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Genetic Predictors of Adenosine Monophosphate Deaminase Deficiency

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Abstract

In the majority of the population, during high intensity exercise, Adenosine Monohosphate Deaminase (AMPD) Converts Adenosine Monophosphate (AMP) to Inosine Monophosphate (IMP), with the liberation of ammonia in the process. The AMPD reaction displaces the adenylate kinase equilibrium in the direction of ATP formation during exercise, providing additional energy and preventing a large increase in ADP. AMPD deficiency has been proposed to result in faster fatigue development and earlier inhibition of muscle contractions. This review considers a number of genetic mutations that lead to skeletal muscle AMPD deficiency, their pathology and likely symptoms of the disorder.

Keywords: Exercise intolerance; ADP; AMP; AMPD; ATP

Introduction

During high intensity exercise, Adenosine Triphosphate (ATP) hydrolysis exceeds the rate of Adenosine Diphosphate (ADP) rephosphorylation in the muscle cell, leading to excessive formation of the latter. This results in a shift in the adenylate kinase equilibrium (2 ADP ↔ ATP + AMP) towards increased Adenosine Monophosphate (AMP) content. In the majority of the population, such conditions activate the enzyme Adenosine Monophosphate Deaminase (AMPD) also known as myoadenylate deaminase. AMPD converts AMP to Inosine Monophosphate (IMP), with the liberation of ammonia in the process. The AMPD reaction displaces the adenylate kinase equilibrium in the direction of ATP formation during exercise, providing additional energy and preventing a large increase in ADP. Together with adenylosuccinate synthase and adenylosuccinate lyase, it forms the purine nucleotide cycle which produces fumarate, an intermediate of the Krebs cycle, and therefore yields energy (Figure 1). As AMP deamination displaces the adenylate kinase equilibrium toward ATP resynthnesis, a proposed role for AMPD is to alleviate the exercise-induced decrease in the ATP/ADP ratio and its inhibitory effect on muscle contraction [1].

The aforementioned accumulation of ADP has been shown in rabbit and rodent skeletal muscle to reduce maximal shortening velocity and slow relaxation time [3-4]. Therefore, AMPD deficiency has been proposed to result in faster fatigue development and earlier inhibition of muscle contractions. Support for this proposal derives from reports of exercise-related symptoms such as early fatigue, muscle cramps and pain in AMPD deficient individuals [5]. Nevertheless, the combination of symptomatic variability and high incidence has resulted in some authors referring to AMPD deficiency as a "harmless genetic variant" [6]. In contrast, Isackson et al. reported symptoms of AMPD deficiency to include German measles, restless leg syndrome, cramps, abdominal discomfort, increasing joint hypermobility, chronic muscle pain, lack of aerobic stamina, chronic fatigue and deep burning in muscles with any repetitive movement [7]. These authors recognise that AMPD deficiency can potentially be a debilitating and crippling disease resulting in exercise intolerance. Skeletal muscle AMPD activities are varied across a wide array of neuromuscular disorders, owing to a combination of genetic and pathological factors that influence the expression of this enzyme [8]. Approximately 1-2% of the general Caucasian population exhibits a skeletal muscle AMPD deficiency however, only a few develop symptoms [9].

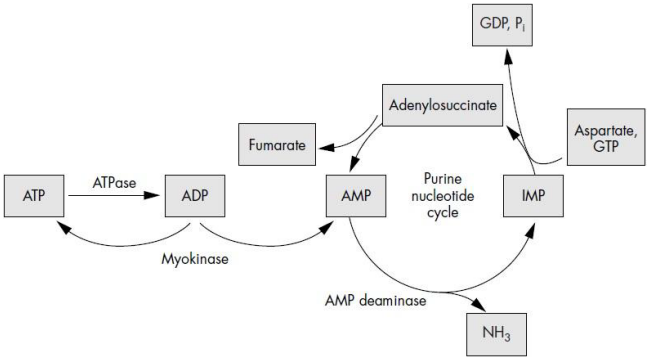

Figure 1: The purine nucleotide cycle (adapted from Lucia et al. [2]).

AMPD deficiency was first reported by Fishbein et al. [5]. There are no specific diagnostic criteria, and no clinical or biochemical definition for this disorder [10]. However, AMPD deficiency should be considered with any presented exercise-induced muscular symptoms especially premature fatigue and/or cramps [11]. The subsequent diagnosis would be based on the biochemical detection of AMPD deficiency in a muscle biopsy or detection of a disease-inducing genetic mutation. The disorder exclusively affects skeletal muscle as the disorder is a specific lack of the skeletal muscle type of AMPD activity. Unfortunately, there is no medical cure for this disorder. The only treatment possibility is the administration of D-ribose which is easily absorbed in the gut and rapidly cleared by metabolic pathways [12]. It serves as an additional energy source and is only effective as long as it is present in the blood. Due to its short half-life, it must be taken constantly to be beneficial. 0.1- 0.15 $mg \cdot kg^{-1}$ body mass must be taken per hour for the treatment to be beneficial. Any less yields no effect and doses above this result in diarrhoea. These limitations suggest it is only reasonable to take ribose at times of increased workload. Ribose is not approved by any organisation for the treatment of patients and must be bought from the chemical industry. So far, there is no explanation for the clinical

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symptomatic variability of AMPD deficient individuals. Presumably, there are alternative pathways for energy production in asymptomatic homozygote patients however, these have not been established.

The muscle-specific isoform AMPD is coded by the *AMPD1* gene and the majority of AMPD deficient cases are due to a C to T transition at nucleotide 34 in exon 2 of the gene, creating a nonsense codon that prematurely terminates translation [7,13]. The frequency of the mutant allele is 8–11% in sedentary Caucasians, with only 2% of the population being homozygous for this mutation (20% are heterozygous) [14]. AMPD muscle activity is greatly diminished even in heterozygous individuals, reaching only ~39% activity of healthy controls [11]. Furthermore, in some heterozygotes, AMPD activity has been reported to be as low as 16% of its normal activity [15]. As a result, deleterious effects on exercise capacity associated with the C34T mutation have been reported, even in heterozygotes. For instance, Norman reported that young heterozygotes for the c.34C>T mutation had reduced capacity to deplete ATP and accumulate IMP during high intensity exercise, demonstrated by higher and lower concentrations of ATP and IMP (+18% and -32% respectively) when compared to normal homozygotes [15].

A number of studies have examined different performance parameters or markers of muscle metabolism in relation to the expression of *AMPD1* with varied findings [14-18]. Fischer et al. examined metabolic differences across *AMPD1* genotypes and the influence on muscle power [9]. It was hypothesised that AMPD deficient individuals would have an earlier onset of fatigue during the Wingate test (30 second (s) cycle ergometer sprint). Blood samples for DNA isolation were collected and genotyped for three different *AMPD1* mutations: c.468G>T, c.34C>T, and c.404delT. The c.404delT was described for the first time in two of three siblings. Investigators then analysed the newly identified c.404delT and previously described c.468G>T mutations in DNA samples from the Swedish population (n = 704), finding one c.404delT allele. Notably, this individual was compound heterozygous for the common c.34C>T mutation. AMPD assays performed using skeletal muscle biopsies showed that both compound heterozygotes for the c.404delT mutation lacked enzymatic activity. Fischer et al [9]. sampled 139 participants, of which 89 homozygous for the normal C34 allele (CC), 38 were heterozygotes (CT) and 9 were homozygotes for the mutant c.34C>T allele (TT). Due to their lack of AMPD activity, the three heterozygotes harbouring the c.34C>T and c.404delT mutations were included in the latter group and were referred to collectively as TT+. No differences were observed in anthropometric data or training index across genotypes.

The TT+ group were older than the CC group; however a regression analysis showed that age did not contribute significantly to any of the power variables measured. Figure 2 shows relative power outputs for the different *AMPD1* genotypes during the 30s Wingate.

Power output profiles normalized for Body weight (Bw) (means ± SE) during 30 2 wingate cycling in different *AMPD1* genotypes; CC, normal homozygotes; CT, heterozygotes; TT+, mutant homozygotes and compound heterozygotes. TT+ group consists of nine subjects that are homozygous for the exon 2c.34C>T mutation and three subjects that are compound heteozygotes for the c.34C>T mutation in exon 2 and c.40delT mutation in exon 5. *A*: men and women; *B*: men; *C*: women. *P* value for the interactopm term (genotype x time) in the ANOV analysis indicates level of significance for the comparison of performance between genotypes

There appeared to be no difference in Peak Power (PP) across genotypes, however a premature decrease in power in TT+ individuals

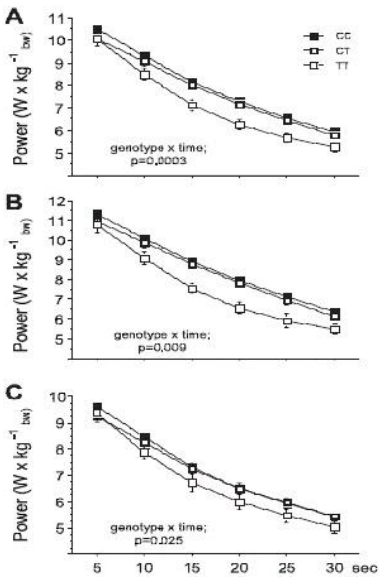

Figure 2: From Fischer et al. [9].

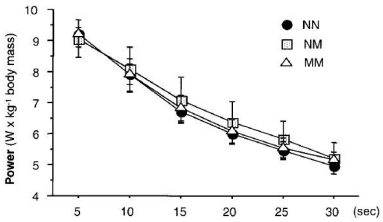

Figure 3: From Norman, Sabina and Jansson [15].

resulted in a difference in Mean Power (MP) across the genotypes (P=0.020). The decrease in power output at 15s was significantly greater in the TT+ group compared to the CT and CC groups (P=0.0006). This difference remained after 30s but was not as pronounced (P=0.046) and was more apparent in males. With regards to metabolic responses, the TT+ group showed almost no increase in plasma ammonia after completion of the Wingate test, which is consistent with a lack of AMPD activity. Fischer et al. concluded that AMPD deficiency was associated with diminished Wingate performance in otherwise healthy individuals and although the MP was only decreased by approximately 10% in TT+ individuals, this indicates a clear functional role for AMPD in skeletal muscle. Fischer et al. failed to detail the mechanism for deleterious effects of AMPD deficiency, and were unclear on how a small subset of the AMPD deficient population experience exercise-related cramping. Fischer et al. were successful in reporting significant findings despite the inherent difficulties when examining AMPD deficiency i.e. the small sample size and magnitude of expected differences.

Norman, also investigated AMPD deficient patients during a Wingate cycle ergometer sprint with contrasting findings to Fischer et al. [9,15]. As shown in Figure 3, no differences in power were observed between genotypes for the *AMPD1* gene unlike the Fischer et al. study.

Power output profiles (mean ± SD) during 30s Wingate cycling normalized for body weight in Normal Homozygotes (NN), Heterozygotes (NM), and Mutant Homozygotes (MM). Mutant allele = c.34C>T mutation in exon 2. In fact, no differences were observed in fiber type, peak power, mean power or ADP, AMP, lactate or creatine phosphate concentrations between groups. There was a

pronounced difference between AMPD activity as expected; with normal homozygotes exhibiting the highest values and homozygotes for the mutation, the lowest. Unlike the other genotypes, the mutant homozygotes did not exhibit a decrease in ATP levels after exercise compared to baseline. Other metabolic discrepancies between genotypes include homozygotes for the mutant allele not showing any significant post-exercise increase in venous plasma ammonia, which is constant with the absence of IMP accumulation. Energy charge exhibited no change in the mutant homozygotes whereas both other genotypes experienced a decrease. Norman et al. summarised by suggesting that due to the high incidence of asymptomatic cases, AMPD deficiency does not commonly cause exercise intolerance. This is consistent with the prediction that, when individuals present severe exercise intolerance and are found to have an isolated deficiency in AMPD, the disorder may not be due to this enzymatic deficiency alone [17]. It is unclear as to why Fischer et al. and Norman et al. reported conflicting findings as the basic characteristics of the subjects (age, height, mass and training status) as well as the sample size were similar between studies. One characteristic of AMPD deficiency is the symptomatic variability. This, coupled with small sample sizes may have caused the discrepancy in findings. A difference in statistical analysis between papers may have caused differences in the findings. Norman et al. conducted an ANCOVA with fibre type as the nuisance variable as this factor is known to affect metabolism despite finding no significant difference between genotypes for fibre type [17]. Contrastingly, Fishcer et al. conducted an ANOVA, which would not take into consideration inconsistencies in fiber type between genotypes despite none being found.

Ruiter et al. also analysed muscle function in individuals homozygous for the C34T mutation [16]. However, Ruiter et al. reported that all AMPD deficient individuals were symptomatic; reporting muscle aches and early fatigue during and after intense exertion. The main methodical difference between the Ruiter et al. paper and the two previously mentioned is that unlike the previous two studies, Ruiter et al. attempted to exclude the influence of central fatigue by maximally stimulating the ulnar nerve electrically and measuring force output at the thumb [9,15]. In accordance with the previous findings by Norman et al. and Fischer et al. PP showed a greater rate of decrease in AMPD deficient individuals (Figure 4).

Change in power during repetitive contractions. Peak power (γ-axis) of 60 repetitive isovelocity (153°s) concentric contractions (χ-axis) with occluded blood flow is shown for AMPD deficient (clear circle) and control (filled square) human adductor pollicis muscle. Note that the unusual unit for power (N°s) is the result of multiplying shortening velocity (°s) and force applied by the thumb at the transducer (N).

However, there were no observed differences between genotypes for

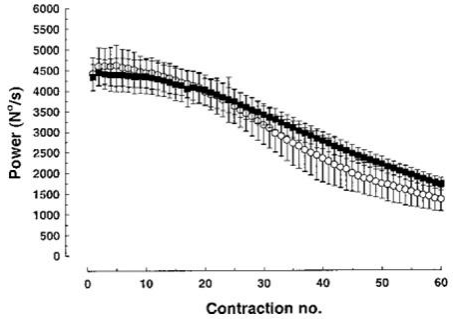

Figure 4: From Ruiter et al. [16].

maximal shortening velocity (V_{max}), maximal isometric force (F_{max}) or optimal shortening velocity (V_{opt}) defined as the velocity of shortening giving the highest power output on the velocity/power curve. Therefore, Ruiter et al. concluded that changes in contractile properties of the human adductor pollicis muscle in response to 60 electrically induced shortening contractions were similar for AMPD deficient individuals and the control group. In other words, the absence of AMPD appeared to have no functional consequences despite the decrease in PP [16].

Although the C34T mutation in exon 2 of *AMPD1* is the most common mutation causing AMPD deficiency, Isackson et al. and Gross et al. have described rarer genetic mutations [7,19]. Isackson et al. examined two Caucasian brothers with AMPD deficiency reporting exercise intolerance and muscle cramps who did not exhibit the common mutations (C34T in exon 2 or C143T in exon 3) in the *AMPD1* gene. However, both brothers were compound heterozygotes for a previously unreported deletion within intron 2 (IVS2-(4-7) del-CTTT) and an A860T mutation in exon 7. The IVS2-(4-7) delCTTT appeared to be accountable for the presence of numerous aberrantly spliced *AMPD1* mRNA species. In the IVS2-(4-7) delCTTT mutation, four pyrimidine bases (bold) were deleted from the middle of the pyrimidine-rich tract (cct**cttt**tagA). Genetta et al. identified two regions in the centre of intron 2 that are necessary for splicing to generate the predominant *AMPD1* transcript apparent in skeletal muscle that includes exon 2 [18]. The IVS2-(4-7) delCTTT mutation, at the 3' end of intron 2, did not fall inside either of those regions and exon 2 was present in all transcripts required. Isackson et al. noted that although both brothers were absent in the common C34T mutation of the *AMPD1* gene, AMPD activity was still reduced to 20% of the reference mean (137 Caucasian adults). Although the precise nature of the changes in AMPD activity resulting from these two mutations is unidentified, exercise intolerance in both brothers along with the decreased enzymatic activity observed in one of the brothers suggests that the impact of heterozygosity is similar to that observed with homozygosity for the common co-segregating Q12X/P48l mutations. Additional genetic mutations probably contributed to the symptomatic problems of these brothers relative to the asymptomatic AMPD deficient members of the general population. In addition, the more severe symptoms of one sibling suggest further genetic variation may be present in this patient in view of his complex phenotype. One limitation of the above study and the conclusions drawn from it is the small sample size (n=2) expressing the IVS2-(4-7) delCTTT mutation. However, this is unavoidable as the intronic deletion has not previously been reported, with the size of the control group suggesting IVS2-(4-7) delCTTT to be a rare mutation and therefore not commonly expressed. This characteristic would make the intronic deletion problematic to investigate as finding other heterozygotes would require large sampling. Studying any possible linkage with other genetic mutations would prove very challenging.

Despite AMPD deficiency being shown to limit exercise capacity (albeit inconsistently) and the physiology of the disorder suggesting muscle metabolism is inhibited, there are reports of AMPD deficient individuals achieving sporting excellence. One such example of this is a case report by Lucia et al. who studied the case of an elite Spanish white runner, who was in the top eight in the 5,000 m at the Word Championships despite being heterozygous for the common C34T mutation in *AMPD1* [2]. Lucia et al. reported the test subject to have lower ammonia and lactate concentrations than the control group (four world class elite East-African runners and five of the best Spanish runners) [2]. His VO_2 cost of running was higher at all three velocities measured than the control group yet he still managed to compete at the highest level of athletics. This was suggested to be due to a number of compensatory mechanisms (which have been reported in other

carriers), for example, a higher oxidative capacity as a result of higher adenosine concentrations stimulating enhanced blood flow. Lucia et al. also suggested more research into the area of central fatigue in AMPD deficient individuals is necessary, as during prolonged endurance exercise, increased blood ammonia concentrations result in increased cerebral uptake of this metabolite, leading to central fatigue [2]. In summary, the paper by Lucia et al. suggests that endurance running at world class competition can be achieved despite partial deficiency in AMPD activity [2]. One criticism of the Lucia et al. study in particular was that although the subject was indeed heterozygous for the mutant allele C34T, AMPD activity was not actually quantified. Rubio et al. sampled a group of top-level Caucasian Spanish endurance athletes (runners and cyclists) and found that the expression of the C34T mutant allele was significantly lower ($P<0.05$) in the athlete group that the control group (4.3% vs 8.5%) with no individuals homozygous for the mutant allele [14]. However, when comparing performance measures within the group of athletes, no significant differences were found in VO_{2max} or ventilation threshold between genotypes. Rubio et al. concluded by suggesting that the C34T *AMPD1* genotype was expressed less in elite endurance athletes compared to controls, however, once elite status has been achieved, this mutation does not appear to affect endurance performance [14].

Conclusion

In conclusion, the often asymptomatic nature of AMPD deficiency makes it a difficult disorder to investigate. The disorder can limit exercise capacity and cause physical pain, as shown by the study of Isackson et al. [7]. However, elite endurance athlete status can be achieved despite a decreased level of AMPD enzyme activity, due to physiological adaptations [2]. The differences in the severity of the disorder have been attributed to additional genetic defects that influence muscle metabolism [7]. To better understand the genetic predictors of AMPD deficiency, additional research could focus on any potential linkage between mutations or additional mutations that determine the symptomatic variance.

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