DD = 0.24, 0.50, 0.26, respectively; I allele = 0.49) (22). Within-group analysis of allele frequency was also performed across the 12 distances run. These were grouped according to the type of muscle metabolism involved (24): ≤200 m (predominantly anaerobic or power generating), 400–3,000 m (mixed aerobic and anaerobic), and ≥5,000 m (predominantly aerobic...
or endurance trained). This segregation was confirmed by two independent sources (D. Jones, Professor of Sport and Exercise Physiology, Birmingham University, and R. Godfrey, Chief Physiologist, British Olympic Medical Centre). Finally, the allele frequency for the remaining 404 elite athletes was compared with that of the controls.

Statistical methods. Frequency of the I alleles across the 12 competitive distances was compared by χ² test for linear trend by using the distance run as the categorical variable. Genotype and allele frequencies between groups were compared by χ² test. P values of <0.05 were considered statistically significant.

RESULTS

Analysis revealed a linear trend of increasing I allele frequency with distance run with groups of ≤200 m (predominantly anaerobic), 400–3,000 m (mixed aerobic and anaerobic), and ≥5,000 m (predominantly aerobic); see Table 1. The proportion of I alleles increased from 0.35 to 0.53 and 0.62 for those athletes running ≤200 m (n = 20), 400–3,000 m (n = 37), and ≥5,000 m (n = 34), respectively (P = 0.009 for linear trend; Fig. 1). This association remained when the 13 runners who competed in hurdles events were excluded (I allele proportions were 0.32, 0.62, and 0.62 for n = 14, 30, and 34, respectively, for the three distance groups; P = 0.020 for linear trend).

When analyzed with a conventional χ² test, the observed numbers of both genotypes and alleles in each group were significantly different from expected values (P = 0.019 and 0.012 for genotypes and alleles, respectively). This was due to a skew toward the I allele in the ≥5,000-m group and an opposite skew toward the D allele in the ≤200-m group.

DISCUSSION

These results support the hypothesis that the I allele of the ACE gene may be associated with improved endurance performance. We have previously demonstrated an overrepresentation of the I allele in elite

Table 1. Relative insertion (I) allele frequency of 91 Olympic-standard runners and of 79 Caucasians alone

<table>
<thead>
<tr>
<th>Distance Run</th>
<th>ACE Genotype</th>
<th>I Allele Frequency (95% confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All runners</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤200 m</td>
<td>20 D 0.45, I 0.15</td>
<td>0.35* (0.20–0.50)</td>
</tr>
<tr>
<td>400–3,000 m</td>
<td>37 D 0.19, I 0.24</td>
<td>0.53* (0.41–0.64)</td>
</tr>
<tr>
<td>≥5,000 m</td>
<td>34 D 0.18, I 0.41</td>
<td>0.62* (0.50–0.73)</td>
</tr>
<tr>
<td>All distances</td>
<td>91 D 0.24, I 0.29</td>
<td>0.52 (0.45–0.59)</td>
</tr>
</tbody>
</table>

Caucasians:

<table>
<thead>
<tr>
<th>Distance Run</th>
<th>ACE Genotype</th>
<th>I Allele Frequency (95% confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤200 m</td>
<td>13 D 0.31, I 0.23</td>
<td>0.46* (0.27–0.65)</td>
</tr>
<tr>
<td>400–3,000 m</td>
<td>32 D 0.16, I 0.28</td>
<td>0.56* (0.44–0.69)</td>
</tr>
<tr>
<td>≥5,000 m</td>
<td>34 D 0.18, I 0.41</td>
<td>0.62* (0.50–0.73)</td>
</tr>
<tr>
<td>All distances</td>
<td>79 D 0.19, I 0.33</td>
<td>0.57 (0.49–0.65)</td>
</tr>
</tbody>
</table>

n, No. of subjects. ACE, angiotensin I-converting enzyme; I and D, insertion and deletion, respectively. For all runners, relative I allele frequency in controls was 0.49. For Caucasians alone, overall, I allele frequency was 0.57 vs. 0.49 for controls (P = 0.039). *P = 0.009 and †P = 0.180 for linear trend.

mountaineers and an I allele-associated improvement in the training response for loaded repetitive biceps flexion (23). Our new data suggest that I allele frequency is greater among longer distance runners than controls and rises with the distance run.

The racial mix of the cohort studied may have influenced our results, as ACE genotype distribution is influenced by race (1). The association of the I allele with endurance performance is, however, unlikely to have been due to the effects of race alone. First, only blacks and Caucasians were represented among the athletes studied, and it has been shown that ACE genotype distributions in healthy African and Caribbean blacks in the UK are no different from those of healthy Caucasians in a British population sample (5) (II, ID, DD = 0.18, 0.50, 0.32, respectively, for both ethnic groups; I allele frequency = 0.43). Second, when only the 79 Caucasian runners are included in the χ² analysis, allele frequency still differs significantly from that of controls (P = 0.039). This was again due to an increasing frequency of the I allele in those running longer distances, with the proportion of I alleles rising from 0.46 for those running ≤200 m to 0.56 and 0.62 in those running 400–3,000 m and ≥5,000 m, respectively (P = 0.180 for linear trend). The χ² for Caucasian genotypes was not significantly different from that of controls (P = 0.091). The association of I allele frequency with running distance among those of different races requires further study, given the small number of non-Caucasians represented in this sample on which no conclusion can be drawn.

Men and women were included together in the groups, as the ACE gene is not gender linked, and allele frequency is, therefore, the same in men and women. However, again due to limitations of sample size, we
were unable to examine any differential impact of genotype on performance between genders.

The I allele of the ACE gene may thus be associated with improved endurance performance, and such an effect might influence general sporting prowess. We therefore examined genotype distribution and I allele frequency among the potential Olympians engaged in other sporting activities. The I allele frequency in other sports as a whole was no different from that in controls (0.50 vs. 0.49; \( P = 0.526 \)). Individual sports did not show any significant excess of the I allele (Table 2). Caution should be advised, however, as the limitations in sample size imposed by the elite nature of the athletes to be studied reduces the number of athletes represented in each of the 19 sporting disciplines and limits the conclusions that can be drawn. In particular, there may be sports in which endurance is an important but not prime determinant of success. In these, any effect of the I allele may be masked in such a small group. Nonetheless, it is interesting to note the excess of the D allele in swimmers (D allele frequency = 0.60 and 0.51 for swimmers and controls, respectively; \( P = 0.034 \)). Most swimming events are undertaken in under 2 min, and thus power, rather than pure endurance characteristics, may play a key role (R. Godfrey, Chief Physiologist, British Olympic Medical Centre, personal communication). The suggestion of a possible advantageous effect of the D allele in power sports is supported by the presence of an excess frequency of the D allele in sprinters compared with controls and elite endurance runners (D allele frequency = 0.62 in those running \( \leq 200 \) m). ANG II is a recognized cellular growth factor and is in linkage disequilibrium with factors influencing the expression of the neighboring growth hormone gene (21). It is possible that paracrine adipose RASs may play a role in the mobilization of triglycerides as a metabolic fuel (4, 11, 12, 15). In addition, kinins may alter relative glucose-free fatty acid-\( \beta \)-hydroxybutyrate substrate availability (8, 17, 32) and handling of lactate (27). In turn, a local skeletal muscle RAS (10) and kinin system (26) may modify substrate delivery through short-term vasodilation (30), long-term changes in capillary density (29), and uptake (9). Thus ACE inhibition can increase skeletal muscle glucose uptake, glycogen storage, and the adaptation of the enzymes responsible for glucose catabolism (13, 14).

Finally, by modulating kinin levels, tissue ACE activity may also influence metabolic efficiency. Kinin-driven nitric oxide release may strongly influence muscle respiration (33). ANG II may also impair mitochondrial efficiency, causing a (partly) metabolic cachexia in rats (3) and increased glucose oxidation compared with uptake (31). The effect of the local RAS on cellular metabolic efficiency has mostly been studied in the heart. During ischemia, locally administered bradykinin can improve energy-rich phosphate levels (adenosine triphosphate and creatine phosphate), reduce lactate concentration, and preserve glycogen stores while improving cardiodynamics (left ventricular pressure and contractility) (18, 19). It can also limit infant size (20), an effect that is abolished by bradykinin inhibitors. This evidence strongly suggests an improvement in myocardial metabolic efficiency mediated by bradykinin through a local RAS. Similar studies are evidently required in skeletal muscle.

The decreased tissue ACE levels associated with the I allele would increase local bradykinin levels and may improve performance via the mechanisms highlighted above. Under normal circumstances, when nutrient supply is adequate, this efficiency is not necessary, and, therefore, no advantage would accrue. Only under situations of extreme metabolic stress, such as during extreme endurance exercise, would this genotype be of an advantage and a selection bias exist.

The understanding of the mechanisms of cellular efficiency has important applications beyond the world of extreme endurance sports. It would be of major benefit in other situations of extreme metabolic stress, such as unstable angina, adult respiratory distress syndrome, and severe pancreatitis, for which intervention with appropriate pharmacological agents, such as ACE inhibitors, may one day improve outcome.

We acknowledge all of the staff at the British Olympic Association. H. Montgomery, S. Myerson, and S. Humphries were funded by the British Heart Foundation. This program of work has been supported by unconditional educational grants from British United Provident Association (UK).

Address for reprint requests and other correspondence: H. Montgomery, UCL Dept. for Cardiovascular Genetics, 3rd floor, Rayne...
REFERENCES


