Observational Study

Medicine®

OPEN

Common variants in IL-17A/IL-17RA axis contribute to predisposition to and progression of congestive heart failure

Chaugai Sandip (MD)[a,c], Lun Tan (MD)[a], Jin Huang (MD)[a], Qing Li (MD)[a], Li Ni (MD, PhD)[a], Katherine Cianflone (MD, PhD)[b], Dao Wen Wang (MD, PhD)[a,*]

Abstract

Heart failure is characterized by immune activation leading to production and release of proinflammatory cytokines. Interleukin 17A (IL-17A) is a proinflammatory cytokine and multiple lines of evidence from animal and human studies suggest crucial roles of IL-17A in heart failure. Therefore, we investigated whether common polymorphisms of genes *IL17A* and *IL17RA* (coding interleukin 17 receptor A) contribute to genetic predisposition to heart failure and adverse clinical outcomes associated with it.

A total of 1713 adult patients with congestive heart failure and 1713 age- and sex-matched controls were genotyped for promoter single nucleotide polymorphisms (SNPs), rs2275913 and rs8193037 in *IL17A* and rs4819554 in *IL17RA*, to assess the relationship between individual SNPs and the risk of congestive heart failure. Results showed that rs8193037 in *IL17A* was associated with the risk of congestive heart failure (odds ratio [OR] = 0.76; 95% confidence interval [CI] 0.63–0.90, adjusted $P = 0.002$) after adjustment for multiple cardiovascular risk factors including age, sex, smoking status, diabetes, hypertension, and dyslipidemia. This association was evident in both ischemic and nonischemic heart failure ($P = 0.005$ and $P = 0.05$, respectively). Furthermore, prospective follow-up of 12.7 months for the occurrence of adverse clinical outcomes showed that rs4819554 in *IL17RA* was significantly associated with cardiovascular mortality (hazard ratio [HR] = 1.28; 95% CI = 1.02–1.59, adjusted $P = 0.03$) after adjustments for multiple cardiovascular risk factors and New York Heart Association functional class.

This study demonstrated associations of rs8193037 in the promoter of *IL17A* with the risk of congestive heart failure, and of rs4819554 in the promoter of *IL17RA* with the risk of cardiovascular mortality in patients with congestive heart failure. These data lend further support to the notion that immune activation and genetic polymorphisms contribute to heart failure pathogenesis and progression.

Abbreviations: CAD = coronary artery disease, CHF = congestive heart failure, CI = confidence interval, DCM = dilated cardiomyopathy, ESRD = end-stage renal disease, HR = hazard ratio, IL-17A = interleukin 17A, IQR = interquartile range, NFAT = nuclear factor activated T cells, OR = odds ratio, SNP = single nucleotide polymorphism.

Keywords: congestive heart failure, IL-17A, IL-17RA, single nucleotide polymorphism

1. Introduction

Heart failure is a systemic disease with a multifactorial etiology including genetic factors and immune factors.[1–3] Immune activation leads to production and release of proinflammatory cytokines, activation of the complement system, and production of autoantibodies.[2] Yndestad et al[4] have reported that gene expression of chemokines is upregulated in T cells from patients with heart failure, which activates the immune system through binding to the tumor necrosis factor superfamily and their

Editor: Ken Rosenthal.

CS, LT, and JH contributed equally to this article.

CS's current address is Department of Medicine, division of clinical pharmacology, Vanderbilt University Medical Center, Nashville, TN.

Funding: The study was supported by a grant from the National Basic Research Program of China (2009CB521900 and 2012CB518004) and Key Grant of Natural Science Foundation (no. 91439203), National 863 Plan (no. 2012AA02510 and 2011BAI11B04).

Competency in Medical knowledge: Common mutations in IL-17A/IL-17RA account for inter individual variations in the risk of heart failure and the risk of cardiovascular mortality in such patients.

Competency in patient care: Screening for IL-17A/IL17RA may be considered in risk stratification and clinical decision making.

Translational outlook: Further work is needed to develop targeted novel therapeutic strategies for heart failure subpopulations at higher risk of cardiovascular death.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

[a] Division of Cardiology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China, [b] Centre de Recherche Institut Universitaire de Cardiologie & Pneumologie de Québec, Université Laval, QC, Canada, [c] Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee, USA.

[*] Correspondence: Dao Wen Wang, Division of Cardiology, Departments of Internal Medicine and The Institute of Hypertension, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095# Jiefang Ave., Wuhan 430030, People's Republic of China (e-mail: dwwang@tjh.tjmu.edu.cn).

Medicine (2016) 95:27(e4105)

Received: 31 March 2016 / Received in final form: 4 June 2016 / Accepted: 8 June 2016

http://dx.doi.org/10.1097/MD.0000000000004105

receptors, the inflammatory cytokines interferon-gamma, and interleukin-18 with similar pattern in ischemic and idiopathic cardiomyopathy. Th1/Th2 cytokine imbalance has also been identified to play a role in heart failure pathogenesis.[4–8] Circulating levels of proinflammatory cytokines such as TNF-alpha, soluble TNF receptors (sTNF-R1 and sTNF-R2), IL-6 and sCD14 have been shown to be independent predictors of mortality in patients with advanced heart failure.[9,10] Interleukin 17A (IL-17A) is a proinflammatory cytokine produced mainly by Th17 lymphocytes, but also by natural killer T cells, γδ T cells, cytotoxic $CD8^+$ T cells, and neutrophils.[11–13] Multiple lines of evidence from animal and human studies suggest crucial roles of IL-17A and its receptor interleukin 17 receptor A (IL-17RA) in ischemic heart disease.[14–21] Similarly, animal studies have provided evidence that IL-17A plays an important role in dilated cardiomyopathy (DCM).[22–26] Previous studies have identified Th17/Treg imbalance with upregulation of IL-17A that plays important roles in heart failure pathogenesis.[27–31]

Recent studies have identified single nucleotide polymorphisms (SNPs) in IL-17A/IL-17RA axis contribute to various cancers, autoimmune diseases, diabetes, and end-stage renal disease (ESRD). The A allele of rs2275913 (G>A) has been reported to show an association with immune-mediated disorders such as giant cell arteritis,[32] primary antiphospholipid syndrome,[33] ulcerative colitis,[34] etc. A allele carriers of rs4819554 (A>G) has been reported to be associated with immune-mediated clinical phenotypes such as aspirin hypersensitivity in asthma and age of onset in alopecia as well as ESRD and development of new onset diabetes in renal transplant recipients[35–39] in Asian population. The G allele carriers of rs8193037 (G>A) were reported to be at higher risk of metabolic syndrome in a large Mexican study.[40] A large angiography-based study in a Chinese Han population suggested G allele carriers rs8193037 are at significantly higher risk of coronary artery disease (CAD) risk.[41] However, whether these SNPs in IL-17A/IL-17RA axis are associated with risk stratification of heart failure has never been investigated.

Considering the crucial roles of IL-17A and its receptor IL-17RA in heart failure, we enrolled 1713 patients with congestive heart failure (CHF) and 1713 matched control subjects and aimed to investigate whether common polymorphisms of *IL17A* and *IL17RA* gene contribute to genetic predisposition to heart failure and adverse clinical outcomes associated with it.

2. Methods

2.1. Study participants

Patients referred to cardiology department of Tongji Medical College between September 2008 and August 2014 were screened for potential inclusion. Patients diagnosed with CHF based on medical history, physical examination, and relevant investigations were included as cases. Patients with ≥50% stenosis of major coronary arteries on coronary angiography were classified as ischemic heart failure, and those without angiographic evidence of coronary stenosis were classified as nonischemic heart failure. Ethnically and geographically matched individuals without evidence of CHF were included as controls. Anthropometric measurements, clinical characteristics, and clinical events were recorded at planned follow-up clinic visits, from questionnaires, medical records, and telephone calls.

The investigation conformed to the principles of the Declaration of Helsinki. All protocols and methods were approved by the ethics committee of the local hospital (Ethics Committee of Tongji Hospital). Written informed consents were obtained from all the participants.

2.2. Clinical endpoints

The endpoints included all-cause mortality, cardiac mortality, and hospitalization for heart failure.

2.3. SNP Selection and genotyping

Polymorphisms with minor allele frequencies >0.01, based on HapMap Beijing data, located in the promoter region and recently reported to be associated with immune-mediated diseases were selected for genotyping. Polymorphisms were genotyped using the TaqMan assay (Applied Biosystems, Foster City, CA). Primers and probes are presented in Supplementary Table 1, http://links.lww.com/MD/B104. Polymorphism genotyping adheres to a rigorous quality control program including blind replicate genotype assessment on 5% of the total samples.

2.4. Enzyme-linked immunosorbent assay

Blood samples collected in tubes containing EDTA were centrifuged at 3000*g* for 15 min at 4°C to isolate the plasma. Plasma samples were stored at −80°C till further use. Plasma levels of IL-17A were measured in duplicate using Legend Max™ Human IL-17A ELISA kit (No. 433917, BioLegend, San Diego, CA). The detection limit was 0.8 pg/mL and the lowest concentration for standard sample was 3.9 pg/mL. The mean intraassay and the mean interassay coefficients of variation were 5.4% and 7.9%, respectively. High sensitivity C reactive protein (hs-CRP) levels were obtained from medical records.

2.5. Statistical analysis

Continuous variables were compared between groups by Mann–Whitney *U* test or independent sample *t* test. Categorical variables were compared by Chi-square test. The polymorphisms were tested for Hardy–Weinberg equilibrium among the heart failure patients and the controls using a Chi-square test with 1 degree of freedom. The association between the individual SNPs and heart failure risk was estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs)[9] from the multivariate logistic regression analyses. An unconditional logistic model was used to adjust for multiple cardiovascular risk factors. Clinical outcome analyses were performed using Kaplan–Meier curve and Cox proportional hazards regression model. All statistical analyses were performed with SPSS 15.0 (SPSS, Inc., Chicago, IL) for Windows and the level of statistical significance was set at $P \leq 0.05$.

3. Results

3.1. Baseline characteristics of study participants

Baseline characteristics of heart failure cases and control participants are shown in Table 1. Heart failure and control participants were well matched in age and sex. More participants in heart failure group had diabetes, hypertension, dyslipidemia, smoking habit, but slightly lower level of triglycerides, total cholesterol, and low-density lipoprotein (LDL) cholesterol owing to lipid lowering therapy. The distribution of allele and genotype frequencies of the 3 SNPs is shown in Table 2. All the SNPs investigated are in Hardy–Weinberg equilibrium.

Table 1

Baseline characteristics of the study population.

Characteristics	Controls (N = 1713)	Cases (N = 1713)
Men, %	64.4	65.7
Age, y	57.47 ± 10.28	57.98 ± 13.63
Glucose, mmol/L	4.75 ± 1.9	6.64 ± 3.01*
TG, mmol/L	1.61 ± 1.33	1.4 ± 1.09*
TC, mmol/L	4.42 ± 0.98	3.92 ± 1.18*
LDL-C, mmol/L	2.52 ± 0.82	2.36 ± 0.88*
Hypertension, %	26.3	59.5*
Hyperlipidemia, %	20.7	33.9*
Diabetes, %	4.6	19.3*
Smoking status, %	30.2	37.7*

Data are expressed as means ± SD or percentages.
LDL-C = low-density lipoprotein cholesterol, TC = total cholesterol, TG = triglyceride.
* $P < 0.05$ vs control.

3.2. Association between IL-17A/IL-17RA polymorphisms and risk of congestive heart failure

No significant difference was detected in genotype and allele frequency of rs2275913 and rs4819554 between cases and controls suggesting these SNPs are not associated with the risk of heart failure. However, a statistically significant association was observed for rs8193037 (G>A). The frequency of GG and GA genotype was significantly higher in heart failure subjects than controls (Table 2). The distribution of genotype frequency was similar across ischemic and nonischemic heart failure and differed significantly from the controls (Table 3 and Supplementary Tables 2 and 3, http://links.lww.com/MD/B104). A allele was associated with a decreased risk of heart failure compared with G allele (OR = 0.76; 95% CI = 0.63–0.90, $P = 0.002$) after adjustment for multiple cardiovascular risk factors including age, sex, smoking status, diabetes, hypertension, and dyslipidemia. Results of the stratified analysis suggested the effect size to be comparable between ischemic (OR = 0.79; 95% CI = 0.65–0.97, $P = 0.021$) and nonischemic heart failure (OR = 0.80; 95% CI = 0.65–0.99, $P = 0.044$). After adjustment for age, sex, smoking status, diabetes, hypertension, and dyslipidemia, these associations were still highly significant for ischemic heart failure (OR = 0.74; 95% CI = 0.60–0.91, $P = 0.005$) and retained borderline significance for nonischemic heart failure (OR = 0.80; 95% CI = 0.61–1.00, $P = 0.050$).

3.3. Association between IL-17A/IL-17RA polymorphisms and clinical outcomes of heart failure

In total, 1515 CHF patients were prospectively followed for a mean of 12.7 months. Survival analyses were conducted on these patients stratified by the genotype of SNPs in IL17-A/IL-17RA axis. No significant differences were noted between the investigated SNPs and all-cause mortality (Supplementary Figure 1, http://links.lww.com/MD/B104). Among these investigated SNP, only rs4819554 (G being the minor allele, with a frequency of 45.2% in this CHF cohort) exhibited a highly significant association with the diversity of cardiovascular mortality (log rank = 0.027) (Fig. 1). The deaths from cardiovascular causes occurred in 69 patients (14.3%) in AA genotype group, 77 patients (11.3%) in AG genotype group, and 29 patients (8.3%) in GG genotype group. Cox-regression analysis showed that the A allele is significantly associated with a increased risk of cardiovascular mortality (hazard ratio

Table 2

Association of the *IL17A* and *IL17RA* polymorphisms with chronic heart failure.

	Genotype, n (%)				Per copy of the minor allele	
Groups	MM	Mm	mm	MAF (%)	Unadjusted OR (95% CI), *P*	Adjusted OR (95% CI), *P*
IL17A rs2275913						
Controls	461 (26.9)	876 (51.1)	376 (21.9)	47.5	Reference	
Cases	494 (28.8)	840 (49.0)	379 (21.1)	46.6	0.97 (0.88–1.06), 0.47	0.95 (0.85–1.05), 0.30
IL17A rs8193037						
Controls	1389 (81.1)	303 (17.7)	21 (1.2)	10.1	Reference	
Cases	1445 (84.4)	256 (14.9)	12 (0.7)	8.2	0.80 (0.68–0.94), 0.007	0.76 (0.63–0.90), 0.002
IL17RA rs4819554						
Controls	560 (32.7)	780 (45.5)	373 (21.8)	44.5	Reference	
Cases	554 (32.3)	769 (44.9)	390 (22.8)	45.2	1.03 (0.94–1.12), 0.59	1.04 (0.94–1.46), 0.46

Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by logistic regression, with and without adjustment for age, sex, hypertension, diabetes, hyperlipidemia, and smoking status. rs22759123, M = G, m = A; rs8193037, M = G, m = A; rs4819554 M = A, m = G.
MAF = minor allelic frequency.

Table 3

Association of rs8193037 (G>A) with chronic heart failure with an ischemic or nonischemic etiology.

	Genotype, n (%)				Per copy of the risk allele	
Groups	GG	GA	AA	MAF (%)	Unadjusted OR (95% CI), *P*	Adjusted OR (95% CI), *P*
Ischemic etiology						
Controls	1389 (81.1)	303 (17.7)	21 (1.2)	10.1	Reference	
Cases	649 (84.2)	117 (15.2)	5 (0.6)	8.2	0.79 (0.65–0.97), 0.021	0.74 (0.60–0.91), 0.005
Nonischemic etiology						
Controls	1389 (81.1)	303 (17.7)	21 (1.2)	10.1	Reference	
Cases	796 (84.5)	139 (14.8)	7 (0.7)	8.1	0.80 (0.65–0.99), 0.044	0.80 (0.61–1.00), 0.050

Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by logistic regression, with and without adjustment for age, sex, hypertension, diabetes, hyperlipidemia, and smoking status.
MAF = minor allelic frequency.

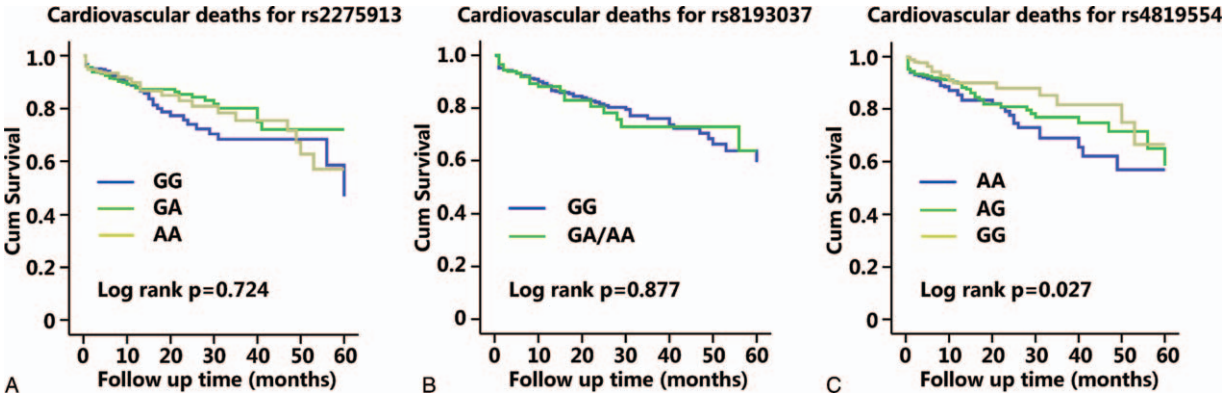

Figure 1. Effects of SNPs of IL-17A/IL-17RA axis on cardiovascular mortality in heart failure patients. Kaplan–Meier survival analysis showing no association between genotypes of rs2275913 (A) or rs8193037 (B) with cardiovascular mortality but A-allele dose-dependent effect of rs4819554 on cardiovascular mortality (C). Heart failure patients with AA genotype had worse survival compared with GA or GG genotypes.

[HR] = 1.32, 95% CI = 1.07–1.62; *P* trend = 0.008) (Table 4). This higher risk of cardiovascular mortality retained statistical significance in multivariate analysis after adjustments for age, sex, smoking status, diabetes, hypertension, hyperlipidemia, baseline New York Heart Association (NYHA) functional class, and left ventricular ejection fraction (HR = 1.28, 95% CI = 1.02–1.59; *P* = 0.03) (Table 4). The association of rs4819554 with cardiovascular mortality reached statistical significance in all the genetic models tested (Table 4). However, no significant association was detected in rehospitalization for heart failure for any of the SNPs tested, except that rs4819554 exhibited a modest trend toward higher rate of hospitalization for heart failure (Supplementary Table 4, http://links.lww.com/MD/B104).

3.4. Effect of rs8193037 genotypes on plasma IL-17A levels

Plasma IL-17A levels were tested in100 GG, 100 GA, and 12 AA heart failure samples stratified by rs8193037 genotype. IL-17A was detected in 54 GG, 46 GA, and 1 AA genotype patients, while IL-17A level in remaining samples was not credible as the concentration was beyond the minimum threshold. The rate of IL-17A detection did not differ between GG and GA genotypes (*P* = 0.258). Overall, the difference in circulating IL-17A level between GG (median, 5.30 pg/mL; interquartile range [IQR], 0–8.76 pg/mL) and GA genotypes (median, 0 pg/mL; IQR, 0–7.13 pg/mL) failed to reach statistical significance (*P* = 0.062). When only samples with detectable level of IL-17A were included in the analysis, plasma IL-17A levels were normally distributed and significantly higher in homozygous GG group compared with GA genotype group (8.94 ± 2.98 pg/mL vs 7.64 ± 2.77 pg/mL; *P* = 0.028) (Fig. 2A). From medical records, we obtained the data of plasma high sensitivity C-reactive protein (hs-CRP), an inflammatory biomarker, in 78 patients (47 patients with GG genotype and 31 patients with GA genotype) and stratified these patients according to the level of plasma hs-CRP. Results showed that the genotype-dependent diversity of IL-17A level was more distinct in the subgroup of patients with increased level of hs-CRP (8.53 ± 1.84 vs 7.11 ± 1.72; *P* = 0.015), but not statistically significant in patients with normal level of hs-CRP (9.05 ± 2.36 vs 8.24 ± 2.52; *P* = 0.342) (Fig. 2B and C). Plasma IL-17A levels did not differ between three genotypes of rs2275913 (Supplementary Figure 2, http://links.lww.com/MD/B104).

4. Discussion

We performed the first case–control study involving 3426 Chinese Han participants to investigate a potential association of rs2275913 and rs8193037 in *IL17A* and rs4819554 in *IL17RA* with the risk of heart failure and major adverse clinical outcomes associated with it. Our results demonstrated that single-base substitution of G to A of rs8193037 seemed to be

Table 4

Association of the *IL17A* and *IL17RA* polymorphisms with cardiac mortality in chronic heart failure.

SNPs	MM vs mm		Dominant model		Recessive model		Additive model	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
IL17A rs2275913								
Unadjusted	0.93 (0.76–1.15)	0.519	0.88 (0.64–1.21)	0.433	0.97 (0.81–1.16)	0.735	0.93 (0.76–1.14)	0.491
Adjusted	0.94 (0.75–1.17)	0.574	0.86 (0.62–1.19)	0.368	0.98 (0.83–1.18)	0.897	0.93 (0.75–1.16)	0.526
IL17A rs8193037								
Unadjusted	1.28 (0.48–3.43)	0.621	1.04 (0.70–1.55),	0.854	1.28 (0.48–3.42)	0.624	1.05 (0.72–1.54)	0.799
Adjusted	1.47 (0.54–3.98)	0.445	1.07 (0.72–1.60)	0.730	1.42 (0.53–3.81)	0.483	1.01 (0.66–1.55)	0.949
IL17RA rs4819554								
Unadjusted	1.35 (1.08–1.67)	0.007	1.57 (1.05–2.34)	0.027	1.18 (1.02–1.38)	0.029	1.32 (1.07–1.62)	0.008
Adjusted	1.25 (0.98–1.56)	0.053	1.51 (1.01–2.25)	0.045	1.21 (1.04–1.41)	0.014	1.28 (1.02–1.59)	0.030

Hazard ratio (HR) and 95% confidence intervals (CIs) were obtained by Cox regression, with and without adjustment for sex, age, BMI, hypertension, diabetes, hyperlipidemia, smoking status, NYHA class, and left ventricular ejection fraction. rs22759123, M = G, m = A; rs8193037, M = G, m = A; rs4819554 M = A, m = G.
SNP = single nucleotide polymorphism.

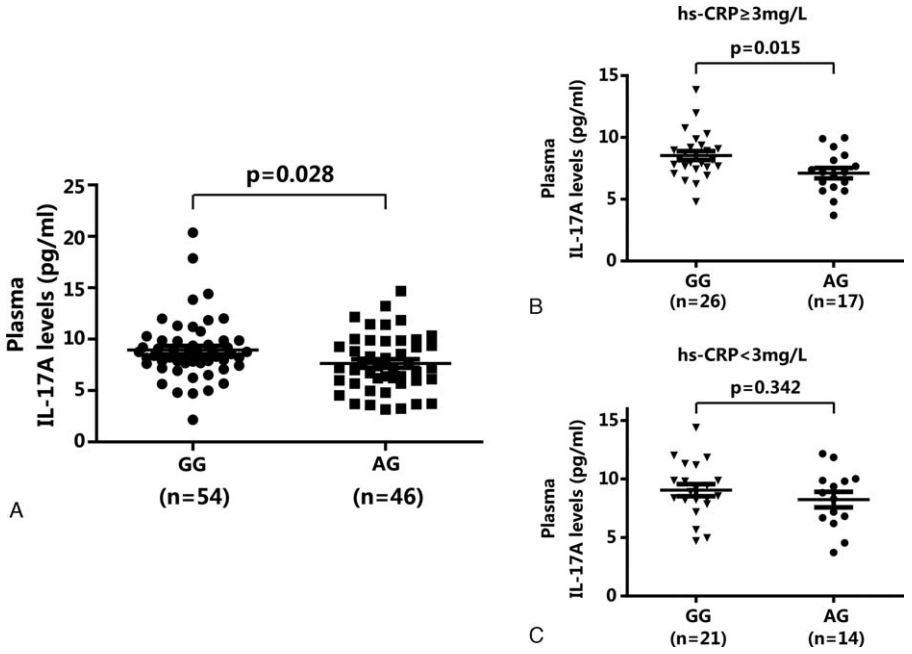

Figure 2. Effect of rs8193037 genotypes on plasma IL-17A levels. Plasma IL-17A levels were significantly higher in homozygous GG compared to GA genotype of rs8193037 (8.94 ± 2.98 vs 7.64 ± 2.77; $P = 0.028$) (A); subgroup analyses show that the diversity of plasma IL-17A levels related to genotype of rs8193037 results from patients in inflammation condition (8.53 ± 1.84 vs 7.11 ± 1.72; $P = 0.015$) (B), but not from those in noninflammation condition (9.05 ± 2.36 vs 8.24 ± 2.52; $P = 0.342$) (C) stratified by plasma levels of high sensitivity C-reactive protein (normal range: 0.1–3 mg/L).

associated with lower IL-17A level in plasma, which suggested patients carrying A allele exhibited lower inflammatory response in CHF. This, at least, partly explain why A allele is significantly associated with a decreased risk of heart failure. Stratified analysis revealed that this SNP was associated with ischemic as well as nonischemic heart failure, which was in line with the crucial roles of IL-17A in heart failure of various forms and etiologies. Further, A allele of rs4819554 exhibited a statistically significant association with higher cardiovascular mortality in cases of heart failure after adjustment for multiple cardiovascular risk factors, baseline NYHA class and left ventricular ejection fraction suggesting an important role of this SNP in the occurrence of fatal cardiac events for heart failure patients.

4.1. IL-17A/IL-17RA axis in heart failure

Heart failure is the end stage of adverse cardiac remodeling initiated by a wide variety of cardiac injuries. DCM, ischemic cardiomyopathy, and hypertensive heart disease are among the leading causes of heart failure. IL-17A is the prototype cytokine in IL-17 family that acts through IL-17RA to activate proinflammatory signaling pathways.[42] There is a vast body of evidence from previous studies demonstrating crucial roles of IL-17A/IL-17RA axis in atherosclerosis suggesting that it may contribute to the risk of ischemic heart failure.[14–19] In a T-cell receptor transgenic mouse model of DCM, IL-17A in cooperation with interferon-gamma was essential for transition from autoimmune myocarditis to DCM.[26] These data suggest that IL-17A plays an essential role in progression of myocarditis to DCM. IL-17A was shown to contribute to inflammatory DCM pathogenesis by stimulating cardiac fibroblast-GM CSF-monocyte macrophage pathway.[43] Collectively, these findings suggest crucial roles of IL-17A in heart failure of various forms and etiologies.

4.2. IL17A/IL17RA genes and SNPs

The most extensively investigated polymorphism of IL-17A/IL-17RA axis is rs2275913 with an association reported with acute graft versus host disease,[44] childhood asthma,[45] age related macular degeneration,[46] infections,[47,48] autoimmune disorders,[32,33,49,50] inflammatory bowel disease,[34] and various cancers,[51–54] albeit usually in smaller studies. However, there is no evidence to support its role in cardiovascular diseases. Two of the previous studies investigating rs2275913 showed no association with CAD in a Chinese Han population[40] or with premature CAD in a Mexican population.[41] Another small scale study in a Chinese Han population failed to detect any association with DCM.[55] Our findings provide further confirmation of a lack of association of rs2275913 with ischemic or nonischemic heart failure. Collectively, these results argue against a significant role of this polymorphism in heart failure, at least in Chinese Han population.

Another SNP rs8193037 represents G to A single-base substitution in the promoter at position 121 of *IL17A* gene.[40] To our knowledge, only 2 studies have investigated this SNP with the risk of CAD. Zhang et al[40] studied 5 IL-17A polymorphisms (rs4711998, rs3819024, rs2275913, rs8193037, and rs3819025) in 1031 CAD cases and 935 controls. They observed a significant association of rs8193037 with the risk of CAD in the Han population from northern China. Vargas-Alarcon et al[41] studied four *IL17A* gene polymorphisms (rs8193036, rs3819024, rs2275913, and rs8193037) in 900 patients with premature CAD and 667 controls in a Mexican population. Interestingly, rs8193037 was associated with metabolic syndrome, rs3819024 with increased levels of visceral abdominal fat and rs8193036 with central obesity, metabolic syndrome, and hypertriglyceridemia. Two haplotypes (CAGG and TAGA) were significantly associated with increased risk of premature CAD

though the individual SNPs showed no direct association with premature CAD. In our study with 1713 CHF cases and 1713 controls, rs8193037 was found to be significantly associated with the risk of CHF in a Han population from southern China. This association holds true in both ischemic and nonischemic heart failure suggesting that rs8193037 could play a central role in cardiac remodeling induced by diverse stimuli. To our knowledge, this is the first study demonstrating an association between rs8193037 and the risk of heart failure, although there was no significant effect of this SNP on major adverse clinical outcomes in CHF.

Rs4819554 represents A to G single-base substitution in the promoter at position 947 of *IL17RA* gene. Rs4819554 is in linkage disequilibrium with 2 other promoter polymorphisms, rs4819553 and rs917864, defining an *IL17RA* risk haplotype.[35] It has been recently linked to immune-mediated clinical phenotypes such as aspirin hypersensitivity in asthma, alopecia, papillary carcinoma of thyroid, ESRD and risk of new onset diabetes in ESRD.[35–39,56,57] Interestingly, the homozygous AA genotype showed a statistically significant higher risk of cardiovascular mortality compared to GG homozygotes, suggesting this polymorphism could have a prognostic role in CHF. Although a statistically significant association with rehospitalization for heart failure was not observed, differences in treating physician's preferences and socioeconomic condition of the patient may have influenced the results.

4.3. Functional studies

Gene expression is sophistically regulated by a complex of transcriptional activators or inhibitors with transcriptional machinery, and is subjected to influence by SNPs contributing to inter individual variations.[58] Kim et al[58] identified *IL17A* variants exerting allele-specific effects on gene expression in peripheral blood mononuclear cells by regulating their binding affinities for transcription factor complexes or CpG methylation profiles in inflammatory bowel disease. In this study, we have observed that GA heterozygotes of rs8193037 have lower circulating levels of IL-17A than GG homozygotes, which is consistent with previous study based on a CAD population.[40] Our data provide further confirmation of an IL-17A-mediated adaptive immune response in a subpopulation of heart failure patients suggesting that the presence of G allele of rs8193037 could potentially contribute to trigger this response especially during inflammation. Elevated levels of inflammatory mediators have been identified in patients with heart failure, and experimental studies have shown repeatedly that activation of inflammation in the heart provokes left ventricular remodeling and left ventricular dysfunction.[59] Lower circulating level of IL-17A in A allele carriers of rs8193037 could explain, at least in part, the lower risk of heart failure as observed in this study. Our findings also suggest that subtle differences in expression of IL-17A due to differences in promoter sequences make a significant difference over a lifetime, and potentially the difference between a balanced inflammatory response and a slightly too enthusiastic response that will exacerbate the inflammation rather than transition to a healing response following a cardiac event.

Other polymorphisms in IL-17A/IL-17RA system have also been reported to modulate the expression of the inflammatory cytokine IL-17A and its receptor IL-17RA, such as rs2275913 G>A locating within a binding motif for the nuclear factor-activated T cells (NFAT) which shows a higher affinity to this transcription factor and regulates IL-17A expression.[44,60] However, data on rs2275913 and IL17A secretion remain conflicting and we did not detect the association between rs2275913 and plasma IL-17A level.[44–48] Park et al have provided evidence that rs4819554 predisposes to asthma by upregulating *IL17RA* gene expression. IL-17RA mRNA and protein expressions in CD14(+) peripheral blood monocytes and mononuclear cells are significantly higher in AA homozygotes compared with the GG homozygotes in line with the lower frequency of the minor allele G in asthmatics.[35] However, it is great challenge to access the effect of a single SNP on the level of gene expression and regulation of cytokines is a complex network. Further studies are needed to define the underlying molecular mechanisms regulating IL-17A and IL-17RA expression by rs8193037 and rs4819554, respectively.

Drawbacks pertinent to the current study include: Low event rate and lower frequency of AA genotype of rs8193037 limited the power to assess the effect of this polymorphism on clinical outcomes and IL-17A levels. Relatively short mean follow-up period. Single center study.

5. Conclusions

The current study provides the first clinical evidence for an association of rs8193037 in IL-17A with the risk of heart failure, and of rs4819554 in IL17RA with the risk of cardiovascular mortality. Collectively, these data lend further support to the notion that immune activation and genetic polymorphisms contribute to heart failure pathogenesis and progression. These findings require replication in diverse ethnicities.

Acknowledgments

We thank all the study participants and the medical staff of Tongji hospital for their kind assistance in sample collection.

References

[1] Morita H, Seidman J, Seidman CE. Genetic causes of human heart failure. J Clin Invest 2005;115:518–26.
[2] Torre-Amione G. Immune activation in chronic heart failure. Am J Cardiol 2005;95:3C–8C. discussion 38C–40C.
[3] Ruppert V, Meyer T, Struwe C, et al. Evidence for CTLA4 as a susceptibility gene for dilated cardiomyopathy. Eur J Hum Genet 2010;18:694–9.
[4] Yndestad A, Holm AM, Muller F, et al. Enhanced expression of inflammatory cytokines and activation markers in T-cells from patients with chronic heart failure. Cardiovascul Res 2003;60:141–6.
[5] Athanassopoulos P, Vaessen LM, Maat AP, et al. Peripheral blood dendritic cells in human end-stage heart failure and the early post-transplant period: evidence for systemic Th1 immune responses. Eur J Cardiothorac Surg 2004;25:619–26.
[6] Yamaoka-Tojo M, Tojo T, Inomata T, et al. Circulating levels of interleukin 18 reflect etiologies of heart failure: Th1/Th2 cytokine imbalance exaggerates the pathophysiology of advanced heart failure. J Card Fail 2002;8:21–7.
[7] Cheng X, Liao YH, Ge H, et al. TH1/TH2 functional imbalance after acute myocardial infarction: coronary arterial inflammation or myocardial inflammation. J Clin Immunol 2005;25:246–53.
[8] Yu Q, Watson RR, Marchalonis JJ, et al. A role for T lymphocytes in mediating cardiac diastolic function. Am J Physiol Heart Circ Physiol 2005;289:H643–51.
[9] Rauchhaus M, Doehner W, Francis DP, et al. Plasma cytokine parameters and mortality in patients with chronic heart failure. Circulation 2000;102:3060–7.
[10] Deswal A, Petersen NJ, Feldman AM, et al. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). Circulation 2001;103:2055–9.
[11] Chakir J, Shannon J, Molet S, et al. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta,

IL-11, IL-17, and type I and type III collagen expression. J Allergy Clin Immunol 2003;111:1293–8.
[12] Hamada S, Umemura M, Shiono T, et al. IL-17A produced by gammadelta T cells plays a critical role in innate immunity against listeria monocytogenes infection in the liver. J Immunol 2008;181: 3456–63.
[13] Taylor PR, Roy S, Leal SMJr, et al. Activation of neutrophils by autocrine IL-17A-IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, RORgammat and dectin-2. Nat Immunol 2014;15:143–51.
[14] Gao Q, Jiang Y, Ma T, et al. A critical function of Th17 proinflammatory cells in the development of atherosclerotic plaque in mice. J Immunol 2010;185:5820–7.
[15] Butcher MJ, Gjurich BN, Phillips T, et al. The IL-17A/IL-17RA axis plays a proatherogenic role via the regulation of aortic myeloid cell recruitment. Circ Res 2012;110:675–87.
[16] Erbel C, Chen L, Bea F, et al. Inhibition of IL-17A attenuates atherosclerotic lesion development in apoE-deficient mice. J Immunol 2009;183:8167–75.
[17] Usui F, Kimura H, Ohshiro T, et al. Interleukin-17 deficiency reduced vascular inflammation and development of atherosclerosis in Western diet-induced apoE-deficient mice. Biochem Biophys Res Commun 2012;420:72–7.
[18] Xie JJ, Wang J, Tang TT, et al. The Th17/Treg functional imbalance during atherogenesis in ApoE(-/-) mice. Cytokine 2010;49:185–93.
[19] Smith E, Prasad KM, Butcher M, et al. Blockade of interleukin-17A results in reduced atherosclerosis in apolipoprotein E-deficient mice. Circulation 2010;121:1746–55.
[20] de Boer OJ, van der Meer JJ, Teeling P, et al. Differential expression of interleukin-17 family cytokines in intact and complicated human atherosclerotic plaques. J Pathol 2010;220:499–508.
[21] Eid RE, Rao DA, Zhou J, et al. Interleukin-17 and interferon-gamma are produced concomitantly by human coronary artery-infiltrating T cells and act synergistically on vascular smooth muscle cells. Circulation 2009;119:1424–32.
[22] Yuan J, Yu M, Lin QW, et al. Th17 cells contribute to viral replication in coxsackievirus B3-induced acute viral myocarditis. J Immunol 2010; 185:4004–10.
[23] Xie Y, Chen R, Zhang X, et al. Blockade of interleukin-17A protects against coxsackievirus B3-induced myocarditis by increasing COX-2/ PGE2 production in the heart. FEMS Immunol Med Microbiol 2012; 64:343–51.
[24] Xie Y, Li M, Wang X, et al. In vivo delivery of adenoviral vector containing interleukin-17 receptor a reduces cardiac remodeling and improves myocardial function in viral myocarditis leading to dilated cardiomyopathy. PloS One 2013;8:e72158.
[25] Baldeviano GC, Barin JG, Talor MV, et al. Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. Circ Res 2010;106:1646–55.
[26] Nindl V, Maier R, Ratering D, et al. Cooperation of Th1 and Th17 cells determines transition from autoimmune myocarditis to dilated cardiomyopathy. Eur J Immunol 2012;42:2311–21.
[27] Huang Y, Wu W, Wang Y. Expression or secretion of IL-17 in the peripheral blood mononuclear cells from patients with dilated cardiomyopathy. Acta Cardiol 2009;64:201–5.
[28] Yi A, Jian L, Xiaojing H, et al. The prevalence of Th17 cells in patients with dilated cardiomyopathy. Clin Invest Med 2009;32: E144–50.
[29] Li J, Wang L, Wang S, et al. The Treg/Th17 imbalance in patients with idiopathic dilated cardiomyopathy. Scand J Immunol 2010;71:298–303.
[30] Li N, Bian H, Zhang J, et al. The Th17/Treg imbalance exists in patients with heart failure with normal ejection fraction and heart failure with reduced ejection fraction. Clin Chim Acta 2010;411:1963–8.
[31] Tang TT, Ding YJ, Liao YH, et al. Defective circulating CD4CD25 +Foxp3+CD127(low) regulatory T-cells in patients with chronic heart failure. Cell Physiol Biochem 2010;25:451–8.
[32] Marquez A, Hernandez-Rodriguez J, Cid MC, et al. Influence of the IL17A locus in giant cell arteritis susceptibility. Ann Rheum Dis 2014;73:1742–5.
[33] Popovic-Kuzmanovic D, Novakovic I, Stojanovich L, et al. Increased activity of interleukin-23/interleukin-17 cytokine axis in primary antiphospholipid syndrome. Immunobiology 2013;218:186–91.
[34] Hayashi R, Tahara T, Shiroeda H, et al. Influence of IL17A polymorphisms (rs2275913 and rs3748067) on the susceptibility to ulcerative colitis. Clin Exp Med 2013;13:239–44.
[35] Park JS, Park BL, Kim MO, et al. Association of single nucleotide polymorphisms on interleukin 17 receptor A (IL17RA) gene with aspirin hypersensitivity in asthmatics. Hum Immunol 2013;74:598–606.
[36] Coto E, Gomez J, Suarez B, et al. Association between the IL17RA rs4819554 polymorphism and reduced renal filtration rate in the Spanish RENASTUR cohort. Hum Immunol 2015;76:75–8.
[37] Kim YG, Kim EY, Ihm CG, et al. Gene polymorphisms of interleukin-17 and interleukin-17 receptor are associated with end-stage kidney disease. Am J Nephrol 2012;36:472–7.
[38] Lew BL, Cho HR, Haw S, et al. Association between IL17A/IL17RA gene polymorphisms and susceptibility to alopecia areata in the Korean population. Ann Dermatol 2012;24:61–5.
[39] Lee YC, Chung JH, Kim SK, et al. Association between interleukin 17/ interleukin 17 receptor gene polymorphisms and papillary thyroid cancer in Korean population. Cytokine 2015;71:283–8.
[40] Zhang X, Pei F, Zhang M, et al. Interleukin-17A gene variants and risk of coronary artery disease: a large angiography-based study. Clin Chim Acta 2011;412:327–31.
[41] Vargas-Alarcon G, Angeles-Martinez J, Villarreal-Molina T, et al. Interleukin-17A gene haplotypes are associated with risk of premature coronary artery disease in Mexican patients from the Genetics of Atherosclerotic Disease (GEA) study. PLoS ONE 2015;10:e0114943.
[42] Han Q, Das S, Hirano M, et al. Characterization of lamprey IL-17 family members and their receptors. J Immunol 2015;195:5440–51.
[43] Wu L, Ong S, Talor MV, et al. Cardiac fibroblasts mediate IL-17A-driven inflammatory dilated cardiomyopathy. J Exp Med 2014;211:1449–64.
[44] Espinoza JL, Takami A, Nakata K, et al. A genetic variant in the IL-17 promoter is functionally associated with acute graft-versus-host disease after unrelated bone marrow transplantation. PLoS ONE 2011;6: e26229.
[45] Chen J, Deng Y, Zhao J, et al. The polymorphism of IL-17 G-152A was associated with childhood asthma and bacterial colonization of the hypopharynx in bronchiolitis. J Clin Immunol 2010;30:539–45.
[46] Zhang S, Liu Y, Lu S, et al. Genetic variants of interleukin 17A are functionally associated with increased risk of age-related macular degeneration. Inflammation 2015;38:658–63.
[47] Ocejo-Vinyals JG, de Mateo EP, Hoz MA, et al. The IL-17 G-152A single nucleotide polymorphism is associated with pulmonary tuberculosis in northern Spain. Cytokine 2013;64:58–61.
[48] Stappers MH, Thys Y, Oosting M, et al. Polymorphisms in cytokine genes IL6, TNF, IL10, IL17A and IFNG influence susceptibility to complicated skin and skin structure infections. Eur J Clin Microbiol Infect Dis 2014;33:2267–74.
[49] Bogunia-Kubik K, Swierkot J, Malak A, et al. IL-17A, IL-17F and IL-23R gene polymorphisms in polish patients with rheumatoid arthritis. Arch Immun Ther Exp 2015;63:215–21.
[50] Nordang GB, Viken MK, Hollis-Moffatt JE, et al. Association analysis of the interleukin 17A gene in Caucasian rheumatoid arthritis patients from Norway and New Zealand. Rheumatology (Oxford) 2009;48:367–70.
[51] Li N, Zhu Q, Li Z, et al. IL17A gene polymorphisms, serum IL-17A and IgE levels, and hepatocellular carcinoma risk in patients with chronic hepatitis B virus infection. Mol Carcinog 2014;53:447–57.
[52] Zhou B, Zhang P, Wang Y, et al. Interleukin-17 gene polymorphisms are associated with bladder cancer in a Chinese Han population. Mol Carcinog 2013;52:871–8.
[53] Liu J, Xu Q, Yuan Q, et al. Association of IL-17A and IL-17F polymorphisms with gastric cancer risk in Asians: a meta-analysis. Hum Immunol 2015;76:6–12.
[54] Quan Y, Zhou B, Wang Y, et al. Association between IL17 polymorphisms and risk of cervical cancer in Chinese women. Clin Dev Immunol 2012;2012:258293.
[55] Peng Y, Zhou B, Wang YY, et al. Analysis of IL-17 gene polymorphisms in Chinese patients with dilated cardiomyopathy. Hum Immunol 2013;74:635–9.
[56] Kim YG, Ihm CG, Lee TW, et al. Association of genetic polymorphisms of interleukins with new-onset diabetes after transplantation in renal transplantation. Transplantation 2012;93:900–7.
[57] Lima L, Oliveira D, Ferreira JA, et al. The role of functional polymorphisms in immune response genes as biomarkers of bacille Calmette-Guerin (BCG) immunotherapy outcome in bladder cancer: establishment of a predictive profile in a Southern Europe population. BJU Int 2015;116:753–63.
[58] Kim SW, Kim ES, Moon CM, et al. Genetic polymorphisms of IL-23R and IL-17A and novel insights into their associations with inflammatory bowel disease. Gut 2011;60:1527–36.
[59] Mann DL. Innate immunity and the failing heart: the cytokine hypothesis revisited. Circ Res 2015;116:1254–68.
[60] Liu XK, Lin X, Gaffen SL. Crucial role for nuclear factor of activated T cells in T cell receptor-mediated regulation of human interleukin-17. J Biol Chem 2004;279:52762–71.