Effects of Ninjurin 2 Polymorphisms on Susceptibility of Coronary Heart Disease

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Abstract
Objective The aim of this study was to explore the effects of NINJ2 polymorphisms on susceptibility of coronary heart disease (CHD). Methods We conducted a case-control study with 499 CHD cases and 505 age- and sex- matched controls. Five single nucleotide polymorphisms (SNP) in NINJ2 (rs118050317, rs75750647, rs7307242, rs10849390 and rs11610368) were genotyped by Agena MassARRAY platform. Odd ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression to assess the association of NINJ2 polymorphism and CHD risk adjusting for age and gender. Results NINJ2 rs118050317 significantly increased CHD risk among people older than 60 years old (allele: P = 0.010; heterozygote: P = 0.016; dominant: P = 0.015; additive: P = 0.021) and women (allele: P = 0.026; heterozygote: P = 0.015; dominant: P = 0.018; additive: P = 0.030). Rs118050317 and rs7307242 had strong relationship with hypertension risk in CHD patients. Additionally, rs75750647 significantly increased diabetes risk in multiple models among cases (allele: P = 0.014; homozygote: P = 0.037; heterozygote: P = 0.044; dominant: P = 0.019; additive: P = 0.013), whereas rs10849390 could protect CHD patients from diabetes in allele ( P = 0.035), homozygote ( P = 0.047) and additive ( P = 0.037) models. We also observed two block (block 1: rs118050317 and rs75750647; block 2: rs7307242, rs10849390 and rs11610368) in NINJ2. Conclusion Our results suggest that NINJ2 polymorphisms are associated with CHD risk.

Introduction
Coronary heart disease (CHD) is a common cardiovascular disorder (CVD) with rapidly increased incidence rate in the recent year[1]. Moreover, the incidence of CHD varies by age and sex[2]. The World Health Organization (WHO) reported that approximately 7.4 million people died from CHD in 2012 [3]. In China, CHD has affected more than 10 million people[4]. CHD is thought to be a complex disease resulting from the interaction between genetic and environmental factors[5]. The risk factors of CHD include hypertension, diabetes, lifestyle and clinical indicators (the level of cholesterol, triglyceride and uric acid)[6]. Nevertheless, increasing studies revealed that genetic variants contribute to CHD susceptibility[7].

Ninjurin 2 (NINJ2) is a transmembrane protein belongs to the ninjurin family, expressed in glia that
plays a role in neurite outgrowth[8]. This gene encodes NINJ2 is located on chromosome 12p13. NINJ2 interacts with several substance involved in leukocyte and endothelial cells and thus plays a vital role on inflammation regulation[9, 10]. Previous studies have reported that NINJ2 are associated with many diseases, including ischemic stroke, large artery atherosclerotic stroke, Alzheimer’s disease and vascular dementia[11, 12]. Rs11833579 and rs12425791 are the most two studied NINJ2 polymorphism, which are considered as potential risk variants for ischemic stroke in the four large cohorts[13]. These evidences showed that NINJ2 might be associated with pathology of atherosclerosis, suggesting a linkage of NINJ2 and CHD. However, no genetic polymorphism had yet shown convincing evidence for an association with CHD. Hence, we hypothesized that NINJ2 polymorphisms might be associated with susceptibility of CHD. In order to clarify the role of NINJ2 polymorphisms in the etiology of CHD, We carried out a genetic association study to examine the relationship between CHD and NINJ2 polymorphisms (rs118050317, rs75750647, rs7307242, rs10849390, and rs11610368) among the Chinese Han population.

Materials And Methods
Study population
This study consisted of 499 patients with CHD and 505 healthy controls. All of the participants were recruited from First Affiliated Hospital of Xi’an Jiaotong University in Shaanxi province, China. CHD patients were diagnosed by at least two cardiovascular physicians based on angiographically demonstrated stenosis (> 50%) in a major or main branch of the coronary artery[14]. CHD Patients with stroke, cardiomyopathy, chronic diseases or other serious physical diseases were excluded from this study. Controls were healthy people who receiving physical examinations in the same hospital with cases. This study was approved by the Ethical Committee of First Affiliated Hospital of Xi’an Jiaotong University and conducted in accordance with the Declaration of Helsinki. And, informed consents were collected from all participants in this study.

SNP selection and genotyping
Based on the Han Chinese in Beijing (CHB) population data from 1000 Genomes Project (http://www.internationalgenome.org/) and dbSNP database (https://www.ncbi.nlm.nih.gov/snp/), we
selected five SNPs (rs118050317, rs75750647, rs7307242, rs10849390 and rs11610368) of NINJ2 with minor allele frequency (MAF) > 5% for genotyping. Then, we used DNA extraction kit (GoldMag Co. Ltd., Xi’an, China) to isolated genomic DNA from peripheral blood. Five SNPs were genotyped using the Agena MassARRAY platform Agena, San Diego, CA, USA). Genotypic primers for NINJ2 polymorphisms were designed by The Agena MassARRAY Assay Design 3.0 Software (San Diego, California, USA) and were presented in Supplemental table 1. Data were managed and analyzed using Agena Typer 4.0 Software (San Diego, CA, USA).

Statistical analysis
Statistical analysis was done using Microsoft Excel and SPSS version 21.0 software (SPSS, Chicago, IL, USA). Continuous data and categorical variables were compared by Student’s t-test and chi-square test. We used Fisher’s exact test to analyze the Hardy-Weinberg equilibrium (HWE) for all SNPs in the control group. The association of NINJ2 polymorphisms and CHD risk in genetic models (co-dominant, dominant, recessive and additive) was assessed by logistic regression, and the relationship strength was defined as the odd ratios (OR) and 95% confidence intervals (CI). Then, we did linkage disequilibrium (LD) and haplotype analysis by Haplovieview software and the PLINK software[15]. All tests were two-sided, and P < 0.05 was considered as statistical significance.

Results
Characteristics of study subjects
The characteristics of study subjects were listed in Table 1. The distribution of age and sex was similar for cases and controls (age: P = 0.191, sex: P = 0.896). The mean ages of cases (318 men and 181 women) and controls (323 men and 182 women) were 61.34 ± 11.69 and 60.51 ± 8.11, respectively. Additionally, we collected clinical characteristics of all subjects, including uric acid (UA), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), platelet (PLT), platelet distribution width (PDW), mean platelet volume (MPV), and plateletcrit (PCT). There were significantly different between two groups in the level of UA, TC, LDL, PLT and PCT. In CHD patients, 294 (59%) had hypertension and 100 (20%) had diabetes.
Table 1
Characteristics of study subjects

| Variables | Case (N = 499) | Control (N = 505) | P       |
|-----------|---------------|-------------------|---------|
| Age       | 61.34 ± 11.69 | 60.51 ± 8.11     | 0.191   |
| > 60      | 270(54%)      | 308(61%)         |         |
| ≤ 60      | 229(46%)      | 197(39%)         |         |
| Sex       | 0.896         |                   |         |
| Man       | 318(64%)      | 323(64%)         |         |
| Woman     | 181(36%)      | 182(36%)         |         |
| UA (umol/L) | 292.67 ± 87.62 | 326.17 ± 80.95 | < 0.001 |
| TC (mmol/L)  | 4.09 ± 1.15    | 4.78 ± 1.00     | < 0.001 |
| TG (mmol/L)  | 1.78 ± 1.48    | 1.70 ± 1.18     | 0.418   |
| HDL (mmol/L) | 1.13 ± 0.25    | 1.12 ± 0.25     | 0.862   |
| LDL (mmol/L) | 1.92 ± 0.82    | 2.64 ± 0.76     | < 0.001 |
| PLT (10^9/L) | 182.43 ± 60.35 | 207.51 ± 56.19 | < 0.001 |
| PDW (%)    | 14.32 ± 2.79  | 14.10 ± 2.79    | 0.272   |
| MPV (FL)   | 10.88 ± 1.09  | 10.73 ± 1.06    | 0.051   |
| PCT (%)    | 0.21 ± 0.07   | 0.23 ± 0.05     | < 0.001 |
| Hypertension | Yes 294(59%) | No 205(41%)    |         |
| Diabetes   | Yes 100(20%)  | No 399(80%)     |         |

Bold values indicate a statistically significant P value (P < 0.05).

UA, uric acid; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; PLT, platelet; PDW, platelet distribution width; MPV, mean platelet volume; PCT, plateletcrit.

Association of NINJ2 polymorphisms and CHD risk

The genotype distribution of NINJ2 polymorphisms was presented in Table 2. All SNPs are in HWE (P > 0.05). In allelic model, no significant association of NINJ2 polymorphisms and CHD risk was observed (P > 0.05). We further performed the association of NINJ2 polymorphisms and CHD risk in multiple models and stratification analysis (Table 3, Table 4, Table 5 and Supplemental table 2). The effects of NINJ2 rs118050317 on CHD risk are dependent on age and sex. For the individuals elderly than 60 years old, rs118050317 was significantly associated with CHD risk in allele (OR = 1.64, 95%CI = 1.13–2.40, P = 0.010), heterozygote (OR = 1.73, 95%CI = 1.11–2.70, P = 0.016), dominant (OR = 1.71, 95%CI = 1.11–2.65, P = 0.015) and additive (OR = 1.63, 95%CI = 1.08–2.46, P = 0.021) models.

Similarly, rs118050317 significantly increased CHD risk among women (allele: OR = 1.75, 95%CI = 1.07–2.86, P = 0.026; heterozygote: OR = 1.99, 95%CI = 1.14–3.45, P = 0.015; dominant: OR = 1.92, 95%CI = 1.12–3.28, P = 0.018; additive: OR = 1.74, 95%CI = 1.06–2.86, P = 0.030). In CHD patients, rs118050317 increased the risk of hypertension (allele: OR = 1.66, 95%CI = 1.09–2.53, P = 0.017; heterozygote: OR = 1.72, 95%CI = 1.08–2.76, P = 0.024; dominant: OR = 1.70, 95%CI = 1.08–2.70, P = 0.023; additive: OR = 1.60, 95%CI = 1.04–2.46, P = 0.031), whereas rs7307242 was significantly
associated with decreased hypertension risk (homozygote: OR = 0.35, 95%CI = 0.13–0.99, P = 0.049; recessive: OR = 0.35, 95%CI = 0.13–0.99, P = 0.047). In addition, rs75750647 and rs10849390 had strong association with diabetes risk in CHD patients (P < 0.05).

Table 2
Genotype distribution of NINJ2 polymorphisms and Hardy-Weinberg equilibrium test for the healthy controls

| SNP     | Chromosome | Allele | Group | Group A | Group B | OR(95%CI) | P       |
|---------|------------|--------|-------|---------|---------|-----------|---------|
| rs118050317 | 12: 634980 | C/G    | CHD   | 6       | 102     | 1.16(0.87–1.54) | 0.310   |
| rs75750647  | 12: 638831 | A/G    | CHD   | 44      | 224     | 0.93(0.77–1.12)  | 0.440   |
| rs730724    | 12: 641529 | A/T    | CHD   | 17      | 110     | 1.05(0.81–1.34)  | 0.729   |
| rs10849390  | 12: 646086 | A/G    | CHD   | 66      | 232     | 1.18(0.98–1.42)  | 0.084   |
| rs11610368  | 12: 662624 | A/G    | CHD   | 11      | 104     | 1.43(0.86–1.48)  | 0.384   |

CHD, coronary heart disease; A means minor allele; B means major allele; OR, odd ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

Table 3
Association of NINJ2 polymorphisms and CHD risk

| SNP     | Homozygote OR(95%CI) | P       | Heterozygote OR(95%CI) | P       | Dominant model OR(95%CI) | P       | Recessive model OR(95%CI) | P       | Additive model OR(95%CI) | P       |
|---------|-----------------------|---------|------------------------|---------|--------------------------|---------|---------------------------|---------|--------------------------|---------|
| rs118050317 | 1.22(0.3–7.40)       | 0.748   | 1.17(0.85–1.60)       | 0.341   | 1.17(0.86–1.59)          | 0.323   | 1.18(0.93–1.53)          | 0.785   | 1.15(0.82–1.53)          | 0.329   |
| rs75750647  | 0.76(0.4–9.18)        | 0.228   | 1.01(0.78–1.31)       | 0.937   | 0.96(0.75–1.23)          | 0.750   | 0.76(0.53–1.01)          | 0.198   | 0.92(0.66–1.26)          | 0.406   |
| rs730724    | 1.34(0.6–2.80)        | 0.437   | 0.98(0.72–1.32)       | 0.900   | 1.02(0.70–1.35)          | 0.906   | 1.35(0.60–2.81)          | 0.428   | 1.05(0.72–1.33)          | 0.715   |
| rs10849390  | 1.38(0.92–2.08)       | 0.121   | 1.18(0.90–1.54)       | 0.221   | 1.22(0.95–1.57)          | 0.123   | 1.27(0.83–1.86)          | 0.228   | 1.18(0.84–1.42)          | 0.086   |
| rs11610368  | 2.27(0.78–8.61)       | 0.132   | 1.03(0.76–1.40)       | 0.852   | 1.00(1.08–1.46)          | 0.585   | 2.26(0.79–8.65)          | 0.134   | 1.13(0.87–1.48)          | 0.364   |

CHD, coronary heart disease; OR, odd ratio; CI, confidence interval.

Table 4
Association of NINJ2 rs118050317 and CHD risk after age and sex stratification

| Model | Age > 60 | ≤ 60 | Sex | Man | Woman |
|-------|----------|------|-----|-----|-------|
| OR(95%CI) | P       | OR(95%CI) | P       | OR(95%CI) | P       |
| Allele  | 1.64(1.13–2.40) | 0.010 | 0.73(0.47–1.12) | 0.146 | 0.94(0.66–1.33) | 0.713 |
| Dominant | 1.45(0.23–9.02) | 0.691 | 0.72(0.12–4.40) | 0.725 | 1.33(0.29–6.02) | 0.709 |
| Heterozygote | 1.73(1.11–2.70) | 0.016 | 0.75(0.46–1.24) | 0.265 | 0.89(0.60–1.31) | 0.542 |
| Recessive | 1.71(1.11–2.65) | 0.015 | 0.75(0.46–1.22) | 0.250 | 0.90(0.62–1.33) | 0.607 |
| Additive | 1.63(1.08–2.46) | 0.021 | 0.77(0.49–1.21) | 0.261 | 0.93(0.66–1.33) | 0.704 |

CHD, coronary heart disease; OR, odd ratio; CI, confidence interval.
Table 5
Association of NINJ2 polymorphisms and CHD risk after disease stratification

| SNP      | Model          | Hypertension vs Non-hypertension | Diabetes vs Non-diabetes |
|----------|----------------|----------------------------------|--------------------------|
|          |                | OR(95%CI)                         | OR(95%CI)                | P  |
| rs118050317 | Allele         | 1.66(1.09–2.53)                  | 0.17                     | 0.83(0.50–1.39) | 0.479 |
|          |                | 1.44(0.25–8.12)                  | 0.22(0.53–1.60)          | 0.780 |
|          |                | 1.70(1.08–2.70)                  | 0.023                    | 0.86(0.50–1.48) | 0.581 |
|          |                | 1.29(0.23–7.27)                  | 0.776                    | 0.80(0.48–1.34) | 0.404 |
| rs75750647 | Allele         | 1.13(0.86–1.48)                  | 0.391                    | 1.50(1.09–2.07) | 0.014 |
|          |                | 0.98(0.51–1.90)                  | 2.20(1.05–4.63)          | 0.037 |
|          |                | 1.36(0.93–1.98)                  | 1.63(1.01–2.62)          | 0.044 |
|          |                | 1.28(0.89–1.84)                  | 1.72(1.09–2.71)          | 0.019 |
|          |                | 1.32(0.45–1.60)                  | 1.71(0.85–3.41)          | 0.130 |
| rs7307242 | Allele         | 0.79(0.56–1.13)                  | 0.199                    | 0.86(0.54–1.36) | 0.512 |
|          |                | 0.35(0.13–0.99)                  | 0.049                    | 0.83(0.23–2.99) | 0.781 |
|          |                | 1.01(0.65–1.56)                  | 0.974                    | 0.85(0.49–1.46) | 0.551 |
|          |                | 0.88(0.58–1.32)                  | 0.530                    | 0.85(0.50–1.42) | 0.524 |
|          |                | 0.35(0.13–0.99)                  | 0.047                    | 0.87(0.24–3.08) | 0.823 |
|          |                | 0.81(0.57–1.14)                  | 0.218                    | 0.87(0.56–1.35) | 0.543 |
| rs10849390 | Allele         | 1.07(0.82–1.39)                  | 0.616                    | 0.70(0.50–0.98) | 0.035 |
|          |                | 1.10(0.62–1.95)                  | 0.739                    | 0.44(0.20–0.99) | 0.047 |
|          |                | 1.18(0.80–1.74)                  | 0.410                    | 0.75(0.47–1.20) | 0.232 |
|          |                | 1.16(0.80–1.68)                  | 0.428                    | 0.68(0.43–1.06) | 0.088 |
|          |                | 1.01(0.59–1.72)                  | 0.973                    | 0.51(0.23–1.11) | 0.089 |
|          |                | 1.08(0.83–1.42)                  | 0.557                    | 0.70(0.50–0.98) | 0.037 |
| rs11610368 | Allele         | 0.89(0.61–1.30)                  | 0.544                    | 0.82(0.50–1.34) | 0.432 |
|          |                | 1.93(0.50–7.45)                  | 0.341                    | 1.43(0.37–5.53) | 0.607 |
|          |                | 0.80(0.51–1.24)                  | 0.313                    | 0.69(0.38–1.25) | 0.226 |
|          |                | 0.86(0.56–1.32)                  | 0.492                    | 0.76(0.43–1.31) | 0.321 |
|          |                | 2.03(0.53–7.82)                  | 0.303                    | 1.54(0.40–5.93) | 0.534 |
|          |                | 0.95(0.65–1.38)                  | 0.781                    | 0.85(0.52–1.37) | 0.500 |

CHD, coronary heart disease; OR, odd ratio; CI, confidence interval.

Haplotype analysis

We also conducted haplotype analysis of NINJ2 polymorphisms and CHD risk (Supplemental table 3).

We did not find significant association between haplotype of NINJ2 polymorphisms and susceptibility to CHD (P > 0.05). As it shown in Fig. 1, there are two blocks in NINJ2 (block 1: rs118050317 and rs75750647; block 2: rs7307242, rs10849390 and rs11610368).

Association of genotypes of NINJ2 polymorphisms and clinical indicators of CHD patients

In Supplemental table 4, we showed the association of different genotypes of NINJ2 polymorphisms (rs118050317, rs75750647, rs7307242, rs10849390 and rs11610368) and clinical indicators (UA, TC, TG, HDL, LDL, PLT, PDW, MPV and PCT) of CHD patients. All patients with different genotypes had significantly difference in rs118050317, rs11610368 and PLT level (P_{rs118050317} = 0.041, P_{rs11610368} = 0.040). There were no strong relationships between other polymorphisms and clinical indicators in
Discussion

In this study, we revealed that NINJ2 polymorphisms were associated with susceptibility of CHD. NINJ2 rs118050317 significantly increased CHD risk in elderly person (age > 60) and woman. Among CHD patients, NINJ2 polymorphisms (rs118050317, rs75750647, rs7307242 and rs10849390) had strong relationship with risk of hypertension and diabetes. In addition, we observed two blocks (block 1: rs118050317 and rs75750647; block 2: rs7307242, rs10849390 and rs11610368).

NINJ2 is a cell surface adhesion protein that is upregulated after nerve injury[16]. NINJ2 play an important role in nerve, stroke and inflammation[17–20]. We explored the effects of NINJ2 polymorphism on susceptibility of CHD. It is the first study to show the strong association of NINJ2 polymorphisms and CHD risk among the Chinese Han population. Aging and sex differences are obvious in the development of CHD. Advancing age is a non-modifiable risk factor for CHD in both men (age > 46) and women (age > 55)[21]. The death rate of CHD is higher among men than women at all stages, and it accelerate for women older than 60 years old[22]. To investigate the effects of NINJ2 polymorphisms on CHD risk in different populations, we did stratification analysis. Our results showed the effect of rs118050317 on CHD risk was related to age and sex. Rs118050317 significantly increased risk of CHD for the elderly people and women in multiple genetic models, it may attribute to the level of hormone. The exact mechanism of this influences need further studies. It gives us a clue for diagnosis, treatment and prevention of CHD in clinic.

Hypertension and diabetes are closely related to CHD. Previous studies showed each 10 mm Hg increase in systolic blood pressure is associated with an increased risk of CHD[23]. Moreover, people are more likely to have hypertension or diabetes with aging. Our study confirmed the association of CHD and some diseases (hypertension and diabetes). For CHD patients, rs118050317 and rs75750647 were associated with higher risk of hypertension and diabetes, individually. Rs7307242 and rs10849390 could protect CHD patients from hypertension and diabetes in genetic models, respectively. Additionally, many clinical factors are significantly different between cases and controls. We further explored the association of NINJ2 polymorphisms and clinical indicators among CHD
patients. We found the level of PLT is different in the genotypes of rs118050317 and rs11610368, it suggesting PLT may play a role in CHD. However, the mechanism of CHD is needed to explore in the future study.

Several limitations in the present study should be noted. Firstly, we recruited all study subjects from hospital, a selection bias may exist. Secondly, we only focused five polymorphisms of NINJ2, the association of other NINJ2 polymorphisms and CHD risk should be further studied. Thirdly, we did not perform association analysis stratified by smoking and drinking status due to the limitation of information. Hence, further investigation in larger populations and functional experiments is necessary to validate our findings.

Conclusions
In conclusion, NINJ2 polymorphisms were nominally associated with CHD susceptibility, which dependents on age, sex and complications (hypertension and diabetes). And, NINJ2 polymorphisms may be involved in the development of CHD. However, further studies are needed to confirm the results obtained in this study.

List Of Abbreviations
Coronary heart disease (CHD)
Single nucleotide polymorphisms (SNP)
Odd ratios (OR)
Confidence intervals (CI)
Ninjurin 2 (NINJ2)
Minor allele frequency (MAF)
Han Chinese in Beijing (CHB)
Hardy-Weinberg equilibrium (HWE)
Linkage disequilibrium (LD)
Uric acid (UA)
Total cholesterol (TC)
Triglyceride (TG)
High-density lipoprotein (HDL-C)
Low-density lipoprotein (LDL-C)
Platelet (PLT)
Platelet distribution width (PDW)
Mean platelet volume (MPV)
Plateletcrit (PCT)

Declarations

Ethical Approval and Consent to participate (Mandatory)

This study was approved by the Ethical Committee of First Affiliated Hospital of Xi’an Jiaotong University and conducted in accordance with the Declaration of Helsinki. And, informed consents were collected from all participants in this study.

Consent for publication

We are agree to publish our study.

Availability of data and material

All data and material are available.

Competing interests

The authors declare no conflict of interest.

Funding

No.

Authors’ contributions

Yuping Yan and Gang Tian designed this study, Yuping Yan, Gang Tian, Xiaoyan Du and Xiaoxi Liu mainly performed this study, Zichao Xiong, Jiamin Wu and Yao Sun collected samples, Jingjie Li analyzed data, Xiaoxi Liu wrote the draft.

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Figures
Figure 1

Haplotype block map for the SNPs of the NINJ2 gene. Block 1 includes rs118050317 and rs75750647. Block 2 includes rs7307242, rs10849390 and rs11610368. The numbers inside
the diamonds indicate the D for pairwise analyses.

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