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Genetic Variants Are Not Associated with Outcome in Patients with Coronary Artery Disease and Left Ventricular Dysfunction: Results of the Genetic Substudy of the Surgical Treatment for Ischemic Heart Failure (STICH) Trials

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Key Words
Heart failure · Coronary artery disease · Genotype

Abstract
Objectives and Background: We evaluated the ability of 23 genetic variants to provide prognostic information in patients enrolled in the Genetic Substudy of the Surgical Treatment for Ischemic Heart Failure (STICH) trials. ***Methods:*** Patients assigned to STICH Hypothesis 1 were randomized to medical therapy with or without coronary artery bypass grafting (CABG). Those assigned to STICH Hypothesis 2 were randomized to CABG or CABG with left ventricular reconstruction. ***Results:*** In patients assigned to STICH Hypothesis 2 (n = 714), no genetic variant met the prespecified Bonferroni-adjusted threshold for statistical significance ($p < 0.002$); however, several variants met nominal prognostic significance: variants in the β_2-adrenergic receptor gene (β_2-AR Gln27Glu) and in the A_1-adenosine receptor gene (A_1-717 T/G) were associated with an increased risk of a subject dying

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or being hospitalized for a cardiac problem (p = 0.027 and 0.031, respectively). These relationships remained nominally significant even after multivariable adjustment for prognostic clinical variables. However, none of the 23 genetic variants influenced all-cause mortality or the combination of death or cardiovascular hospitalization in the STICH Hypothesis 1 population (n = 532) by either univariate or multivariable analysis. ***Conclusion:*** We were unable to identify the predictive genotypes in optimally treated patients in these two ischemic heart failure populations.

Introduction

Heart failure is a disease of epidemic proportions that affects over 5 million individuals in the US and accounts for over 250,000 deaths and 1 million hospitalizations each year [1]. Heart failure is attributable to coronary artery disease in over 70% of these individuals. There is great variability in the progression of heart failure in different individuals as well as in their response to various therapies including drugs or devices. These differences have been attributable at least in part to genetic variation [2, 3].

Genetic variants in genes that encode proteins that effect cardiac remodeling and that encode proteins that are targets of pharmacologic therapy have been associated with the progression of heart failure. However, the results of studies that have assessed the association of these genetic variations with outcomes have often provided disparate results. For example, genetic variations in genes encoding the β_1-adrenergic receptor [4, 5], the β_2-adrenergic receptor [6], the angiotensin-converting enzyme (ACE) [7–9], aldosterone synthase [10, 11], matrix metalloproteinase type 9 [12], tumor necrosis factor-α [13, 14], endothelial nitric oxide synthase [15, 16], and adenosine monophosphate deaminase-1 [17, 18] have all been associated with varying outcomes in patients with heart failure. The disparities across these studies have been attributed in part to the small size or genetic heterogeneity of the study populations, the inclusion of patients with both ischemic and idiopathic dilated cardiomyopathy, differences in baseline heart failure therapies, and/or statistical noise due to the absence of replicable results in a separate population with the same phenotype.

We evaluated the relationship between genotype and outcome in patients enrolled in the Genotype Substudy of the Surgical Treatment for Ischemic Heart Failure (STICH) trials to assess the role of genetic variants in predicting outcome in a group of patients with heart failure secondary to ischemic heart disease [19]. Funded by the National Institutes of Health, this multicenter international study enrolled 2,136 patients with ischemic heart failure into one of two studies, STICH Hypothesis 1 and STICH Hypothesis 2, thereby providing two independent studies in which to prospectively evaluate the ability of genotype to predict outcome. Hypothesis 1 assessed whether coronary artery bypass grafting (CABG) with intensive medical therapy could improve long-term survival when compared with intensive medical therapy alone. There was no statistically significant difference in the primary outcome of death from any cause between the two treatment groups; CABG relative to medical therapy alone led to a significant reduction in cardiovascular deaths and survival free of cardiovascular hospitalizations [20]. Hypothesis 2 evaluated the benefits of left ventricular surgical reconstruction (SVR) combined with CABG when compared with CABG alone. The addition of SVR had no effect on the primary outcome variable of death from any cause or hospitalization for cardiac cause [21]. Patients enrolled in STICH were carefully phenotyped, received optimal medical therapy including a β-blocker and an ACE inhibitor or an angiotensin receptor blocker, and were followed for a median of 48 months.

To test the hypothesis that genotype is associated with outcome in patients with ischemic heart failure being considered for surgical revascularization, we genotyped patients enrolled in the two STICH studies that participated in the STICH genotype substudy. Genetic variants were chosen that had been found in other studies to be relevant in predicting either outcomes or response to therapy and that represented important neuropharmacologic targets including hormonal signaling pathways (β_1-AB, β_2-AR, and adenosine-R) [4–6], neurohormone levels (ACE, aldosterone synthase, and adenosine monophosphate deaminase) [7–11, 17, 18], inflammatory mediators (tumor necrosis factor-α and matrix metalloproteinases) [12–14], and vascular reactants (endothelial nitric oxide synthase) [15–16].

Methods

Study Design

The rationale and design of the STICH trial as well as the results of both Hypothesis 1 and Hypothesis 2 were presented previously in detail [19–21]. In brief, STICH enrolled 2,136 patients with a left ventricular ejection fraction ≤35% and coronary artery disease that was amenable to CABG. Patients were excluded if they had a recent myocardial infarction, a need for aortic valve replacement,

a planned percutaneous coronary intervention, and coexisting noncardiac disease that would shorten their life expectancy. All patients underwent cardiac imaging for assessment of left ventricular function and wall motion.

The enrolling physician assigned patients to one of three strata. Stratum A included patients who were eligible for either medical therapy alone or medical therapy plus CABG. Patients were eligible for medical therapy alone if they did not have significant left main coronary artery disease or Canadian Cardiovascular Society class III or IV angina. Stratum B included patients who were eligible for medical therapy alone, medical therapy plus CABG or medical therapy plus CABG and SVR. Patients were eligible for SVR if they had anterior left ventricular akinesia or dyskinesia. Stratum C patients were eligible for medical therapy plus CABG or medical therapy plus CABG and SVR. Patients were then randomly assigned to one of the treatment options for which they were eligible. All of the patients in stratum A and some of the patients in stratum B were randomly assigned to medical therapy or medical therapy plus CABG (STICH Hypothesis 1). All of the patients in stratum C and some of the patients in stratum B were randomly assigned to medical therapy and CABG or to medical therapy, CABG and SVR (STICH Hypothesis 2). Seventy-six patients randomized to CABG in stratum B fit the criteria for assignment to either Hypothesis 1 or Hypothesis 2. Those patients and their genetic data were analyzed with the Hypothesis 2 cohort.

Patients received pharmacologic therapy based on consensus guideline recommendations; however, the use of device therapy was highly variable across different countries [22]. All countries and study centers were given the opportunity to participate in the STICH genotype substudy; however, a number of centers declined because of local or national laws that prohibited them from transporting blood products or genetic material out of the country. Approval from the institutional review board was obtained from each institution participating in the genetic substudy of the STICH trial and all patients provided written informed consent.

Genotyping

The planning for the STICH trials began in 2000 and enrollment into the two studies began shortly after receipt of the notice of funding from the National Institutes of Health in 2002 [19]. Our ability to assess the effects of a limitless number of genetic variables on outcome in the STICH trial was restricted by the technology available and its attendant costs at the time the study was begun. Therefore, we used the following criteria to select the specific variants for study: (1) discrete genetic variants that alter the function of genes that modify cardiac remodeling; (2) variants in genes that encode proteins that are targets of heart failure pharmacologic therapy, and (3) genetic variants that had previously been associated with changes in the risk of developing heart failure or in response to pharmacologic therapy. We did not proactively eliminate any genetic variants that met at least one of these criteria. In fact, we added several variants as new information became available prior to the start of genotyping. Once genotyping began, we did not alter the panel of variants. Approximately 10 ml of blood was obtained from each subject prior to randomization and shipped to the core laboratory at Thomas Jefferson University, Philadelphia, Pa., USA, within 7 days. The core laboratory was blinded to the treatment arm to which the individual patients were assigned. Total genomic DNA was extracted from these samples using a genomic DNA extraction kit (Promega, Madison, Wisc., USA). We assessed the presence of genetic variants using the PCR-based restriction fragment length polymorphism method that had been reported previously by either our own laboratory or by others. As new technologies became available, several of the genetic variants were assessed using high-throughput analysis. In all cases, we confirmed the identity of each restriction enzyme-based product by sequence analysis prior to utilizing the technology on the sample population. Specific details regarding the technique and primers used for each genotype can be found in the online supplementary material (for all online supplementary material, see www.karger.com/doi/10.1159/000368221).

Statistical Analysis

A unique feature of STICH is that it consisted of two different studies in patients that had similar severity and etiology of their heart failure (Hypothesis 1 and Hypothesis 2), thus enabling an examination of the relationships of the genetic markers with clinical outcomes in two separate studies. Because blood samples for genotyping could not be obtained in every randomized patient (as described above), we examined the baseline characteristics and outcomes of patients in the genetic substudy compared to the patients where samples for genotyping could not be obtained. Data are descriptively summarized using the median and interquartile range for continuous variables and frequencies and percentages for categorical variables. The distributions of continuous variables and ordinal categorical variables were compared between groups using the Wilcoxon rank-sum test, and nominal categorical variables were compared using conventional χ^2 statistics. The incidence of the primary endpoint in each trial (mortality in Hypothesis 1 and death or cardiovascular hospitalization in Hypothesis 2) was also compared between patients in the genetic substudy compared to the patients who were not included. Because homozygous variants were rare, heterozygote and homozygote variants were combined as a single endpoint.

The relationships of each of the 23 genetic markers with the clinical outcomes of (a) death and (b) death or cardiac hospitalization were examined separately in the Hypothesis 1 and Hypothesis 2 cohorts using the Cox regression model [23]. We assessed the univariate relationship of each genetic marker with the clinical outcomes and also examined the extent to which any of the genotypes contributed significant independent prognostic information beyond the baseline clinical variables routinely available in these patients. The clinical variables were the prognostic variables identified through separate multivariable Cox regression analyses for death and for death or cardiovascular hospitalization in the Hypothesis 1 and Hypothesis 2 patient cohorts and included age, New York Heart Association heart failure classification, creatinine, hemoglobin, end-systolic volume index (ESVI), mitral regurgitation, and history of myocardial infarction, stroke, and atrial fibrillation. The specific variables for each cohort and endpoint are listed in tables 1 and 2. In the Hypothesis 1 cohort, we examined whether CABG plus medical therapy had a greater (or lesser) effect on clinical outcomes compared to medical therapy alone depending on the genetic variant. This assessment was performed by examining treatment by genetic marker interactions using the Cox model. Identical analyses for CABG versus CABG plus SVR were performed in the Hypothesis 2 cohort.

Hazard ratios (HR), 95% confidence intervals (CI), and p values were generated in both univariate and multivariable analyses using the Cox model. Because of the number of genetic markers examined [23] and the inherent multiplicity of comparisons, we

Table 1. Comparison of STICH Hypothesis 2 patients with versus without genetic data

Characteristics	No genetic data (n = 286)	Genetic data (n = 714)	p value
Median age (25th, 75th), years	60.5 (53.6, 68.0)	62.1 (55.2, 69.3)	0.108
Female	50 (17.5)	97 (13.6)	0.116
White race	250 (87.4)	661 (92.6)	0.010
Minority (Hispanic or racial minority)	56 (19.6)	68 (9.5)	<0.001
BMI	27.4 (24.5, 30.5)	27.0 (24.5, 30.0)	0.358
Previous MI	253 (88.5)	619 (86.7)	0.450
Previous stroke	10 (3.5)	46 (6.4)	0.067
Diabetes	96 (33.6)	248 (34.7)	0.725
Hypertension	177 (61.9)	408 (57.1)	0.169
Hyperlipidemia	208 (73.0)	510 (71.6)	0.667
Current smoker	66 (23.1)	151 (21.1)	0.504
Chronic renal insufficiency	33 (11.6)	52 (7.3)	0.029
Peripheral vascular disease	53 (18.5)	93 (13.0)	0.026
Atrial fibrillation/flutter	38 (13.3)	79 (11.1)	0.323
Previous PCI	36 (12.6)	159 (22.3)	<0.001
Previous CABG	7 (2.4)	17 (2.4)	0.950
Current CCS angina class			0.843
No angina	85 (29.7)	164 (23.0)	
I	15 (5.2)	56 (7.8)	
II	39 (13.6)	149 (20.9)	
III	114 (39.9)	294 (41.2)	
IV	33 (11.5)	51 (7.1)	
Current NYHA heart failure class			0.004
I	30 (10.5)	56 (7.8)	
II	90 (31.5)	339 (47.5)	
III	147 (51.4)	281 (39.4)	
IV	19 (6.6)	38 (5.3)	
Creatinine, mg/dl	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)	0.656
Risk at randomization*	12 (6, 21)	12 (5, 21)	0.488
Diseased vessels (>75% stenosis)			0.231
0–1	49 (17.1)	156 (21.8)	
2	123 (43.0)	288 (40.3)	
3	114 (39.9)	270 (37.8)	
Left main artery (≥50% stenosis)	57 (19.9)	140 (19.6)	0.908
Proximal LAD (≥75% stenosis)	232 (81.4)	525 (73.5)	0.009
Median LV ejection fraction (25th, 75th), %	28.0 (23.0, 34.0)	28.0 (22.1, 34.0)	0.672
Median ESVI (25th, 75th), ml/m^2	80.2 (61.0, 100.2)	77.5 (59.0, 98.4)	0.227
Mitral regurgitation			0.371
None or trace	92 (32.7)	271 (38.2)	
Mild (≤2+)	141 (50.2)	308 (43.4)	
Moderate or severe	48 (17.0)	130 (18.3)	
Region			<0.001
Europe	158 (55.2)	415 (58.1)	
USA	49 (17.1)	151 (21.1)	
Canada	50 (17.5)	104 (14.6)	
Other	29 (10.1)	44 (6.2)	
Cardiovascular medications			
β-Blocker	221 (77.3)	637 (89.2)	<0.001
ACE inhibitor or ARB	246 (86.0)	633 (88.7)	0.247
Statin	198 (69.2)	573 (80.3)	<0.001
Antiarrhythmic	40 (14.0)	93 (13.0)	0.686
Digoxin	47 (16.4)	110 (15.4)	0.687
Aspirin or warfarin	217 (75.9)	603 (84.5)	0.001
Clopidogrel	21 (7.3)	60 (8.4)	0.579
Diuretic	195 (68.2)	481 (67.4)	0.804
Nitrate	188 (65.7)	399 (55.9)	0.004

Table 1 (continued)

Characteristics	No genetic data (n = 286)	Genetic data (n = 714)	p value
Previous ICD	9 (3.1)	25 (3.5)	0.780
Pacemaker for heart rate	6 (2.1)	11 (1.5)	0.590
Pacemaker for resynchronization	3 (1.0)	2 (0.3)	0.145
Clinical endpoints[a]			
Death	71 (24.8)	208 (29.1)	0.497
Death or CV hospitalization	148 (51.7)	433 (60.6)	0.031

Values are medians and interquartile ranges for continuous variables and frequencies and percentages for categorical variables. BMI = Body mass index; MI = myocardial infarction; PCI = percutaneous coronary intervention; CCS = Canadian Cardiovascular Society; NYHA = New York Heart Association; LAD = left anterior descending artery; LV = left ventricular; ARB = angiotensin II receptor blocker; ICD = implantable cardioverter defibrillator; CV = cardiovascular. [a] Comparisons based on log-rank test. * The risk of randomization score ranges from 1 to 32 with higher numbers indicating a higher predictive rate of death.

used a Bonferroni-corrected level of (0.05/23) = 0.002 as a guide for interpreting the statistical significance of the prognostic value of each genetic marker. Nominal p values are reported, however, for each of the assessments.

Results

A total of 1,212 patients were enrolled in STICH Hypothesis 1 between July 24, 2002, and May 5, 2007, and randomly assigned to receive medical therapy alone or medical therapy plus CABG. Samples from 532 Hypothesis 1 patients were included in the genotype analysis. This number did not include the patients with genetic data who were enrolled in both the Hypothesis 1 and Hypothesis 2 studies. Those patients were included in the Hypothesis 2 analyses. Between September 12, 2002, and January 24, 2006, clinical sites randomized 1,000 patients to STICH Hypothesis 2: treatment with CABG alone (n = 499) or CABG plus SVR (n = 501). Follow-up continued through December 31, 2008. Samples from 714 of the Hypothesis 2 patients were included in the genotype analysis. Because enrollment into STICH Hypothesis 2 was completed before enrollment into STICH Hypothesis 1, we completed genotyping and analysis of the Hypothesis 2 cohort before completion of the Hypothesis 1 cohort, and the data are therefore presented in that order.

Overall, the majority of genotype substudy participants in STICH were from North America (34%) or Europe (59%). A smaller percentage of individuals assigned to STICH Hypothesis 1 participated in the genetic analysis substudy than did patients assigned to STICH Hypothesis 2. This difference was attributable to the fact that a higher percentage of patients assigned to Hypothesis 1 came from countries that did not participate in the genetic substudy.

STICH Hypothesis 2

As seen in table 1, the baseline characteristics of the subjects enrolled in STICH Hypothesis 2 who participated in the genetic study were generally similar to the Hypothesis 2 patients who did not participate. In particular, there were no significant differences in terms of age, gender, left ventricular ejection fraction, left ventricular ESVI, or history of a previous myocardial infarction, hypertension, or diabetes. The patients who participated in the genetic substudy had a lower incidence of chronic renal disease, peripheral vascular disease, and less symptomatic heart failure. There was a modestly higher incidence of the primary composite endpoint of death or cardiovascular hospitalization among the patients in the genetic substudy.

Figure 1a shows the relationship between the 23 genetic variants on one or both alleles of 11 cardiac genes and the primary endpoint of all-cause death or cardiovascular hospitalization of patients enrolled in Hypothesis 2 of the trial. No genetic variant met the threshold for statistical significance ($p < 0.002$); however, several met nominal significance. The presence of an informative single nucleotide polymorphism (SNP) on one or both alleles of the β_2-AR gene that results in a shift of the amino acid at position 27 from a glutamine to a glutamic acid (β_2-AR Gln27Glu) contributed nominal prognostic information in univariate analysis regarding a subject either

23 genetic markers	Wild type (patients/ events)	Variant (patients/ events)	HR	95% CI	p value
1) β_1-adrenergic receptor –arg389gly	368/227	346/206	0.94	(0.78, 1.13)	0.511
2) β_1-adrenergic receptor –ser49gly	559/338	154/95	1.02	(0.81, 1.28)	0.874
3) β_2-adrenergic receptor –thre64ile	693/420	20/13	1.02	(0.59, 1.78)	0.937
4) β_2-adrenergic receptor –arg16gly	107/56	607/377	1.25	(0.95, 1.66)	0.116
5) β_2-adrenergic receptor –gln27glu	230/125	484/308	1.27	(1.03, 1.56)	0.027
6) ACE –i/d	145/97	569/336	0.83	(0.66, 1.04)	0.106
7) Adenosine 1 receptor A_1 –717t/g	322/182	391/250	1.23	(1.02, 1.49)	0.031
8) Adenosine 1 receptor A_1 –Del36	702/424	12/9	1.34	(0.69, 2.59)	0.388
9) Adenosine 1 receptor A_1 –1689c/a	660/404	54/29	0.83	(0.57, 1.21)	0.329
10) Adenosine 1 receptor A_1 –2206Tdel	659/402	54/30	0.87	(0.60, 1.26)	0.469
11) Angiotensin –thr235met	209/135	504/297	0.84	(0.69, 1.03)	0.095
12) Adenosine monophosphate deaminase 1 –cyt/thy	508/320	205/113	0.84	(0.67, 1.04)	0.101
13) Cyp11b2 (aldosterone synthase gene) –344c/t	160/91	552/341	1.19	(0.94, 1.50)	0.146
14) Matrix metalloproteinase type 9 –arg668/gln	518/311	196/122	1.10	(0.89, 1.35)	0.382
15) Matrix metalloproteinase type 9 –arg270gln	493/304	221/129	0.92	(0.75, 1.13)	0.433
16) Matrix metalloproteinase type 9 –1562c/t	499/297	214/136	1.12	(0.92, 1.38)	0.263
17) Matrix metalloproteinase type 9 +6/ct	180/108	534/325	1.00	(0.80, 1.24)	0.980
18) Nitric oxide synthase type 3 –4b/4a	513/312	200/121	0.94	(0.76, 1.16)	0.550
19) Nitric oxide synthase type 3 –786t/c	102/63	611/370	1.00	(0.77, 1.31)	0.982
20) Nitric oxide synthase type 3 –glu298asp	338/196	376/237	1.19	(0.98, 1.44)	0.075
21) Monocyte chemoattractive protein –2136a/t	475/285	239/148	1.06	(0.87, 1.30)	0.552
22) Monocyte chemoattractive protein –2578a/g	375/228	339/205	0.99	(0.82, 1.20)	0.948
23) Tumor necrosis factor –308a/g	15/8	699/425	1.10	(0.55, 2.22)	0.787

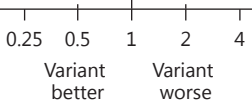

Fig. 1. a Relationship of 23 genetic markers and clinical outcomes (nonadjusted; Hypothesis 2: endpoint = death/cardiovascular hospitalization).

dying or having a cardiovascular hospitalization during the course of the study (HR 1.27; 95% CI 1.03–1.56; p = 0.027). This finding remained significant in multivariable analysis that examined the prognostic effect of this SNP after adjusting for prognostic clinical variables (HR 1.24; 95% CI 1.01–1.53; p = 0.045). The presence of a noninformative shift on one or both alleles in the nucleotide at position 717 in the coding region of the A_1-adenosine receptor gene from a thymine to a guanine (A_1-717 T/G) was associated with an increased risk of a subject either dying or being hospitalized for a cardiac problem by univariable analysis (HR 1.23; 95% CI 1.02–1.49; p = 0.031) and by multivariable analysis (HR 1.29; 95% CI 1.07–1.57; p = 0.009); however, these associations only met nominal significance when assessed using the Bonferroni-corrected significance level. The presence of a variant on one or both alleles of the A_1-adenosine receptor gene was also nominally associated with the endpoint of all-cause mortality in both univariate (p = 0.048) and multivariable (p = 0.015) analysis, but again did not meet the Bonferroni-adjusted significance criterion. None of the other genetic variants provided significant predictive information with respect to the secondary endpoint of mortality in the STICH Hypothesis 2 patients (fig. 1b, online suppl. fig. 1B). In addition, it is noteworthy that the treatment effect of CABG or the combination of CABG and ventricular reconstruction was not consistently modified by genotype (online suppl. fig. 2A, B). Furthermore, the representation of each variant in the STICH population was consistent with earlier reports. Including the presence or absence of an implantable cardioverter defibrillator or the inclusion of the treatment itself in the adjusted model had no effect on the results.

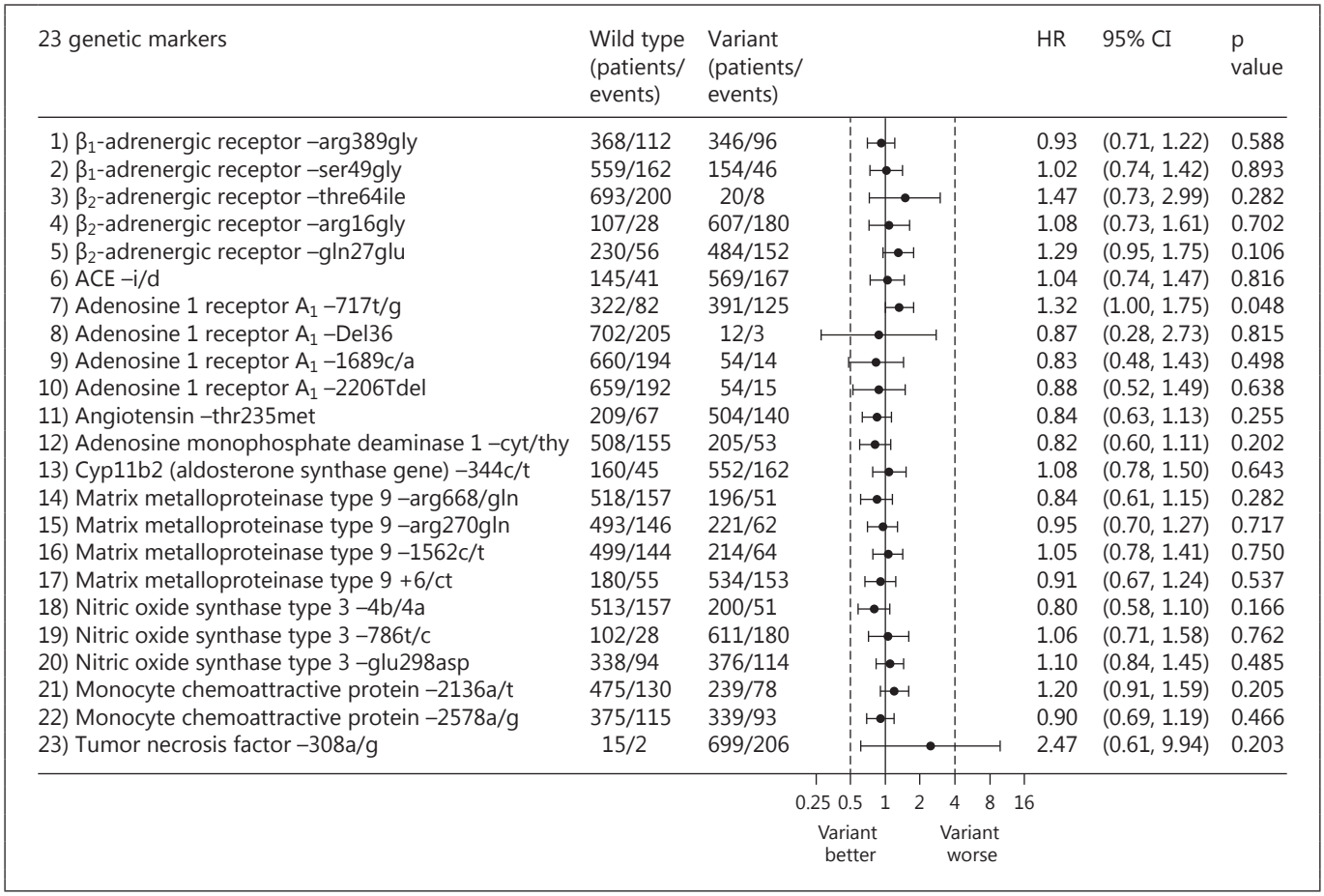

Fig. 1. b Relationship of 23 genetic markers and clinical outcomes (nonadjusted; Hypothesis 2: endpoint = death).

STICH Hypothesis 1

Among the 1,212 patients enrolled in Hypothesis 1, there were 76 patients who were also in Hypothesis 2. Those patients were analyzed with the Hypothesis 2 cohort, leaving a completely independent Hypothesis 1 cohort of 1,136 patients. Table 2 shows the baseline characteristics of the 532 Hypothesis 1 patients who were included in the genetic substudy cohort compared to the 604 patients who were not included. The patients with genetic data were older on average, had less racial diversity, had a higher frequency of a history of stroke, hyperlipidemia, and atrial fibrillation, and higher average ESVI, although there was a lower percentage with angina. The risk at randomization of the Hypothesis 1 genetic cohort was moderately higher than in the patients who were not included, as evidenced by an increased incidence of death or cardiovascular hospitalization.

When assessing the relationship between the 23 genetic variants on one or both alleles of the 11 cardiac genes, no single genotype was even nominally associated with the primary outcome variable of all-cause mortality by either univariate or multivariable analysis (fig. 2a, online suppl. fig. 3A). Similar results were observed when assessing the secondary endpoint of death or cardiovascular hospitalization, that is, no single genetic variant was predictive of the secondary outcome in the STICH Hypothesis 1 population (fig. 2b, online suppl. fig. 3B). The treatment effect of CABG and optimal medical treatment versus medical therapy alone was not significantly modified by the genotype with the exception of the A_1-adenosine receptor gene (A_1-717T/G) where there was a nominally significant interaction with treatment ($p = 0.032$ for death and $p = 0.037$ for death or cardiovascular hospitalization), with CABG having a greater effect in patients

Table 2. Comparison of STICH Hypothesis 1 patients with versus without genetic data

Characteristics	No genetic data (n = 604)	Genetic data (n = 532)	p value
Median age (25th, 75th), years	57.8 (52.5, 65.8)	61.2 (54.8, 69.1)	<0.001
Female	67 (11.1)	72 (13.5)	0.210
White race	281 (46.5)	476 (89.5)	<0.001
Minority (Hispanic or racial minority)	347 (57.5)	66 (12.4)	<0.001
BMI	26.1 (23.3, 29.5)	27.4 (24.5, 30.2)	<0.001
Previous MI	460 (76.2)	410 (77.1)	0.718
Previous stroke	29 (4.8)	56 (10.5)	<0.001
Diabetes	229 (37.9)	226 (42.5)	0.117
Hypertension	353 (58.4)	338 (63.5)	0.080
Hyperlipidemia	313 (51.8)	361 (68.0)	<0.001
Current smoker	128 (21.2)	100 (18.8)	0.308
Chronic renal insufficiency	39 (6.5)	48 (9.0)	0.105
Peripheral vascular disease	86 (14.2)	89 (16.7)	0.246
Atrial fibrillation/flutter	58 (9.6)	85 (16.0)	0.001
Previous PCI	55 (9.1)	82 (15.4)	0.001
Previous CABG	20 (3.3)	16 (3.0)	0.771
Current CCS angina class			<0.001
No angina	175 (29.0)	242 (45.5)	
I	80 (13.2)	94 (17.7)	
II	322 (53.3)	170 (32.0)	
III	21 (3.5)	22 (4.1)	
IV	6 (1.0)	4 (0.8)	
Current NYHA heart failure class			0.012
I	58 (9.6)	73 (13.7)	
II	304 (50.3)	281 (52.8)	
III	230 (38.1)	161 (30.3)	
IV	12 (2.0)	17 (3.2)	
Creatinine, mg/dl	1.1 (1.0, 1.2)	1.1 (0.9, 1.3)	0.587
Risk at randomization*	10.5 (5.0, 19.0)	13.0 (5.0, 20.0)	0.027
Diseased vessels (>75% stenosis)			0.151
1	136 (22.5)	143 (26.9)	
2	236 (39.1)	197 (37.1)	
3	232 (38.4)	191 (36.0)	
Left main artery (>50% stenosis)	14 (2.3)	16 (3.0)	0.466
Proximal LAD (>75% stenosis)	432 (71.5)	333 (62.7)	0.002
LV ejection fraction, median (25th, 75th), %	28.0 (22.9, 34.0)	27.0 (22.0, 33.4)	0.260
Median ESVI (25th, 75th), ml/m^2	76.6 (58.4, 97.0)	81.8 (63.0, 105.3)	0.005
Mitral regurgitation			0.326
None or trace	197 (32.7)	207 (39.1)	
Mild (≤2+)	306 (50.7)	218 (41.1)	
Moderate or severe	100 (16.6)	105 (19.8)	
Region			<0.001
Europe	312 (51.7)	319 (60.0)	
US	33 (5.5)	74 (13.9)	
Canada	22 (3.6)	89 (16.7)	
Other	237 (39.2)	50 (9.4)	
Cardiovascular medications			
β-Blocker	497 (82.3)	471 (88.5)	0.003
ACE inhibitor or ARB	518 (85.8)	494 (92.9)	<0.001
Statin	471 (78.0)	444 (83.5)	0.020
Antiarrhythmic	64 (10.6)	55 (10.3)	0.888
Digoxin	129 (21.4)	98 (18.4)	0.217
Aspirin or warfarin	523 (86.6)	479 (90.0)	0.072
Clopidogrel	139 (23.0)	63 (11.8)	<0.001
Diuretic	419 (69.4)	434 (81.6)	<0.001
Nitrate	364 (60.3)	245 (46.1)	<0.001

Table 2 (continued)

Characteristics	No genetic data (n = 604)	Genetic data (n = 532)	p value
Previous ICD	7 (1.2)	18 (3.4)	0.011
Pacemaker for heart rate	9 (1.5)	9 (1.7)	0.786
Pacemaker for resynchronization	4 (0.7)	2 (0.4)	0.690
Clinical endpoints[a]			
Death	226 (37.4)	215 (40.4)	0.234
Death or CV hospitalization	330 (54.6)	379 (71.2)	<0.001

Values are medians and interquartile ranges for continuous variables and frequencies and percentages for categorical variables. BMI = Body mass index; MI = myocardial infarction; PCI = percutaneous coronary intervention; CCS = Canadian Cardiovascular Society; NYHA = New York Heart Association; LAD = left anterior descending artery; LV = left ventricular; ARB = angiotensin II receptor blocker; ICD = implantable cardioverter defibrillator; CV = cardiovascular. [a] Comparisons based on log-rank test. * The risk of randomization score ranges from 1 to 32 with higher numbers indicating a higher predictive rate of death.

with no genetic variant present on either allele of the A_1-adenosine receptor (online suppl. fig. 4A, B). However, given the number of comparisons performed, this result should be interpreted cautiously.

Discussion

Our finding that genotype was not consistently predictive of outcome in two distinct heart failure populations is in conflict with some previous literature from single population studies. Polymorphisms in the β-adrenergic receptor (β_2-AR) gene have been extensively studied because of the critical role that the β_2-AR plays in cardiac homeostasis [24]. Informative SNPs have been variably associated with changes in the function of the receptor; however, in most instances, no relationship has been found. For example, de Groote et al. [25] found that neither the Arg16Gly, Gln27Glu, or Thr16Ile polymorphism affected survival in a group of 444 consecutive patients with left ventricular systolic dysfunction, although a significant effect was seen when looking at the haplotype. Similarly, Shin et al. [6] were not able to identify an association between a β_2-AR genotype and outcome in a group of 227 patients followed at a single center. Furthermore, a study of 637 patients enrolled in 2 US cardiovascular genetic registries with heart failure and left ventricular dysfunction and discharged on β-blockers, ACE inhibitors, or angiotensin II receptor blockers and diuretics, failed to identify a relationship between β-AR genotypes and heart failure outcomes [5]. By contrast, a study of 122 patients demonstrated that those homozygous for the β_2-AR Glu27 genotype were over five times more likely to have maladaptive ventricular remodeling after a myocardial infarction [26], and a study of 183 patients with heart failure demonstrated that this same group would have a more robust response to β-blocker therapy [27].

Our study differed from the finding that a missense mutation at nucleotide 145 in the β_1-AR gene was associated with decreased mortality in 184 patients with idiopathic dilated cardiomyopathy [28]. Our results are also disparate from earlier studies demonstrating that an SNP in the adenosine monophosphate deaminase 1 gene predicted outcome in 132 patients with both ischemic and nonischemic advanced heart failure [17], in 144 patients with heart failure after myocardial infarction [28], and in 367 patients with coronary artery disease [29]. Our finding that the –1562 C/T variant in the matrix metalloproteinase type 9 gene was not associated with outcome in patients with ischemic heart failure also conflicts with results of a previous study of 443 patients with both ischemic and nonischemic heart failure, less than half of whom were receiving β blockade, which suggested that the –1562 T/T genotype was an independent predictor of survival [12].

It was also surprising that we were unable to demonstrate a relationship between the presence of ACE D/D or I/D genotypes and outcome in the STICH trial. The D/D allele has been associated with increased production of angiotensin II, a neurohormone widely associated with a poor prognosis in patients with heart failure [8]. While an early study in 99 patients with heart failure failed to identify an association between ACE genotype and outcome [8], we found in relatively large populations of heart fail-

23 genetic markers	Wild type (patients/ events)	Variant (patients/ events)	HR	95% CI	p value
1) β_1-adrenergic receptor –arg389gly	296/110	235/104	1.16	(0.89, 1.52)	0.282
2) β_1-adrenergic receptor –ser49gly	417/172	115/43	0.97	(0.69, 1.36)	0.861
3) β_2-adrenergic receptor –thre64ile	518/209	13/5	1.19	(0.49, 2.89)	0.699
4) β_2-adrenergic receptor –arg16gly	82/37	450/178	0.82	(0.58, 1.17)	0.281
5) β_2-adrenergic receptor –gln27glu	186/82	346/133	0.81	(0.62, 1.07)	0.139
6) ACE –i/d	128/53	404/162	0.94	(0.69, 1.28)	0.705
7) Adenosine 1 receptor A_1 –717t/g	257/92	273/121	1.25	(0.96, 1.65)	0.101
8) Adenosine 1 receptor A_1 –Del36	517/209	14/5	0.89	(0.37, 2.16)	0.796
9) Adenosine 1 receptor A_1 –1689c/a	485/199	45/14	0.71	(0.41, 1.22)	0.212
10) Adenosine 1 receptor A_1 –2206Tdel	491/200	38/12	0.65	(0.36, 1.16)	0.141
11) Angiotensin –thr235met	149/54	383/161	1.24	(0.91, 1.69)	0.164
12) Adenosine monophosphate deaminase 1 –cyt/thy	390/152	142/63	1.14	(0.85, 1.53)	0.379
13) Cyp11b2 (aldosterone synthase gene) –344c/t	107/46	425/169	0.88	(0.63, 1.22)	0.431
14) Matrix metalloproteinase type 9 –arg668/gln	374/154	158/61	0.92	(0.68, 1.24)	0.579
15) Matrix metalloproteinase type 9 –arg270gln	394/159	138/56	0.99	(0.73, 1.34)	0.942
16) Matrix metalloproteinase type 9 –1562c/t	394/155	138/60	1.11	(0.83, 1.50)	0.475
17) Matrix metalloproteinase type 9 +6/ct	115/48	417/167	0.84	(0.61, 1.16)	0.296
18) Nitric oxide synthase type 3 –4b/4a	365/142	166/72	1.11	(0.83, 1.47)	0.483
19) Nitric oxide synthase type 3 –786t/c	74/35	458/180	0.84	(0.58, 1.20)	0.336
20) Nitric oxide synthase type 3 –glu298asp	262/101	270/114	1.08	(0.83, 1.41)	0.563
21) Monocyte chemoattractive protein –2136a/t	360/142	172/73	1.05	(0.79, 1.40)	0.719
22) Monocyte chemoattractive protein –2578a/g	267/112	265/103	0.93	(0.71, 1.22)	0.594
23) Tumor necrosis factor –308a/g	15/6	517/209	0.92	(0.41, 2.08)	0.849

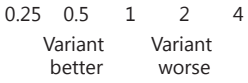

Fig. 2. a Relationship of 23 genetic markers and clinical outcomes (nonadjusted; Hypothesis 1: endpoint = death).

ure patients that the presence of the deletion on even one allele is associated with a significantly worse prognosis [9]. However, many of these patients were either receiving a low dose of an ACE inhibitor or no ACE inhibitor at all. Our results also differ from our recent finding that the C>T SNP in the aldosterone synthase gene may predict outcome in African-Americans with heart failure, although this group was poorly represented in the STICH trial [10]. The finding that a noninformative SNP in the adenosine receptor gene was nominally associated with a worse outcome in patients in STICH Hypothesis 2 was intriguing; however, we could not confirm this finding in the STICH Hypothesis 1 population suggesting that the finding was a statistical aberration.

Several important factors may explain the disparity between the present studies and earlier reports. First, and foremost, we performed analysis of multiple genetic markers in two separate and distinct but very similar patient populations. This gave us the opportunity to confirm or, in our case, refute findings from a single study in a comparable patient population. Second, by protocol, each of the patients enrolled in STICH Hypothesis 1 and STICH Hypothesis 2 was receiving optimal medical therapy. We have shown previously that optimal medical therapy can obviate differences seen in untreated or undertreated populations when assessing genetic variants in drug targets (β-blockers or ACE inhibitors) [30]. Over 90% of patients enrolled in STICH were treated with both a β-blocker and an ACE inhibitor or an angiotensin receptor antagonist, and pharmacologic dosages were optimized. Thus, the medical regimen of the patients was substantially more robust than in many of the earlier genotyping studies. The size of the trial was also of potential importance. The number of patients enrolled in the substudy for both STICH Hypothesis 1 and STICH Hypothesis 2 was greater than the number of individuals enrolled

23 genetic markers	Wild type (patients/ events)	Variant (patients/ events)	HR	95% CI	p value
1) β_1-adrenergic receptor –arg389gly	296/198	235/180	1.23	(1.00, 1.50)	0.046
2) β_1-adrenergic receptor –ser49gly	417/305	115/74	0.90	(0.70, 1.16)	0.404
3) β_2-adrenergic receptor –thre64ile	518/368	13/10	1.14	(0.61, 2.14)	0.679
4) β_2-adrenergic receptor –arg16gly	82/60	450/319	0.94	(0.71, 1.24)	0.661
5) β_2-adrenergic receptor –gln27glu	186/135	346/244	0.88	(0.72, 1.09)	0.248
6) ACE –i/d	128/90	404/289	1.01	(0.80, 1.28)	0.938
7) Adenosine 1 receptor A_1 –717t/g	257/182	273/195	0.95	(0.77, 1.16)	0.589
8) Adenosine 1 receptor A_1 –Del36	517/368	14/10	1.00	(0.53, 1.88)	1.000
9) Adenosine 1 receptor A_1 –1689c/a	485/347	45/30	0.92	(0.63, 1.33)	0.646
10) Adenosine 1 receptor A_1 –2206Tdel	491/350	38/26	0.90	(0.60, 1.34)	0.609
11) Angiotensin –thr235met	149/106	383/273	1.00	(0.80, 1.25)	0.986
12) Adenosine monophosphate deaminase 1 –cyt/thy	390/282	142/97	0.95	(0.75, 1.19)	0.641
13) Cyp11b2 (aldosterone synthase gene) –344c/t	107/79	425/300	0.90	(0.70, 1.15)	0.387
14) Matrix metalloproteinase type 9 –arg668/gln	374/262	158/117	1.12	(0.90, 1.39)	0.311
15) Matrix metalloproteinase type 9 –arg270gln	394/278	138/101	1.04	(0.83, 1.31)	0.737
16) Matrix metalloproteinase type 9 –1562c/t	394/279	138/100	1.01	(0.80, 1.27)	0.950
17) Matrix metalloproteinase type 9 +6/ct	115/80	417/299	0.93	(0.73, 1.20)	0.589
18) Nitric oxide synthase type 3 –4b/4a	365/258	166/120	1.06	(0.85, 1.32)	0.593
19) Nitric oxide synthase type 3 –786t/c	74/55	458/324	0.94	(0.71, 1.25)	0.679
20) Nitric oxide synthase type 3 –glu298asp	262/178	270/201	1.16	(0.94, 1.41)	0.160
21) Monocyte chemoattractive protein –2136a/t	360/251	172/128	1.06	(0.85, 1.31)	0.616
22) Monocyte chemoattractive protein –2578a/g	267/195	265/184	0.89	(0.73, 1.09)	0.267
23) Tumor necrosis factor –308a/g	15/12	517/367	0.71	(0.40, 1.26)	0.245

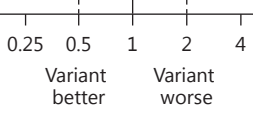

Fig. 2. b Relationship of 23 genetic markers and clinical outcomes (nonadjusted; Hypothesis 1: endpoint = death/cardiovascular hospitalization).

in many earlier studies, thereby reducing the risk of aberrant findings. And finally, the STICH population was relatively homogenous. All of the subjects had heart failure secondary to ischemic heart disease and the vast majority of the patients were Caucasian with a Northern-European ethnic background. This is in contrast to most earlier studies that assessed genotype in patients with idiopathic disease or with the idiopathic and ischemic cardiomyopathy.

A limitation of this study was that we took a reductionist approach to identifying genetic variants that could predict risk in patients with left ventricular dysfunction secondary to ischemic heart disease who were enrolled in the STICH trials. This approach was necessitated by the fact that the technology required for genome-wide association studies (GWAS) or whole-exome or whole-genome sequencing was not available during either the planning phase or the implementation stage of the STICH trials. Consistent with the present results, neither GWAS studies nor whole-exome sequencing have identified the genetic variants that were evaluated in STICH despite the fact that many of these variants had been demonstrated to influence treatment outcome in large studies of patients with heart failure, the Arg389Gly variant in the β_1-adrenergic receptor gene being a good example [31]. For example, Meder et al. [32] found a close association of genetic variants on chromosome 6p21 with the development of idiopathic dilated cardiomyopathy and an association of HLA-C gene expression with this locus, a finding that suggested a link between susceptibility to idiopathic dilated cardiomyopathy and autoimmune mechanisms that lead to myocardial inflammation. Similarly, GWAS identified a genetic susceptibility locus on chromosome 10q26 within the BCL2-associated athanogene 3 (BAG3) gene. Variants in this gene have been shown by our own group and by others to be a mono-

genic cause of dilated cardiomyopathy [33–35]. Because most GWAS and whole-exome sequencing studies have focused on patients with idiopathic dilated cardiomyopathy, it is difficult to compare our results with those obtained from GWAS or other newer methodologies applied to individuals with idiopathic or familial heart failure. Further studies in patients with ischemic heart disease using GWAS or whole-exome/genome sequencing will be useful in furthering our understanding of the linkage between genotype and outcomes in this group of patients.

Our study has several additional limitations. First, while the overwhelming majority of the patients were from North America and Europe, significant genetic differences and population stratification could have occurred in this international trial. Second, the study was begun in 2002. Therefore, we were not able to take advantage of new and less costly technology that might have allowed us to pursue genome-wide association in haplotype identification. Third, since most of the patients in STICH were considered for cardiac surgery, the results may not be able to be extrapolated to the overall ischemic group or to the overall STICH group that differs in some baseline characteristics from the genetic substudy population. Finally, because heart failure patients in general do well on optimal medical therapy, a longer period of follow-up might have revealed an association between genetic variants and outcomes that were not obvious in the present analysis.

Nonetheless, our failure to identify and confirm genetic markers of outcome in these two heart failure populations points out the need to confirm genotypic findings in a comparable population, the importance of study size in genetic analysis, the importance of optimizing medical therapy, and the need to study populations that are homogenous. These lessons will be of particular importance as new technologies increase our ability to readily measure multiple genetic markers in populations as well as to sequence a subject's entire exome or genome.

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