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The Methionine 196 Arginine Polymorphism of the TNF Receptor 2 Gene (TNFRSF1B) is Not Associated with Worse Outcomes in Heart Failure

Charles F McTiernan, Ph.D^a, Ravi Ramani, MD^a, Benjamin Burkhead^a, and Dennis McNamara, MD^a

^aDivision of Cardiology, Dept of Medicine University of Pittsburgh, Pittsburgh PA

Abstract

Tumor necrosis factor α (TNF α) may contribute to the pathologic process of congestive heart failure (CHF). TNF α signaling occurs through two receptors; TNFR1 (TNFRSF1A) and TNFR2 (TNFRSF1B). In humans a single nucleotide polymorphism (rs1061622 in TNFRSF1B exon 6; T587G) encodes two different amino acids (M196R) in the transmembrane region. The 587G allele is associated with greater severity and/or prevalence of some inflammatory diseases, but its role in CHF is unknown. This study sought to test the hypothesis that the 587G allele is associated with a worse outcome or more severe phenotype in CHF. Peripheral blood DNA was isolated and genotyped from 379 heart failure patients enrolled in a genetic outcome study (GRACE); (44.7% ischemic, 70.4% male, 8.5% black race, age 55.6 $\pm$ 11.7 yr (SD), LVEF 0.245 $\pm$ 8.3%, NYHA 2.53 $\pm$ 0.64). Genotyping was performed by PCR-RFLP. Cardiac function was assessed from medical records at study entry. The distribution of genotypes in this population was 54% T/T, 38.4% G/T, and 7.7% G/G. Mean LV ejection fraction (T/T 24.4 $\pm$ 8.2, T/G 25.0 $\pm$ 8.4, G/G 23.3 $\pm$ 8.6, $n=352$, $p=ns$) and LV end-diastolic dimensions (T/T 6.57 $\pm$ 0.93, T/G 6.53 $\pm$ 1.0, G/G 6.57 $\pm$ 0.78, $n=211$, $p=ns$) were comparable in all groups. Transplant-free survival (median 23 months (range 1–62 months) did not vary by genotype ($p=0.95$). A lack of effect ($p=0.74$) on transplant-free survival was also observed in a subset of patients with ischemic heart failure ($n=169$). The TNFRSF1B 587G allele is not associated with the severity of heart failure phenotype or clinical outcomes in patients with chronic CHF.

Keywords

Tumor Necrosis Factor α ; TNF receptor; polymorphisms; heart failure

1. Introduction

Elevated serum and/or tissue levels of multiple cytokines, including tumor necrosis factor alpha (TNF α), can induce (in experimental models), or are associated with (in human studies), diminished cardiac function and worsened cardiac remodeling in heart failure and inflammatory cardiac diseases [1]. In experimental animal and isolated heart or cell systems,

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Address for Correspondence: Charles F. McTiernan, PhD, 200 Lothrop St., BST 1744.1, Cardiovascular Institute, University of Pittsburgh, Pittsburgh PA 15213, Phone 1 412 648-3016, Fax 1 412 383-8857, mctiernanc@upmc.edu.

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TNF α diminishes cardiomyocyte calcium transients and contractile function [2–4], and increases cardiomyocyte and cardiac hypertrophy [3–5], cardiac fibrosis [3, 6], oxidative stress [7], and apoptosis [8, 9].

Cells of the myocardium, including cardiomyocytes, express two transmembrane TNF receptors, TNFR1 (TNFRSF1A) and TNFR2 (TNFRSF1B) [10, 11]. TNFR1 shows variable expression in response to pathologic and physiologic signaling, and has been linked to both pro- and anti-apoptotic pathways and the activation of multiple downstream pathways [12–14]. TNFR2 shows a less variable level of expression. While TNFR2 cannot directly mediate apoptosis it modulates TNFR1 -induction of apoptosis, and can directly activate NF κ B [14–17]. Several studies have shown that, in cardiovascular pathologies, TNFR1 can mediate many of the ‘adverse’ responses to TNF α exposure, and that the absence of TNFR2 leads to an enhancement of the pathophysiology induced by TNFR1 activation [11, 18–21]. Both TNF receptors can also be cleaved from cells by proteases including ADAM17, and are present in elevated levels in the serum of heart failure patients [22–24]. Various studies have found significant correlations between elevations in soluble TNF receptors and the severity of heart failure [23, 24]. The soluble receptors may serve as either a circulating pool of bioavailable TNF α , or conversely, may serve to limit the bioactive levels of soluble TNF α .

The human TNFRSF1B gene contains two coding sequence alleles (coding nucleotides 587 T/G in exon 6, (GenBank dbSNP Short genetic variations; rs1061622), encoding amino acid residues 196) that encode differing amino acid sequences of TNFR2 (M196R) [25]. This amino acid difference occurs close to the cleavage site that leads to the shedding of TNFR2 receptors. Some studies observe decreased sTNFR2 levels with the R196 receptor form [26, 27]. The M196 and R196 forms differ in their ability to mediate TNF signaling and activate downstream pathways and pathologic processes (such as apoptosis), such that the R196 form allows less activation of NF κ B and recruitment of TRAF2, and greater induction of apoptosis after activation of the TNFR1 pathway [28]. Additional studies have observed elevated frequencies of the R196 TNFR2 allele in various inflammatory and/or autoimmune diseases (including SLE, Crohn’s disease, and familial rheumatoid arthritis), suggesting a role for this allele in inflammatory diseases [25, 29, 30], although conflicting reports exist [31–33]. In this study we examined a population of patients with severe heart failure so as to test the hypothesis that the TNFRSF1B 587G allele is linked to worse survival or worse function in severe heart failure patients.

1. Methods

2.1 Patient Population

This study was approved by the Institutional Review Board of the University of Pittsburgh Medical Center, and was performed in accordance with *The Code of Ethics of the World Medical Association* (Declaration of Helsinki). The study population (n=378) consisted of heart failure patients with systolic dysfunction enrolled in a genetic outcome study (study of Genetic Risk Assessment of Cardiac Events; GRACE) at the University of Pittsburgh Medical Center between April 1996 and January 2001, and for whom DNA was available [34]. Upon enrollment, demographic information, New York Heart Association classification, data from cardiac evaluation for systolic function and ischemic vessel disease, and peripheral blood (for DNA isolation) were collected. Cardiac function was assessed by review of medical records at baseline. Patients were followed until an event of orthotopic cardiac transplantation or death. Medical therapy was classified from review of medical records at the time of enrollment.

Of the 378 patients, most (n=357) had a quantitative demonstration of LV systolic dysfunction (LVEF 0.45) as determined by echocardiography (n=202), radionuclide scan (n= 134), or LV angiography (n=21). A qualitative assessment of moderate to severe LV dysfunction was reported in a minority of cases (n=26). All patients had undergone evaluation for coronary artery disease; >90% by coronary angiography, with the rest by noninvasive assessments. Patients were classified as ischemic as previously described [34]; >50% stenosis of major epicardial artery, positive noninvasive assessment for ischemia, or prior history of myocardial infarction).

2.2 Genotype analyses

Peripheral blood DNA was isolated (Puregene Kit, Gentra Systems Inc). and PCR-RFLP used to genotype for the T587G polymorphism (dbSNP ID: rs1061622). The primers *TNFR2 snp196* for 5' act ctc cta tcc tgc ctg ct 3' and *TNFR2 snp196* rev 5' ttc tgg agt tgg ctg cgt gt 3' were used to PCR amplify a 242 base pair fragment arising from exon 6 and which included the T587G site using conditions previously described [31]. PCR products were purified and digested with and NLAIII. Digestion with NLAIII generates two products from the 587G allele of 133 and 109 base pair, whereas the T587 allele remains undigested. Digestion products were size fractionated on a 3.5% agarose gel and DNA fragment identified after staining with ethidium bromide and visualization under UV light.

2.3 Statistical Analyses

Patients were grouped as 587TT homozygotes (group T/T) encoding M196) and compared to heterozygotes (587TG) and 587GG homozygotes (grouped together as K/G) which contained at least one allele encoding R196. Testing for Hardy-Weinberg equilibrium utilized the Pearson χ^2 Test. Continuous variables (age, EF, LVEDD, VO2 MAX) are presented as mean $\pm$ SD, and were compared between genotype groups by Mann-Whitney U-test. Categorical variables were compared by Pearson χ^2 (unordered variables), or Mantel-Haentzel χ^2 (ordered categorical variables). For outcome analysis, Kaplan-Meier freedom from event curves were computed for genotype subgroups, and compared between subgroups by using the log rank test. To ascertain whether the 587T allele had greater impact in patients with heart failure arising from an ischemic etiology, outcome measures were repeated on the ischemic subset of patients.

2. Results

3.1 Baseline Demographics and Medical Therapy

Cohort (n=378) characteristics were; mean age 55.6 $\pm$ 11.7 yr (range 20–81), 70.4% male, 91% Caucasian race, 44.7% ischemic etiology (Table 1), and NYHA class distribution I/II/III/IV of 5%/46.3%/43.4%/4.8%. The distribution of genotypes in this population was 54% T/T, 38.4% T/G, and 7.7% G/G. There was no evidence for a different distribution of genotypes (T/T vs. K/G between Caucasians (n= 344; 54% T/T, 39.3% T/G, 7.6% G/G) and non-Caucasians (n=34; 58.8% T/T, 32.4% T/G, 8.8% G/G) (Mantel-Haentzel χ^2 =0.354, p=0.55). There was no evidence for a different distribution of genotypes (T/T vs. K/G) between males (266) and females (112) (Mantel- Haentzel χ^2 =0.333, p=0.564). There was also no evidence for a different distribution of genotypes between non-ischemic (n=209; 52.6% T/T, 38.3%T/G, 9.1% G/G and ischemic (n=169; 55.6% T/T, 38.5% T/G, 5.9% G/G) etiologies. (Mantel- Haentzel χ^2 =0.478, p=0.489).

Testing for Hardy-Weinberg equilibrium demonstrated no statistical evidence of disequilibrium overall (prevalence of the 587T allele 73.3%, χ^2 = 0.209, NS for one degree of freedom), or when analyzed by race (Caucasian, 587T allele 73%, χ^2 =0.055, NS for one degree of freedom; non-Caucasian; 587T allele 75%, χ^2 =0.641, NS for one degree of

freedom), or heart failure etiology (ischemic, 587T allele 74.9%, $\chi^2=0.82$, NS for one degree of freedom; non-ischemic etiology, 587T allele 71.7%, $\chi^2=2.58$, NS for one degree of freedom).

There was no evidence for a different distribution of genotypes (T/T vs K/G) between those patients receiving treatment with angiotensin converting enzyme inhibitor or angiotensin receptor blockers (n=354; 94.1% T/T, 93.1% T/G; 93.1% G/G; Mantel-Haentzel $\chi^2=0.162$, p=0.687) β -adrenergic receptor blockers (n=164; 40.2% T/T, 46.2% T/G; 51.7% G/G; Mantel-Haentzel $\chi^2=1.83$, p=0.176), or aldosterone-receptor antagonist (n=40; 13.2% T/T, 5.5% T/G; 17.2% G/G; Mantel-Haentzel $\chi^2=3.288$, p=0.07) at the time of enrollment, and those not receiving such medications.

3.2 Clinical Characteristics

Comparisons of LVEF, LVEDD, baseline heart rate, blood pressure, and functional assessment by metabolic stress test (VO₂ MAX) demonstrated no significant differences according to the presence of the 587G allele (196R) (Table 2). Patients with at least one 587G allele (196R) also did not differ in NYHA class distribution from those with only the 587T allele (Pearson $\chi^2=8.28$, 3 degrees of freedom, p=NS).

3.3 Outcomes; Event-Free Survival

The median follow-up time for all patients (n=378) was 23 months (range 1–62 months); for patients alive and transplant-free at last follow-up, median follow-up was 30 months (range 6–67 months). During follow-up there were 148 events (98 deaths and 50 cardiac transplants; see Table 3). For the entire cohort, there was no difference (p<0.947) in survival according to genotype (Kaplan-Meier plot, Figure 1). A similar analyses in the subsets of patients with non-ischemic (n=209) or ischemic etiology (n=179) also failed to show a significant relationship between genotype and survival (p=0.88 and p=0.89, respectively, data not shown). As multiple factors can impact the timing of transplantation in those listed for need of cardiac transplantation, cardiac transplantation as an endpoint may confound the effect of the TNFR1 587 allele on survival. We also calculated survival according to genotype only in those patients that did not receive cardiac transplantation (n=326). While the overall survival was longer (as expected from removal of those receiving transplants as an endpoint), no statistical differences were apparent amongst genotypes in survival of non-transplant recipients. (p<0.7, data not shown).

3. Discussion

This study examined a cohort of heart failure patients with systolic dysfunction to determine the relationship between the 587G allele of the TNFRSF1B gene and the severity of heart failure or survival. TNF α has been proposed as a mediator of heart failure pathophysiology in animal and human studies [1]. The TNFRSF1A and TNFRSF1B receptors mediate different signaling pathways and co-operatively modulate TNF α responses [13, 14, 35]. Several studies in humans have found correlations between the presence of the 587G allele encoding R196 and the increased incidence or severity of various inflammatory/immune diseases [25, 29, 30]. Thus the TNFRSF1B 587G allele is a plausible modulator of heart failure severity.

Two forms of TNF receptors (TNFR1, or TNFRSF1A and TNFR2, or TNFRSF1B) show closely conserved extracellular ligand-binding domains, but highly divergent intracellular regions reflecting differences in the interaction with intracellular signaling adaptor proteins. While the TNFR1 form has an intracellular death-domain region that can interact with signaling proteins (TRADD/FADD) leading to induction of apoptosis, TNFR2 lacks any death-domain binding proteins but is shown to bind to TRAF2 and activate the transcription

factor NF κ B, which can signal cell replication and survival pathways [12, 15, 16, 17]. In actuality, the activation of apoptosis versus survival signaling pathways via TNF receptor binding of TNF α also depends upon the cell type and other physiologic/pathologic parameters. Indeed a functional interaction of TNFRI with TNFRII to modulate cellular responses specific to differing cell types and physiologic states is proposed, with either receptor capable of mediating apoptosis and/or inflammatory responses under varying conditions [17, 35, 36]. The TNFRII R196 form does not impact soluble TNF α binding kinetics, but does lead to diminished NF κ B activation and activation of NF κ B-regulated pro-survival pathways and inflammatory cytokines [28]. Also, this residue exists in a transmembrane/sheddase cleavage site responsible for formation of soluble TNFRII, and some studies report a relationship between the 587 polymorphism and sTNFII levels [26, 27]. While the level of soluble TNFRII correlates strongly with the severity of heart failure, the pathophysiologic role of elevated sTNFRII in heart failure is unclear [23, 24].

TNF alpha is a prominent mediator of inflammatory processes and is associated with multiple autoimmune and inflammatory diseases [37, 38]. An allelic variation in exon 6 of the TNFRSF1B gene which encodes the 196R variant was first observed to be more prevalent in a Japanese population with SLE. [25]. However such an association was not observed in Caucasian population of SLE patients. [31]. Additional studies have variably observed an increased prevalence of the 196R allele in familial RA [27, 29, 32, 33], and Crohn's disease [30]. Other polymorphisms (but not R196M) of the TNFRSF1B locus have also been associated with coronary artery disease or familial hyperlipidemia [39, 40], which are risk factors for development of heart failure.

TNF α may play a role in the pathophysiology of cardiac dysfunction and heart failure. Circulating and cardiac levels of TNF α are elevated in human heart failure, and both TNFRI and TNFRII are expressed in the myocardium and cardiomyocytes [10, 11]. Elevated levels of TNF α induce structural (hypertrophy) and functional (contractile and calcium kinetics) changes in cardiomyocytes that resemble heart failure [2–4]. Transgenic over-expression of TNF α induces cardiac structural (cardiac fibrosis, cardiomyocyte and cardiac hypertrophy, cardiac dilation), functional (decreased fractional shortening and developed pressures), organ level (cellular infiltrates, increased apoptosis, cardiac arrhythmias), and cellular/molecular changes (diminished calcium transients, increased NF κ B activation, MMP and ANP expression, and a 'fetal gene expression' profile) consistent with heart failure pathophysiology [3, 4, 6].

TNFRI and TNFRII play different roles in mediating the effects of TNF α in heart failure and cardiac dysfunction. In a transgenic model of heart failure arising from cardiomyocyte-specific over-expression of TNF α , genetic ablation of the TNFRSF1A gene prevented development of cardiac functional and structural remodeling and heart failure, whereas ablation of the TNFRSF1B gene led to an exacerbation of heart failure development [18]. Similarly in mice subjected to chronic ischemic injury, ablation of the TNFRSF1A gene moderated heart failure severity whereas ablation of the TNFRSF1B gene exacerbated remodeling and heart failure [19, 20]. In isolated cardiomyocytes, use of TNF receptor-subtype specific blocking antibodies demonstrated that signaling through TNFRI had a greater adverse effect when the TNFRII form was blocked, consistent with the in vivo studies [11]. The protective effect of TNFRII signaling in ischemic/reperfusion injury is more robust in female mice [21]. The sum total of these observations suggest that the balance of cardiac TNFRI and TNFRII signaling impacts the severity of heart failure/remodeling, with the presence of TNFRII signaling serving to ameliorate the adverse effects of signaling via the TNFRI form. Thus a decrease in TNFRII signaling (such as reported for the human TNFRII R196 form) could plausibly worsen heart failure severity.

However, despite the reported observations that would support a plausible differential influence of the two TNFR2 forms (R196M) on heart failure severity, our results did not find any significant relationship between presence of the 587G allele and severity of heart failure (determined by systolic function and LV dimensions, classification of heart failure severity by NYHA category, or VO2MAX) or outcomes. Consideration of sex, race, or ischemic etiology also failed to reveal any influence of SNP rs1061622 in the TNFRSF1B and heart failure severity or outcomes.

We acknowledge limitations of the current study. First, the analysis of outcomes was limited to the median followup interval of 23 months; a longer followup may have revealed a greater number of events. We did not have sufficient serum samples to analyze the relationship between sTNFR2, the 587G allele, and CHF severity or outcome. We also did not analyze a healthy cohort to determine whether the frequency of the R196 allele was correlated to heart failure incidence, although it was not associated with outcomes of those with severe systolic dysfunction. We also did not examine other TNFRSF1B polymorphisms (promoter CA repeats, and 3'UTR snps) that have been associated with heart failure risk factors (hyperlipidemia, coronary artery disease) [39, 40]. However, this study focused on a well-characterized TNFRSF1B allele that encodes a functionally variant form of the TNFR2 protein that is plausibly related to pathophysiologic processes (such as apoptosis and NFκB activation) relevant to heart failure severity and outcomes. Finally, inflammation may play a more prominent role in a subset of heart failure etiologies such as myocarditis. These rare diseases would require a substantially larger cohort of patients to assess the role of R196 in the cases with more pronounced inflammatory cardiac disease.

In summary, we did not find a significant relationship between the TNFRSF1B T587G polymorphism and the severity of heart failure or clinical outcomes in the GRACE cohort of patients with systolic heart failure. The lack of association remained when the patient population was analyzed by sex, race, or ischemic/non-ischemic etiology. The relationship between the TNFRSF1B 587G allele and heart diseases with a more prominent inflammatory etiology, or between other TNFRSF1B variants and heart failure severity/outcomes remains unexplored.

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ABBREVIATIONS

CHF	congestive heart failure
GRACE	genetic risk assessment of cardiac events
LVEF	left ventricular ejection fraction
LVEDD	left ventricular end-diastolic dimension
NYHA	New York heart Association functional class
RFLP	restriction fragment length polymorphism
SLE	Systemic lupus erythematosus
TNFα	tumor necrosis factor alpha
TNF receptor I	TNFR1 or TNFRSF1A

TNF receptor II

TNFRII or TNFRSF1B

VO2max

maximum oxygen consumption during metabolic stress testing

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Highlights

- TNF α contributes to the pathophysiology of CHF
- A polymorphism in TNF α Receptor-II (the 587G allele) encodes two different amino acids (M196R)
- The 587G allele is associated with greater severity of some inflammatory diseases
- We demonstrate that the 587G allele is not associated with increased severity of heart failure

Figure 1.

Event free survival by TNFRSF1B 587 polymorphism. Dashed line indicates patients homozygous for 587T allele (n=204); dotted line indicates patients heterozygous for 587G/T allele (n=145); solid line indicates patients homozygous for 587G allele (n=29). p= NS for survival differences.

TABLE 1

Demographics and Medical Therapy at Enrollment

	T/T (n=204)	K/G (n=174)	All Pts (n=378)	p
Age	56+/-11	55+/-13	56+/-12	NS
Female %	30.9	28.1	29.6	NS
Caucasian %	90.2	92.0	91.0	NS
Ischemic %	46.1	43.1	44.7	NS
Initial Medical Therapy				
ACEI or ARB%	94.1	93.1	93.7	NS
β-Blocker %	40.2	47.1	43.4	NS
Aldos. Inhib.	13.2	7.5	10.6	NS

T/T refers to homozygous 587T allele; K/G refers to patients homozygous or heterozygous for 587G allele. ACEI; angiotensin converting enzyme inhibitor. ARB: angiotensin receptor blocker. Aldos. Inhib.; aldosterone inhibitor

TABLE 2

Clinical Characteristics by Genotype

	T/T (n=204)	K/G (n=174)	All Pts (n=378)	p
LVEF (n=352)	24+/-8.2	25+/-8.4	24.5+/-8.3	NS
LVEDD (n=211)	6.57+/-0.93	6.57+/-1.0	6.57+/-1	NS
NYHA, %I/II/III/IV	3.9/43.6/47.1/6.4	6.3/49.4/40.2/2.9	5/46.3/43.4/4.8	NS
BP sys (n=374)	114+/-18	112+/-16	113+/-17	NS
BP dias (n=374)	71+/-11	71+/-10	71+/-11	NS
Basal HR (n=373)	78+/-13.4	78+/-12.4	78+/-13	NS
VO2 Max (n=159)	16.3+/-4.8	17.7+/-5.3	16.9+/-5.1	NS

LVEF indicates left ventricular ejection fraction (%); LVEDD, left ventricular end diastolic dimension (cm); NYHA refers to New York Heart Association functional class; BP sys, systolic blood pressure (mm Hg); BP dias, diastolic blood pressure (mm Hg); HR, heart rate; VO2max, maximal oxygen consumption during metabolic stress testing (mL/kg/minute)

TABLE 3

Events by Genotype

	T/T (n=204)		K/G (n=174)		All Pts (n=378)	
	n	%	n	%	N	%
Alive	122	59.8	106	60.9	228	60.3
Transplanted	31	15.2	19	8.6	50	13.2
Deceased	49	24.0	49	28.2	98	25.9