ErbB4 gene polymorphism is associated with the risk and prognosis of congestive heart failure in the Northern Han Chinese population

YUNHONG WANG, MM,¹ YUHUI ZHANG, MD,¹ TAO AN, MD,¹ RONGCHENG ZHANG, MD,¹¹ XUEMEI ZHAO, MB,¹ NINI LIU, MB,¹ SHIJIE YIN, MM,¹ TIANYI GAN, MD,¹ TUO LIANG, MM,¹ YAN HUANG, MM,¹ QIONG ZHOU, MB,¹ JIAN ZHANG, MD, PhD,¹

¹ From the State Key Laboratory of Cardiovascular Disease, Heart Failure Center Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Correspondence author: Jian Zhang, MD, PhD, 167 Beilishi Road, Beijing, 100037, China. Tel: 0086-13911102015; Fax: 0086 (10) 8839 6180. E-mail: fwzhangjian62@126.com

The first 2 authors contributed equally to this work.

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Graphical Abstract

HF: heart failure; SNP: single nucleotide polymorphism; NRG-1: neuregulin-1; CV: cardiovascular.

Abstract: Background: There has been no research evaluating the association between human Neuregulin-1(NRG-1)/ErbB2/ErbB4 gene polymorphisms and heart failure risk.

Methods and Results: Genotyping of thirteen single nucleotide polymorphisms (SNPs) in the NRG-1/ ErbB2/ ErbB4 genes was performed in 569 unrelated heart failure patients and 682 healthy controls from the Northern Han Chinese population using iPLEX SNP Genotyping analysis on a Sequenom MassARRAY® System. In the ErbB4 gene, the variants rs10932374 and rs1595064 were associated with reduced risk of heart failure under allelic, recessive and additive genetic models, and the variants rs13003941 and rs1595065 were associated with increased risk of heart failure under allelic, dominant and additive models. The G-G-C-C-T haplotype of rs10932374-rs13003941-rs1595064-rs1595065-rs3748960 in the ErbB4 gene increased the risk of heart failure (OR=1.35, 95%CI [1.06-1.70], P=0.014). The T variant of rs13003941 was associated with larger left ventricle (dominant model, P=0.014; additive model, P=0.048), and increased risk of overall death (RR=1.48, 95%CI [1.01-2.18], P=0.045) and cardiovascular death (RR=1.56, 95%CI [1.04-2.33], P=0.03) after adjusting for age and gender. NRG-1/ErbB2 gene polymorphisms were not associated with heart failure risk or prognosis.

Conclusion: ErbB4 gene polymorphisms were associated with the risk, severity and prognosis of heart failure in the Northern Han Chinese population.

Keywords: ErbB4, polymorphism, heart failure.

INTRODUCTION

Congestive heart failure is a complex clinical syndrome that is caused by damage to the heart or increased myocardial stress stemming from various cardiovascular diseases. The clinical phenotype of heart failure that presents in an individual patient is determined not only by the underlying causes but also by genetic factors ^[1]. Due to the complexity of heart failure, it is difficult to characterize its genetic background for sporadic cases. However, understanding the genetic mechanism of heart failure is helpful for personalized diagnosis and treatment of the disease.

Neuregulin-1 (NRG-1) is a member of the epidermal growth factor (EGF) family synthesized by endothelial cells of the cardiac microvasculature and of the endocardium. The ErbB2/ ErbB4 proteins are members of the EGF family of tyrosine kinase receptors. NRG-1 can bind to ErbB4 receptor on cardiomyocytes, then induces ErbB4–ErbB2 heterodimerization, and finally activates subsequent downstream signaling cascades ^[2]. Previous studies suggest that the endogenous NRG-1/ErbB2/ErbB4 signaling system might play an important role in restoring cardiac function after initial injury and might thus participate in the progression of heart failure ^[3]. To date, the major investigations have focused on the use of recombinant NRG-1 beta in heart failure treatment and its mechanism in myocardial reverse remodeling ^[4,5]. Given the critical role of the NRG-1/ErbB2/ErbB4 signaling pathway in maintenance of normal heart structure and function, the genes encoding NRG-1/ErbB2/ErbB4 protein are appropriate candidates for the genetic study of heart failure.

The basis of our hypothesis was that NRG-1/ErbB2/ErbB4 signaling mediates cellular adaptations to stress and restores myocardial function after injury through a series of pathways; therefore, different functional genotypes might lead to different presentations of heart failure and might be associated with susceptibility to heart failure. In this respect, we undertook this study to examine whether polymorphisms in the NRG-1/ErbB2/ErbB4 genes were associated with the progression and prognosis of heart failure.

METHODS

Study subjects

The protocol was approved by the Ethics Committee of Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union of Medical College. All subjects provided written informed consent. This study complies with the Declaration of Helsinki.

Heart failure patients who agreed to participate in the genetic study were recruited from the heart failure center of Fuwai Hospital between March 2009 and April 2014.

The inclusion criterion for enrollment was a clinical diagnosis of heart failure with reduced ejection fraction (<50%) according to the guideline ^[6,7]. The exclusion criteria were primary valvular heart disease, congenital heart disease, pulmonary heart disease, pericarditis, tumor or immune system disorders. Etiological diagnoses were determined by clinical assessment, echocardiography and coronary angiography (or coronary CT angiography), as well as cardiac magnetic resonance imaging and cardiac radionuclide examination if clinically indicated. The etiological categories included non-ischemic cardiomyopathy and ischemic heart disease (IHD). IHD was defined by at least one of the following conditions: $\geq 50\%$ coronary luminal obstruction, history of myocardial infarction, history of coronary stent implantation and/or coronary artery bypass graft. Cardiomyopathy was classified as nonischemic when coronary heart disease was excluded. All the patients' electronic medical records included their familial history. Their relatives' hospitalization information in our hospital were also recorded. Thus we could know whether they were related or not. A total of 1044 patients were screened, and finally, 569 unrelated patients met the enrollment criteria and were included in the present study.

Control subjects with comparable age and gender were selected from a community-based echocardiographic survey study, which collected detailed demographic and medical information to investigate the prevalence and risk factors of cardiovascular disease in the Beijing area between March 2012 and August 2012. According to the inclusion criteria, these individuals should be unrelated to one another and were apparently healthy as assessed by questionnaire, physical examination, serum biochemical testing and echocardiography. The exclusion criteria were history or symptoms of cardiovascular disease, on any medications for cardiovascular disease, and diagnosis of diabetes mellitus. A total of 682 individuals with comparable age and gender were enrolled.

All subjects were of Northern Han Chinese origin and we ascertained the subjects' ethnicity according to their resident identity card.

Echocardiography was performed by independent ultrasound staff who were blinded to the polymorphism analysis results. The echo parameters, such as left ventricular ejection fraction and internal dimensions, were measured according to standard methods.

Single nucleotide polymorphism (SNP) genotyping

Blood was taken into EDTA-containing receptacles. Genomic DNA was extracted from white blood cells in peripheral venous blood according to standard salting-out method.

A total of 6 SNPs encompassing the 1.163 MB region of the ErbB4 gene, 2 SNPs encompassing the 40.52kB region of the ErbB2 gene, and 5 SNPs encompassing the 1.126 MB region of the NRG-1 gene were selected for genotyping, and they were identified from the SNP database in NCBI (<http://www.ncbi.nlm>) and the international HapMap project (<http://www.hapmap.org/index.html>. Release No.24/phase II Nov08, on NCBI B 36 assembly). All the SNPs were selected based on the following principles: the SNPs are from possible functional regions of each gene (i.e., exons,

gene flanking regions and the junctional area between exon and intron); the minimum allele frequency is greater than 0.05. Tagging SNPs were selected for analysis based on $r^2 > 0.8$ by running the Haploview tagger program.

SNP genotyping was performed by an iPLEX SNP Genotyping analyzer using the Sequenom MassARRAY® System in 384-well plates. (Sequenom, Inc., San Diego, CA, <http://www.sequenom.com/>).

All assays for the PCR and associated extension reactions were designed by on-line Assay Design Suite software (Sequenom), and the primers were synthesized by Invitrogen (Thermo Fisher Scientific). PCR conditions, allele specific primer extension and analysis of their products were as performed according to the standard method provided by the Sequenom MassARRAY® SNP technology. The resulting mass spectra were processed and analyzed for peak identification and allele determination by the MassARRAY Typer 4.0 software (Sequenom). In order to ensure that the obtained genotypes were valid, each of the 13 SNPs was genotyped in duplicate in 5% of the DNA samples. All results were in full agreement. Genotyping was performed blindly to all other data. Genotyping call rates were between 92.6% and 100%, and most were higher than 99%.

Statistical analysis

Continuous variables were tested for normal distribution by means of the Kolmogorov-Smirnov test and are described as mean $\pm$ SD for normally distributed variables and median (interquartile range [IQR]) for variables with skewed distribution. Two-tailed independent t test was used to compare the difference of continuous variables between heart failure (HF) and control groups. Kruskal-Wallis H test was used to compare the difference of nonnormal continuous variables among three genetic models. Comparison of categorical variables was performed by the chi-square test.

Deviation from Hardy–Weinberg Equilibrium (HWE) for each SNP in the control group was assessed by a chi-square goodness of fit test. The chi-square test was used to evaluate differences in the allelic and genotypic frequencies of cases and controls. Logistic-regression methods were used to analyze the effect of genotype specified by different genetic models or allele on the risk of heart failure. For outcome analysis, Kaplan–Meier plots and log-rank tests were used to assess whether survival differed significantly among subgroups defined according to genotype. Cox regression analysis was used to quantify the relative risk of an event over time by genotype or allele class. All the above analyses were performed using SPSS (Version 19.0; SPSS, IBM company). Linkage disequilibrium (LD) and haplotype blocks were identified using Haploview4.2. The Haplo.stats program developed by R language version 3.1.2 (<http://www.r-project.org>) was used to perform haplotype analysis. The threshold of significance was set as $p < 0.05$. False discovery rate (FDR) control was utilized to adjust for multiple testing^[8].

RESULTS

Characteristics of the subjects

A total of 1251 unrelated participants comprising 569 heart failure patients (442 men and 127 women, mean age= 60y; SD±10.0) and 682 controls (532 men and 150 women, mean age=60.4y; SD±7.4) were recruited for this study. The clinical characteristics of the patients and controls are presented in Table 1. The percentage of hypertension was not different between the two groups. Compared with the control group, systolic and diastolic blood pressure were lower in the heart failure group. Biochemical measurements such as NT-proBNP (amino-terminal pro-brain natriuretic peptide), blood uric acid, and creatinine were higher in the heart failure group. Significant differences in body mass index (BMI), left atrial diameter (LAD), left ventricular end diastolic diameter (LVEDD) and left ventricular ejection fraction (LVEF) were also observed between patients and controls. Etiologies consist of ischemic (46.9%) and non- ischemic (53.1%) heart failure.

Association between gene polymorphism and heart failure

There was no departure from HWE for any of the SNPs in the control group (Supplementary Table1). The allele frequencies of the 13 polymorphisms in the NRG-1/ ErbB2/ ErbB4 genes between heart failure patients and controls are shown in Table 2. Subgroup analysis indicated that the allele frequency was not different between ischemic and nonischemic patients for all SNPs (Supplementary Table2). The frequencies of these gene polymorphisms in other populations were shown in Supplementary Table3.

Univariate analysis showed that the A allelic polymorphism of rs10932374 and the G allelic polymorphism of rs1595064 in the ErbB4 gene were significantly associated with reduced risk of heart failure (OR=0.84, 95%CI[0.71-0.99], P=0.039; OR=0.80, 95%CI[0.69-0.94], P=0.007, respectively); whereas the T allelic polymorphism of rs13003941 and the C allelic polymorphism of rs1595065 in the ErbB4 gene were significantly associated with increased risk of heart failure (OR=1.25, 95%CI[1.05-1.50], P=0.014; OR=1.30, 95%CI[1.08-1.56], P=0.005, respectively). After adjusting for the potential confounding effects of age, gender, BMI, smoking, drinking, hypertension history and uric acid, three allelic polymorphisms of rs10932374, rs1595064 and rs13003941 were still significantly associated with risk of heart failure, suggesting that the A allele of rs10932374 and G allele of rs1595064 might be protective factors, and the T allele of rs13003941 might be a risk factor, for heart failure in the Northern Han Chinese (Table 2).

The distributions of genotypes in different genetic models in the case and control groups are summarized in Table 3. Four polymorphisms in the ErbB4 gene were associated with risk of heart failure. Two polymorphisms, rs10932374 and rs1595064, were associated with reduced risk of heart failure in both a recessive genetic model (P=0.009 and P=0.001, respectively) and an additive genetic model (P=0.042 and P=0.007, respectively). The other two polymorphisms, rs13003941 and rs1595065, were associated with increased risk of heart failure in both a dominant genetic model (P=0.004 and P=0.000, respectively) and an additive genetic model (P=0.013 and P=0.004, respectively). After adjusting for the confounding variables of age, gender, BMI, smoking, drinking, hypertension history and uric acid, the association between

heart failure and the gene polymorphisms rs10932374, rs1595064, rs13003941 and rs1595065 was still significant, except for rs1595065 analyzed in additive model (Table 3).

One polymorphism of rs12674586 in the NRG-1 gene was significantly associated with heart failure, but the association disappeared after adjusting for confounding factors. No association was found between other SNPs in the NRG-1 and ErbB2 genes and heart failure (Table 3).

FDR control was shown in Supplementary Table 4 & 5.

Haplotype analysis

Haploview was used to conduct linkage disequilibrium evaluation (LD). LD evaluation of NRG-1/ErbB2/ErbB4 gene polymorphisms is presented in Figure 1. No complete LD was found for any of the 13 SNPs. Five SNPs (rs10932374, rs1595064, rs13003941, rs1595065 and rs3748960) in the ErbB4 gene were in strong LD and in the same LD block. Therefore, haplotypes were constructed on the basis of the five SNPs because rs2128321 was too far away from the other SNPs. All of the SNPs in the ErbB2 gene and NRG-1 gene were used for haplotype analysis.

Five haplotypes with frequency greater than 1% were detected for the ErbB4 gene in haplotype analysis. The A-G-G-T-T haplotype of rs10932374-rs13003941-rs1595064-rs1595065-rs3748960 in the ErbB4 gene was the most prevalent. Its frequency was significantly lower in the patients than in the controls (case vs. control = 33% vs. 37%, permutation p value = 0.045). Compared with the A-G-G-T-T haplotype, haplotype G-G-C-C-T increased the risk of heart failure (OR=1.35, 95%CI [1.06-1.70], P=0.014) (Table 4). After adjusting for age and gender, this haplotype was still significantly associated with risk of heart failure (P=0.013). FDR control was shown in Supplementary Table 6. None of the haplotypes constructed from the NRG-1/ErbB2 genes was found to be significantly associated with heart failure (Supplementary Table 7 & 8).

In silico analysis of ErbB4 polymorphism

A freely available website (<http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm>) were utilized to predict functional characteristics of the related ErbB4 SNPs [9]. Rs1595064 was located in the miRNA-binding site, but did not affect miRNA-binding site activity because the miRanda scores for the two alleles differed by <16 [9]. However, rs10932374 was not found to be located in miRNA-binding site. Mutant alleles of both rs1595065 and rs13003941 differed from their wild-type alleles in binding miRNA and might affect miRNA-binding site activity (Table 5).

Association of ErbB4 polymorphism with clinical characteristics in heart failure patients

Association analysis revealed that 4 SNPs in the ErbB4 gene (rs10932374, rs13003941, rs1595064, rs1595065) were significantly associated with risk of heart failure, thus an analysis of the influence of the 4 SNPs on the clinical features of heart

failure patients was performed and is summarized in Table 6. The presence of the T variant of rs13003941 was associated with increased LVEDD (dominant model, $P=0.014$; additive model, $P=0.048$). The A variant of rs10932374 did not consistently relate with increased LVEDD (additive model, $P=0.10$, dominant model, $P=0.037$). Other indexes were not significantly different among the different genotype groups of all the other SNPs.

Effect on prognosis of heart failure

Kaplan–Meier survival analysis indicated that the T variant of rs13003941 in the ErbB4 gene tends to lower overall survival rate compared with GG homozygotes in heart failure patients, but without statistical significance (global log rank test: $P=0.065$) (Figure 2A). Corresponding Cox regression analysis revealed that the relative risk of overall death became significantly increased after adjusting for age and gender ($RR=1.48$, 95%CI [1.01-2.18], $P=0.045$).

Moreover, the T variant of rs13003941 was also associated with increased risk of cardiovascular death (unadjusted $RR=1.50$, 95%CI [1.01-2.25], $P=0.047$; adjusted $RR=1.56$, 95%CI [1.04-2.33], $P=0.03$, the adjusting factors: age and gender), suggesting that the T allele of rs13003941 might be a potential risk factor for poor heart failure prognosis. No other SNPs were observed to be associated with heart failure prognosis (Supplementary Table 9 & 10).

No difference of prognosis was observed across the ischemic and nonischemic groups (nonischemic v.s. ischemic: for overall death, $P=0.739$; for cardiovascular death, $P=0.617$). The T variant of rs13003941 was still associated with increased risk of overall death ($RR=1.801$, 95%CI [1.013-3.202], $P=0.045$) and cardiovascular death ($RR=1.915$, 95%CI [1.046-3.505], $P=0.035$) after adjusting for age, gender and the etiology of HF. Subset analysis revealed that the impact of the T variant of rs13003941 on survival was primarily evident in patients with ischemic cardiomyopathy (global log rank test: $P=0.05$ for ischemics [$n=267$] and $P=0.533$ for nonischemics [$n=302$]) (Figure 2B & 2C). After adjusting for age and gender, Cox regression analysis showed an increased relative risk of 1.81 for overall death in the ischemic subgroup with the T variant (95% CI [1.02-3.23]; $P=0.043$). In the ischemic subgroup, increased relative risk was also observed for cardiovascular death both before and after adjustment for age and gender ($RR=1.86$, 95%CI [1.02-3.41], $P=0.044$; $RR=1.93$, 95%CI [1.05-3.53], $P=0.034$, respectively). This variant did not affect the risk of cardiovascular death in nonischemics ($P=0.462$).

The G-T-C-T-T haplotype of rs10932374-rs13003941-rs1595064-rs1595065-rs3748960 in the ErbB4 gene was associated with increased risk of overall death (unadjusted $RR=1.44$, 95%CI [0.98-2.12], $P=0.062$; adjusted $RR=1.49$, 95%CI [1.01-2.19], $P=0.044$) and cardiovascular death (unadjusted $RR=1.51$, 95%CI [1.01-2.26], $P=0.045$; adjusted $RR=1.56$, 95%CI [1.04-2.34], $P=0.031$) (the adjusting factors: age and gender; Table 7).

Discussion

In this study, we found that four genetic single nucleotide polymorphisms in the ErbB4 gene were associated with heart failure, with the T variant of rs13003941 and C variant of rs1595065 increasing risk of heart failure, and AA homozygotes of rs10932374 and GG homozygotes of rs1595064 showing decreased risk. The T variant of rs13003941 was also associated with enlargement of the left ventricle. Finally, the T variant of rs13003941 indicated poor prognosis after heart failure, increasing the risk of overall death and cardiovascular death. We did not observe any association between NRG-1 or ErbB2 gene polymorphisms and heart failure.

We selected the NRG-1/ErbB2/ErbB4 genes as candidate risk factors for heart failure because the NRG-1/ErbB2/ErbB4 signaling pathway plays a critical role in the regulation of cardiac myocyte biology and normal heart function^[3,10-12].

NRG-1 beta, expressed the most abundantly by cardiac endothelial cells in humans, interacts with the tyrosine kinase family receptors (ErbB2/ErbB4) to induce the formation of homologous or heterologous dimerization^[2]. The protein's conformational changes further cause intracellular kinase receptor phosphorylation and activate intracellular downstream signal transduction pathways, including mitogen activated protein kinase pathways (MAPK), phosphatidylinositol-3 kinase/serine threonine kinase (PI3K/AKT) signal pathways, and they launch a series of biological reactions^[13], taking part in the differentiation, proliferation, growth, survival and apoptosis of cardiomyocytes. In addition, the NRG-1/ErbB2/ErbB4 signal system also functions similar to the M cholinergic system. It can inhibit excessive activation of the sympathetic nervous system, and thus it might reverse ventricular remodeling^[14,15]. The relationship between the NRG-1/ErbB2/ErbB4 system and cardiovascular disease is mainly embodied in two aspects^[16]: Firstly, it is crucial for the normal development of the embryonic heart. Secondly, it maintains the normal structure and function of the heart after birth and plays a role in restoring cardiac function after injury.

Experimental rats in which the genes for ErbB2 had been inactivated presented with a series of dilated cardiomyopathy manifestations, such as ventricular dilatation and wall thinning, as well as decreased contractility^[17]. Moreover, NRG-1/ErbB2/ErbB4 gene-defective myocardial cells were also more susceptible to anthracycline's toxicity, and myocardial fibers were abnormally arranged^[17,18]. These studies suggest that factors that depress the NRG-1/ErbB2/ErbB4 signaling pathway might predispose patients to develop heart failure. On the other hand, in both heart failure animal models and heart failure patients, including ischemic, viral, and anthracycline-induced heart failure, recombinant NRG-1 was found to be beneficial in heart failure treatment by reducing myocardial necrosis and improving cardiac function and survival rate^[19]. This finding indicates that an activated NRG-1/ErbB2/ErbB4 signaling pathway prevents the progression of heart failure.

Therefore, it is meaningful to determine whether polymorphisms in the NRG-1/ErbB2/ErbB4 genes are associated with heart failure. Previous studies mainly focused on association studies between schizophrenia and NRG-1/ErbB^[10]. Recently, an investigation discovered a comorbid SNP that links schizophrenia and sudden cardiac death^[21]. However, to date, there have been no studies conducted to directly

address this hypothesis in heart failure.

The selected SNPs obtained from the Hapmap represented tagged SNPs in functional areas (exons, gene flanking regions and the junction area between exon and intron) of the NRG-1/ErbB2/ErbB4 genes. All of the four significant SNPs are located in the 3'-UTR (untranslated region) of the ErbB4 gene. Although they have not been reported by other studies, on the basis of this result and the molecular properties of the ErbB4 receptor ^[22,23], we consider that the significant SNPs found in this study might be tagSNPs or functional SNPs that play a role in the development and progression of heart failure. Besides, through in silico analysis, we found rs1595065 and rs13003941 were located in miRNA-binding site and their mutant alleles might affect miRNA-binding site activity. Therefore, our work indicated that it might be worth exploring the underlying cellular biological mechanism of these SNPs in HF in the future.

Because haplotypes contain genetic information on multiple SNPs and haplotype analysis has stronger statistical power than analysis of single SNPs, we further analyzed haplotypes of SNPs in the NRG-1/ErbB2/ErbB4 genes. This analysis revealed that one haplotype of the ErbB4 gene increased the risk of heart failure, but no haplotype of the NRG-1/ErbB2 genes was associated with heart failure, in accordance with the single SNP analysis. Further analysis demonstrated that the gene variant rs13003941 was associated with severity of heart failure and poor prognosis, indicating a potential role of rs13003941 in progression of heart failure. However, this phenomenon was primarily observed in ischemic cardiomyopathy, rather than nonischemics. One study showed that increased activity of the NRG-1/ErbB4 signaling pathway promotes myocardial regeneration by inducing differentiated cardiomyocytes to proliferate and regenerate after myocardial infarction ^[12]. Nevertheless, the exact mechanism underlying the association between rs13003941 and heart failure prognosis remains to be defined.

To sum up, our study provided some clues regarding the potential clinical association of the ErbB4 gene with heart failure. It is reasonable to initiate a more comprehensive survey, and functional experiments to explore the exact mechanism need to be performed in the future.

The current study was not designed to ascertain effects of gene polymorphisms on the response to treatment. Some clinical studies using recombinant NRG-1 beta treatment have observed improvement in indexes of cardiac output and hemodynamic changes ^[10,19]. Whether treatment effects differ according to the presence of NRG-1/ErbB2/ErbB4 gene polymorphisms remains unknown. Further studies could focus on the role of ErbB4 gene polymorphisms in tailoring pharmacologic therapy.

This study has several limitations. Firstly, although we found four SNPs in the ErbB4 gene, we still could not exclude false positive results, thus replication studies should be performed in another independent sample set in the future. Secondly, this study did not assess the expression of NRG-1/ErbB2/ErbB4 gene in the tissue or serum, therefore it could not be withdrawn from this study that the four SNPs in the ErbB4 gene affected the gene expression. Thirdly, we did not perform functional studies to analyze the effects of the risk alleles and protective alleles of ErbB4 gene.

Therefore, the exact relationship between ErbB4 gene polymorphisms and heart failure could not be elucidated from the current study.

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Figure 1. A: Block structure of linkage disequilibrium (LD) for genotyped single nucleotide polymorphisms (SNPs) in the NRG-1 gene. B: Block structure of LD for SNPs in the ErbB2 gene. C: Block structure of LD for SNPs in the ErbB24 gene. Stronger correlations between these SNPs are noted by red color in the intersecting squares linking each pair of SNPs

Figure 2. Overall survival by rs13003941 polymorphism. The solid line indicates subjects with the T variant; the dashed line, GG homozygotes. A, All heart failure patients (n=485): T variants (n=276), GG homozygotes (n=209). B, Patients with ischemic cardiomyopathy (n=231): T variants (n=126), GG homozygotes (n=105). C, Patients with nonischemic cardiomyopathy (n=254): T variants (n=150), GG homozygotes (n=104).

Table 1. Characteristics of participants

Variable	HF(n=569)	Controls(n=682)	P
Age(years)	60.0±10.0	60.4±7.4	0.259
Male (n, %)	442(77.7%)	532(78.0%)	0.89
History (n, %)			
Hypertension	241(42.4%)	325(47.7%)	0.061
Smoking	311(54.7%)	250(36.7%)	<0.001
Drinking	213(37.4%)	172(25.2%)	<0.001
Physical examination			
Body mass index (kg/m ²)	24.2±4.0	25.85±3.55	<0.001
Systolic blood pressure (mmHg)	115.2±19.3	129.4±19.0	<0.001
Diastolic blood pressure (mmHg)	71.8±12.5	80.9±10.7	<0.001
Echocardiography parameter			
LAD (mm)	44.7±8.1	32.6±4.3	<0.001
LVEDD (mm)	66.1±10.7	45.2±4.2	<0.001
LVEF (%)	32.0±8.2	68.6±6.5	<0.001
Laboratory parameter			
Creatine(umol/L)	101.8±43.1	69.6±17.2	<0.001
Uric acid(umol/L)	466.5±149.2	296.6±82.9	<0.001
NT-proBNP(pg/ml)	2924.4±3303.7	73.2±155.6	<0.001
Etiology of HF (n, %)			
Ischemic	267(46.9%)	-	
Non-ischemic	302(53.1%)	-	
NYHA class (n, %)			
II	110(19.3%)	-	
III	258(45.3%)	-	
IV	201(35.3%)	-	

HF: heart failure; LAD: left atrial diameter; LVEDD: left ventricular end diastolic diameter; LVEF: left ventricular ejection fraction; NT-proBNP: amino-terminal pro-brain natriuretic peptide; Values are mean±SD.

Table 2. Allele frequency of 13 *NRG-1/ErbB2/ErbB4* gene polymorphisms in Northern Han Chinese heart failure patients and controls

SNP Allele	HF (n,%)	Controls (n,%)	OR(95%CI)	P	OR*(95%CI)	P*
NRG-1 gene						
rs12674586						
A	226(22.2%)	328(24.0%)	0.90(0.74-1.09)	0.280	0.95(0.73-1.23)	0.684
G	794(77.8%)	1036(76.0%)	1.00	-	1.00	-
rs3735774						
A	67(5.9%)	73(5.4%)	1.11(0.79-1.56)	0.562	0.93(0.58-1.47)	0.743
G	1071(94.1%)	1291(94.6%)	1.00	-	1.00	-
rs3735775						
A	368(32.4%)	423(31.2%)	1.06(0.89-1.25)	0.522	1.02(0.81-1.28)	0.875
G	768(67.6%)	933(68.8%)	1.00	-	1.00	-
rs3808368						
T	489(43.3%)	619(45.4%)	0.92(0.78-1.08)	0.292	0.86(0.69-1.07)	0.167
A	641(56.7%)	745(54.6%)	1.00	-	1.00	-
rs3924999						
C	266(23.4%)	328(24.3%)	0.95(0.79-1.14)	0.591	0.95(0.74-1.23)	0.706
T	872(76.6%)	1022(75.7%)	1.00	-	1.00	-
ErbB2 gene						
rs1058808						
G	495(43.5%)	612(45.1%)	0.94(0.80-1.10)	0.432	0.87(0.70-1.08)	0.202
C	643(56.5%)	746(54.9%)	1.00	-	1.00	-
rs2517956						
A	480(44.3%)	607(45.4%)	0.96(0.82-1.12)	0.593	0.86(0.70-1.08)	0.189
G	604(55.7%)	731(54.6%)	1.00	-	1.00	-
ErbB4 gene						
rs10932374						
A	380(33.7%)	511(37.7%)	0.84(0.71-0.99)	0.039	0.76(0.61-0.95)	0.017
G	746(66.3%)	843(62.3%)	1.00	-	1.00	-
rs13003941						
T	324(33.4%)	385(28.6%)	1.25(1.05-1.50)	0.014	1.35(1.06-1.72)	0.015
G	646(66.6%)	961(71.4%)	1.00	-	1.00	-
rs1595064						
G	505(44.5%)	669(49.9%)	0.80(0.69-0.94)	0.007	0.74(0.60-0.92)	0.007
C	631(55.5%)	671(50.1%)	1.00	-	1.00	-
rs1595065						
C	305(26.8%)	300(22.1%)	1.30(1.08-1.56)	0.005	1.26(0.98-1.62)	0.069
T	831(73.2%)	1060(77.9%)	1.00	-	1.00	-
rs2128321						
T	101(8.9%)	120(8.8%)	1.01(0.77-1.34)	0.933	1.21(0.83-1.76)	0.322
G	1033(91.1%)	1242(91.2%)	1.00	-	1.00	-
rs3748960						
C	81(7.2%)	81(5.9%)	1.22(0.89-1.68)	0.223	1.27(0.81-1.97)	0.297

T	1051(92.8%)	1281(94.1%)	1.00	-	1.00	-
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NRG-1: neuregulin-1; SNP: single nucleotide polymorphism; HF: heart failure; * adjusted for age, gender, BMI, smoking, drinking, hypertension history and uric acid.

Table 3. Distribution of *NRG-1/ErbB2/ErbB4* genotypes in Northern Han Chinese heart failure patients and controls

SNP	Model	Contrast	HF	Controls	OR(95%CI)	P	OR*(95%CI)	P*
NRG-1 gene								
rs12674586	Dominant	AA+AG/GG	187/323	292/390	0.77(0.61-0.98)	0.032	0.80(0.58-1.09)	0.160
	Recessive	AA/AG+GG	39/471	36/646	1.49(0.93-2.37)	0.097	1.73(0.94-3.19)	0.077
	Additive	AA/AG/GG	39/148/323	36/256/390	0.90(0.75-1.09)	0.292	0.95(0.74-1.22)	0.693
rs3735774	Dominant	AA+AG/GG	67/502	71/611	1.15(0.81-1.64)	0.443	0.98(0.60-1.59)	0.932
	Recessive	AA/AG+GG	0/569	2/680	0	0.999	0	0.999
	Additive	AA/AG/GG	0/67/502	2/69/611	1.11(0.79-1.57)	0.556	0.92(0.58-1.48)	0.74
rs3735775	Dominant	AA+AG/GG	306/262	367/311	0.99(0.79-1.24)	0.928	0.95(0.70-1.29)	0.754
	Recessive	AA/AG+GG	62/506	56/622	1.36(0.93-1.99)	0.112	1.26(0.75-2.10)	0.378
	Additive	AA/AG/GG	62/244/262	56/311/311	1.06(0.89-1.26)	0.516	1.02(0.81-1.29)	0.873
rs3808368	Dominant	TT+AT/AA	393/172	479/203	0.97(0.76-1.24)	0.795	0.89(0.64-1.24)	0.479
	Recessive	TT/AT+AA	96/469	140/542	0.79(0.59-1.06)	0.113	0.72(0.48-1.06)	0.098
	Additive	TT/AT/AA	96/297/172	140/339/203	0.92(0.78-1.08)	0.284	0.85(0.68-1.06)	0.159
rs3924999	Dominant	CC+CT/TT	238/331	289/386	0.96(0.77-1.20)	0.726	0.97(0.71-1.32)	0.839
	Recessive	CC/CT+TT	28/541	39/636	0.84(0.51-1.39)	0.505	0.82(0.41-1.63)	0.567
	Additive	CC/CT/TT	28/210/331	39/250/386	0.95(0.79-1.15)	0.588	0.95(0.74-1.23)	0.702
ErbB2 gene								
rs1058808	Dominant	GG+GC/CC	392/177	470/209	0.99(0.77-1.25)	0.901	0.85(0.61-1.18)	0.319
	Recessive	GG/GC+CC	103/466	142/537	0.84(0.63-1.11)	0.213	0.80(0.55-1.18)	0.266
	Additive	GG/GC/CC	103/289/177	142/328/209	0.94(0.80-1.10)	0.432	0.87(0.70-1.08)	0.198
rs2517956	Dominant	AA+AG/GG	373/169	463/206	0.98(0.77-1.25)	0.884	0.81(0.58-1.13)	0.208
	Recessive	AA/AG+GG	107/435	144/525	0.90(0.68-1.19)	0.447	0.85(0.58-1.24)	0.393
	Additive	AA/AG/GG	107/266/169	144/319/206	0.96(0.82-1.12)	0.598	0.87(0.70-1.08)	0.193
ErbB4 gene								
rs10932374	Dominant	AA+AG/GG	319/244	403/274	0.89(0.71-1.12)	0.308	0.74(0.55-1.02)	0.062
	Recessive	AA/AG+GG	61/502	108/569	0.64(0.46-0.90)	0.009	0.61(0.39-0.97)	0.037
	Additive	AA/AG/GG	61/258/244	108/295/274	0.85(0.72-0.99)	0.042	0.76(0.61-0.95)	0.017
rs13003941	Dominant	TT+TG/GG	276/209	326/347	1.41(1.11-1.78)	0.004	1.52(1.11-2.09)	0.009
	Recessive	TT/TG+GG	48/437	59/614	1.14(0.77-1.71)	0.513	1.30(0.76-2.25)	0.342
	Additive	TT/TG/GG	48/228/209	59/267/347	1.25(1.05-1.50)	0.013	1.35(1.06-1.73)	0.015
rs1595064	Dominant	GG+GC/CC	399/169	490/180	0.87(0.68-1.11)	0.261	0.78(0.56-1.10)	0.155
	Recessive	GG/GC+CC	106/462	179/491	0.63(0.48-0.83)	0.001	0.58(0.40-0.83)	0.003
	Additive	GG/GC/CC	106/293/169	179/311/180	0.81(0.69-0.94)	0.007	0.75(0.61-0.93)	0.008
rs1595065	Dominant	CC+CT/TT	279/289	267/413	1.49(1.19-1.87)	0.000	1.38(1.02-1.87)	0.040
	Recessive	CC/CT+TT	26/542	33/647	0.94(0.56-1.59)	0.819	1.15(0.56-2.36)	0.712

rs2128321	Additive	CC/CT/TT	26/253/289	33/234/413	1.32(1.09-1.60)	0.004	1.28(0.99-1.66)	0.061
	Dominant	TT+TG/GG	96/471	112/569	1.04(0.77-1.40)	0.819	1.22(0.82-1.84)	0.328
	Recessive	TT/TG+GG	5/562	8/673	0.75(0.24-2.30)	0.613	1.32(0.27-6.39)	0.729
rs3748960	Additive	TT/TG/GG	5/91/471	8/104/569	1.01(0.77-1.33)	0.934	1.21(0.83-1.75)	0.324
	Dominant	CC+CT/TT	80/486	81/600	1.22(0.88-1.70)	0.241	1.26(0.80-2.00)	0.325
	Recessive	CC/CT+TT	1/565	0/681	-	1.0	-	1.0
	Additive	CC/CT/TT	1/79/486	0/81/600	1.23(0.89-1.71)	0.210	1.28(0.81-2.02)	0.286

NRG-1: neuregulin-1; SNP: single nucleotide polymorphism; HF: heart failure; * adjusted for age, gender, BMI, smoking, drinking, hypertension history and uric acid.

Table 4. ErbB4 haplotype distribution and effects on heart failure

Haplotype	Total	HF(n =569)	Contr ols(n= 682)	Score	P	Psim	Score *	P *	Psim*	OR#	P#	P#*
AGGTT	0.35	0.33	0.37	-2.01	0.045	0.037	-2.00	0.045	0.051	Base	-	-
GTCTT	0.29	0.29	0.28	0.52	0.605	0.592	0.45	0.655	0.656	1.14 (0.94-1.39)	0.178	0.193
GGCCT	0.17	0.19	0.16	2.10	0.036	0.05	2.13	0.033	0.031	1.35 (1.06-1.70)	0.014	0.013
GGGTT	0.11	0.10	0.12	-1.34	0.181	0.171	-1.25	0.212	0.192	0.95 (0.72-1.25)	0.694	0.746
GGCCC	0.06	0.07	0.06	1.24	0.217	0.216	1.18	0.238	0.228	1.38 (0.98-1.95)	0.068	0.075

Haplotype constructed in the sequence: rs10932374-rs13003941-rs1595064-rs1595065-rs3748960; score: haplo,score test; sim: simulation, number of simulation=1000; #: haplo.glm analysis.

* adjusted for age and gender. HF: heart failure.

Table 5. MiRNA-binding site of ErbB4 gene polymorphisms by in silico analysis

SNP	Allele	Binding miRNA	Score	Energy
rs1595064	C	hsa-miR-513b	143.00	-14.93
	G	hsa-miR-513b	148.00	-18.15
	C	hsa-miR-520g	149.00	-11.25
	G	hsa-miR-520g	145.00	-11.55
	C	hsa-miR-520h	147.00	-11.25
	G	hsa-miR-520h	145.00	-9.77
rs1595065	T	hsa-miR-1244	151.00	-11.92
	C	hsa-miR-196a	143.00	-11.50
	T	hsa-miR-199a-3p	153.00	-13.19
	T	hsa-miR-199b-3p	153.00	-13.19
rs13003941	G	hsa-miR-1244	140.00	-15.97

Predicted by software miRanda, with default parameter values (score cutoff $S \geq 140$; energy cutoff $E \leq -7.0$; gap opening: -9.0; gap extension -4.0; 5' scaling: 4).

Table 6. Association of ErbB4 gene polymorphisms with clinical characteristics of heart failure patients

SNP	Model	Genotype	NT-proBNP(pg/ml)		LVEF(%)		LAD(mm)		LVEDD(mm)	
			Md±IQR	P	Md±IQR	P	Md±IQR	P	Md±IQR	P
rs10932 374	Additive	AA	2406.6±2406.4	0.562	30.5±14.0	0.368	46.0±12.0	0.612	65.0±15.3	0.100
		AG	1979.2±2789.0		30.0±13.0		44.0±9.0		66.±14.0	
		GG	1880.5±2816.7		32.0±14.0		44.0±11.0		64.0±14.0	
	Dominant	AA+AG	2073.2±2625.1	0.443	30.0±13.0	0.175	44.0±10.0	0.695	66.0±14.0	0.037
		GG	1880.5±2816.7		32.0±14.0		44.0±11.0		64.0±14.0	
	Recessive	AA	2406.6±2406.4	0.342	30.5±14.0	0.974	46.0±12.0	0.324	65.0±15.3	0.891
		AG+GG	1928.9±2800.4		31.0±14.0		44.0±11.0		65.0±14.0	
rs13003 941	Additive	TT	1765.3±3309.5	0.813	30.0±13.5	0.494	45.0±11.0	0.141	66.0±13.5	0.048
		TG	2117.7±2573.3		30.0±13.0		45.0±10.0		66.5±12.3	
		GG	1909.3±2822.0		32.0±14.0		43.0±11.0		63.0±16.0	
	Dominant	TT+TG	2043.8±2556.6	0.848	30.0±13.0	0.235	45.0±10.0	0.051	66.0±13.0	0.014
		GG	1909.3±2822.0		32.0±14.0		43.0±11.0		63.0±16.0	
	Recessive	TT	1765.3±3309.5	0.519	30.0±13.5	0.724	45.0±11.0	0.371	66.0±13.5	0.408
		TG+GG	2018.0±2686.1		31.0±14.0		44.0±11.0		65.0±14.0	
rs15950 64	Additive	GG	2022.0±2442.1	0.339	31.0±14.3	0.933	44.5±11.0	0.89	64.0±15.3	0.916
		GC	2123.5±3014.2		31.0±13.8		44.0±11.0		66.±13.8	
		CC	1760.8±2609.7		30.5±13.3		44.0±10.0		65.0±14.0	
	Dominant	GG+GC	2119.9±2838.7	0.145	31.0±14.3	0.721	44.0±11.0	0.633	65.5±14.0	0.677
		CC	1760.8±2609.7		30.5±13.3		44.0±10.0		65.0±14.0	
	Recessive	GG	2022.0±2442.1	0.781	31.0±14.3	0.993	44.5±11.0	0.836	64.0±15.3	0.938
		GC+CC	1940.0±2806.4		31.0±13.3		44.0±10.0		65.5±14.0	
rs15950 65	Additive	CC	1528.6±1909.3	0.183	30.0±14.0	0.887	43.0±12.0	0.299	65.0±19.0	0.629
		CT	1879.5±2950.2		31.0±13.0		44.0±10.0		64.0±14.0	
		TT	2108.1±2671.8		31.0±14.0		44.0±10.8		66.0±13.8	
	Dominant	CC+CT	1808.2±2733.7	0.671	31.0±13.0	0.887	44.0±11.0	0.974	64.0±14.0	0.345
		TT	2108.1±2671.8		31.0±14.0		44.0±10.8		66.0±13.8	
	Recessive	CC	1528.6±1909.3	0.065	30.0±14.0	0.624	43.0±12.0	0.136	65.0±19.0	0.943
		CT+TT	2021.6±2789.5		31.0±13.5		44.0±10.0		65.0±14.0	

SNP: single nucleotide polymorphism; NT-proBNP: amino-terminal pro-brain natriuretic peptide; LVEF: left ventricular ejection fraction; LAD: left atrial diameter; LVEDD: left ventricular end diastolic diameter; Md: median; IQR: interquartile range.

Table 7. Effect of ErbB4 haplotype on overall and cardiovascular death in Northern Han Chinese heart failure patients

haplotype	Log-rank p	Univariate analysis		Multivariate analysis	
		HR(95%CI)	P	HR*(95%CI)	P*
overall death					
GGCCT	0.721	0.934(0.643-1.357)	0.721	0.898(0.617-1.306)	0.573
GTCTT	0.061	1.444(0.982-2.123)	0.062	1.49(1.012-2.195)	0.044
GGGTT	0.979	1.005(0.69-1.465)	0.979	1.012(0.694-1.475)	0.952
GGCCC	0.997	0.99(0.588-1.697)	0.997	1.022(0.602-1.737)	0.935
AGGTT	0.314	0.826(0.569-1.199)	0.314	0.838(0.577-1.216)	0.351
cardiovascular death					
GGCCT	0.839	0.961(0.652-1.415)	0.839	0.928(0.629-1.369)	0.705
GTCTT	0.043	1.511(1.01-2.261)	0.045	1.563(1.043-2.342)	0.031
GGGTT	0.804	0.952(0.645-1.405)	0.804	0.955(0.647-1.41)	0.817
GGCCC	0.972	1.01(0.584-1.746)	0.972	1.031(0.596-1.784)	0.912
AGGTT	0.24	0.794(0.539-1.168)	0.242	0.803(0.545-1.183)	0.267

Haplotype constructed in the sequence: rs10932374-rs13003941-rs1595064- rs1595065-rs3748960.

* adjusted for age and gender.