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Effects of *Ninjurin 2* Polymorphisms on Susceptibility of Coronary Heart Disease

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Primary research

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

Posted Date: August 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-33439/v2

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Version of Record: A version of this preprint was published at Cell Cycle on March 1st, 2024. See the published version at https://doi.org/10.1080/15384101.2024.2330225.

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 the risk of CHD in people over 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 were closely related to the risk of hypertension in CHD patients. Additionally, rs75750647 significantly increased diabetes risk in multiple models among CHD 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 suggested that the relationships of *NINJ2* polymorphisms and CHD risk were dependent on age, sex or complications.

Introduction

Coronary heart disease (CHD) is a common cardiovascular disorder (CVD) with rapidly increasing incidence 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 a complex disease resulting from the interactions 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]. The gene that encodes NINJ2 protein is located on chromosome 12p13. *NINJ2* interacts with several substances (IL-1β, TNF-α, IL-8, IL-6, ICAM-1 and E-selectin) in leukocyte and endothelial cells and thus plays an important role in the regulation of inflammation [9, 10]. *NINJ2* could regulate NF-kB and c-jun through interacting with TLR4[11]. 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 polymorphisms have shown convincing evidence related to 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 risk 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 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. Individuals who had CVD, immune diseases, cancer or disease history were excluded in the control group.

SNP selection and genotyping

Based on previous studies and data from 1000 Genomes Project (http://www.internationalgenome.org/), dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) and HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), 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 isolate genomic DNA from peripheral blood. Five SNPs were genotyped using the Agena MassARRAY platform Agena, San Diego, CA, USA). The primers of *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 analyzed with 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 assess 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 evaluated by 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 years old, 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 differences 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.

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 associations of *NINJ2* polymorphisms and CHD risk were 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 were dependent on age and sex. For the individuals over 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. Meanwhile, rs118050317 significantly increased the risk of CHD 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 associations with diabetes risk in CHD patients ($P < 0.05$).

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 Figure 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 found that *NINJ2* polymorphisms were associated with the 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. Five SNPs were intonic, and they might be affected CHD by regulating Promoter histone marks, Enhancer histone marks, DNAse, Motifs changed and Selected eQTL hits. 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* plays 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. Age 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 mortality of CHD is higher in men than in women at all stages, and it accelerates 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 be attributed to the level of hormone. The exact mechanism of these influences needs 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. Additionally, many clinical factors (UA, TC, LDL, PLT and PCT) were significantly different between CHD cases and controls. Especially, platelet plays an important role in CVD by involving in acute and chronic inflammatory[24]. We further explored the association of *NINJ2* polymorphisms and clinical indicators among CHD patients. We found the level of PLT was different in the genotypes of rs118050317 and rs11610368, it suggests the level of PLT may affect the development of CHD. However, the mechanism of CHD still needs further 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, due to the limitation of information we collected, we could not take more risk factors into this manuscript, such as family history, lifestyle factors, and diet habits. Hence, further investigations in larger populations and functional experiments are necessary to validate our findings.

Conclusions

In conclusion, the effects of *NINJ2* polymorphisms on CHD susceptibility were related to age, sex or complications (hypertension and diabetes). It revealed that *NINJ2* polymorphisms might be involved in the development of CHD among elder people (age > 60) and women. Our results will be helpful for the individualized diagnosis and testing of CHD. Additionally, further studies are needed to confirm the results obtained in this study.

Declarations

Ethics approval and consent to participate

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 all agree to publish this 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

Gang Tian and Yuping Yan designed and supervised this study. Yuping Yan, Xiaoyan Du, Zichao Xiong, Jiamin Wu and Yao Sun mainly performed this study. Xiaoxi Liu wrote and polished this manuscript, and Jingjie Li analyzed data.

Acknowledgements

The authors thank all participants for their support and participation.

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Tables

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.

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

SNP	Chromosome: BP	Position	Allele	Group	AA	AB	BB	OR(95%CI)	*P*	P_{HWE}	HaploReg v4.1
rs118050317	12: 634980	Intronic	C/G	CHD	6	102	391	1.16(0.87-1.54)	0.310	1.000	Enhancer histone marks, DNAse, Motifs changed
				control	6	102	391				
rs75750647	12: 638831	Intronic	A/G	CHD	44	224	231	0.93(0.77-1.12)	0.440	0.763	Promoter histone marks, Enhancer histone marks, DNAse, Motifs changed
				control	56	220	229				
rs7307242	12: 641529	Intronic	A/T	CHD	17	110	371	1.05(0.81-1.34)	0.729	0.261	Enhancer histone marks, DNAse
				control	13	114	376				
rs10849390	12: 646086	Intronic	A/G	CHD	66	232	197	1.18(0.98-1.42)	0.084	0.919	DNAse, Motifs changed
				control	53	220	220				
rs11610368	12: 662624	Intronic	A/G	CHD	11	104	383	1.43(0.86-1.48)	0.384	0.659	Promoter histone marks, Enhancer histone marks, DNAse, Motifs changed, Selected eQTL hits
				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.

Figures

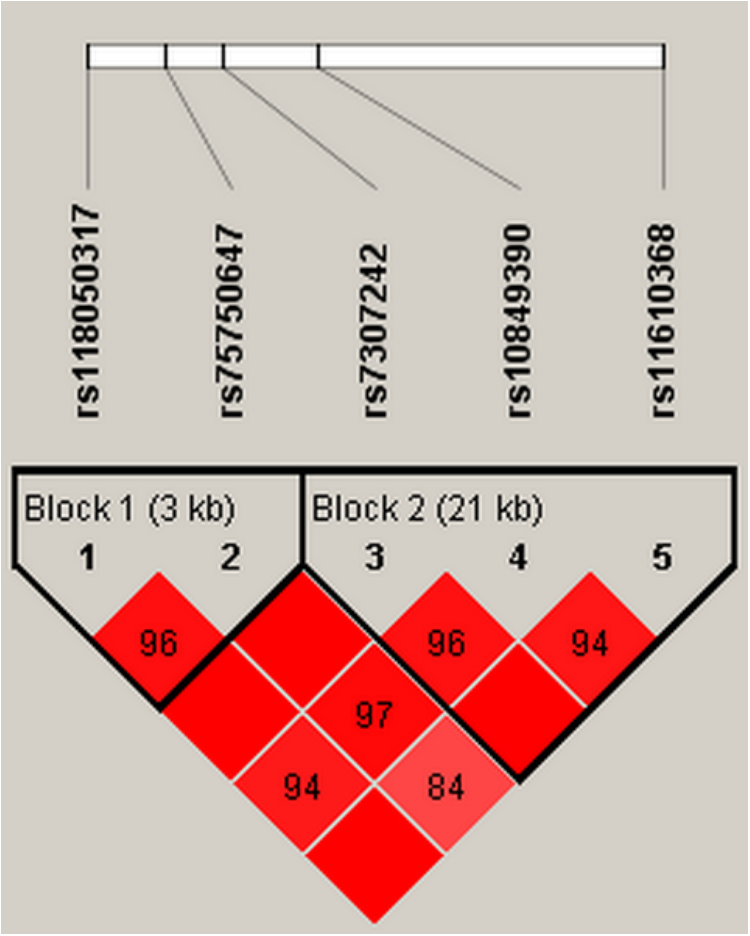


Figure 1

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.