Frequency of the C34T mutation of the AMPD1 gene in world-class endurance athletes: does this mutation impair performance?

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AMP deaminase; cycling; running

DURING INTENSE EXERCISE CAUSING adenosine monophosphate (AMP) accumulation, the enzyme AMP deaminase (AMPD; EC 3.5.4.6) is activated in skeletal muscle. This enzyme is a very important regulator of muscle energy metabolism during exercise, and its expression is highly regulated (11). By converting AMP to inosine monophosphate (IMP) with liberation of ammonia, AMPD displaces the equilibrium of the myokinase reaction toward ATP production (27). Also, the AMPD reaction is the initial reaction of the purine nucleotide cycle, which plays a central role in the salvage of adenine nucleotides and in determining energy charge (15). Other possible important functions of the purine nucleotide cycle are the deamination of amino acids (aspartate) and the regulation of the glycolytic pathway by the formation of ammonia and IMP (11, 15).

The skeletal muscle-specific isofrom (M) of AMPD is encoded by the AMPD1 gene, located on the chromosome 1p13-p21 (30). A nonsense mutation [C to T transition in nucleotide 34 (C34T)] in exon 2 of AMPD1 converting the codon CAA into the premature stop-codon TAA, and thus resulting in premature stop of protein synthesis, appears to be the main cause of AMPD deficiency (21). Approximately 2% of the general Caucasian population is homozygous (TT) and nearly 20% heterozygous (CT), for the aforementioned mutation (21, 22, 27, 28, 37). Although some sedentary individuals with this defect (mostly TT) present with easy fatigability, cramps, or myalgia after exercise, the mutation is also present in asymptomatic individuals (31). On the other hand, both heterozygous and homozygous statuses for the C34T mutation have been associated with increased severity of coexisting disorders (29).

Since the original report by Fishbein et al. (9), who first proposed that a deficiency of AMPD causes exercise limitation, several studies using different exercise models have analyzed the extent to which this condition alters functional capacity and the mechanisms behind this potential functional limitation (6, 23, 27, 32, 34, 36). Some controversy arises from these excellent reports. On the other hand, these studies were conducted in nonathletes. No study has yet analyzed the frequency distribution of C34T AMPD1 genotypes among top-level endurance athletes, e.g., Olympic-class runners or professional cyclists able to successfully complete 3-wk tour races, nor whether the C34T mutation in the AMPD1 gene might affect their performance. Although one would expect that AMPD deficiency might affect performance mainly during short-term, supramaximal [≥100% maximal oxygen uptake (V˙O₂ max)] exercise inducing depletion of phosphocreatine (PCr) and fall in the total adenine nucleotide pool (e.g., 400-m track races or short velodrome events), several studies have noted the accumulation of IMP to occur at fatigue during prolonged, submaximal exercise (e.g., ~1 h at 70–75% V˙O₂ max), particularly in the presence of low intramuscular glycogen stores by the end of exercise (2, 24, 25, 33). The subsequent decrease in ATP provision from carbohydrate sources may lead to a transient increase in ADP concentration, stimulating the myokinase reaction. This reaction results in the formation of AMP, which must be rapidly deaminated to IMP and ammonia via the activity of AMPD (35). The aforementioned phenomena at the muscle level are likely to occur at the end of top-level endurance competitions, e.g., marathon races (13), or each daily stage throughout 3-wk cycling races, each of which lasts 5 or more hours but can include some sprints (>20–30

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Performance would be overall unaffected in heterozygous athletes (23, 27, 36), common indexes of endurance performance (V\textsubscript{O2 max} or ventilatory thresholds) within the group of athletes depending on their C34T AMPD genotype. Our hypothesis was that, in elite endurance athletes, the frequency distribution of the mutant T allele is lower than in the general population, and particularly the TT genotype makes achievement of elite status unlikely. Nevertheless, on the basis of the results of previous research (23, 27, 36), common indexes of endurance performance would be overall unaffected in heterozygous athletes (CT) who have reached the status of “elite athlete.”

**METHODS**

**Subjects.** Written consent was obtained from each subject and the study was approved by the institutional ethics committee (Universidad Europea de Madrid, Spain).

Our sample comprised 104 elite endurance athletes, i.e., 50 unrelated top-level male Spanish riders from the four best professional cycling teams who ranked among the top 65–70 Spanish cyclists in terms of a 3-wk stage race performance in this country and 54 runners who were the best Olympic-class Spanish male runners [specialists in middle-distance events (1,500 m), 5,000 and 10,000 m track races, 3,000-m steeplechase events, or marathon] as determined by actual performance during international competitions for the 1999–2004 period.

We specifically chose professional cyclists for this study who met the following criteria: 1) being enrolled in a professional cycling team, 2) having at least 2 yr experience in the professional category of the International Cycling Union, and 3) having participated in (and finished) one or more classic 3-wk stage races between 1999 and 2004. During this period, 11 cyclists won at least one mass-start stage or time trial of the Tour, Giro, or Vuelta, and 7 and 11 were among top-3 and top-10 finishers, respectively, in these main 3-wk races.

All of the runners participate in cross-country races (usual distance of 10,000–12,000 m) during winter months (including world cross-country championships for some of them). Irrespective of their specialty, the training loads of these 54 subjects typically include >150 km/wk (150–200 km/wk) and approach 250 km/wk in marathoniens during some periods of the year. Among the most important competition awards of our runners during the last years are the following: European champion (1,500 m, 3,000-m steeplechase, 5,000 m, or marathon), world champion, and top 3 in world championships (e.g., marathon, 1,500 m, or 3,000-m steeplechase), or Olympic medalist and finalist in Olympic Games (1,500 m, 3,000-m steeplechase, 5,000 m, 10,000 m, or marathon).

The mean (±SD) age, height, and mass of all the athletes was: 27 ± 4 yr, 176.6 ± 6.2 cm, and 64.3 ± 6.8 kg, respectively. A group of 100 sedentary unrelated, healthy Spanish nonathletes (60 men, 40 women, aged 18–50 yr) without symptoms of metabolic muscle diseases served as controls.

**Ethnic and geographic origin of subjects.** All the control subjects are Spanish Caucasians of the same ethnic and geographic origin (i.e., from Castile (the main, central area of Spain)). Concerning athletes, 58.7% are also from Castile (and 3.8% are from areas geographically located next to Castile as Aragón or Extremadura), 12.5% are from the Mediterranean area, 10.6% are from the Southern part of Spain (Andalucía), 5.8% are non-Basques from the Atlantic, Northern part of Spain (e.g., Asturias), and 8.6% are of Basque origin. Although previous research has evidenced some peculiar genetic characteristics in the Basque population compared with the rest of Spaniards and other European Caucasians, e.g., in human leukocyte antigen (HLA) gene frequencies (4), to the best of our knowledge no study has reported differences in the genes regulating muscle metabolism between Basque and non-Basque Spaniards, nor between different Spanish, non-Basque subpopulations. Thus both groups of controls and athletes were comparable in terms of ethnic origin, especially when considering that the percent of Basque subjects was 0% and <9% in controls and athletes, respectively.

**Determination of indexes of endurance performance.** During the 2001 period, we measured the gas exchange of each athlete during exercise tests until exhaustion (see below) using a face mask apparatus attached to a continuous, breath-by-breath monitoring system (Oxycon Champion System; Jaeger, Wuerzburg, Germany). The cyclists and runners were evaluated while pedaling on a cycle ergometer (Ergometrics 900; Ergo-line; Bitz, Germany) or running on a treadmill (Technogym Run Race 1400 HC, Gambettola, Italy), respectively, after an incremental protocol until exhaustion, i.e., workload increases of 25 W/min starting at 25 W for the cyclists and of 1 km/h starting at 8 km/h (with constant 1% upgrade) for the runners.

The following variables were measured during each test: oxygen uptake (V\textsubscript{O2}), pulmonary ventilation (V\textsubscript{E}), ventilatory equivalents for oxygen (V\textsubscript{E}/V\textsubscript{O2}) and carbon dioxide (V\textsubscript{E}/V\textsubscript{CO2}), and end-tidal partial pressure of oxygen (P\textsubscript{ETO2}) and carbon dioxide (P\textsubscript{ETCO2}).

V\textsubscript{O2 max} was recorded as the highest V\textsubscript{O2} value obtained for any continuous 1-min period during the tests. At least two of the following criteria were also required for the attainment of V\textsubscript{O2 max}: a plateau in V\textsubscript{O2} values despite increasing workload, a respiratory exchange ratio \(>1.15\) or the attainment of a maximal heart rate value above 95% of the age-predicted maximum. The ventilatory threshold (VT) was determined by using the criteria of an increase in both V\textsubscript{E}/V\textsubscript{O2} and P\textsubscript{ETO2} with no increase in V\textsubscript{E}/V\textsubscript{CO2}, whereas the respiratory compensation threshold (RCT) was determined by using the criteria of an increase in both V\textsubscript{E}/V\textsubscript{O2} and V\textsubscript{E}/V\textsubscript{CO2} and a decrease in P\textsubscript{ETCO2} (19).

**Genotype determinations.** Genomic DNA was extracted from peripheral blood anticoagulated with EDTA according to standard phenol-chloroform procedures followed by alcohol precipitation. To detect the C34T (C to T transition in nucleotide 34) in exon 2 of AMPD1, a PCR fragment containing the mutation was amplified by using the primers and PCR conditions indicated by Tsujino et al. (37). The fragment was digested with Mae II and electrophoresed through 2% agarose gel. The wild-type PCR product is cleaved by the enzyme, whereas the mutant is not.

In those athletes showing the C34T mutation we also studied the G468T mutation of AMPD1 as previously described by Gross et al. (12). [This mutation has also been associated with deficiency of muscle AMPD and exercise-induced myalgia in Caucasians (12)].

**Statistical analysis.** The frequency of both the mutant T allele and heterozygous subjects with the C34T mutation was compared between the two groups (athletes and nonathletes) by use of a z-test for binomial population proportions. A Mann-Whitney’s test was used to compare V\textsubscript{O2 max}, VT, and RCT levels in carriers and noncarriers of the C34T mutation, both within the total group of athletes and within each of both subgroups of athletes (cyclists, on one hand, and runners, on the other). The level of significance was set at 0.05.

**RESULTS**

**C34T AMPD1 genotype distributions.** The T allelic frequency in our control population (8.5%) was overall similar to that previously reported in large samples of white Caucasians, e.g., 10.8% of 503 subjects in the study by Rico-Sanz et al. (27), but significantly higher (~50%) than in the group of...
athletes (4.3%) \((P < 0.05)\) (Fig. 1). No homozygosity (TT) individual for the C34T mutation was found among the two groups. [Previous research from our laboratory, however, has revealed a 1.5% prevalence of homozygosity for this mutation in a larger population sample \((n = 400)\) of individuals with clinical suspicion of metabolic myopathy (28)]. The distribution frequency of heterozygous (CT) genotypes for the C34T mutation in the control group \((17\%)\) was similar to the distribution reported in the literature for Caucasian populations, i.e., ~1 of every 5 individuals \((21, 22, 27, 38)\), but was ~50% higher \((P < 0.05)\) than in athletes \((8.7%)\), i.e., ~1 of every 12 athletes.

Clinical and performance history in genotyped athletes. Nine athletes (4 cyclists and 5 runners) were heterozygous for the C34T mutation. None carried the G468T mutation of AMPD1 previously described by Gross et al. (12). All of them had successfully pursued their sporting careers, e.g., winner of the Young Riders’ classification in the Tour de France, top 9 (1st non-African) in 5,000 m (World Championships) and 2nd in European cross-country championships, or European champion in 3,000-m steeplechase. During the last years, their resting blood levels of total creatine kinase levels measured several times during the season \((in\ foot:\text{ noncompetition}\ days)\) have consistently ranged within normal limits \((< 167\ \text{IU/L})\), i.e., reflecting no excessive muscle damage (7).

One of the runners had suffered an episode of acute liver and renal failure \([due\ to\ a\ combination\ of\ both\ excessive\ training\ (overtraining)\ and\ severe\ viral\ infection]\) that required hospitalization. He did recover adequately \((i.e., to\ reach\ an\ Olympic\ final)\) after a resting period of a few months.

Six subjects had reported no previous cramps, abnormal myalgia, and/or delayed muscle soreness or limited exercise capacity during their career except two cyclists. One of them complained of powerless feeling and mild leg pain \((with\ no\ cramps)\) during the last season of his career. These symptoms were due to endofibrosis of the external iliac artery, a disease that has been described in highly trained athletes, resulting in significantly reduced blood flow to working muscles (1). Another cyclist reported both increased myalgia and muscle spasms in several competitions and frequent episodes of painful contractions in the dorsum of the feet. His levels of thyroid hormones \((total\ triiodothyronine,\ total\ thyroidin)\) and thyroid-stimulating hormone, growth hormone, cortisol, and testosterone were consistently within normal limits during the last seasons. Thus the possibility that his clinical symptoms had been induced by partial AMP deficiency is not to be ruled out. Further physiological evaluations were performed in this cyclist, including a surface EMG record of his vastus lateralis and rectus femoris muscles during the aforementioned type of incremental cycle-ergometer test and a 20-min constant-load test at 400 W. Both tests, however, showed excellent adaptation to endurance exercise, i.e., a significant upward shift in EMG activity after a power output of 400 W was surpassed \([indicating\ neuromuscular\ fatigue\ (17)\ to\ clearly\ occur\ only\ after\ 400\ W\ was surpassed\ during\ this\ test]\, and a high value of gross mechanical efficiency \((24\%)\) (18).

\(\dot{V}_O_{2\max}, VT,\ and\ RCT\). The main characteristics of laboratory indexes of endurance performance are shown in Table 1. No significant differences in age, height, body mass, \(\dot{V}_O_{2\ max}\), VT, or RCT were found between homozygous or heterozygous athletes \((P > 0.05)\). Similarly, no significant differences were found in these variables between homozygous and heterozygous athletes within each group of cyclists and runners, respectively \((P > 0.05)\).

**DISCUSSION**

In this study, we have studied for the first time the frequency distribution of C34T AMPD1 genotypes in top-level endurance athletes. The main finding of our investigation was twofold: 1) in Caucasian elite endurance athletes the frequency distribution of the mutant T allele is lower (~50%) than in nonathletes, but, 2) in general, the T allele does not seem to be associated to limited endurance performance once the elite-level status has been reached in sports.

One potential limitation from our investigation stems from the fact that we did not study athletes engaging in short-term, supramaximal \((\geq 110\%\ \dot{V}_O_{2\ max})\) exercise \((e.g.,\ 400-m\ track\ running\ races\ or\ short\ cycling\ events\ in\ a\ velodrome)\). Indeed, the activity of AMPD is especially important during short, supramaximal efforts \((e.g.,\ 110\%\ \dot{V}_O_{2\ max})\), inducing a marked depletion of muscle PCr and a fall in the total adenine nucleotide pool \((\text{ATP} + \text{ADP} + \text{AMP})\) (8). Nevertheless, several studies on healthy humans have noted the accumulation of IMP to occur at fatigue during prolonged, submaximal exercise \((e.g.,\ \sim 1\ h\ at\ 70–75\%\dot{V}_O_{2\ max})\), particularly in the presence of low intramuscular glycogen stores \((e.g.,\ 2, 24, 25, 33)\). For instance, Norman et al. (25) studied changes in muscle energy state during prolonged exercise \((\sim 70\%\dot{V}_O_{2\ max})\) until exhaustion in healthy men. Muscle biopsies were obtained at rest, after 15 and 45 min of exercise, and at exhaustion and analyzed for ATP, ADP, AMP, IMP, hypoxanthine, and PCr content. Glycogen content at exhaustion was ~30% of the preexercise level. The PCr content decreased steeply during the

**Table 1. Main characteristics of athletes homozygous with no mutation (CC) or heterozygous (CT) for the C34T mutation of AMPD1**

<table>
<thead>
<tr>
<th>Characteristik</th>
<th>CC (n = 95)</th>
<th>CT (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27±4</td>
<td>26±4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176.7±6.1</td>
<td>175.9±7.2</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>64.5±5.6</td>
<td>62.5±5.9</td>
</tr>
<tr>
<td>%\dot{V}<em>O</em>{2\ max} at VT</td>
<td>73.5±5.9</td>
<td>72.3±4.6</td>
</tr>
<tr>
<td>\dot{V}<em>O</em>{2\ max} at VT, ml·kg(^{-1})·min(^{-1})</td>
<td>68.4±6.4</td>
<td>67.1±4.9</td>
</tr>
<tr>
<td>\dot{V}<em>O</em>{2\ max} at RCT</td>
<td>50.3±5.3</td>
<td>48.5±5.0</td>
</tr>
<tr>
<td>%\dot{V}<em>O</em>{2\ max} at RCT</td>
<td>86.9±5.1</td>
<td>85.9±4.9</td>
</tr>
<tr>
<td>\dot{V}<em>O</em>{2\ max} at RCT, ml·kg(^{-1})·min(^{-1})</td>
<td>63.9±6.6</td>
<td>62.1±4.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. \dot{V}_O_{2}, oxygen uptake; \dot{V}_O_{2\ max}, maximal \dot{V}_O_{2}; VT, ventilatory threshold; RCT, respiratory compensation threshold. No significant differences between means were found in \dot{V}_O_{2\ max}, VT, or RCT \((P > 0.05)\).
first 15 min of exercise and continued to decrease during the rest of the exercise period ($P < 0.05$). Pronounced increases (64%) in contents of IMP ($P < 0.001$) were found when exhaustion was approaching. Furthermore, energy charge [($ATP + 0.5 ADP)/(ATP + ADP + AMP)$] was decreased at exhaustion ($P < 0.05$). The increases in IMP and hypoxanthine that occurred when exhaustion was approaching during prolonged submaximal exercise together with the decrease in energy charge during this phase of exercise suggest a failure of the exercising skeletal muscle to regenerate ATP at exhaustion. Glycogen depletion and the aforementioned phenomena at the muscle level could also occur at the end of top-level endurance competitions, e.g., daily stages throughout 3-wk cycling races, each of which lasts 5 or more hours but might include some bouts ($>20–30$ min) of very intense ($\geq 90\%$ $V_{O2}$ max) exercise, particularly high mountain stages (16). In our athlete sample, there were also world-class specialists in middle-distance track events (1,500 m). In this competition, elite runners must perform well above 100% $V_{O2}$ max (i.e., with a total anaerobic contribution as high as 20%) (14). Among our subjects were Olympic-level specialists in 3,000-m steeplechase, 5,000 and 10,000 m, which are events requiring a continuous near-maximal effort ($\geq 90\%$ $V_{O2}$ max) (5, 39). Even elite marathoners such as the ones studied here must run consistently at $\sim 80–90\%$ of $V_{O2}$ max to achieve successful competitive performances (13).

On the other hand, the AMPD reaction is also the major contributor to the production of ammonia, a biochemical indicator of the intensity of exercise (10, 15). In this regard, previous research using different workloads has reported significant increases in ammonia levels to occur at high, yet submaximal intensities (i.e., $>VT$) (26). In fact, the so-called “ammonia threshold” (i.e., nonlinear increases in ammonia levels once the VT or lactate threshold has been reached) has been described during gradual testing (40). Dudley et al. (8) reported significant increases in ammonia levels after exercise above 85% $V_{O2}$ max. Thus AMPD could also play a very important role in regulating muscle metabolism during the endurance, yet intense events in which our elite athletes selected as subjects participate.

The lack of significant difference in indicators of endurance performance between CT and CC genotypes found here is in overall agreement with previous research with nonathletes. Norman et al. (23) showed short-term, high-intensity exercise performance (30-s Wingate test) to be unaffected by AMPD1 genotypes. At least in their exercise model, AMPD deficiency would cause higher oxidative metabolism during exertion, as a result of the increased adenosine levels enhancing blood flow and increased ADP levels stimulating oxidative phosphorylation, respectively, which in turn would compensate for the decreased purine nucleotide cycling associated with the T allele (23). Tarnopolsky et al. (36) found no significant differences among AMPD1 genotypes in time to exhaustion during incremental cycle ergometry tests; e.g., the mean value of heterozygous subjects was 94% of CC homozygous individuals. It was concluded that complete (TT) or partial AMPD deficiency (CT) does not affect tricarboxylic acid cycle anaplerosis, PCr hydrolysis, or cellular energy charge during exhaustive exercise. In contrast, the capacity for repetitive submaximal isometric muscle contractions (determined during a 20-min test of repetitive voluntary isometric contractions at 40% of maximal force-generating capacity) has been shown to be reduced in AMPD-deficient subjects (TT genotype) (6). In line with our findings, Rico-Sanz et al. (27) found no significant differences between CC and CT genotypes in the $V_{O2}$ max values attained by previously sedentary individuals during incremental testing. Their pioneer study was the only one to describe the effects of endurance exercise training (20 wk) among the different C34T AMPD1 genotypes. Interestingly, the training-induced increase in $V_{O2}$ max was significantly higher ($P = 0.006$) in heterozygous than in homozygous with no C34T mutation, although no explanation for this finding was mentioned in their report. In this regard, one could hypothesize that, during the natural selection process to reach the status of elite athletic competition, the partial metabolic deficiency in heterozygous elite athletes (i.e., decreased purine nucleotide cycling) is compensated for by other training adaptations such as increased blood flow and oxidative phosphorylation in working muscles because of higher levels of adenine and ADP, respectively. Another explanation for the lack of differences in $V_{O2}$ max among homozygous and heterozygous athletes might derive from the fact that, in endurance-trained humans, maximal endurance performance and $V_{O2}$ max are largely constrained by the oxygen delivery to working muscles (particularly, cardiac pump capacity), more so than by peripheral metabolic factors (i.e., at the muscle level) (3). Thus any partial metabolic limitation at the peripheral muscle level (e.g., decreased purine nucleotide cycling) might not significantly affect the $V_{O2}$ max values of top-level athletes. On the other hand, we did not find any evidence of alteration in two other powerful indicators of endurance capacity, VT and RCT (20). Both thresholds are good indicators of the adaptations that occur after endurance training, mostly at the peripheral muscle level, i.e., improved buffer capacity, increased muscle oxidative capacity, or higher fatigue tolerance of type I (oxidative) fibers before type II (glycolytic) fibers are recruited. Extensive research has shown that both variables are related with competitive performance in endurance sports as cycling or running [see Meyer et al. (20) for a review]. Although our design is limited by the fact that we did not perform muscle biopsies, the high $V_{O2}$ max values (mean of $\sim 73$ ml·kg$^{-1}$·min$^{-1}$ and consistently $\geq 65$ ml·kg$^{-1}$·min$^{-1}$) and the very high workloads at which both VT and RCT occurred in heterozygous athletes ($\sim 67\%$ $V_{O2}$ max and 86% $V_{O2}$ max, respectively) seem to suggest that partial AMPD deficiency does not impair aerobic energy production. No such high values of VT, RCT, or $V_{O2}$ max have been reported to date in carriers of the T allele. Finally, some hypotheses that explain the fact that AMPD deficiency is not necessarily associated with clinical symptoms or reduced exercise capacity could also help explain the lack of detrimental effect of this mutation in the performance of our heterozygous athletes. The most likely hypothesis is alternative splicing of exon 2 harboring the mutation (11). [Because exon 2 is only 12 nucleotides in length (4 times 3), skipping of exon 2 would not affect the reading frame (11)].

Although no significant differences were found in common indicators of endurance performance between genotypes, caution must be taken when extrapolating laboratory data to actual competition. Indeed, small physiological differences might result in significant changes in competition performance, as small differences ($<2–3\%$ in terms of performance time) usually make the difference between winning and losing an Olympic final or a cycling main race. In addition, the distri-
bution frequency of the T allele was lower in athletes than in nonathletes. In this regard, further research is necessary to corroborate that the C34T is harmless in terms of top-level performance. For instance, future studies might discern whether humans who are homozygous for the C34T mutation can also reach the status top-level endurance performance. Further research is also needed with elite athletes participating in shorter, supramaximal (>100% \( \dot{V} \text{O}_2 \max \)) events.

In conclusion, although the frequency distribution of the mutant T allele of the C34T AMPD1 genotype seems to be lower in Caucasian elite endurance athletes than in controls, this mutation does not appear to affect endurance performance once the elite-level status has been reached in sports.

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