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The Human G147D-Protein Phosphatase 1 Inhibitor-1 Polymorphism Is Not Associated with Altered Clinical Characteristics in Heart Failure

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Key Words
Heart failure · Polymorphism · Protein phosphatase inhibitor-1

Abstract
Objectives: A human protein phosphatase inhibitor-1 polymorphism, G147D (c.440G>A, p.147G>D), has been previously demonstrated to blunt the contractile responses of cardiomyocytes to β-adrenergic agonists. The present study sought to examine whether the G147D inhibitor-1 polymorphism may be associated with specific clinical characteristics of heart failure carriers. ***Methods:*** Clinical information of 963 heart failure patients was analyzed according to race, inhibitor-1 genotype, treatment with β-blockers and mortality patterns. ***Results:*** The G147D inhibitor-1 genetic variant was found almost exclusively in black subjects and its frequency was similar between normals and heart failure patients, indicating that it was not a primary risk factor for developing heart failure. Comparison of the major cardiac functional parameters and transplant-free survival patterns between carrier and noncarrier patients did not reveal any significant differences. Furthermore, echocardiographic evaluation showed similar outcomes of β-blocker treatment between G147D carriers and noncarriers. ***Conclusions:*** The present findings indicate that the human inhibitor-1 G147D polymorphism, found almost exclusively in blacks, may act as a modifier rather than risk factor in heart failure development.

Introduction

Heart failure is a pleiotropic disease reflecting complicated interactions between individual genetic backgrounds and environmental factors. Nevertheless, depressed β-adrenergic signaling, resulting from defects in its receptor and/or downstream components, is one of the most prominent and common molecular mechanisms underlying the pathogenesis of heart failure [1, 2].

The functional effects of β-adrenergic agonists on cardiomyocyte contractility depend on PKA-mediated phosphorylation of multiple key intracellular proteins, including ryanodine receptor, phospholamban, L-type calcium channel and troponin I [3]. The reversal of these stimulatory effects is mediated by dephosphorylation of these

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regulatory proteins through protein phosphatases 1 and 2. The fine balance of protein kinase and phosphatase activities is essential for proper calcium cycling and functional homeostasis in the heart. However, depressed adrenergic signaling cascade and elevated phosphatase activity tilt this balance in favor of dephosphorylation in experimental and human heart failure [4–7]. Particularly, abnormal elevation of protein phosphatase 1 (PP1) activity has been associated with depressed calcium homeostasis in failing hearts [4, 8].

One potential mechanism underlying the pathologically elevated PP1 activity in heart failure is the diminished restriction from its endogenous inhibitor, inhibitor-1. Inhibitor-1 potently inhibits PP1 activity upon its phosphorylation by β-adrenergic stimulation, resulting in increased cardiac contractility [5, 9–11]. Consequently, cardiac overexpression of a constitutively active form of inhibitor-1 (AA 1–65, T35D) resulted in higher resistance to stress-induced cardiac remodeling and heart failure [8]. In contrast, decreased activity and/or reduced protein levels of inhibitor-1 have been identified in animal models with cardiac dysfunction and human heart failure patients [4, 7, 11–14], suggesting that defects in inhibitor-1 activity may contribute to depressed cardiac function.

To determine whether there exist genetic variants of human inhibitor-1 that may act as direct or conjunct factors in heart failure, we sequenced the inhibitor-1 gene in heart failure patients. A human inhibitor-1 polymorphism, G147D (c.440G>A, p.147G>D), was identified almost exclusively in black subjects. Notably, expression of this polymorphism in isolated adult rat cardiomyocytes interfered with the contractile response to β-adrenergic agonists [15]. Therefore, we further analyzed the clinical information of the heart failure patients to assess whether carrying this inhibitor-1 genetic variant contributed to heart failure. The nearly equal distribution of this polymorphism in normal subjects and heart failure patients indicated that it did not independently alter the risk of developing heart failure. Furthermore, the inhibitor-1 polymorphism did not appear to modify cardiac function, the responses to β-blocker treatment or the overall mortality pattern.

Materials and Methods

Study Subjects

As described previously [15, 16], the human study protocols were approved by the Institutional Review Board of the University of Cincinnati. Subjects provided written informed consent. Heart failure subjects were aged 18–80 years and had New York Heart Association heart failure class II–IV. The study included 999 heart failure patients followed at the University Hospital, Cincinnati, between 1999 and 2004 and 357 nonaffected controls recruited from the greater Cincinnati area between 2003 and 2005. Racial classification as whites, blacks or others was self-reported. The heart failure study endpoint was death or cardiac transplantation.

Genotyping

Exons 1–6 of inhibitor-1 1 [PPP1R1A according to National Center for Biotechnology Information, PPP1R1A = protein phosphatase 1 regulatory (inhibitor) subunit 1A *(Homo sapiens)*, gene ID 5502, in chromosome 12] were screened, as described previously [15].

Statistics

For the clinical characteristics of the patients with heart failure, continuous variables are presented as mean ± SD, and comparisons between ethnic groups, between genotype classes within ethnic groups and between β-blocker usage groups are conducted by unpaired Student's t tests. Categorical variables are presented as proportions, and comparisons between ethnic groups and between genotype classes within ethnic groups are conducted by χ^2 tests. Hardy-Weinberg equilibrium is assessed in each ethnic group separately by χ^2 test. Subjects, either heterozygous (G/D) or homozygous (D/D) for the G147D polymorphism of inhibitor-1 gene, are taken together into one group, because the mutant homozygotes were too few for separate analysis. χ^2 or Fisher's exact tests are used to test for associations between heart failure and genotype or allele within each ethnic group. Differences in time from heart failure diagnosis to endpoint were assessed using Kaplan-Meier curves and log-rank tests. Relative risks were obtained using Cox proportional hazards modeling. In all analyses, $p < 0.05$ was considered statistically significant.

Results

Identification of Genetic Variants in the Human Inhibitor-1 Gene and Clinical History of Heart Failure Patients

The PP1 inhibitor-1 gene was sequenced in 181 unrelated heart failure patients recruited from the University Hospital and Cincinnati Heart Failure/Transplant Program. We identified several genetic variants in the inhibitor-1 gene (p109G>E, p110T>I and p147G>D). Interestingly, the G147D genetic variant was identified only in black heart failure patients. Moreover, a previous study in isolated adult rat cardiomyocytes has demonstrated that overexpression of this polymorphism interfered with the contractile response of cardiomyocytes to β-adrenergic agonists [15]. Therefore, we further assessed the functional parameters in 963 heart failure patients with detailed information. These include 288 blacks, 671 whites and 4 others recruited from the University Hospital and

Table 1. Clinical characteristics of heart failure patients by race

Variable	Whites		Blacks		p value
	n	mean ± SD	n	mean ± SD	
Age at onset of heart failure, years	671	55.73 ± 12.78	288	52.66 ± 13.33	0.001
Weight, kg	656	85.85 ± 21.46	281	90.68 ± 25.99	0.006
Height, cm	649	172.56 ± 14.68	272	171.58 ± 10.42	0.314
Systolic BP, mm Hg	540	117.73 ± 21.25	231	119.88 ± 23.65	0.234
Diastolic BP, mm Hg	540	72.96 ± 13.49	230	75.00 ± 14.55	0.061
LVEDD, cm	526	6.43 ± 1.18	254	6.12 ± 1.02	<0.001
LVESD, cm	502	5.08 ± 1.38	251	4.80 ± 1.29	0.007
Septal wall, cm	476	1.07 ± 0.28	246	1.13 ± 0.28	0.018
Posterior wall, cm	476	1.01 ± 0.24	246	1.04 ± 0.27	0.239
LVEF, %	502	45.83 ± 16.25	251	44.25 ± 13.88	0.220
Fractional shortening, %	502	21.74 ± 11.19	251	22.54 ± 11.81	0.368
MVO_2	409	17.39 ± 5.83	137	15.74 ± 5.08	0.003
Variable	Whites		Blacks		p value
	n	%	n	%	
Male	464	69.2	157	54.5	<0.001
Primary diagnosis					
Idiopathic cardiomyopathy	332	52.2	175	63.4	
Coronary artery disease	222	34.9	44	15.9	
Other	82	12.9	57	20.7	<0.001
Cardiac-related death or received transplant	279	42.1	80	28.6	<0.001
Other risk factors or coexisting conditions					
Hypertension	313	47.2	227	79.4	<0.001
Diabetes mellitus	199	33.5	93	34.8	0.703
Hypercholesterolemia	166	31.4	56	25.3	0.099
Tobacco use					
Present user	294	63.4	144	68.9	
Past only	96	20.7	36	17.2	
Never	74	15.9	29	13.9	0.374
Medications at entry					
β-Blocker	453	70.6	228	81.7	<0.001
ACE inhibitor	487	88.9	223	93.7	0.035
ARB	80	38.8	40	34.8	0.472
Aldosterone blocker	83	32.8	39	31.5	0.792

BP = Blood pressure; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction; MVO_2 = maximum amount of oxygen that can be removed from circulating blood and used by the working tissues during a specified period; ACE inhibitor = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor-blocking agent.

Cincinnati Heart Failure/Transplant Program. The clinical characteristics of the patients are shown in table 1 according to race. Significant differences in age of clinical presentation, body weight, LVEDD, LVESD, thickness of septal wall, MVO_2, gender distribution, primary diagnosis and coexistence of hypertension were identified between white and black patients. Comorbid conditions in the cohorts included hypertension (47 and 79% in whites and blacks), diabetes (34 and 35% in whites and blacks) and hypercholesterolemia (31 and 25% in whites and blacks). The medications used by the patients were: β-blocker (71 and 82% in whites and blacks), ACE inhibitor (89 and 94% in whites and blacks), angiotensin receptor-blocking agent (39 and 32% in whites and blacks) and aldosterone blocker (33 and 32% in whites and blacks).

Table 2. Racial distribution of inhibitor-1 G147D polymorphism

	Patients with HF[1]	Normal controls[1]	OR (95% CI)	p value
Blacks				
GG	259 (89.9)	35 (87.5)	1	
GD + DD	28+1 (10.1)	5+0 (12.5)	0.784 (0.285–2.158)	0.636
Total	288 (100)	40 (100)		
Frequency of allele D, %	5.21	6.25	0.824 (0.310–2.189)	0.603
Whites				
GG	671 (100)	311 (99.7)	1	
GD + DD	0	1+0 (0.3)	0.155 (0.006–3.806)	0.142
Total	671 (100)	312 (100)		
Frequency of allele D, %	0	0.16	0.155 (0.006–3.806)	0.404
Others				
GG	4	9		
GD + DD	0	0		
Total	4	9		
Frequency of allele D, %	0	0		

HF = Heart failure; GG = major form of inhibitor-1 gene; GD/DD = heterozygous/homozygous inhibitor-1 G147D genetic variant.

[1] Unless otherwise indicated values represent numbers with the percentages in parentheses.

Characterization of Inhibitor-1 G147D in Heart Failure Patients

Consistently, the G147D genetic variant of inhibitor-1 was identified only in black heart failure patients. There were 28 heterozygous and 1 homozygous carriers among 288 patients, while this variant was not observed in 671 white patients (table 2). The black patients exhibited a frequency of 10.1% and an allele frequency of 5.21%, while the black normal population indicated a similar distribution, with a frequency of 12.5% and an allele frequency of 6.25%. There was 1 white normal subject out of 312 screened, who exhibited the G147D polymorphism (0.16% allele frequency) (table 2). The inhibitor-1 genetic variant was found at allele frequencies consistent with the prediction of Hardy-Weinberg equilibrium (p = 1.000 in normal black subjects, 0.549 in black patients).

Association Analysis of Inhibitor-1 G147D with Human Heart Failure

Analysis of the clinical data of black heart failure patients revealed that carriers and noncarriers are compatible with age, gender, weight, height, blood pressure, primary diagnosis, other risk factors and medications (table 3). As mentioned above, there was only one homozygous carrier (DD), which precluded comparison of specific characteristics between heterozygous and homozygous carriers. Thus, the clinical parameters in G147D and D147D carriers were combined and compared to wild types (GG). This polymorphism was not directly associated with deteriorated cardiac function, as was evaluated by echocardiographic parameters including LVEF, LVEDD, LVESD, fractional shortening, septal thickness and postal wall thickness.

Kaplan-Meier analysis revealed that there were no significant differences in the pattern of endpoint event occurrence (we defined endpoint events as cardiac-associated death or cardiac transplantation) between G147D carriers and noncarriers in black heart failure patients by a 10-year follow-up since the first diagnostic date (fig. 1, log-rank p = 0.334). Unfortunately, too few cases, especially for the G147D carriers, were observed beyond 10 years, which precluded us from further exploring longer-term survival. In addition, Cox regression analysis was conducted to reveal a potential association between any of four variables including genotype, gender, body weight and β-blocker usage and the occurrence of cardiac-associated death or heart transplantation. It appears that only body weight was significantly associated with the frequency of the endpoint events, when effects from the other three factors were excluded based on the patient information (data not shown).

Based on the fact that overexpressing this polymorphism in isolated adult rat cardiomyocytes interfered with the contractile responses to β-adrenergic agonists

Table 3. Clinical characteristics of black heart failure patients by inhibitor-1 genotype

Variable	GG		GD/DD		p value
	n	mean ± SD	n	mean ± SD	
Age at onset of heart failure, years	251	52.97 ± 13.06	29	50.38 ± 16.47	0.326
Weight, kg	245	89.34 ± 25.07	28	99.66 ± 34.09	0.131
Height, cm	236	171.83 ± 10.79	28	170.5 ± 7.67	0.525
Systolic BP, mm Hg	198	120 ± 23.96	27	113.85 ± 23.06	0.157
Diastolic BP, mm Hg	198	75.45 ± 14.49	27	72.42 ± 15.37	0.32
LVEDD, cm	217	6.10 ± 1.04	29	6.06 ± 0.88	0.839
LVESD, cm	215	4.82 ± 1.29	29	4.56 ± 1.38	0.318
Septal wall, cm	212	1.13 ± 0.29	28	1.14 ± 0.29	0.807
Posterior wall, cm	212	1.03 ± 0.27	28	1.10 ± 0.26	0.164
LVEF, %	215	44.76 ± 14.09	29	43.64 ± 13.78	0.735
Fractional shortening, %	215	22.02 ± 11.40	29	25.95 ± 14.70	0.094
MVO_2	117	15.66 ± 5.03	13	15.88 ± 5.94	0.881
Variable	GG		GD/DD		p value
	n	%	n	%	
Male	136	54.2	16	55.2	0.919
Primary diagnosis					
Idiopathic cardiomyopathy	154	64.2	17	58.6	
Coronary artery disease	37	15.4	6	20.7	
Other	49	20.4	6	20.7	0.75
Cardiac-related death or received transplant	59	33.9	8	36.4	0.791
Other risk factors or coexisting conditions					
Hypertension	195	78.3	26	89.7	0.152
Diabetes mellitus	75	32.3	13	48.1	0.1
Hypercholesterolemia	48	24.7	8	38.1	0.185
Tobacco use					
Present user	127	69	12	63.2	
Past only	33	17.9	3	15.8	
Never	24	13	4	21.1	0.627
Medications at entry					
β-Blocker	198	81.8	23	79.3	0.94
ACE inhibitor	194	94.2	21	87.5	0.414
ARB	35	34.7	2	22.2	0.698
Aldosterone blocker	33	31.4	4	26.7	0.482

GG = Major form of inhibitor-1 gene; GD/DD = heterozygous/homozygous inhibitor-1 G147D genetic variant; BP = blood pressure; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction; MVO_2 = maximum amount of oxygen that can be removed from circulating blood and used by the working tissues during a specified period; ACE inhibitor = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor-blocking agent.

[15], it was interesting to assess whether the G147D inhibitor-1 carriers exhibited different contractile parameters in response to treatment with β-adrenergic blockers. However, no differences in terms of cardiac function or wall thickness were observed following β-blocker treatment in either G147D carriers or noncarriers (table 4).

Discussion

As a leading cause of mortality and morbidity worldwide, heart failure has been well-recognized as a multifactorial disease. Indeed, an increasing number of polymorphisms and mutations in signaling components regulating cardiac contractile function have been found to

Table 4. Response to β-blocker treatment in black heart failure patients by inhibitor-1 genotype

Variable	GD/DD				p	GG				p
	non-BB takers		BB takers			non-BB takers		BB takers		
	n	mean ± SD	n	mean ± SD		n	mean ± SD	n	mean ± SD	
Age at enrollment, years	6	52.32 ± 15.78	23	49.86 ± 16.91	0.751	45	51.05 ± 14.03	206	53.41 ± 12.84	0.273
Weight, kg	6	100.64 ± 26.66	23	103.78 ± 36.89	0.296	45	85.93 ± 24.64	206	89.89 ± 24.52	0.327
Height, cm	6	165.74 ± 8.02	23	167.04 ± 21.97	0.888	45	170.72 ± 10.94	206	170.85 ± 15.65	0.957
LVEDD, cm	5	6.60 ± 0.70	22	6.00 ± 0.90	0.183	36	6.10 ± 1.28	190	6.13 ± 0.99	0.883
LVESD, cm	5	5.26 ± 1.39	22	4.55 ± 1.32	0.294	36	4.78 ± 1.39	189	4.85 ± 1.24	0.750
Septal wall, cm	4	0.90 ± 0.24	22	1.19 ± 0.29	0.078	36	1.13 ± 0.24	190	1.12 ± 0.28	0.838
Posterior wall, cm	4	1.03 ± 0.26	21	1.10 ± 0.27	0.570	35	1.04 ± 0.23	177	22.25 ± 0.27	0.645
Fractional shortening, %	4	21.32 ± 13.30	16	25.19 ± 13.61	0.570	29	22.68 ± 10.18	160	23.71 ± 12.51	0.849
LVEF, %	4	40.52 ± 19.19	16	44.42 ± 12.79	0.626	29	45.52 ± 13.88	160	44.62 ± 14.17	0.754

GD/DD = Heterozygous/homozygous inhibitor-1 G147D genetic variant; GG = major form of inhibitor-1 gene; BB takers = β-blocker takers; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction.

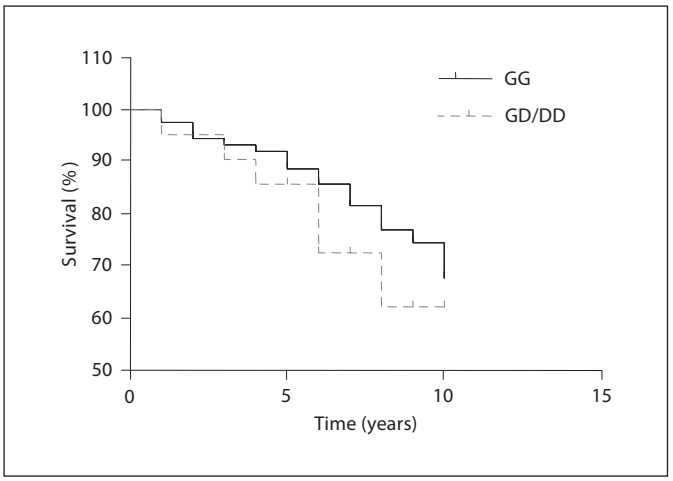

Fig. 1. Kaplan-Meier estimates of transplant-free survival. The survival rates were compared between G147D carriers (GD/DD) and noncarriers (GG) in black heart failure patients for a 10-year term. Curves were compared using the log-rank test. The overall pattern of event-free survival was not different (p = 0.334) between the two groups.

be linked to dilated cardiomyopathy or propensity to arrhythmias [17–21]. For example, multiple human polymorphisms on adrenergic signaling pathways have been reported to be direct or conjunct factors affecting development, prognosis or therapy of heart failure [17, 22–25]. PP1 inhibitor-1 has recently emerged as an important regulator for cardiac contractile function by maintaining an appropriate phosphorylation level of cardiac proteins and serving as a downstream mediator in the β-adrenergic signaling pathway. Moreover, reduced protein levels or activity of this molecule have been linked to experimental and human heart failure [4, 12–14]. In this study, we screened the inhibitor-1 gene in heart failure patients and cohort normal subjects in order to identify genetic variants, possibly associated with the onset, progression and/or prognosis of heart failure. Interestingly, the G147D inhibitor-1 genetic variant was found almost exclusively in the black population.

Notably, the human inhibitor-1 variant, G147D, is located close to the C-terminus of the protein, which may be important for its activity [26]. A recent study in isolated adult rat cardiomyocytes revealed that overexpression of this human inhibitor-1 polymorphism blunted the cell's responses to β-adrenergic agonists in regard to contractility and Ca cycling by reducing the phosphorylation levels of phospholamban [15]. The present study analyzed the patients' clinical history, but there were no alterations in any of the evaluated functional parameters or their responses to β-blocker treatment elicited by the inhibitor-1 polymorphism. However, this apparent discrepancy between findings in isolated cardiomyocytes [15] and heart failure patients should be interpreted with caution, given the complexities in translating a cellular response to an in vivo phenotype. An additional confounding factor in defining the clinical significance of the G147D inhibitor-1 may be the low prevalence of this polymorphism in the human population.

While the inhibitor-1 variant clearly had physiological significance in isolated cardiac myocytes, it was not an independent risk factor for heart failure in humans, as the prevalence was similar in normal subjects and the heart failure cohort and major cardiac functional parameters were not significantly altered in G147D carriers compared to noncarriers in the heart failure patients. However, this genetic variant may elicit measurable consequences in human disease in the context of other polymorphisms that already impact cardiac function. For example, polymorphisms of β_1- (Arg89) and α_{2c}- (Del22-325) adrenergic receptors act synergistically to increase the risk of human heart failure [27]. In addition, increasing evidence has indicated that the increased prevalence of genetic variants may contribute to the development and outcome of heart failure in blacks [28–30]. In particular, multiple polymorphisms of the adrenergic signaling pathways have been linked to the increased risk and worse prognosis of heart failure for black patients [22, 27]. Therefore, given the lack of statistical power in identifying an apparent correlation between the G147D inhibitor-1 polymorphism and clinical parameters, we can speculate that the G147D genetic variant may have a rather small adverse biological effect on its own. However, it may contribute to a multigenetic predisposition to adverse heart failure outcomes in blacks. Alternatively, the presence of other frequent mutant alleles found in the black population [22, 27–30], as outlined above, may act as confounding factors in the development and/or progression of heart failure. However, lack of information on the presence of other functionally significant mutations or polymorphisms in our cohort prevents further evaluation of this hypothesis. To further define such potential gene-gene interactions, studies in a much larger population with genotyping of entire functional clusters of β-adrenergic signaling factors and their downstream signaling effectors and targets will be required.

Overall, the present findings suggest that the G147D genetic variant of inhibitor-1 may not play an independent role in human heart failure, but may be a contributing factor to the deteriorated cardiac function acting as one of the modifiers for this disease.

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