Human Performance: A Role for the ACE Genotype?

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INTRODUCTION

The polymorphism of the human angiotensin-converting enzyme (ACE) gene discussed here is defined by the presence (insertion, I allele) or the absence (deletion, D allele) of a 287 bp fragment. Hence, three variants exist: II, ID, and DD, the distributions of which within a Caucasian population are roughly 25%, 50%, and 25% respectively.

In the selection of candidate genes that might influence performance, several basic criteria must first be satisfied; in essence, a reasonable hypothesis should be formulated (to avoid the reporting of spurious association).

THE ACE I/D POLYMORPHISM: A LINK TO ANGIOTENSIN II AND BRADYKININ METABOLISM

Firstly, it is important that the polymorphism of the gene under study is functional, or functionally associated. Although this polymorphism occurs in an intron, and is therefore thought not to be functional, it is an exceptionally strong and consistent marker for ACE activity in Caucasians. It not only accounts for half the variation in serum ACE but also relates to ACE activity in local tissues such as the heart, kidney, and adipose tissue (previously reviewed in (12)).

Secondly, it is a logical prerequisite that the relation between the polymorphism and the protein (ACE) has a potential physiological role in the development of the phenotype. Increasing ACE activity is associated with the D allele, which in turn may affect the degradation of bradykinin and synthesis of angiotensin II, both of which have a crucial role in circulatory homeostasis and a significant effect on cell growth (Fig. 1).

Increased ACE activity in the myocardium of rats significantly increases local angiotensin II production. This effect is replicated in human right atrial appendages and in DD subjects who show increased conversion of angiotensin I to angiotensin II. The classical pressor actions of angiotensin II are mediated directly through vasoconstriction, and indirectly through renal salt and water retention via aldosterone. Other effects that may influence the human training-response include the stimulatory effect of angiotensin II on endothelial, cardiac, and smooth muscle cell growth (hypertrophic and hyperplastic). These effects may contribute to a potential hypertrophic training response that may then confer an advantage in power sports.

Conversely, DD subjects demonstrate a significant increase in bradykinin degradation with an inverse relationship between the half-life of bradykinin and serum ACE activity. These are intriguing effects because skeletal muscle cells contain a complete kallikrein-kinin system and bradykinin is an endothelial-dependent vasodilator that has important effects on substrate metabolism, stimulating glucose extraction from the extracellular fluid. In the heart, bradykinin mediates improved myocardial metabolic efficiency, with increased coronary flow and contractility in addition to conservation of glycogen and adenosine triphosphate stores during ischemia (previously reviewed in (12)).
In summary then, the lower ACE activity and increased half-life of bradykinin associated with II genotype may favorably alter substrate metabolism. This may produce improvements in the efficiency of mitochondrial respiration and perhaps contractile function in both cardiac and skeletal muscle, beneficial effects in endurance exercise. Conversely, the effect of increased angiotensin II with the DD genotype may have a predominant effect on cell growth, perhaps favoring the development of muscle bulk that may be of benefit in more power-oriented sports.

THE ACE I/D POLYMORPHISM AND POPULATION-ASSOCIATION STUDIES

Population-association studies attempt to relate whether a genetic marker (polymorphism or allele) occurs more frequently in cases than in controls. In regard to the ACE I/D polymorphism and elite athletic performance this in effect means examining whether the I or D allele occurs more commonly in elite athletes than in control populations. If an association is found then either the polymorphism itself is responsible, or the locus within which the polymorphism occurs. Alternatively, the polymorphism may be in linkage disequilibrium (i.e., is tightly linked) with another locus that is in fact responsible.

All such studies are prone to difficulties in comparison because there may be variable definitions of what constitutes a “case” or phenotype (in this regard, elite athlete status). In addition, variation within the control group used can be another confounding factor, especially if the frequency of the polymorphism under investigation varies between control populations. Thirdly, selection bias may occur in the inclusion of cases (elite athletes), and confusion due to the heterogeneity of, in this case, the elite athlete phenotype. Finally, the influence of the varying genetic backgrounds in different populations may obscure an association. Notwithstanding these difficulties there has been a plethora of intriguing investigations reported to date.

THE ACE I/D POLYMORPHISM AND ELITE PERFORMANCE

The discovery of local renin-angiotensin systems (RAS) in the heart and muscle that may affect tissue growth led to the subsequent association of the D allele with increased exercise-induced left ventricular hypertrophy (LVH). This then prompted Montgomery et al. to examine the relationship between the ACE polymorphism and performance (12). In attempting to investigate a gene-environment interaction, in this case the interaction of the ACE polymorphism with exercise training, it is crucial that as many other environmental factors as possible are kept constant. The cohort of 78 British Army recruits examined by Montgomery et al. before and after an identical 10-wk general physical training program satisfies such criteria. The maximum duration for which they could perform repetitive biceps flexion with a 15-kg barbell increased 11-fold more among those of II genotype compared with DD.

Consistent with this, Gayagay et al. (3) found a significant excess of the I allele and the II genotype in Australian national rowers attending their pre-Olympics selection trial. This cohort certainly satisfy the criteria of being elite athletes, 41 of the 64 studied went on to win more rowing medals in the 1996 Olympics than any other nation. Selection bias was kept to a minimum owing to the Australian national identification program screening for talented individuals. Rowers demonstrate outstanding cardiorespiratory efficiency in terms of work done for oxygen consumed. Their sport has one of the highest energy costs of any predominantly aerobic-type sport (75% of the energy cost of the standard 2000-m distance is met by aerobic metabolism). They also exhibit a preponderance of Type I muscle fibers similar to endurance runners. Further, they are capable of achieving very high ventilation volumes, a feature they have in common with a cohort of elite high altitude (HA) climbers who had ascended Everest, K2, Kanchenjunga, or Lhotse without supplemental oxygen (14). These physiological findings are
compatible with the report of a relative excess of II genotype compared with controls in a small cohort of elite mountaineers who had ascended beyond 7000 m without the use of supplemental oxygen (14).

A somewhat larger study (5) of the 495 respondents who were potential Olympic competitors identified by the British Olympic Association found a significant excess of both the I allele ($P = 0.01$) and II genotype ($P = 0.019$) among the 91 runners compared with controls (Fig. 2). Although a mixed racial cohort was studied it included only Blacks and Caucasians, the distribution of the ACE genotype within which is the same in U.K. populations (5). As this cohort included runners competing over 100 m to 100 km it gave opportunity for the examination of the gene frequency within a sport over a range of endurance. This therefore permitted the study of the ACE genotype influence on a spectrum from the sprint to the endurance phenotype rather than just the elite athlete. This approach revealed a significant linear trend ($P = 0.009$) of increasing I allele frequency with distance run (0.35, $N = 20$, 0.53, $N = 37$, 0.62, $N = 34$ for $\leq$200 m, 400–3000 m, and $\geq$5000 m, respectively). Indeed, the significant excess of the I allele in the runners as a whole was due to a skew toward the I allele in the $\geq$5000 m group, with an interesting skew in favor of the D allele (D allele frequency, 0.65) among the sprinters ($\leq$200 m). I allele frequency remained significantly greater when only the 79 Caucasian runners were included (I allele frequency 0.57, $P = 0.039$).

Examination of the gene frequency within a single sporting discipline with a spectrum from power-orientated short, to more endurance-based longer distances is a preferred strategy that has been further employed in a study of swimmers (13). A comparison of gene frequency by similar distances as that used for runners by Myerson et al. would, however, be meaningless. An elite 5000 m runner completes the event in around 13.5 min (current world record, 12 min 39 s) whereas an elite swimmer will have completed less than 1500 m (current world 1500 m record, 14 min 41 s).

The D allele, with increased ACE and angiotensin II production and previously associated with greater training-related strength gain and sprinting ability, could reasonably be expected to favor performance in the shorter, power-oriented distances. Swimmers were subdivided into 400 m and below (a strong relationship between upper body power and performance over 50–400 m has previously been demonstrated) and $>400$ m, according to their main discipline. They were also subdivided by standard as being either elite (competitors at the European or Commonwealth championships, $N = 56$) or nonelite (American college-standard team, $N = 47$). Only Caucasian swimmers were included, and compared with several Caucasian-only control groups. There was a significant excess of the D allele and DD genotype ($P = 0.004–0.028$) compared with the large control groups only in the elite swimmers. This was due to an excess of the D allele (frequency, 0.69) and DD genotype in those swimming over the shorter distances. Combining the elite swimmers of the European and Commonwealth championships with the college-standard swimmers demonstrated no difference in allele frequency compared with controls; diluting a cohort with nonelite athletes may result in failure to find an association with the ACE I/D polymorphism. The control group in a case-control study is vital and it is interesting to note that any associations were lost when using two small control groups previously reported in the literature. To test whether a genetic marker occurs more frequently in elite athletes than controls requires a homogenous cohort from the same sporting discipline with large, matched control groups.

These results are consistent with those of Myerson et al. (5), who also documented an excess of the D allele (D allele frequency, 0.6; $P = 0.034$ compared with controls) in 64 swimmers composing part of their Olympic-standard cohort. Similarly, a recent report regarding Russian athletes (6) demonstrated an excess of the D allele ($P = 0.042$) in 52 elite swimmers competing over short distances ($<1$ min) (Fig. 3). Once again, combining the elite swimmers with those of a lesser standard, the mixed cohort of average and elite Russian swimmers demonstrated no difference in ACE genotype distribution.

In contrast to these findings several other reports have found no association between the ACE polymorphism and elite athletic performance. Another Australian cohort (10),

Figure 2. ACE genotype frequency in 91 Olympic-standard runners. Control group genotype frequency was 0.24, 0.5, and 0.26 for II, ID, and DD, respectively. The proportion of I alleles increased with distance run from 0.35 to 0.53 and 0.62 among those running $\leq$200 m ($N = 20$), 400–3000 m ($N = 37$), and $\geq$5000 m ($N = 34$), respectively ($P = 0.009$ for linear trend). The excess of I alleles ($P = 0.012$) and the II genotype ($P = 0.019$) in the runners as a whole compared with controls was due to a skew toward the I allele in the $\geq$5000 m group, with a skew in favor of the D allele among the sprinters.
composed of a 120 Caucasian national representatives, was recruited from sports deemed to demand a high level of aerobic fitness (26 hockey players, 25 cyclists, 21 skiers, 15 track and field athletes, 13 swimmers, 7 rowers, 5 gymnasts, and 8 "other"). No association with the ACE polymorphism was found. Swimmers may demonstrate an excess of the D allele, rowers an excess of the I allele; their inclusion together inevitably reduces the likelihood of finding an association. A Finnish cohort (4) did not find an excess of the I allele or II genotype in endurance athletes from their national teams compared with a Finnish control population. The 80 subjects were elite endurance athletes but the phenotype under investigation was confused by the inclusion of long-distance runners, orienteers, cross-country skiers, and triathletes. If the I allele does enhance endurance performance we have no knowledge of any confounding effect or otherwise on navigational ability in the orienteer, or technique in cross-country skiing.

192 national and international athletes from Finland, Canada, Germany, and the United States constitute the GENATHLETE cohort, which has demonstrated no relationship between elite endurance status and the I allele (8). Selection criteria for the GENATHLETE cohort are also based on a minimum VO2max of 75 mL·kg⁻¹·min⁻¹. If an assumption that the I allele mediates its effect on endurance via improvements in the central cardiorespiratory response to training were to be correct, then this approach may increase the likelihood of finding an association. However, it has been clearly shown that this is not the mechanism (7,9,15). In concert with other negative reports the athletes were recruited from different sporting disciplines including cross-country and Nordic combined skiing, the biathlon, running, and road cycling. Over 20% of the athletes were from the biathlon, although rifle marksmanship is an unlikely phenotypic characteristic to be influenced by the ACE genotype. Further subgroup analysis by greater levels of VO2max still bore no relationship to the prevalence of the I allele or II genotype. Although a high level of aerobic fitness is an essential requirement for prowess in endurance activity, endurance performance can vary greatly among individuals inevitably reduces the likelihood of finding an association.

The ACE I/D polymorphism should not be considered a "gene for human performance," but a marker of modulation such that one would expect an excess of the I allele in the truly elite endurance athlete, with a concordant excess of the D allele represented in the more power-oriented events. Therefore, the study of a mixed cohort of athletes is unlikely to prove fruitful. There is, however, always the exception that proves the rule: Alvarez et al. found a significant excess (P = 0.0009) of the I allele in a mixed cohort of 60 elite athletes compared with controls (6).

More recently a mixed cohort of 217 Russian athletes from various disciplines would appear to substantiate the notion that mixed cohorts of athletes obscure any association with the ACE I/D polymorphism (6). The athletes (track and field, triathlon, swimming, and cross-country skiing) were prospectively stratified by performance as "outstanding" or "average" and compared with a control group. The "elite" athletes (N = 141) were all national representatives and included 81 European and Russian champions and 19 Olympic or World champions. "Average" athletes (N = 76) were regional competitors. The athletes were also prospectively stratified into groups according to event duration, covering a spectrum from the more power-oriented to the more endurance-based. Duration of their sporting event classified them as SDA (<1 min, predominantly anaerobic energy production), MDA (1 to 20 min, mixed anaerobic and aerobic), and LDA (>20 min, aerobic): short-, middle-, and long-distance athletes respectively.

ACE genotype frequency among the whole cohort, or all the outstanding athletes alone, was no different than that among sedentary controls. However, when only outstanding athletes were considered, ACE genotype was indeed associated with event duration: an excess of D alleles (P = 0.001) being noted in the SDA group, and an elevated frequency of the I allele (P = 0.032) in the MDA group. As discussed above, these findings were replicated in the outstanding swimmers: a significant excess of the D allele evident in the SDA (P = 0.042) and an excess of the I allele in the MDA (P = 0.042). Outstanding, but not average, track and field SDA similarly demonstrated an excess of the D allele (P = 0.01) with an excess of the DD genotype (P = 0.018). No relationship between the ACE genotype and cross-country skiing or the triathlon was seen, which may be relevant to
the inclusion of such athletes in the cohorts of negative studies (4,8,10).

These data not only confirm an excess of the D allele in elite SDA and I allele in elite MDA, but also offer an explanation as to why any such association may be hard to detect among a heterogeneous cohort of mixed athletic abilities and disciplines. These findings are compatible with those of Myerson et al. (5), who found no gene association when the 404 nonrunner athletes from diverse disciplines such as judo and pole vaulting were lumped together.

It is interesting that this study of Russian athletes failed to demonstrate an excess of the I allele in the outstanding long-distance athletes, nor an increasing linear trend of I allele with increasing aerobic component as has previously been described (5). This may be a function of the categorization of the athletes; Myerson et al. examined allele frequency by distance (200 m, 400–3000 m, and ≈5000 m), whereas Nazarov et al. categorized athletes by duration of event (<1, 1–20, and >20 min). The latter may permit reasonable comparison between outstanding athletes of different disciplines and take account of the interdiscipline variation in the time taken to complete the same distance but does not facilitate direct comparison. Elite cross-country skiers cover 5000 m in approximately 12 min, and elite runners in a little longer, whereas a swimmer will have completed less than 1500 m by similar time points. Hence, a 5000 m runner or skier would be categorized as a MDA by Nazarov et al., but a long-distance competitor by the criteria of Myerson et al. Nevertheless, both groups (≈5000 m and MDA) show an excess of the I allele, with similar frequencies (0.63 and 0.62, respectively), and both studies confirm an excess of the D allele in the power-oriented events.

**TRAINING STUDIES—THE SEARCH FOR A PHYSIOLOGICAL LINK**

The I Allele and Endurance

The ACE genotype has never been associated with endurance performance in the untrained state. Any effect appears to require a period of gene-environment interaction. A high level of aerobic fitness is an essential, but not sole, requirement for elite endurance. VO\(_{2}\)\(_{\text{max}}\), the standard measure of aerobic capacity, is a multifactorial phenotype influenced by genetic and environmental factors. Marked interindividual differences in the trainability of the VO\(_{2}\)\(_{\text{max}}\) phenotype exist: identical programs can improve VO\(_{2}\)\(_{\text{max}}\) by between almost nothing and 1 L·min\(^{-1}\), with maximal heritability estimated at 47% in response to 20 wk of training (7). Although after removal of a shared environment the genetic component is likely to be considerably less, it remains a reasonable starting point in the search for a mechanism.

As the most important environmental factor in determining VO\(_{2}\)\(_{\text{max}}\) is regular physical activity it is vital that any genetic study is charged with standardizing the training protocol within and between groups. This was most admirably acquitted in the HERITAGE study (9) of sedentary Caucasians (N = 476) and Black (N = 248) subjects. This cohort was assessed for a number of cardiorespiratory fitness pheno-

Types, including VO\(_{2}\)\(_{\text{max}}\) before and after 20 wk of strictly supervised training. The exercise program was tailored individually with subjects exercising to a target heart rate corresponding to their baseline VO\(_{2}\)\(_{\text{max}}\). There was no difference in VO\(_{2}\)\(_{\text{max}}\) by genotype at baseline. The training was effective, the four groups of subjects increasing their VO\(_{2}\)\(_{\text{max}}\) by 16.7 ± 9.4%, 17.0 ± 8.9%, 22.6 ± 11.3%, and 17.4 ± 8.9% (mean ± SD for Caucasian parents, Caucasian offspring, Black parents, and Black offspring, respectively). A significantly greater increase with training occurred in the DD subjects compared with II and ID, but only in the Caucasian offspring; this was not seen in the other groups.

In contrast, Hagberg and colleagues found that postmenopausal women homozygous for the I allele had a significantly greater VO\(_{2}\)\(_{\text{max}}\) (6.3 mL·kg\(^{-1}\)·min\(^{-1}\), or 23%) than DD homozygotes. This was entirely due to increased maximal arteriovenous oxygen difference rather than cardiac output (15). A cross-sectional study of postmenopausal women does not readily translate into understanding a specific gene-environment interaction and is contradictory to the findings of several training studies (7,9,15).

In determining factors that may enhance performance we must examine change in that factor with training as an environmental stimulus (as Rankinen and colleagues (7) have done) while keeping as many environmental factors as possible constant. The British military recruit is therefore particularly suitable. They are all sleeping in the same location for a similar duration, eating the same diet, and exercising to identical supervised targets at identical times. They are also often of one sex, from a very narrow age-band, and from a similar racial background.

One such study assessed 58 recruits before and after 11 wk of physical training (11,15). VO\(_{2}\)\(_{\text{max}}\) and \(\delta\) efficiency (DE, \(\delta\) work accomplished·min\(^{-1}\) divided by \(\delta\) energy expended·min\(^{-1}\), expressed as a percentage) were determined before and after training. There was no significant intergroup difference in VO\(_{2}\)\(_{\text{max}}\) at baseline or after training (15). This correlates with the work by Rankinen et al. (7) and is corroborated by a genome-wide scan for markers linked with VO\(_{2}\)\(_{\text{max}}\), finding none on chromosome 17, the location of the ACE gene (8). It is very unlikely that any effect of the I allele on enhanced endurance performance is mediated via differences in the VO\(_{2}\)\(_{\text{max}}\) response to training.

If no difference in the training response of VO\(_{2}\)\(_{\text{max}}\) exists perhaps a local muscle effect may be the underlying mechanism? The increased arteriovenous oxygen difference that accounted for the greater VO\(_{2}\)\(_{\text{max}}\) among postmenopausal women with the II genotype may give an insight. In the United Kingdom recruit study (15) VO\(_{2}\) at all work-rates in the submaximal test was lower for all subjects after training with a significantly greater reduction at 80W for II subjects compared with DD, suggesting more work for less oxygen. Examination of the DE for each recruit before and after training is more robust and revealed baseline DE to be independent of genotype (24.5% and 24.9%, II and DD subjects, respectively). However, DE rose significantly with training only among those of II genotype (absolute change, 1.87% for DD), a proportional increase in efficiency of 8.62%, enough to have a significant biological impact (11). If the benefit conferred by the I allele is due to
improved muscle efficiency then a relative sparing of energy stores over time with a period of training could be expected. Conservation of fat mass and nonfat mass has been shown by bioimpedance in 123 U.K. recruits undergoing training. II subjects demonstrated a greater anabolic response than ID or DD subjects for fat mass (0.55 vs −0.2 kg) and nonfat mass (1.31 vs −0.15kg). Magnetic resonance imaging (MRI) confirmed a significantly greater increase in total and nonfat mass in the mid-thigh of II subjects (14). This relative sparing of fat stores and anabolic effect on nonfat mass is consistent with an improvement in muscle efficiency.

The D Allele and Power-Oriented Performance

The response to strength training also varies widely between individuals but few studies have revealed genetic variables underlying this. One report documents the effects of 9 wk of strength training on quadriceps muscle strength in 33 healthy male volunteers (2). A significant interaction between ACE genotype and isometric training occurred with greater strength gains shown by subjects with the D allele (DD, 14.9%, ID, 17.6%, II, 9%, mean increase over baseline, \( P = 0.05 \) for presence of D allele). This increase in strength may account for the apparent excess of the D allele among power-oriented events.

A recent study in American recruits, however, does not support this (9). Sonna et al. specifically set out to assess whether an association between the ACE genotype and performance exists in a heterogenous, mixed-sex, multi-ethnic population of U.S. military recruits. One hundred forty-seven recruits were studied before and after 8 wk of basic training, which, in marked contrast to U.K. recruits, is partly performed in streamed quartiles according to baseline fitness. All subjects were assessed by peak \( \dot{V} \text{O}_2 \) and scored on the U.S. Army Physical Fitness Test (APFT). The APFT consists of three events: as many standard push-ups in 2 min, sit-ups in 2 min, and a 2-mile run. The National Guard defines the APFT as a “measure of strength,” and the U.S. Army APFT protocol itself considers these tests to be measures of combined endurance and strength. Given that the D allele is associated with a strength-training response and the I allele with endurance these tests are unlikely to be effective discriminators.

U.S. military training is evidently successful in improving the fitness of recruits as assessed by the APFT score as these significantly improved in the whole group. Those in the fittest quartile at baseline, however, showed no improvement in the 2-mile run or push-up event suggesting an insufficient training stimulus. Peak \( \dot{V} \text{O}_2 \) improved but, concordant with other studies (7,15), there was no difference between genotype groups. The authors conclude, quite reasonably, that the magnitude of any genotype effect is smaller than the power of their study (80% power to detect a 20% difference). The application of heterogenous training to a heterogenous group is likely to prevent identification of candidate allele-associated changes in performance unless each race-sex combination is itself substantial. An association is even less likely if, as discussed above, the relationship between the gene under investigation and the protein in relation to it that may mediate the effects of the gene does not hold in the population under study. The relationship between serum ACE activity and the ACE gene has been found lacking in African-Americans.

CONCLUSION

This research is inevitably confined by the confounding variables of population association studies. One could criticize the sample sizes in the positive studies finding a relationship between the ACE I/D polymorphism and elite athletes, but identifying and recruiting a cohort of elite athletes will, by this very definition, never yield massive numbers. However, as the common denominator among the negative studies is the inclusion of athletes from mixed sporting disciplines, thereby involving phenotypic heterogeneity, it is probably more appropriate to remain focused on elite groups of athletes from single sporting disciplines. A population association study testing whether a genetic marker (the ACE I/D polymorphism) occurs more frequently in cases (elite athletes) than controls requires a homogenous cohort of subjects from the same sporting discipline with due regard to the relative power (anaerobic) or endurance (aerobic) component of the event.

Taken together the data would suggest that the D allele is associated with elite power-oriented athletic performance, perhaps via an angiotensin II-mediated increase in muscle growth and strength. Conversely, a local increase in muscle efficiency, rather than a central cardiorespiratory effect, contributes to the enhanced endurance associated with the I allele.

There will still be many elite endurance athletes who are of the ACE DD genotype, and many champions in anaerobic sports of the II genotype. Whatever the data may conclude, elite athletes are still made not born, though perhaps some may be made elite in one discipline more easily than others.

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References