Effects of Ninjurin 2 Polymorphisms on Susceptibility of Coronary Heart Disease

Yuping Yan

First affiliated hospital of xi'an jiaotong university

Xiaoyan Du

First hospital of yulin city

Xiaoxi Liu

Key laboratory of resource biology and biotechnology in western China

Jingjie Li

Laboratory of resource biology and biotechnology in western China

Zichao Xiong

Laboratory of resource biology and biotechnology in Western China

Jiamin Wu

Northwest university

Yao Sun

Northwest University

Gang Tian (✉ tiangang0802@163.com)

Xi'an jiaotong university

Research

Keywords: Coronary heart disease; NINJ2; Stratification analysis; Haplotype; Clinical indicators

Posted Date: February 26th, 2020

DOI: <https://doi.org/10.21203/rs.2.24554/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

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 Haploview 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: BP	Allele	Group	AA	AB	BB	OR(95%CI)	P	P _{HWE}
rs118050317	12: 634980	C/G	CHD	6	102	391	1.16(0.87–1.54)	0.310	1.000
			control	6	102	391			
rs75750647	12: 638831	A/G	CHD	44	224	231	0.93(0.77–1.12)	0.440	0.763
			control	56	220	229			
rs7307242	12: 641529	A/T	CHD	17	110	371	1.05(0.81–1.34)	0.729	0.261
			control	13	114	376			
rs10849390	12: 646086	A/G	CHD	66	232	197	1.18(0.98–1.42)	0.084	0.919
			control	53	220	220			
rs11610368	12: 662624	A/G	CHD	11	104	383	1.43(0.86–1.48)	0.384	0.659
			control	5	105	395			
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		Heterozygote		Dominant model		Recessive model		Additive model	
	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P
rs118050317	1.22(0.37–4.03)	0.748	1.17(0.85–1.60)	0.341	1.17(0.86–1.59)	0.323	1.18(0.36–3.9)	0.785	1.15(0.87–1.53)	0.329
rs75750647	0.76(0.49–1.18)	0.228	1.01(0.78–1.31)	0.937	0.96(0.75–1.23)	0.750	0.76(0.50–1.15)	0.198	0.92(0.76–1.12)	0.406
rs7307242	1.34(0.64–2.80)	0.437	0.98(0.73–1.32)	0.900	1.02(0.76–1.35)	0.906	1.35(0.65–2.81)	0.428	1.05(0.82–1.33)	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(0.86–1.86)	0.228	1.18(0.98–1.42)	0.086
rs11610368	2.27(0.78–6.61)	0.132	1.03(0.76–1.40)	0.852	1.09(0.81–1.46)	0.585	2.26(0.78–6.56)	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		Sex					
	> 60		≤ 60		Man		Woman	
	OR(95%CI)	P	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	1.75(1.07–2.86)	0.026
Homozygote	1.45(0.23–9.02)	0.691	0.72(0.12–4.40)	0.725	1.33(0.29–6.02)	0.709	1.09(0.15–7.97)	0.935
Heterozygote	1.73(1.11–2.70)	0.016	0.75(0.46–1.24)	0.265	0.89(0.6–1.31)	0.542	1.99(1.14–3.45)	0.015
Dominant	1.71(1.11–2.65)	0.015	0.75(0.46–1.22)	0.250	0.90(0.62–1.33)	0.607	1.92(1.12–3.28)	0.018
Recessive	1.31(0.21–8.12)	0.773	0.77(0.13–4.65)	0.772	1.37(0.30–6.16)	0.685	0.95(0.13–6.92)	0.957
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	1.74(1.06–2.86)	0.030
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)	P	OR(95%CI)	P
rs118050317	Allele	1.66(1.09–2.53)	0.017	0.83(0.50–1.39)	0.479
	Homozygote	1.44(0.25–8.12)	0.682	-	-
	Heterozygote	1.72(1.08–2.76)	0.024	0.92(0.53–1.60)	0.780
	Dominant	1.70(1.08–2.70)	0.023	0.86(0.50–1.48)	0.581
	Recessive	1.29(0.23–7.27)	0.776	-	-
	Additive	1.60(1.04–2.46)	0.031	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
	Homozygote	0.98(0.51–1.90)	0.961	2.20(1.05–4.63)	0.037
	Heterozygote	1.36(0.93–1.98)	0.116	1.63(1.01–2.62)	0.044
	Dominant	1.28(0.89–1.84)	0.175	1.72(1.09–2.71)	0.019
	Recessive	0.85(0.45–1.60)	0.615	1.71(0.85–3.41)	0.130
	Additive	1.13(0.85–1.50)	0.404	1.53(1.09–2.14)	0.013
rs7307242	Allele	0.79(0.56–1.13)	0.199	0.86(0.54–1.36)	0.512
	Homozygote	0.35(0.13–0.99)	0.049	0.83(0.23–2.99)	0.781
	Heterozygote	1.01(0.65–1.56)	0.974	0.85(0.49–1.46)	0.551
	Dominant	0.88(0.58–1.32)	0.530	0.85(0.50–1.42)	0.524
	Recessive	0.35(0.13–0.99)	0.047	0.87(0.24–3.08)	0.823
	Additive	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
	Homozygote	1.10(0.62–1.95)	0.739	0.44(0.20–0.99)	0.047
	Heterozygote	1.18(0.80–1.74)	0.410	0.75(0.47–1.20)	0.232
	Dominant	1.16(0.80–1.68)	0.428	0.68(0.43–1.06)	0.088
	Recessive	1.01(0.59–1.72)	0.973	0.51(0.23–1.11)	0.089
	Additive	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
	Homozygote	1.93(0.50–7.45)	0.341	1.43(0.37–5.53)	0.607
	Heterozygote	0.80(0.51–1.24)	0.313	0.69(0.38–1.25)	0.226
	Dominant	0.86(0.56–1.32)	0.492	0.76(0.43–1.31)	0.321
	Recessive	2.03(0.53–7.82)	0.303	1.54(0.40–5.93)	0.534
	Additive	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 CHD patients.

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.

Acknowledgements

The authors thank all participants for their support and participation.

References

1. Tang X, Laskowitz DT, He L, Østbye T, Bettger JP, Cao Y, Li N, Li J, Zhang Z, Liu J: Neighborhood socioeconomic status and the prevalence of stroke and coronary heart disease in rural China: a population-based study. *International Journal of Stroke* 2015, 10(3):388-395.
2. Sun Y-X, Gao C-Y, Lu Y, Fu X, Jia J-G, Zhao Y-J, Li L-D, Dui H-z, Zhang X-Y, Li Z-Y: Association between PPAP2B gene polymorphisms and coronary heart disease susceptibility in Chinese Han males and females. *Oncotarget* 2017, 8(8):13166.
3. Pan R-Y, Liu P, Zhou H-T, Sun W-X, Song J, Shu J, Cui G-J, Yang Z-J, Jia E-Z: Circular RNAs promote TRPM3 expression by inhibiting hsa-miR-130a-3p in coronary artery disease patients. *Oncotarget* 2017, 8(36):60280.
4. Wei-Wei C, Run-Lin G, Li-Sheng L, Man-Lu Z, Wen W, Yong-Jun W, Zhao-Su W, Hui-Jun L, Dong-Feng G, Yue-Jin Y: China cardiovascular diseases report 2015: a summary. *Journal of geriatric cardiology: JGC* 2017, 14(1):1.
5. Fujimaki T, Horibe H, Oguri M, Kato K, Yamada Y: Association of genetic variants of the α -kinase 1 gene with myocardial infarction in community-dwelling individuals. *Biomedical reports* 2014, 2(1):127-131.
6. Becker RC, Meade TW, Berger PB, Ezekowitz M, O'Connor CM, Vorchheimer DA, Guyatt GH, Mark DB, Harrington RA: The primary and secondary prevention of coronary artery disease: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest* 2008, 133(6):776S-814S.
7. Xu L, Chen X, Ye H, Hong Q, Xu M, Duan S: Association of four CpG-SNPs in the vascular-related genes with coronary heart disease. *Biomedicine & Pharmacotherapy* 2015, 70:80-83.
8. Dimou L, Schnell L, Montani L, Duncan C, Simonen M, Schneider R, Liebscher T, Gullo M, Schwab ME: Nogo-A-deficient mice reveal strain-dependent differences in axonal regeneration. *Journal of Neuroscience* 2006, 26(21):5591-5603.
9. Albelda SM, Smith CW, Ward P: Adhesion molecules and inflammatory injury. *The FASEB Journal* 1994, 8(8):504-512.
10. Granger DN: Leukocyte-endothelial cell interactions: molecular mechanisms and implications in gastrointestinal disease. *Gastroenterology* 1998, 114(5):1066-1090.
11. Lin K-P, Chen S-Y, Lai L-C, Huang Y-L, Chen J-H, Chen T-F, Sun Y, Wen L-L, Yip P-K, Chu Y-M: Genetic polymorphisms of a novel vascular susceptibility gene, Ninjurin2 (NINJ2), are associated with a decreased risk of Alzheimer's disease. *PloS one* 2011, 6(6):e20573.
12. Wang J, Fa J, Wang P, Jia X, Peng H, Chen J, Wang Y, Wang C, Chen Q, Tu X: NINJ2—A novel regulator of endothelial inflammation and activation. *Cellular signalling* 2017, 35:231-241.
13. Wan X-h, Li S-j, Cheng P, Zhang Q, Yang X-c, Zhong G-z, Hu W-l, Jin L, Wang X-f: NINJ2 polymorphism is associated with ischemic stroke in Chinese Han population. *Journal of the neurological sciences* 2011, 308(1-2):67-71.
14. Li X, Ma H, Li F, Li Y, Xie Z, Bai H, Gao B, Liang Q, Gao F, Jin T: CDKN2BAS polymorphisms are associated with coronary artery disease in Chinese Han population. *Int J Clin Exp Pathol* 2017, 10(1):804-810.
15. Chang W, Ma R, Zhang X, Yan Y, Zhang W, Zhao B, Li G, Wang H, Zhu Y, Song Y: Impact of PPAP2B and TCF21 gene polymorphisms on risk of coronary heart disease in Chinese Han population. *Int J Clin Exp Pathol* 2016, 9(2):1968-1974.
16. Araki T, Milbrandt J: Ninjurin2, a novel homophilic adhesion molecule, is expressed in mature sensory and enteric neurons and promotes neurite outgrowth. *Journal of Neuroscience* 2000, 20(1):187-195.
17. Zhang Z, Xu G, Wei Y, Zhu W, Fan X, Liu X: Impact of chromosome 12p13 variants on ischemic stroke risk. *International Journal of Neuroscience* 2016, 126(9):856-862.
18. Zhang Z, Ni G, Xu G, Xu J, Liu X: A novel functional polymorphism in the NINJ2 promoter predicts risk of large artery atherosclerotic stroke. *Molecular neurobiology* 2016, 53(10):7178-7183.
19. Bis JC, DeStefano A, Liu X, Brody JA, Choi SH, Verhaaren BF, Debette S, Ikram MA, Shahar E, Butler Jr KR: Associations of NINJ2 sequence variants with incident ischemic stroke in the Cohorts for Heart and Aging in Genomic Epidemiology (CHARGE) consortium. *PloS one* 2014, 9(6):e99798.
20. Zhu Y, Liu K, Tang X, Wang J, Yu Z, Wu Y, Chen D, Wang X, Fang K, Li N: Association between NINJ2 gene polymorphisms and ischemic stroke: a family-based case-control study. *Journal of thrombosis and thrombolysis* 2014, 38(4):470-476.

21. Tsang TS, Barnes ME, Gersh BJ, Hayes SN: Risks of coronary heart disease in women: current understanding and evolving concepts. In: *Mayo Clinic Proceedings: 2000*: Elsevier; 2000: 1289-1303.

22. La Croix A, Newton KM, Leveille SG, Wallace J: Healthy aging. A women's issue. *Western Journal of Medicine* 1997, 167(4):220.

23. Bush TL: The epidemiology of cardiovascular disease in postmenopausal women. *Annals of the New York Academy of Sciences* 1990, 592(1):263-271.

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplementaltables.pdf](#)