Common Variant in \textit{AMPD1} Gene Predicts Improved Clinical Outcome in Patients With Heart Failure

Evan Loh, MD; Timothy R. Rebbeck, PhD; Paul D. Mahoney, MD; David DeNofrio, MD; Judith L. Swain, MD; Edward W. Holmes, MD

\textbf{Background}—This study was undertaken to identify gene(s) that may be associated with improved clinical outcome in patients with congestive heart failure (CHF). The adenosine monophosphate deaminase locus (\textit{AMPD1}) was selected for study. We hypothesized that inheritance of the mutant \textit{AMPD1} allele is associated with increased probability of survival without cardiac transplantation in patients with CHF.

\textbf{Methods and Results}—\textit{AMPD1} genotype was determined in 132 patients with advanced CHF and 91 control reference subjects by use of a polymerase chain reaction–based, allele-specific oligonucleotide detection assay. In patients with CHF, those heterozygous (n = 20) or homozygous (n = 1) for the mutant \textit{AMPD1} allele (\textit{AMPD1}+/− or −/−, respectively) experienced a significantly longer duration of heart failure symptoms before referral for transplantation evaluation than CHF patients homozygous for the wild-type allele (\textit{AMPD1}+/+; n = 111; 7.6 ± 6.5 versus 3.2 ± 3.6 years; \(P<0.001\)). The OR of surviving without cardiac transplantation ≥ 5 years after initial hospitalization for CHF symptoms was 8.6 times greater (95\% CI: 3.05, 23.87) in those patients carrying ≥ 1 mutant \textit{AMPD1} allele than in those carrying 2 wild-type \textit{AMPD1} +/+ alleles.

\textbf{Conclusions}—After the onset of CHF symptoms, the mutant \textit{AMPD1} allele is associated with prolonged probability of survival without cardiac transplantation. The mechanism by which the presence of the mutant \textit{AMPD1} allele may modify the clinical phenotype of heart failure remains to be determined. (\textit{Circulation}. 1999;99:1422-1425.)

Key Words: heart failure ■ genes ■ survival

Although family studies have suggested that there may be a genetic basis for inherited cardiomyopathy,\textsuperscript{1-5} few studies have attempted to define genetic determinants of disease duration in the general population of patients with heart failure.

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The gene selected for evaluation in this congestive heart failure (CHF) population was the adenosine monophosphate deaminase 1 (\textit{AMPD1}) gene. A nonsense mutation in the \textit{AMPD1} gene is arguably one of the most common inherited defects in whites and blacks. Approximately 20\% of individuals in these ethnic groups are heterozygous for a single mutant allele that leads to premature peptide chain termination and production of a truncated, catalytically inactive enzyme.\textsuperscript{6,7} The \textit{AMPD1} gene is expressed at high levels in skeletal myocytes, and individuals who are either homozygous or heterozygous for this mutant allele have reduced AMPD activity in their skeletal muscle.\textsuperscript{6-8} AMPD is located at a central position in adenine nucleotide catabolism, and reduced activity of this enzyme would be expected to enhance adenosine (a potent cardioprotective agent) production in skeletal muscle after ATP catabolism (Figure 1). Increased skeletal muscle adenosine production has been demonstrated in individuals without known cardiovascular disease who are homozygous for the \textit{AMPD1} mutant allele.\textsuperscript{9}

We hypothesized that inheritance of the mutant \textit{AMPD1} allele might prove advantageous to patients with CHF and tested the hypothesis that inheritance of this mutant allele was associated with an increase in the probability of survival without cardiac transplantation after the onset of CHF symptoms.

\textbf{Methods}

\textbf{Heart Failure Subjects}

Clinical data were collected on 132 consecutive patients with advanced CHF referred for cardiac transplantation evaluation at the Hospital of the University of Pennsylvania between June 1995 and March 1997. Informed consent to participate was obtained from each patient.

\textbf{Genetic Analysis}

Genomic DNA was prepared from peripheral white blood cells of each patient. \textit{AMPD1} genotype was determined by use of a polymerase chain reaction (PCR)–based, allele-specific oligonucleotide detection assay as reported previously\textsuperscript{6} (Figure 2).

\textbf{Statistical Analysis}

The frequencies of the \textit{AMPD1} +/+, \textit{AMPD1} +/−, and \textit{AMPD1} −/− genotypes in the control reference group and in patients with CHF were compared by Fisher’s exact test. Only 1 CHF subject was...
Table 1. Clinical Characteristics Across AMPD1 Genotype

<table>
<thead>
<tr>
<th>AMPD1 Genotype</th>
<th>AMPD1 +/- or -/- (n=21)</th>
<th>AMPD1 +/+ (n=111)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56.8±7.1</td>
<td>52.1±11.6</td>
</tr>
<tr>
<td>Cause, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>8 (38)</td>
<td>55 (50)</td>
</tr>
<tr>
<td>IDCMP</td>
<td>11 (52)</td>
<td>38 (34)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (10)</td>
<td>18 (16)</td>
</tr>
<tr>
<td>Medical therapy, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>19 (90)</td>
<td>99 (89)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>15 (71)</td>
<td>103 (93)*</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>18 (86)</td>
<td>93 (85)</td>
</tr>
<tr>
<td>Cardiac index, L·min⁻¹·m⁻²</td>
<td>1.8±0.6</td>
<td>1.8±0.8</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>21.6±10.9</td>
<td>22.0±9.0</td>
</tr>
<tr>
<td>PVR, Wood units</td>
<td>2.9±2.1</td>
<td>2.9±2.4</td>
</tr>
<tr>
<td>V̇O₂max, mL·kg⁻¹·min⁻¹</td>
<td>15.1±6.7</td>
<td>13.7±4.6</td>
</tr>
<tr>
<td>Symptom duration, y</td>
<td>7.6±6.5</td>
<td>3.2±3.6*</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; IDCMP, idiopathic cardiomyopathy; LVEF, left ventricular ejection fraction; PCWP, pulmonary capillary wedge pressure; and PVR, pulmonary vascular resistance.

*P<0.001; AMPD1 +/- or -/- vs AMPD1 +/+ . All other comparisons P>0.05.

Plus/minus values are mean±SD.

We included coronary artery disease (n=69), idiopathic cardiomyopathy (n=48), and “other” (n=15; 4 valvular, 2 infiltrative, 3 congenital, 2 myocarditis, and 4 alcohol). (See Table 1). No Asians were included among these patients, an important exclusion because the mutant AMPD1 allele is not present in this population.⁶

Clinical Characteristics of Patients With CHF

The mean age of the 132 CHF patients was 52.8±11.2 years; left ventricular ejection fraction (LVEF) was 19.7±6.7%; and peak V̇O₂ max was 13.9±4.9 mL·kg⁻¹·min⁻¹. Causes of CHF included coronary artery disease (n=69), idiopathic cardiomyopathy (n=48), and “other” (n=15; 4 valvular, 2 infiltrative, 3 congenital, 2 myocarditis, and 4 alcohol). (See Table 1). No Asians were included among these patients, an important exclusion because the mutant AMPD1 allele is not present in this population.⁶

Clinical Characteristics of Heart Failure Across AMPD1 Genotype

Of the 132 patients enrolled in this study, 111 were homozygous for the wild-type allele (+/+) and 1 individual was homozygous for the mutant AMPD1 allele (−/−). LVEF, cardiac index, pulmonary capillary wedge pressure, V̇O₂ max, and pulmonary vascular resistance were not significantly different between CHF patients with the +/+ versus +/- and −/− genotypes (Table 1).

AMPD1 Genotype and Clinical Outcome

The time from first hospital admission for CHF symptoms to the clinical end point of evaluation for cardiac transplantation was markedly different between AMPD1 +/- or -/- and AMPD1 +/+ patients (7.6±6.5 years, AMPD1 +/- or -/- versus 3.2±3.6 years, AMPD1 +/+; P<0.0001). There was a trend for patients with the mutant AMPD1 allele (+/- or -/-) to be older than the AMPD1 +/+ homozygotes (56.8±7.1 versus 52.1±11.6 years; P=0.07; t test assuming

Figure 1. Pathways of adenosine nucleotide catabolism.
unequal variances) at the time of initial referral for cardiac transplantation evaluation.

Kaplan-Meier analysis demonstrated that individuals with 1 or 2 mutant AMPD1 alleles (1/2 or 2/2) had an increased probability of survival without cardiac transplantation for a significantly longer time after the first hospitalization for CHF symptoms than patients homozygous for the wild-type allele (Figure 3; P<0.001). In the individuals with the mutant AMPD1 allele, there were 2 deaths (both of progressive heart failure), and 3 heart transplants were performed over the observation period after enrollment into the study. In the individuals without the mutant AMPD1 allele, there were 11 deaths (8 of progressive heart failure, 2 of sudden cardiac death, and 1 of multisystem organ failure), and 40 heart transplants were performed. The proportional hazards model indicates a risk ratio of 4.44 (95% CI: 1.59, 12.35) for age-and sex-adjusted heart failure symptom and disease duration associated with the mutant AMPD1 genotype (Table 2).

To determine the relationship of AMPD1 genotype and other clinical factors to the probability of survival without cardiac transplantation (Table 3), we fit a multivariate proportional hazards model that considered AMPD1 genotype in addition to race, cause of heart failure (ischemic versus nonischemic), adjuvant medical therapy (ACE inhibitor, digoxin, or diuretics), V˙O₂ max, and LVEF. The proportional hazards model indicates a hazard ratio of 4.65 (95% CI: 1.48, 14.66) for adjusted heart failure symptom and disease duration associated with the mutant AMPD1 genotype. This result is nearly identical to the unadjusted heart failure symptom and disease duration associated with the mutant AMPD1 genotype (Table 2), which suggests that these other factors did not modify the hazard ratio point estimate of AMPD1 genotype as a predictor of survival.

**AMPD1 Genotype Frequency in Heart Failure Patients**

The frequency of the AMPD1 +/− or −/− genotype in CHF patients might be expected to vary depending on the elapsed time between the onset of CHF symptoms and the performance of genotyping. Among patients who presented <5 years from the first hospitalization for CHF, 7 (16.7% genotype frequency) of the 97 carried the mutant AMPD1 allele. In the group that presented ≥5 years after the first hospitalization for CHF, 14 (43% genotype frequency) of the 35 carried the mutant AMPD1 allele (χ²=20.66; P<0.001 compared with CHF patients with symptom duration <5 years). With the Mantel-Haenszel method of analysis, a patient carrying the mutant AMPD1 allele (+/− or −/−) had an 8.6 OR (relative to an individual homozygous for the wild-type allele ++/) of living ≥5 years with CHF before dying or requiring cardiac transplantation.

When the AMPD1 genotype frequency of these 2 subgroups of CHF patients was compared with a group of normal volunteers (n=91), the CHF patients who presented <5 years after first hospitalization for CHF were found to have a lower

![Figure 3. Probability of survival without cardiac transplantation in CHF patients stratified across AMPD1 genotype by Kaplan-Meier analysis. These 2 curves are statistically different (P=0.002; log-rank; Table 2).](image-url)
frequency of the mutant allele than the control population (16.7% versus 25.3%; P<0.001). In contrast, CHF patients who presented ≥5 years after initial hospitalization for CHF demonstrated a trend toward a higher frequency of the mutant allele than the control population (43% versus 25.3%; P=NS).

**Discussion**

The results presented in this study provide evidence for an association between inheritance of a mutant allele in the *AMPD1* gene and improved clinical outcome in patients with CHF. The frequency of the mutant *AMPD1* allele appears higher in the patient population with a longer duration of CHF symptoms before presentation for cardiac transplantation evaluation. Individuals with CHF who carry this mutant allele (*AMPD1* +/− or −/−) progress more slowly to end-stage cardiac symptomatology and appear to survive longer without death or need for cardiac transplantation after the first hospitalization for CHF symptoms.

One possible mechanism that may underlie the association between inheritance of the mutant *AMPD1* allele and improved clinical outcomes in patients with CHF could be a direct consequence of the reduction in AMPD activity. As illustrated in Figure 1, reduced AMPD activity could lead to enhanced production of adenosine in skeletal muscle and could accentuate the increased circulating levels of adenosine observed in patients with CHF.12 Adenosine has the potential to be a potent cardioprotective agent, leading to increased regional coronary blood flow,13,14 induction of the ischemic preconditioning response,15–17 suppression of arrhythmias,16 and suppression of cytokine production.18,19 An additional cardioprotective effect of adenosine, mediated via adenosine receptors, is attenuation of release of catecholamines, β-adrenoreceptor–mediated myocardial hypercontraction, and Ca2+ overload.20–22 Recently, other investigators have observed myocardial protection provided for by ischemia in noncardiac tissue, yielding the concept of “ischemic preconditioning at a distance.”23,24 Because the population of inference for these current results are patients with advanced CHF referred specifically for cardiac transplantation evaluation, we cannot at this time extrapolate these observations to patients with less-advanced New York Heart Association symptom classifications of heart failure or asymptomatic patients with milder degrees of left ventricular dysfunction.

Limitations of this study include the lack of genotype information at the time of first onset of CHF symptoms, the combined end-point outcome used, and its retrospective nature. Finally, there are no skeletal or circulating adenosine levels to determine whether heterozygosity at the *AMPD1* allele is associated with clinically significant alterations in adenosine production.

Nevertheless, these observations will serve as the impetus for future prospective studies to examine these hypotheses and to determine the specific pathophysiological mechanisms that may explain these differences in clinical outcomes.

**Acknowledgments**

This work was sponsored in part by grants from the National Institutes of Health (NIH-5 M01 RR00040 to Dr Loh and Dr Mahoney and NIH DK12413 to Dr Holmes). We thank Catherine D. Stolle, PhD, for her expertise in performing the *AMPD1* PCR assays.

**References**


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_Circulation_. 1999;99:1422-1425
doi: 10.1161/01.CIR.99.11.1422

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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