Common Variants in Neuraminidase Genes Contribute to Predisposition to and Progression of Chronic Heart Failure

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Keywords
Heart failure · Single-nucleotide polymorphisms · Neuraminidases · Risk · Prognosis

Abstract
Introduction: The role of neuraminidases in cardiovascular disease has recently gained increasing attention. However, the association between neuraminidase gene polymorphisms and heart failure (HF) has not yet been investigated.

Methods and Results: Genotyping of nine single-nucleotide polymorphisms (SNPs) in the NEU2/NEU3/NEU4 genes was performed in 610 HF patients and 600 healthy controls from the Southwest Han Chinese population using TaqMan SNP Genotyping Assay. Individuals carrying the A allele of rs11545301 had decreased risk of HF (additive model: OR = 0.704, 95% CI = 0.511–0.97; p = 0.032). However, the C allele of rs2293763 increased the risk of HF in the recessive model (OR = 1.486, 95% CI = 1.095–2.012; p = 0.011). Rs2233384, rs2233394, and rs2293763 were significantly associated with the mortality risk of HF in the dominant model, both with and without adjustment for conventional risk factors (HR = 0.686, 95% CI = 0.52–0.906, p = 0.008 for rs2233384, HR = 1.357, 95% CI = 1.035–1.78, p = 0.027 for rs2233384, and HR = 0.76, 95% CI = 0.592–0.975, p = 0.031 for rs2293763).

Conclusion: Our findings demonstrated the association between a series of variants in NEU2/NEU4 genes and the risk or prognosis of HF in the Han Chinese population. These data suggested an important role of NEU2 and NEU4 in the pathogenesis of HF.

Introduction
Cardiovascular diseases (CADs) have been the leading cause of death globally [1]. As a member of CADs, heart failure (HF) is a serious and complex CAD with high morbidity and mortality [2]. A substantial of strategies targeting HF have arisen nowadays, while the prognosis of HF remained unoptimistic owing to various poorly controlled risk factors (hypertension, diabetes, and obesity) and population aging [3].

Sialic acids (Sias) are a family of functional monosaccharides that serve as terminal residues of N- and O-
linked glycoproteins and are widely presented in plants, animals, and microorganisms [4, 5]. Plenty of evidence indicated that sias are involved in the pathogenesis of many cancers including pancreatic cancer [6], breast cancer [7], and ovarian cancer [8]. Sias with different modifications in its core structure of nine carbons displayed different forms [4, 9]. N-acetylneuraminic acid (Neu5Ac), N-glycolyneuraminic acid, and 2-keto-3-deoxynononic acid are among the most abundant naturally occurring sias [10, 11]. Sialidases are responsible for removing the terminal sias with different modifications from glycoproteins, oligosaccharides, and glycolipids [10]. A total of 4 types of mammalian sialidases (neuraminidases) have been identified, and each showed a different cellular location: NEU1, lysosomal [12]; NEU2, cytoplasmic [13]; NEU3, plasma membrane-bound [14, 15]; and NEU4, mitochondrial [16, 17].

The role of neuraminidases in CADs has been revealed recently. Heimerl et al. [18] demonstrated that NEU1 could promote HF after ischemia/reperfusion injury by affecting cardiomyocytes and invading monocytes/macrophages. Besides, silencing or pharmacological inhibition of NEU1 could also protect cardiomyocytes and the heart from myocardial injury [19]. NEU3 was involved in upregulation of HIF-1α in response to chronic hypoxia in cyanotic congenital heart patients [20]. In addition, NEU1 and NEU3 were reported implicated in atherosclerosis through desialylating LDL and increasing their uptake by resident macrophages [21]. However, up to now, the association of variants in neuraminidases with HF remained unclear. Considering the important role of neuraminidases in HF, we speculated that there may exist functional variants in NEU1, 2, 3, 4 that modify the prognosis of HF.

In this study, we selected the common variants in the exon region of NEU genes for genotyping in 610 HF patients and 600 healthy controls. The associations between variants and risk or prognosis of HF were further analyzed.

**Methods**

**Study Participants**

This study conformed with the principles outlined in the Declaration of Helsinki and was approved by the Review Board of Panzhihua Central Hospital. All patients have given informed consent. We recruited a total of 610 HF patients and 600 healthy controls between March 2014 and June 2017 in the Cardiology Division of Panzhihua Central Hospital in Sichuan. Patients diagnosed with chronic HF were based on medical history, physical examination, and relevant investigations. Ethnically and geographically matched participants without evidence of chronic HF were recruited as controls. The detailed clinical characteristics of individuals are listed in Table 1. Details on inclusion and exclusion criteria of HF and the definition of risk factors have been described in online supplementary materials (see www.karger.com/doi/10.1159/000525713 for all online suppl. material).

**End Point Assessment**

The HF population was followed up for an average period of 28.5 months. A standard HF questionnaire was conducted during regular outpatient clinics or by telephone contact. The end points included cardiovascular deaths or cardiac transplantation.

**DNA Extraction, SNP Selection, and Genotyping**

We extracted genomic DNA from peripheral blood leukocytes using the Tiangen commercially available kit (Tiangen, Beijing, China). Detailed procedures of DNA extraction have been described previously [3]. Referring to the Chinese data of the 1000 Genomes, we selected common genetic variants with minor allele frequency >0.05 in the exon region of NEU1, NEU2, NEU3, and NEU4 for further genotyping. The probes for genotyping came from ABI with the following assay ID: rs2233384 (C__15962661_10, 4351379), rs2233385 (C__15962660_10, 4351379), rs2233394 (C__42329_1_, 4351379), rs36111671 (C__57931136_10, 4351379), rs11545301 (C__57931136_10, 4351379), rs2293764 (C__16185067_10, 4351379), rs2293763 (C__16185022_10, 4351379), rs2293759 (C__11536383_1_, 4351379), rs544115 (C__1053082_10, 4351379). The genotyping procedure was conducted according to the TaqMan assay on the TaqMan 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with the following conditions: 10 min at 95°C (enzyme activation) followed by 45 cycles at 95°C for 15 s and 60°C for 1 min (annealing/extension).

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**Table 1. Baseline characteristics of the study population.**

| Characteristics | Sequencing population (N = 610) | Control population (N = 600) |
|----------------|---------------------------------|-------------------------------|
| Men, %         | 65                              | 64                            |
| Age, years     | 57.00±14.19                     | 58.6±10.2                     |
| TC, mmol/L     | 3.91±1.31                       | 4.93±0.96*                    |
| TG, mmol/L     | 1.40±1.13                       | 1.45±1.00*                    |
| HDL, mmol/L    | 0.96±0.31                       | 1.46±0.35*                    |
| LDL, mmol/L    | 2.42±0.87                       | 2.77±0.79*                    |
| Hypertension, n (%) | 392 (39.2)                 | 108 (26)                      |
| Diabetes, n (%) | 175 (17.5)                      | 0                             |
| Hiperlipidemia, n (%) | 50 (5)                   | 4 (2)                          |
| Current smoking, n (%) | 390 (39)                | 0                             |
| β-Blocker use, n (%) | 435 (43.5)            | 0                             |

Data are expressed as means±SD or percentages. TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. * p < 0.05 case versus control.
Statistical Analysis

Statistical analyses were performed with SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA) for Windows (Microsoft Corp., Redmond, WA, USA). Hardy-Weinberg equilibrium (HWE) among the HF patients and the controls were calculated using the χ² test. Haploview version 4.1 was used for calculation of linkage disequilibrium. The Cox proportional hazards regression model was used for analysis of association between variants and the prognosis of HF, with or without adjustment for traditional risk factors including sex, age, hypertension, diabetes, hyperlipidemia, smoking state, and β-blocker use. Besides, we performed logistic regression analyses based on the different genetic models to test the association between the SNPs and HF. Data are expressed as means ± standard deviation. p < 0.05 was considered statistically significant.

Results

Baseline Characteristics of Study Participants

The detailed features of HF patients and controls are shown in Table 1. The proportion of diabetes, hypertension, dyslipidemia, and smoking state is significantly higher in the HF population than in controls. However, the level of total cholesterol, triglycerides, and LDL in HF patients seems slightly lower than in controls, which can be attributed to lipid-lowering therapy.

Association between Variants in Genes Encoding Neuraminidases and Risk of HF

Referring to the Chinese data of the 1000 Genomes, we identified 11 common genetic variants with minor allele frequency >0.05 in the exon region of NEU1, NEU2, NEU3, and NEU4 (Table 2). The average number of SNPs per gene was 2.75, with a minimum of 0 and a maximum of 7 SNPs. Considering the fact that rs11545301 and rs2293760, rs2293763, and rs2293761 were, respectively, in strong LD ($r^2 = 0.96$) with each other, we selected rs11545301 and rs2293763 as the tagged SNPs for further analysis. We evaluated HWE of all SNPs by using the χ² test with one degree of freedom. As shown in Table 2, all variants were in HWE.

First, we investigated the association of variants with the risk of HF. The results showed that only rs11545301 and rs2293763 were associated with the risk of HF in the additive and recessive models, respectively. As demonstrated in Table 3, individuals carrying A allele of rs11545301 had decreased risk of HF (additive model: OR = 0.704, 95% confidence interval [CI] = 0.511–0.97; p = 0.032), while the C allele of rs2293763 increased the risk of HF in recessive model (OR = 1.486, 95% CI = 1.095–2.012; p = 0.011). The remaining 7 variants showed no difference in genotype and allele frequency between cases and controls, which suggested that these variants had no association with the risk of HF.

Association of Variants in Genes Encoding Neuraminidases with the Prognosis of HF

The average follow-up time of HF patients reached up to 28.5 months, during which 161 cardiovascular deaths or cardiac transplantation occurred. We evaluated the effect of these genetic variants on the prognosis of HF. Our results indicated that rs2233384, rs2233394, and rs2293763 were significantly associated with the mortality risk of HF in the dominant model, both with or without adjustment for conventional risk factors (Table 4; Fig. 1). The cardiovascular deaths or cardiac transplantation had occurred in 115 patients (23.9%) in the CC genotype group, 44 patients (35.2%) in the CA

Table 2. The list of genetic variants in genes encoding neuraminidases

| SNPs      | Gene | Chr | Alleles | MAF    | Function       |
|-----------|------|-----|---------|--------|----------------|
| rs2233384 | NEU2 | 2   | C/A     | 0.107  | Missense       |
| rs2233385 | NEU2 | 2   | G/A     | 0.0955 | Missense       |
| rs2233394 | NEU2 | 2   | C/T     | 0.127  | Synonymous     |
| rs544115  | NEU3 | 11  | C/T     | 0.219  | Synonymous     |
| rs36111671| NEU4 | 2   | T/C     | 0.194  | Synonymous     |
| rs11545301| NEU4 | 2   | G/A     | 0.057  | Missense       |
| rs2293764 | NEU4 | 2   | C/T     | 0.2    | Synonymous     |
| rs2293763 | NEU4 | 2   | G/A     | 0.058  | 3′UTR          |
| rs2293761 | NEU4 | 2   | G/A     | 0.405  | Synonymous     |
| rs2293760 | NEU4 | 2   | G/A     | 0.0885 | 3′UTR          |

SNPs, single-nucleotide polymorphisms; Chr, chromosome; MAF, minor allele frequency.
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Table 3. Association of variants with HF

| SNP            | Additive model | Dominant model | Recessive model |
|----------------|----------------|----------------|-----------------|
|                | p value | OR   | 95% CI | p value | OR   | 95% CI | p value | OR   | 95% CI |
| rs2233384      | 0.477   | 0.909| 0.699–1.182 | 0.651   | 0.938| 0.71–1.239 | 0.162   | 0.411| 0.118–1.427 |
| rs2233385      | 0.603   | 1.079| 0.809–1.44 | 0.492   | 1.111| 0.822–1.501 | 0.435   | 0.55 | 0.123–2.469 |
| rs2233394      | 0.487   | 0.918| 0.72–1.17 | 0.703   | 0.95 | 0.729–1.237 | 0.162   | 0.513| 0.201–1.308 |
| rs36111671     | 0.435   | 0.921| 0.749–1.133 | 0.696   | 0.954| 0.752–1.209 | 0.169   | 0.648| 0.349–1.202 |
| rs11545301     | 0.032*  | 0.704| 0.511–0.97 | 0.057   | 0.723| 0.518–1.01  | 0.086   | 0.137| 0.014–1.322 |
| rs2293764      | 0.628   | 1.052| 0.857–1.291 | 0.754   | 1.039| 0.818–1.32  | 0.526   | 1.222| 0.659–2.266 |
| rs2293763      | 0.854   | 0.984| 0.834–1.162 | 0.101   | 1.218| 0.963–1.54  | 0.011*  | 1.486| 1.095–2.012 |
| rs2293759      | 0.501   | 0.91 | 0.692–1.197 | 0.626   | 0.928| 0.687–1.54  | 0.363   | 0.618| 0.218–1.746 |
| rs544115       | 0.269   | 0.896| 0.739–1.088 | 0.202   | 0.86 | 0.681–1.085 | 0.916   | 0.972| 0.568–1.661 |

OR, odds ratio; CI, confidence interval. * p < 0.05

Table 4. Prognosis analysis for genetic loci in genes encoding neuraminidases

| SNP            | Dominant model | Recessive model |
|----------------|----------------|-----------------|
|                | p value | adjusted p | HR   | 95% CI | p value | adjusted p | HR   | 95% CI |
| rs2233384      | 0.008*  | 0.008*      | 0.686| 0.52–0.906 | 0.157   | 0.166| 2.237| 0.716–6.988 |
| rs2233385      | 0.685   | 0.775 | 0.955| 0.69–1.31 | 0.466   | 0.945| 0  | 0–2.95E114 |
| rs2233394      | 0.025*  | 0.027*      | 1.357| 1.035–1.78  | 0.502   | 0.476| 1.433| 0.533–3.851 |
| rs36111671     | 0.11    | 0.075| 0.788| 0.606–1.025 | 0.551   | 0.313| 1.439| 0.71–2.917 |
| rs11545301     | 0.269   | 0.269| 1.22 | 0.858–1.736 | 0.728   | 0.941| 0  | 0–2.06E89 |
| rs2293764      | 0.314   | 0.306| 0.872| 0.671–1.134 | 0.35    | 0.514| 1.213| 0.679–2.169 |
| rs2293763      | 0.047*  | 0.031*      | 0.76 | 0.592–0.975 | 0.992   | 0.932| 0.984| 0.683–1.419 |
| rs2293759      | 0.082   | 0.109| 1.282| 0.947–1.735 | 0.402   | 0.32 | 0.369| 0.052–2.632 |
| rs544115       | 0.15    | 0.15 | 0.827| 0.639–1.071 | 0.14    | 0.167| 0.588| 0.277–1.248 |

Hazard ratios (HRs) were obtained using Cox regression with adjustment for traditional risk factors. CI, confidence interval. * p < 0.05.

genotype group, and 2 (60%) in the AA genotype group for rs2233384; 114 patients (24.5%) in the CC genotype group, 43 patients (31.9%) in the CT genotype group, and 4 patients (40%) in the TT genotype group for rs2233394; 69 patients (31.7%) in the CC genotype group, 72 patients (23.2%) in the CT genotype group, and 20 patients (24.4%) in the TT genotype group for rs2293763. The statistical significance in multivariate analysis remained after adjustments for sex, age, hypertension, diabetes, hyperlipidemia, smoking state, and β-blocker use (hazard ratio [HR] = 0.686, 95% CI = 0.52–0.906, p = 0.008 for rs2233384, HR = 1.357, 95% CI = 1.035–1.78, p = 0.027 for rs2233384, and HR = 0.76, 95% CI = 0.592–0.975, p = 0.031 for rs2293763) (Table 4).

Discussion

In the present study, we observed significant associations of genetic loci in NEU2 and NEU4 genes with the risk or prognosis of HF in the Han Chinese population. All of them are involved in the regulation of Neu5AC, which has been demonstrated to play an important role in coronary artery diseases [19]. The major C allele of rs2293763 is significantly associated with increased risk and poor prognosis of HF. The A allele of rs11545301 showed reduced HF incidence, while the minor A allele of rs2233384 and T allele of rs2233394 displayed higher mortality risk of HF.

The role of neuraminidases in CADs has gained much attention in recent years. NEU1 and NEU3 have been re-
ported to participate in different stimuli-induced myocardial injuries [18, 20]. Produced by neuraminidases, Neu5AC increased during the progression of coronary artery diseases and was capable of triggering myocardial injury in vitro and in vivo [19], which revealed the importance of neuraminidases in myocytes. Plenty of evidence indicated the involvement of NEU2 in myoblast differentiation [22–25]. However, NEU4 showed important function in ganglioside catabolism and neuronal-cell differentiation. Besides, mutations in the NEU1 gene were reported associated with type 1 sialidosis. Luke et al. [26] demonstrated that rs544115 in NEU3 was associated with noncardioembolic stroke. In our study, we identified four variants in the coding and 3′UTR regions of NEU2 and NEU4 that were associated with the risk or prognosis of HF, which indicated the importance of NEU2 and NEU4 in HF. In fact, NEU2 could promote apoptosis by means of desialylation of related proteins in many disease states [27–29]. Besides, NEU4 was reported involved in the mitochondrial apoptotic pathway in neuronal cells [30] and inflammatory activation in vivo [31]. Notably, Neu5AC, which is regulated by sialidases, has been reported to participate in myocardial infarction [19]. Although no study has focused on the role of NEU2 and NEU4 in CAD, their role in apoptosis and inflammation could support a vital role of NEU2 and NEU4 in HF when combined with our genetic results, which need further detailed investigation.

One limitation of this study is that this is a single-center study with only one cohort, which needs to be confirmed in other studies. In summary, we identified four variants in NEU2 and NEU4 genes that were significantly associated with the risk or prognosis of HF. These findings suggested that common variants in genes may contribute to the pathogenesis of HF. Besides, our results indicated that NEU2 and NEU4 might participate in HF.

**Statement of Ethics**

This study protocol was reviewed and approved by the Review Board of Panzhihua Central Hospital. This study conformed with the principles outlined in the Declaration of Helsinki and was approved by the Review Board of Panzhihua Central Hospital. All patients have provided written informed consent to participate in the study.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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Author Contributions

Shiyang Li, Xiaobing Zeng, and Dong Hu developed the study concept and design, interpreted the data, and drafted the manuscript. Yuehong Wang and Yanyu Zhang helped with specimen collection. Jianjun Lan supervised the design of the study and revised the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

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