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Coronary Artery Disease

A Common Variant of the *AMPD1* Gene Predicts Improved Cardiovascular Survival in Patients With Coronary Artery Disease

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OBJECTIVE We tested whether a common *AMPD1* gene variant is associated with improved cardiovascular (CV) survival in patients with coronary artery disease (CAD).

BACKGROUND Reduced activity of adenosine monophosphate deaminase (AMPD) may increase production of adenosine, a cardioprotective agent. A common, nonsense, point variant of the *AMPD1* gene (C34T) results in enzymatic inactivity and has been associated with prolonged survival in heart failure.

METHODS Blood was collected from 367 patients undergoing coronary angiography. Genotyping was done by polymerase chain reaction amplification and restriction enzyme digestion, resulting in allele-specific fragments. Coronary artery disease was defined as ≥70% stenosis of ≥1 coronary artery. Patients were followed prospectively for up to 4.8 years. Survival statistics compared hetero- (+/−) or homozygotic (−/−) carriers with noncarriers.

RESULTS Patients were 66 ± 10 years old; 79% were men; 22.6% were heterozygous and 1.9% homozygous for the variant *AMPD1*(−) allele. During a mean of 3.5 ± 1.0 years, 52 patients (14.2%) died, 37 (10.1%) of CV causes. Cardiovascular mortality was 4.4% (4/90) in *AMPD1*(−) allele carriers compared with 11.9% (33/277) in noncarriers ($p = 0.046$). In multiple variable regression analysis, only age (hazard ratio, 1.11/year, $p < 0.001$) and *AMPD1*(−) carriage (hazard ratio, 0.36, $p = 0.053$) were independent predictors of CV mortality.

CONCLUSIONS Carriage of a common variant of the *AMPD1* gene was associated with improved CV survival in patients with angiographically documented CAD. The dysfunctional *AMPD1*(−) allele may lead to increased cardiac adenosine and increased cardioprotection during ischemic events. Adenosine monophosphate deaminase-1 genotyping should be further explored in CAD for prognostic, mechanistic and therapeutic insights. (J Am Coll Cardiol 2000;36: 1248–52) © 2000 by the American College of Cardiology

The adenosine monophosphate deaminase-1 *(AMPD1)* gene encodes an isoform of AMP deaminase (*AMPD1*, also called myoadenylate deaminase) that is active in muscular tissue (1). Adenosine monophosphate deaminase-1 occupies a central position in adenosine nucleotide catabolism, catalyzing the conversion of AMP to inosine monophosphate, the rate-limiting step for entry into the purine nucleotide cycle. Adenosine monophosphate deaminase-1 deficiency is believed to cause exercise-induced myalgias and early fatigue in skeletal muscle (1–3). A common polymorphism in axon 2 of *AMPD1*, present in about 25% of Caucasians, causes a C to T transition at nucleotide 34 (C34T) (2,3). This nonsense transition encodes for a truncated, inactive enzyme. A reduced activity of *AMPD1* may increase persistence of adenosine (3,4), a cardioprotective molecule (5). Recently, the C34T variant of *AMPD1* has been reported to be associated with prolonged survival in heart failure (6,7). We tested whether it also is more broadly associated with improved cardiovascular (CV) survival in patients with coronary artery disease (CAD) at high risk for future ischemic events.

METHODS

Study objectives. We tested whether carriage of the common variant allele of the *AMPD1* gene, (*AMPD1*[−]) was associated with a reduced risk of CV death in patients with documented CAD. We also tested its association with all-cause mortality.

Study population. Study subjects came from a consecutive series of clinically stable patients of any age and either gender who underwent coronary angiography, were shown to have severe CAD, consented for a blood draw at the time of angiography (for confidential blood bank studies approved by the hospital's institutional review board) and were followed until death or for >2.5 years from entry. Subjects were primarily residents of Utah, a population ethnically

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Abbreviations and Acronyms

AMISTAD = Acute Myocardial Infarction Study of Adenosine
AMPD = adenosine monophosphate deaminase
CAD = coronary artery disease
CV = cardiovascular
HR = hazard ratio
MI = myocardial infarction

primarily of Northern European descent and genetically similar to the general U.S. Caucasian population (8).

Data collection. At the time of angiography, key demographic characteristics were recorded on standard data forms, including age, gender and history of recent or remote myocardial infarction (MI) (9). Determination of presence and severity of CAD was made by each patient's attending cardiologist, who was unaware of *AMPD1* genotypes, using a format modified after the Coronary Artery Surgery Study protocol (9,10). Severe CAD was defined as the presence of ≥1 coronary lesions of ≥70% diameter stenosis in ≥1 major coronary artery or its primary branch. Mild or absent CAD cases were excluded from this study of secondary risk. Index angiography occurred between August 1994 and December 1997.

Assessment of patient outcomes. The study patient cohort was followed until death or December 1998 (mean, 3.5 ± 1.0 years of follow-up, range 2.5 to 4.8 years). Each subject was interviewed through a telephone survey that determined the subject's medical history since the index hospitalization. Deaths were determined when possible from a family member. Deaths were verified and other deaths determined as of March 1999 by a search of a national Social Security database. Subjects unable to be contacted by telephone but not listed as deceased by the national database were considered to be alive. Follow-up using the database allowed for 100% assessment of survival within the cohort.

DNA extraction. Approximately 20 ml to 30 ml of blood was withdrawn by venipuncture at the time of coronary angiography, collected in ethylenediaminetetraacetic acid, refrigerated at 4°C and processed within 24 h. The leukocyte buffy coat was separated by centrifugation, and genomic DNA was extracted using a standard phenol:chloroform method as previously described (11).

DNA genotyping. To identify the *AMPD1* C34T variant genotypes, polymerase chain reaction amplification was performed with the following primers, as previously published (12):

AMPD1 5′ CAT ACA GCT GAA GAG ACA 3′

AMPD2 5′ AAC ACT GCT GAA AAA TAG 3′

Amplification reactions were performed in 15 μl volumes containing the two primers. Genotyping was performed as previously reported (13). The reaction products were visualized by electrophoresis through a 2% agarose gel containing ethidium bromide.

Statistical considerations. Comparisons of characteristics of survivors and nonsurvivors used chi-square (categorical variables) or unpaired *t* testing (continuous variables). Allelic and genotypic frequencies were determined from observed counts. Comparisons between allelic or genotypic frequency distributions used chi-square analysis. Hetero- (+/−) or homozygotic (−/−) carriers were compared with noncarriers (wild type genotype, [+/+]) using survival statistics. The univariate predictive value of *AMPD1*(−) carriage for CV and total survival was tested using Kaplan-Meier analysis and log-rank statistics. Cox logistic regression analysis (stepwise, backward logistic regression approach) was then used to determine univariate and multiple variable hazard ratios (HR) and the multiple variable predictive value of *AMPD1*(−) carriage, conditioned on 10 other major CAD risk factors: age, gender, smoking status, diabetic status, history of hypertension, history of hyperlipidemia, family history, renal failure, presentation and initial therapy (SPSS v 9.0, Chicago, Illinois). The critical value for entering and excluding variables in the model was set at $p = 0.10$.

RESULTS

Baseline patient characteristics. A total of 367 patients with documented CAD was entered, and 52 patients (14.2%) died during the mean of 3.5 ± 1.0 year follow-up, 37 (10.1%) of CV causes. Selected patient characteristics at study entry are summarized in Table 1 by survival status. Patients averaged 66 ± 10 years of age, and 79% were men. Survivors were younger at baseline, had higher ejection fractions and tended to be men and smokers more frequently than nonsurvivors.

Adenosine monophosphate deaminase-1 genotypic distributions are shown in Table 2 for all patients and for survivors, those dying of any cause and those dying of a CV cause. Of entered patients, 22.6% were heterozygous and 1.9% homozygous for the *AMPD1*(−) allele. Thus, 24.5% were carriers of the polymorphic allele. In bivariate correlation analyses, *AMPD1*(−) carriage was unassociated with any other baseline factor, including ejection fraction.

***AMPD1* genotype and survival.** At the end of follow-up, CV mortality was 4.4% (4/90) for *AMPD1*(−) allele carriers compared with 11.9% (33/277) for noncarriers. Figure 1 shows the time-to-event (Kaplan-Meier) CV survival plot as a function of *AMPD1*(−) allele carriage. A significant difference in survival by *AMPD1* genotype was observed (log-rank statistic, 4.0, $p = 0.046$). The HR of death for *AMPD1*(−) carriage was 0.36 (0.13 to 1.0).

Adenosine monophosphate deaminase-1(−) carriage was not associated with a reduction in noncardiovascular deaths (5.5% in carriers, 3.6% in noncarriers). When all-cause mortality was considered (CV plus non-CV), the difference

Table 1. Patient Characteristics at Baseline by Survival Status

Characteristic	All Patients	Survivors	Deaths	CV Deaths	P1	P2
Number	367	315	52	37	—	—
Age (yr) (X ± SD)	66.1 ± 10.1	65.1 ± 9.9	72.0 ± 8.9	73.6 ± 7.0	0.000	0.000
Gender (% male)	79.0	79.7	75.0	67.6	0.44	0.07
Diabetes (%)	19.6	19.0	23.1	24.3	0.50	0.45
Smoker (%)	25.9	27.6	15.4	10.8	0.06	0.03
Fam Hx (%)	36.8	37.5	32.7	32.4	0.51	0.56
h/o HTN (%)	49.9	49.5	51.9	45.9	0.75	0.62
h/o HLip (%)	48.8	50.8	36.5	40.5	0.06	0.29
Chol (mg/dl)	183 ± 47	184 ± 47	177 ± 49	178 ± 47	0.34	0.49
(n Chol)	(359)	(308)	(51)	(37)		
EF (%)	59.5 ± 17.3	61.0 ± 16.4	50.1 ± 19.9	50.4 ± 19.5	0.000	0.006
(n EF)	(273)	(237)	(36)	(25)		
Allele carrier (%)	24.5	26.1	17.3	10.8		
Death, last f/u (mo)	38.6 ± 11.3	42.3 ± 5.4	16.4 ± 12.3	14.8 ± 11.4	—	—

P1 compares patients dying from any cause with survivors. P2 compares patients dying from cardiovascular causes with others.
Allele = *AMPD1*(−) variant allele; Chol = cholesterol; CV = cardiovascular; EF = ejection fraction; Fam Hx = family history; f/u = follow-up; HLip = hyperlipidemia; HTN = hypertension.

was not significant (10.0% in *AMPD1*[−] carriers, 15.5% in noncarriers; $p = 0.19$).

In multiple variable Cox regression analysis, including 12 clinical and laboratory variables (age, gender, smoking, diabetes, hypertension, hyperlipidemia, family history, total cholesterol, renal failure, presenting diagnosis, therapy at index hospitalization and *AMPD1*[−] carriage), only age (HR, 1.11/year, $p < 0.001$) and *AMPD1*(−) carriage (HR, 0.36, confidence interval 0.13–1.02, Wald chi-square $p = 0.053$) were selected as independent predictors of CV mortality (Table 3).

The incomplete database for ejection fraction dissuaded us from doing a formal determination of the relative predictive value of the polymorphism in high and low ejection fraction subgroups. However, the reduction in CV mortality did appear to be prominent in those with low (≤40%) ejection fractions (0/9 with, vs. 13/34 without an *AMPD1*[−] allele and documented low ejection fraction, died).

In contrast with its value for secondary risk prediction, *AMPD1* polymorphism was not useful for prediction of the presence or absence of CAD at initial angiography in an expanded consecutive series that included subjects with normal angiograms (not shown).

DISCUSSION

Study summary. We found that patients with angiographically documented CAD who were carriers of a common genetic variant of the *AMPD1* gene demonstrated improved CV survival. The *AMPD1* variant did not predict development of CAD; rather, the effect appeared to be in prolonging survival when heart disease was already present. Adenosine monophosphate deaminase-1 genotype was unassociated with other risk factors, and its predictive value was undiminished in multiple variable analyses (HR = 0.36). We speculate that the dysfunctional *AMPD1*(−) allele may lead to increased net production of adenosine locally (in cardiac muscle [7]) and/or systemically (skeletal muscle source [6]), affording increased levels of cardioprotection during ischemic events. If these results are verified, *AMPD1* genotyping may provide useful prognostic, mechanistic and therapeutic insights into CAD progression and prognosis.

Previous work. Recently, Loh et al. (6) reported an improved clinical outcome associated with *AMPD1*(−) allele carriage in a group of 132 patients with advanced heart failure referred for cardiac transplant evaluation. The mutant *AMPD1* allele was associated with an extended time

Table 2. Genotypic Distributions and Allelic Frequencies of *AMPD1* Gene Polymorphism Among Patients by Survival Status

Group	Wild type (+/+) n (%)	Heterozygote (+/−) n (%)	Homozygote (−/−) n (%)	WT (+) Allele	Variant (−) Allele
A. All patients/all deaths					
Survivors	234 (74.3)	76 (24.1)	5 (1.6)	544 (86.3)	86 (13.7)
Deaths	43 (82.7)	7 (13.5)	2 (3.8)	93 (89.4)	11 (10.6)
Total	277 (75.5)	83 (22.6)	7 (1.9)	637 (86.8)	97 (13.2)
B. CV deaths/CV survivors					
No CV death	244 (73.9)	79 (23.9)	7 (2.1)	567 (85.9)	93 (14.1)
CV Deaths	33 (89.2)	4 (10.8)	0 (0)	70 (94.6)	4 (5.4)
Total	277 (75.5)	83 (22.6)	7 (1.9)	637 (86.8)	97 (13.2)

For A, death by genotype contingency table gives $p = 0.15$ (chi-square). For B, CV death by genotype gives $p = 0.11$ (chi-square), $p = 0.063$ (likelihood ratio) or $p = 0.038$ (linear-by-linear association).
CV = cardiovascular; WT = wild type.

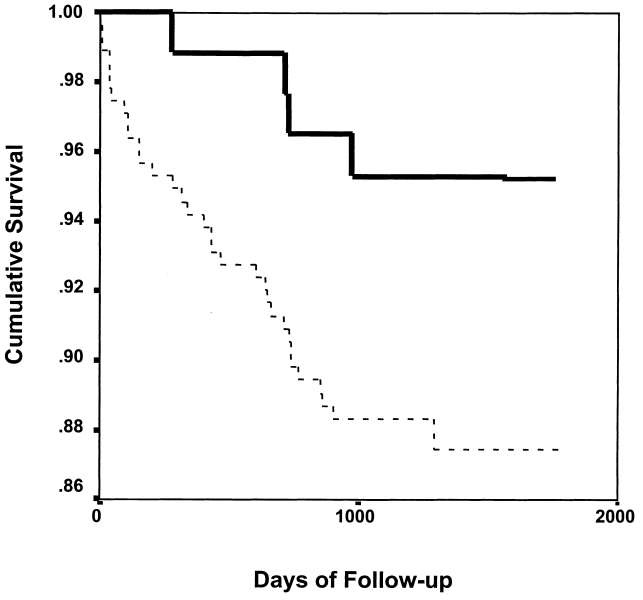

Figure 1. Kaplan-Meier survival-time plot for cardiovascular death by *AMPD1* genotype (wild type vs. variant heterozygote or homozygote). There are four events (4.4%) among 90 patients carrying the mutant polymorphism and 33 events (11.9%) among noncarriers, a statistically significant difference (log-rank statistic 4.0, p = 0.046; Breslow statistic 4.1, p = 0.043). **Solid line** = *AMPD1*(−) variant carrier; **dotted line** = variant noncarrier.

from the first hospitalization for heart failure to evaluation for transplantation, with an HR for transplant-free survival of 4.6. Our study is the first to confirm and extend these findings to patients with CAD who were not selected by ejection fraction or heart failure and who were studied prospectively after angiographic diagnosis.

Mechanisms of benefit. Loh et al. (6) speculated that the mechanism of benefit could be related to enhanced production of adenosine in skeletal muscle that could increase circulating levels of adenosine, leading to cardioprotection. Feldman et al. (7) editorialized that the short circulating half-life of adenosine argued for a primarily local (myocardial) increase in net adenosine and hypothesized that adenosine levels might be increased in cardiac muscle in patients carrying the variant allele (7). Adenosine, released by myocytes during ischemic stress (13), has been studied extensively for a cardioprotective role although this remains to be completely defined (14,15). As reviewed by Mahaffey et al. (15), adenosine has been reported to replenish high-energy phosphates, inhibit oxygen free radical formation and neutrophil activation and accumulation, improve microvascular function and participate in myocardial ischemic preconditioning in experimental models of occlusion/reperfusion, improving cardiac perfusion and function.

Earlier human studies (16,17), promising in themselves, have been followed by a larger (n = 236 patients) controlled study, the Acute Myocardial Infarction Study of Adenosine (AMISTAD) (15). In AMISTAD, adenosine (70 μg/kg/min) infused for 3 h as an adjunct to thrombolytic therapy reduced radionuclide infarct size by 33% (p = 0.03). A larger trial to assess clinical events was proposed.

The role of AMPD1 in cardiac muscle is less well studied than in skeletal muscle although it has been reported to be expressed (together with AMPD2) in mammalian heart (18). To date, neither myocardial nor skeletal muscle adenosine levels have been measured in disease states and by *AMPD1* genotype.

Whatever the precise mechanism of adenosine's benefit, the *AMPD1*(−) allele may provide carriers with an endogenous source of increased myocardial adenosine, improving outcomes in those with CAD at high risk for future ischemic events.

Study strengths and limitations. This study extends previous work on clinical consequences of the *AMPD1* polymorphism (6) by including a larger and broader spectrum of patients and evaluating their clinical course entirely prospectively. Adenosine monophosphate deaminase-1 genotype was unassociated with other risk factors, and its association with CV survival was independent of other tested risk factors in multiple variable analysis. However, the study is only moderate in size, and the number of clinical events is relatively small, so that the confidence intervals for CV survival extension associated with *AMPD1*(−) are broad. Similarly, the database for determining the relative protective effect of the variant as a function of ejection fraction is limited. Also, the study did not directly assess potential mechanisms of apparent benefit. The similarity of genotypic frequencies in our CAD group to that in the general population further supports our observation about the absence of an effect on the development of CAD. Thus, for future studies disease progression or prognosis may be a better focus than disease development. In conclusion, our findings, although promising, should be verified and ex-

Table 3. Cox Multiple Variable Logistic Regression Model* for Cardiovascular Death

Factor	Wald	HR(Exp B)	Lower CI	Upper CI	p Value
AMPD(−)	3.74	0.36	0.13	1.02	0.053
Age/yr	21.96	1.11	1.06	1.16	<0.001

*Using Backward Stepwise Conditional Logistic Regression method (SPSS v 9.0), entering age (year); male gender, h/o hypertension, h/o hyperlipidemia, diabetes, family history, smoking, renal failure, adenosine monophosphate deaminase (−) allele carriage (−/+ or −/−), all yes/no; presentation (stable angina, unstable angina, myocardial infarction), therapy at index hospitalization (medical, angioplasty, or surgery) and total cholesterol (mg/dl). Systolic blood pressure (mm Hg) and diastolic blood pressure (mm Hg) were included in separate analyses (with less complete datasets) with similar results. There were 358 patients with complete datasets entered and 37 events. P to exclude variables stepwise was 0.10 (ejection fraction was not entered because of the large resulting number of incomplete datasets; that is, n = 265).

CI = 95% confidence interval; Exp B = exponential B; HR = hazard ratio.

tended in larger and longer-term studies. If validated, they suggest that *AMPD1* genotyping may provide useful prognostic, mechanistic and therapeutic insights into survival in patients with CAD as well as those with congestive heart failure.

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