No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status

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PERFORMANCE IN ENDURANCE SPORTS is a multifactorial phenotype, influenced by several factors, such as physical, biomechanical, physiological, metabolic, behavioral, psychological, and social characteristics. The effects of environmental factors on endurance performance are well documented, but a considerable amount of data from twin and family studies have shown that cardiorespiratory fitness, for which maximal oxygen consumption (VO2max) is traditionally recognized as the gold standard, is also influenced by genetic factors. For instance, in pairs of monozygotic twins, the VO2max response to standardized training programs in a series of experiments was characterized by six to nine times more variance between genotypes (between pairs of twins) than within genotypes (within pairs of twins) (6). Similarly, in the sedentary state, the intrapair resemblance for cardiorespiratory endurance phenotypes has been significantly higher in monozygotic twins than in dizygotic twins, with heritability estimates ranging from 25 to 66% (7, 11, 21, 40). Family studies have suggested a genetic effect of ~25–40% for VO2max, adjusted for age, gender, and body mass or body composition (18, 20, 25). In the HERITAGE Family Study cohort, maximal heritabilities of 51 and 47% were observed for the VO2max in the sedentary state (5) and its response to 20 wk of endurance training (4), respectively.

Because of the polygenic nature of endurance performance phenotypes, it is unlikely that the genetic component of these traits will be explained by DNA sequence variation at only a few genes. It is more likely that several gene loci, each with a small but significant contribution, will be responsible for this genetic component. A limited number of candidate genes have been tested so far in this context. For example, data from the HERITAGE Family Study have revealed statistically significant associations between the training responses of cardiorespiratory fitness-related phenotypes and skeletal muscle-specific creatine kinase and Na+-K+-ATPase α2 gene polymorphisms (29, 33, 34).

Over the past 2 yr, some studies have suggested that the DNA sequence variation at the gene locus encoding angiotensin-converting enzyme (ACE) is associated with physical performance. A series of papers based on a cohort of British military recruits have found that the insertion (I) allele and the II genotype of the I/deletion (D) polymorphism in intron 16 of the ACE gene were associated with a lower training-induced cardiac hypertrophy (23) and greater increases in performance after a 10-wk physical training program (22, 24). A higher
frequency of the I allele and the II genotype was reported in Australian rowers (12) and British mountaineers (24) than in sedentary controls. In a small group of postmenopausal women, the homozygotes for the I allele had higher VO\textsubscript{2}\text{max} than did the other genotypes (13). In British Olympic-standard runners, an increase in frequency of the I allele as a function of distance run was reported, although the association disappeared when the analysis was repeated only in Caucasian athletes (26).

However, in a cohort of 724 sedentary subjects from the HERITAGE Family Study, we found no support for the hypothesis that the ACE I allele was associated with a greater trainability of fitness-related phenotypes (30). Both in blacks (n = 248) and in Caucasians (n = 476), all of the 26 phenotypes measured in the sedentary state were similar across the ACE ID genotypes. Of the training response phenotypes, statistically significant associations were found only in Caucasian offspring, but in sharp contrast to previous studies, the DD homozygotes showed greater increases in oxygen consumption and power output phenotypes than did the other genotypes. Moreover, in the HERITAGE Family Study, a genomic scan for VO\textsubscript{2}\text{max} in the sedentary state and in its response to training provided no evidence of linkage with the ACE gene locus or any other regions on chromosome 17 (8). Similarly, in a group of 120 Australian endurance athletes (41) and a cohort of 404 British Olympic-standard athletes (26), the ACE ID genotype and allele frequencies did not differ from those of sedentary control groups. In a cohort of 80 Finnish endurance athletes, frequencies of the II, ID, and DD genotypes were 0.225, 0.475 and 0.300, respectively, i.e., identical to the frequencies reported in general population (14, 16).

Because the previous positive findings have emerged mainly from studies with relatively small sample sizes, and because the results derived from sedentary subjects may not necessarily reflect the situation in highly trained athletes, we investigated whether the ACE ID polymorphism was associated with endurance athlete status in the GENATHLETE cohort comprising 192 elite endurance athletes and 189 sedentary controls.

**METHODS**

Subjects. Altogether, 192 male endurance athletes with a VO\textsubscript{2}\text{max} of at least 75 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} [mean 78.6 ± 3.2 (SD) ml·kg\textsuperscript{-1}·min\textsuperscript{-1}], range 75.0–92.9 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}] and 189 sedentary male controls [VO\textsubscript{2}\text{max} 36.4 ± 7.4 (SD) ml·kg\textsuperscript{-1}·min\textsuperscript{-1}, range 23.1–50.0 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}] were available for the present study. The athletes were recruited from Canada (n = 51), Germany (n = 63), Finland (n = 42), and the United States (n = 36), and they represented the following endurance sport: cross-country skiing (n = 59), biathlon (n = 40), nordic combined (n = 2), long-distance running (n = 20), middle-distance running (n = 19), and road cycling (n = 48). All the athletes had been competing at the national or international level for several years. The control group comprised healthy sedentary subjects from the same geographic areas as the athletes. The controls either were derived from the previous studies performed in our laboratories (3, 31) or were recruited specifically for the GENATHLETE study (Germany). The number of athletes and controls from each country was approximately equal. All the subjects were Caucasians. Informed written consent was obtained from all subjects.

The VO\textsubscript{2}\text{max} of the athletes was determined in the course of incremental exercise tests on cycle ergometers (cyclists) or motor-driven treadmills (skiers and runners) when the athletes were at their peak. The VO\textsubscript{2}\text{max} of the controls was assessed during an incremental cycle ergometer test.

Genotype determinations. Genomic DNA was isolated from lymphoblastoid cell lines or white blood cells following a standard protocol (37). The ACE ID polymorphism was typed with a PCR-based method using three primers as previously described (10). The final reaction mixture of 15 μl contained 100 ng of genomic DNA, 3.0 mM MgCl\textsubscript{2}, 200 μM each 2′deoxynucleoside 5′-triphosphates, 300 nM primers flanking the insertion sequence, 140 nM nested primer, 4.7% DMSO, and 1.0 U of Taq polymerase (Pharmacia Biotech, Baie d’Urfé, PQ). The PCR protocol (model 9600 thermal cycler, Perkin Elmer, Norwalk, CT) consisted of one cycle at 94°C for 3 min, 55°C for 1 min, and 72°C for 1 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, and finally 1 cycle at 72°C for 10 min. The PCR products were separated on 3.5% agarose gel and visualized under ultraviolet light after ethidium bromide staining.

Statistical methods. All statistical analyses were done with the version 6.12 of the SAS statistical software package (SAS Institute, Cary, NC). A χ\textsuperscript{2} test was used to confirm that the observed genotype frequencies were in a Hardy-Weinberg equilibrium and to compare the ACE ID allele and genotype frequencies between athletes and controls, as well as between different sports and places of origin. Differences in VO\textsubscript{2}\text{max}, body weight, and height among the athletes from different sports were tested with an analysis of variance by using the general linear model procedure of the SAS package.

**RESULTS**

Among the athletes, VO\textsubscript{2}\text{max} and height were similar across sports (P = 0.246 and 0.715, respectively) and places of origin (P = 0.682 and 0.057, respectively), whereas skiers and cyclists were heavier than runners [70.8 ± 0.7 (SE) and 71.0 ± 1.2 vs. 64.7 ± 1.3 kg, respectively; P = 0.0002]. However, the ACE ID genotype frequencies did not differ among the sports, with values of 0.248, 0.495, and 0.257 in skiers, 0.256, 0.436 and 0.308 in runners, and 0.292, 0.438, and 0.271 in cyclists for the II, ID, and DD genotypes, respectively (χ\textsuperscript{2} = 0.85, df = 4, P = 0.932). The genotype frequencies were also similar across countries of origin for both athletes and controls (0.223, 0.417, and 0.360 in Canada, 0.262, 0.469, and 0.269 in Germany, 0.184, 0.513, and 0.303 in Finland, and 0.250, 0.583, and 0.167 in the United States for the II, ID, and DD genotypes, respectively; χ\textsuperscript{2} = 7.66, df = 6, P = 0.264).

No differences were observed in the allele and genotype frequencies of the ACE ID polymorphism between athletes and controls (Table 1). In both groups, the genotype frequencies were in Hardy-Weinberg equilibrium. The GENATHLETE study has been designed as a case-control study, but because some previous studies have reported that the ACE I allele was associated with a high performance level, we tested whether a similar trend was present among our endurance athletes. For this purpose, two VO\textsubscript{2}\text{max} cutoffs were used to classify
Table 1. Allele and genotype frequencies of the ACE ID polymorphism in elite endurance athletes and sedentary controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Athletes</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>II (0.265)</td>
</tr>
<tr>
<td>II</td>
<td>192</td>
<td>51</td>
</tr>
<tr>
<td>ID</td>
<td></td>
<td>189</td>
</tr>
<tr>
<td>DD</td>
<td></td>
<td>156</td>
</tr>
</tbody>
</table>

Values are absolute and relative (in parentheses) frequencies; n, no. of athletes. ACE, angiotensin-converting enzyme; I, insertion; D, deletion. Genotype frequencies: $\chi^2 = 3.09$, df = 2, $P = 0.214$. Allele frequencies: $\chi^2 = 2.84$, df = 1, $P = 0.096$.

The results of the present study do not support the hypothesis that variation in the ACE gene locus has an influence on human cardiorespiratory endurance performance. The seemingly controversial results reported thus far may be due to factors related to sample sizes, study designs, and phenotype measurements. The positive findings have emerged from relatively small cohorts (n from 25 to 91), whereas studies with large number of subjects (n from 120 to 724) and rigorously controlled phenotype measurements and exercise training programs, such as the HERITAGE Family Study (30), the study by Taylor et al. (41), and the present study, have yielded negative results. A similar effect of sample size has been observed in the studies dealing with the associations between the ACE ID polymorphism and various cardiovascular disorders. The studies based on large numbers of subjects have generally failed to confirm the positive findings arising from smaller cohorts (1, 19, 39, 44). Moreover, two meta-analyses have detected a publication bias toward positive findings from smaller studies (36, 39), a finding that may also be relevant for the topic of the ACE genotype and physical performance.

Other relevant features of the previous studies are that the associations between the ACE ID polymorphism and physical performance were mainly tested post hoc (i.e., the studies were not originally designed to address such questions) and that the phenotypes were not well standardized. An exception is the study by Gayagay et al. (12) where the athletes were selected from a clearly defined group of Australian Olympic-level rowers. In the present study, instead of selecting a specific sport, we employed a preset VO\(_{2max}\) criterion that the athletes had to meet to be included in the study. We were not able to replicate the findings of Gayagay et al. One explanation for the different outcome may be that rowers represent a special group among endurance athletes. Rowing employs mainly upper body musculature, whereas the performance level in other endurance sports is mostly dependent on the function of the muscles of the lower body or both the lower and upper body. Another potential explanation could be the smaller sample size of rowers.

It has been suggested that the ACE I allele is associated with a high level of physical performance (24). Along the lines of this hypothesis, we did a subgroup analysis in the elite endurance athletes group. Even in the athletes with VO\(_{2max}\) values over 83 ml·kg\(^{-1}\)·min\(^{-1}\) (highest decile), we found no trend for an excess of the I allele or a low number of DD homozygotes (Fig. 1). In our opinion, these findings argue against the idea that the ACE ID polymorphism is associated with extraordinary cardiorespiratory endurance performance. However, it remains to be confirmed whether the ACE ID polymorphism is specifically associated with an adaptation to perform at high altitude. In Native South Americans, it does not seem to be the case (35), but similar data in Caucasians are still missing.

Although an association between the ACE ID genotype and circulating ACE levels has been established (32, 42), there are surprisingly few data on the physiological mechanisms for the proposed associations between the ACE ID polymorphism and the various phenotypes. Systemic angiotensin II levels and blood pressure seem to be unaffected by the ACE ID genotype-related variation in plasma ACE levels (17). In addition, treatment with ACE inhibitors causes a drastic
decrease in ACE activity and angiotensin II levels but has no effect on endurance performance in healthy or mildly hypertensive subjects (2, 27, 28). It has been suggested that the greater ACE II genotype frequency in endurance athletes could reflect the selection of individuals with a “healthier” cardiovascular system and, thus a higher aerobic capacity (12, 24, 26). How-ever, the results by Taylor et al. (41), our findings based on a large group of endurance athletes with documented high \( V_{\text{O2max}} \) level, and the controversial reports on the associations between the ACE ID genotype and various cardiovascular phenotypes cast doubts over this hypothesis. Nevertheless, it is possible that an interaction with other genetic, physiological, or environmental factors is required for the expression of the ACE ID genotype effects. This possibility is underlined by the finding that the influence of the ACE ID genotype on plasma angiotensin II levels is detected when the circulating substrate concentrations are increased by angiotensin I infusion (43).

In addition to the endurance performance trait, the data on the effects of the ACE ID polymorphism on various cardiovascular phenotypes in general are far from clear. The interpretation of the data is further complicated by the fact that the studies with positive results have reported that the associations seemed to be restricted to a special subgroup of the cohort, such as subjects with low levels of cardiovascular risk factors (9). Moreover, the reports showing that, unlike one would assume, the D allele and DD genotype were more common in centenarians (38) and were associated with a lower risk of Alzheimer’s disease (15) further add to the confusion. Thus it is obvious that more studies are needed to fully understand the function of the ACE gene and the possible effects of its DNA sequence variation on various biological traits.

In conclusion, the results from the GENATHLETE cohort do not support the hypothesis that the ACE ID genotype is a determinant of cardiorespiratory endurance performance.

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