NPAS4 Polymorphisms Contribute to Coronary Heart Disease (CHD) Risk

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Research article

Keywords: coronary heart disease, NPAS4, polymorphism, susceptibility, case-control

Posted Date: November 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-99035/v1>

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Abstract

Background: As genetic inheritance is an inevitable risk factor in the development of coronary heart disease (CHD), it is critical to identify the single-nucleotide polymorphism (SNP) locus of CHD risk. This case-control study exported whether the *NPAS4* polymorphisms are related to the CHD risk in the Chinese Han population.

Methods: The Agena MassARRAY platform was used to genotype the five SNPs in *NPAS4* from 499 CHD patients and 500 healthy controls. We used χ^2 test to compare the distributions of gender, allele and genotypes frequencies between cases and controls. The logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs).

Results: The rs4466842 in *NPAS4* was found to be correlated with an increased risk of CHD in overall, males and age ≤ 60 . The rs117186164 and rs12785321 in *NPAS4* were significantly related to an increased risk of CHD in the male and the age ≤ 60 , respectively. Moreover, the haplotype AC (rs117186164 and rs4466842) in *NPAS4* was significantly correlated with an increased risk of CHD.

Conclusions: Our results indicated that polymorphisms (rs117186164, rs12785321, and rs4466842) in *NPAS4* were significantly correlated with increased the risk of CHD in the Chinese Han population. Further association and functional studies are required to confirm the results and investigate the role of this SNP in the development of CHD.

Introduction

Coronary heart disease (CHD) is one of the leading causes of disability and death and tremendous social and financial burden worldwide.(1) The Reports of Cardiovascular Diseases showed that the prevalence of CHD disease in China continues to rise.(2) CHD is a complex multifactorial disease that is affected by genetic factors, environmental factors, and their interactions.(3, 4) Epidemiological studies have identified multiple risk factors which contribute to CHD development, such as advanced age, smoking, diabetes, hypertension, hyperlipidemia, obesity, lack of exercise, and dietary factors.(5–8) Previous family- or twin- based epidemiological studies demonstrated that genetic factors contributed to approximately 40%-50% of the risk of CHD.(9, 10) However, the genetic mechanism of CHD has not been fully explained. Recently, a large number of studies have revealed that single nucleotide polymorphisms (SNPs) are related to CHD susceptibility.(11, 12) As genetic inheritance is an inevitable risk factor in the development of CHD, it is critical to identify the SNP locus of CHD risk.

Neuronal Per-Arnt-Sim (PAS) domain protein 4 (Npas4), also known as neuronal transcription factor (NXF) and limbic-enriched PAS domain protein (LE-PAS), is an activity-dependent transcription factor belonging to the basic helix loop helix (BHLH)/PAS transcription factor family.(13) Npas4 is thought to be involved in functional regulation of neurons, because significant expression is found in the mature brain. Previous study suggested that *NXF* induced in response to several neurodegenerative stimuli/excitations for the cell protection, and thus provided an "on demand" cell-protection system in nervous tissue(14) indicate that sensory stimuli, by inducing *NPAS4* and its target gene brain derived neurotrophic factor (*Bdnf*), differentially control spatial features of neuronal inhibition in a way that restricts the output of the neuron while creating a dendritic environment that is permissive for plasticity.(15) Studies have indicated the functional role for *Npas4* in hippocampus-(16) and amygdala-dependent,(17) cognitive and social neuro-behavior (18) and learning and memory formation (19).

However, the contribution of the polymorphisms of *NPAS4* in the risk of CHD remains not reported. Therefore, we performed a case-control study including 499 CHD patients and 505 healthy controls to export the association between *NPAS4* polymorphisms (rs117770654, rs117957381, rs12785321, rs117186164, and rs4466842) of CHD risk in the Chinese Han population.

Subjects And Methods

Subjects

This study was involved with the blood samples of 499 CHD patients and 505 matched healthy controls from the First Affiliated Hospital of Xi'an Jiaotong. All patients were diagnosed with CHD according to standardized electrocardiogram, echocardiography, blood tests and coronary angiography and judged by at least two cardiologists independently. All of the control subjects were randomly recruited from the same hospital during the same period and also underwent a coronary angiogram to confirm no stenosis in their coronary arteries. Individuals were excluded from the study if they had other cardiac diseases (congenital heart disease, cardiomyopathy, concomitant valvular heart disease, or rheumatic heart disease). In addition, patients who had previously received angioplasty, intravenous thrombolysis, coronary artery stents, or coronary artery bypass surgery were also excluded. All the individuals were genetically unrelated Chinese Han people from Shaanxi.

The subject basic characteristics (age, gender and ethic) and clinical indicators information, including levels of total protein, albumin, globulin, total bilirubin, direct bilirubin, indirect bilirubin, uric acid, triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), glucose, white blood cell (WBC) count, neutrophil absolute value (NEUT), lymphocyte absolute value (LYMP), eosinophils absolute value (EO), basophilic absolute value (BASO), red blood cell (RBC) count, hemoglobin (HGB), erythrocyte mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW), red blood cell distribution width coefficient of variation (RDW-CV), platelet count, and plateletcrit was collected from medical record review.

DNA extraction

We collected 5 ml of fasting peripheral venous blood samples from CHD patients and controls, and placed in ethylene diamine tetraacetic acid (EDTA)-containing tubes. We used the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd. Xi'an City, China) to extract DNA from blood samples of the subjects according to the manufacturer's instructions.⁽²⁰⁾ We used the spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, USA) to determine the purity and concentration of the extracted DNA.

SNP selection and genotyping

The five genetic polymorphisms (rs117770654, rs117957381, rs12785321, rs117186164, and rs4466842) in *NPAS4* were screened by HapMap database. The Haploview 4.2 was used to select, and according to Chinese Beijing Han population (CHB), unbalanced r^2 value more than 0.8, and the minor allele frequency (MAF) was greater than 5%. The online software Agena Bioscience Assay Design Suite Version 2.0 (<https://agenacx.com/online-tools/>) was used to design the amplification and unique base extension primers. Applied Biosystems 7500 real-time PCR system was used for the DNA amplification. We used the Agena MassARRAY platform (Agena Bioscience, San Diego, CA, USA) to genotype the polymorphisms of the *NPAS4* gene according to the protocol described. The Agena Bioscience TYPER software (version 4.0) was used to manage and analyze the genotyping results.

Statistical Analysis

We used χ^2 test to compare the distributions of gender between the case group and the control group. The differences in the distribution of gender and age and clinical indicators were analyzed by Student's t-test. We also used χ^2 test to evaluate whether the genotype distribution of each SNP among the control group was in Hardy-Weinberg equilibrium (HWE) and evaluate the differences in the distribution of genotypes and allelic frequencies of these polymorphisms between case and control groups. We used genetic models (co-dominant, dominant, recessive, and additive) and haplotype analyses to evaluate the relationship between *NPAS4* polymorphisms. The logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) by PLINK software (version 1.07). The Haploview 4.2 software was used to conduct linkage disequilibrium (LD) haplotype blocks and calculate the linkage strength between each pair of SNPs with D' and r -squared values. All statistical analyses were two sided and p -value < 0.05 was considered statistically significant. The

SPSS 20.0 statistical package (SPSS, Chicago, IL) and Microsoft Excel (Microsoft Corp., Redmond, WA, USA) was to conduct the basic statistical analysis.

Results

Characteristics of participants

We recruited 499 CHD patients and 505 healthy controls. There were 317 males and 182 females in the case group, and 323 males and 182 females in the control group. The Chi-square test results indicated that no significant difference in the distribution of gender was found between the case and control groups ($P = 0.886$) (Table 1). The mean ages of the case and control groups were 61.34 and 60.51, respectively. No significant difference was found in the distribution of mean age between cases and controls in study populations ($P = 0.191$) (Table 1). There were no significant difference in triglyceride, HDL-C, EO and RDW-CV ($P > 0.05$) between the patients and controls. However, there were significant differences in some biochemistry and blood test indicators (total protein, albumin, globulin, total bilirubin, direct bilirubin, indirect bilirubin, uric acid, triglyceride, total cholesterol, HDL-C, LDL-C, WBC, NEUT, LYMP, EO, BASO, RBC, hemoglobin, MCV, MCH, RDW, RDW-CV, platelet, plateletcrit) between cases and controls ($P < 0.05$) (Table 1). Simultaneously, we also found the mean triglyceride and BASO levels in the case group were higher than the clinical reference values and control group, suggesting that these factors may increase the risk of CHD.

Table 1
Characteristics of the study population

Variables	Case		Control		<i>P</i>
	N	Mean ± SD	N	Mean ± SD	
Age (years)	499	61.34 ± 11.69	505	60.51 ± 8.11	0.191
Gender (M/F)	317/182	—	323/182	—	0.886
Total protein (g/L)	485	66.42 ± 6.36	360	71.65 ± 3.79	< 0.001
Albumin (g/L)	485	41.01 ± 4.33	362	45.58 ± 2.93	< 0.001
Globulin (g/L)	485	25.41 ± 3.3	362	26.21 ± 3.5	0.001
Total bilirubin (umol/L)	485	13.87 ± 6.12	362	16.97 ± 5.86	< 0.001
Direct bilirubin (umol/L)	485	7.76 ± 3.36	362	4.86 ± 2.01	< 0.001
Indirect bilirubin (umol/L)	484	6.04 ± 3.47	361	11.80 ± 3.98	< 0.001
Uric acid (umol/L)	476	292.67 ± 87.62	361	326.17 ± 80.95	< 0.001
Triglyceride (mmol/L)	470	1.78 ± 1.48	362	1.70 ± 1.18	0.418
Total cholesterol (mmol/L)	469	4.09 ± 1.15	361	4.78 ± 1.00	< 0.001
HDL-C (mmol/L)	470	1.13 ± 0.25	296	1.13 ± 0.25	0.862
LDL-C (mmol/L)	470	1.92 ± 0.82	293	2.65 ± 0.76	< 0.001
WBC (*10E9/L)	491	11.67 ± 15.48	360	5.79 ± 1.48	< 0.001
NEUT (*10E9/L)	491	4.97 ± 3.00	360	3.36 ± 1.12	< 0.001
LYMP (*10E10/L)	491	1.65 ± 0.79	360	1.85 ± 0.61	< 0.001
EO (*10E9/L)	433	0.14 ± 0.33	359	0.14 ± 0.15	0.814
BASO (*10E9/L)	414	0.42 ± 1.31	359	0.03 ± 0.02	< 0.001
RBC (10E12/L)	455	4.73 ± 0.66	360	4.83 ± 0.41	0.006
Hemoglobin (g/L)	452	140.11 ± 17.96	360	148.53 ± 14.09	< 0.001
MCV (fL)	455	92.27 ± 6.19	360	90.99 ± 4.25	0.001
MCH (pg)	455	29.57 ± 2.66	359	30.76 ± 1.66	< 0.001
RDW (fL)	454	43.79 ± 4.41	359	44.47 ± 3.56	0.015
RDW-CV (%)	455	13.29 ± 1.19	359	13.43 ± 1.04	0.064
Platelet (*10E9/L)	455	182.43 ± 60.35	359	207.51 ± 56.19	< 0.001
Plateletcrit (%)	440	0.21 ± 0.07	344	0.23 ± 0.05	< 0.001
F: female; M: male; SD: standard deviation; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; WBC: white blood cell count; NEUT: neutrophil absolute value; LYMP: Lymphocyte absolute value; EO: eosinophils absolute value; BASO: basophilic absolute value; RBC: red blood cell count; MCV: erythrocyte mean corpuscular volume; MCH: mean corpuscular hemoglobin; RDW: red blood cell distribution width; RDW-CV: red blood cell distribution width coefficient of variation					
<i>P</i> < 0.05 indicates statistical significance.					

Overall analysis of NPAS4 polymorphisms and CHD risk

The five SNPs (rs117770654, rs117957381, rs12785321, rs117186164, and rs4466842) in *NPAS4* were successfully genotyped, and the call rate of genotyping was more than 95%. The genotypes distributions of all SNPs were in line with HWE in both CHD patients and controls ($P > 0.05$, Table 2). We found that the allele C frequency of the rs4466842 in *NPAS4* in CHD patients was higher than the healthy controls (0.442 vs. 0.389, $P = 0.016$). The rs4466842 was related to an increased risk of CHD (OR = 1.25, 95% CI: 1.04–1.49). However, there was no significant association between other four SNPs and CHD risk.

Table 2
Alleles frequencies distribution and association with CHD risk

SNP-ID	Chr	Position	Role	Alleles A/B	MAF		HWE-p	OR (95% CI)	<i>p</i>
					Case	Control			
rs117770654	11	66410723	Intron	A/G	0.036	0.035	0.457	1.04 (0.65–1.67)	0.863
rs117957381	11	66411414	Intron	A/G	0.065	0.061	0.702	1.08 (0.76–1.56)	0.662
rs12785321	11	66412265	Intron	A/C	0.445	0.408	0.780	1.16 (0.97–1.39)	0.097
rs117186164	11	66412318	Intron	A/G	0.059	0.045	1.000	1.35 (0.90–2.01)	0.141
rs4466842	11	66415549	Intron	C/T	0.442	0.389	0.851	1.25 (1.04–1.49)	0.016
CHD: coronary heart disease; SNP: single nucleotide polymorphism; Chr: chromosome; MAF: minor allele frequency; HWE: hardy-Weinberg equilibrium; OR: odds ratio; CI: confidence interval.									
<i>p</i> < 0.05 indicates statistical significance.									

We performed genetic models analysis to explore the genotype distributions and the correlation between SNPs and CHD risk before and after adjusted with age and gender, as shown in Table 3. The results indicated that individual carrying the TT genotype of rs4466842 in *NPAS4* was correlated with an increased CHD risk, compared with the CC genotype (OR = 1.54, 95% CI: 1.06–2.22, $P = 0.022$; adjusted OR = 1.56, 95% CI: 1.08–2.25, $P = 0.018$). We found that the rs7318578 was related to an increased with CHD risk in the dominant model (TT-TC vs. CC: OR = 1.34, 95% CI: 1.03–1.74, $P = 4.70E-10$; adjusted OR = 1.35, 95% CI: 1.04–1.75, $P = 0.027$) and the additive model (OR = 1.25, 95% CI: 1.04–1.49, $P = 0.016$; adjusted OR = 1.25, 95% CI: 1.05–1.50, $P = 0.013$). However, no significant correlation was found between other four SNPs and CHD risk.

Table 3
Genotypes frequencies distribution and association with CHD risk

SNP-ID	Model	Genotype	Case (%)	Control (%)	OR (95% CI)	<i>p</i>	adjust OR (95% CI)	<i>p</i>
rs117770654	Codominant	GG	463 (92.8)	471 (93.3)	1		1	
		AG	36 (7.2)	33 (6.5)	1.11 (0.68–1.81)	0.677	1.10 (0.67–1.79)	0.708
		AA	0 (0)	1 (0.2)	–		–	
	Dominant	GG	463 (92.8)	471 (93.3)	1		1	
		AA-AG	36 (7.2)	34 (6.7)	1.08 (0.66–1.75)	0.765	1.07 (0.66–1.74)	0.796
		AG-GG	499 (100)	504 (99.8)	–		–	
	Recessive	AA	0 (0)	1 (0.2)	–	–	–	–
		–	–	–	1.04 (0.65–1.68)	0.863	1.03 (0.64–1.66)	0.895
rs117957381	Codominant	AA	436 (87.6)	445 (88.3)	1		1	
		GA	59 (11.8)	57 (11.3)	1.06 (0.72–1.56)	0.781	1.05 (0.71–1.55)	0.812
		GG	3 (0.6)	2 (0.4)	1.53 (0.25–9.21)	0.642	1.46 (0.24–8.80)	0.680
	Dominant	AA	436 (87.6)	445 (88.3)	1		1	
		GG-GA	62 (12.4)	59 (11.7)	1.07 (0.73–1.57)	0.718	1.06 (0.73–1.56)	0.756
		GA-AA	495 (99.4)	502 (99.6)	1		1	
	Recessive	GG	3 (0.6)	2 (0.4)	1.52 (0.25–9.14)	0.647	1.45 (0.24–8.75)	0.685
		–	–	–	1.08 (0.76–1.55)	0.665	1.07 (0.75–1.53)	0.707
rs12785321	Codominant	CC	156 (31.3)	175 (35.4)	1		1	
		AC	242 (48.5)	236 (47.7)	1.15 (0.87–1.52)	0.328	1.16 (0.87–1.53)	0.308
		AA	101 (20.2)	84 (17)	1.35 (0.94–1.94)	0.104	1.37 (0.96–1.97)	0.086
	Dominant	CC	156 (31.3)	175 (35.4)	1		1	
CHD: coronary heart disease; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.								
Adjust OR (95%CI) were calculated by logistic regression analysis with adjustments for age and gender.								
<i>p</i> < 0.05 indicates statistical significance.								

SNP-ID	Model	Genotype	Case (%)	Control (%)	OR (95% CI)	<i>p</i>	adjust OR (95% CI)	<i>p</i>
rs117186164	Recessive	AA-AC	343 (68.7)	320 (64.6)	1.20 (0.92–1.57)	0.171	1.21 (0.93–1.58)	0.152
		AC-CC	398 (79.8)	411 (83)	1		1	
		AA	101 (20.2)	84 (17)	1.24 (0.90–1.71)	0.186	1.26 (0.91–1.74)	0.160
	Additive	–	–	–	1.16 (0.97–1.38)	0.100	1.17 (0.98–1.40)	0.083
	Codominant	AA	445 (89.2)	461 (91.3)	1		1	
		GA	49 (9.8)	43 (8.5)	1.18 (0.77–1.81)	0.449	1.18 (0.77–1.82)	0.444
		GG	5 (1)	1 (0.2)	5.18 (0.6-44.51)	0.134	5.66 (0.65–48.89)	0.115
	Dominant	AA	445 (89.2)	461 (91.3)	1		1	
		GG-GA	54 (10.8)	44 (8.7)	1.27 (0.84–1.93)	0.261	1.28 (0.84–1.95)	0.250
Recessive		GA-AA	494 (99)	504 (99.8)	1		1	
	GG	5 (1)	1 (0.2)	5.1 (0.59–43.82)	0.138	5.57 (0.64–48.09)	0.119	
	Additive	–	–	–	1.32 (0.90–1.95)	0.156	1.34 (0.91–1.97)	0.142
rs4466842	Codominant	CC	155 (31.1)	189 (37.6)	1		1	
		TC	247 (49.5)	237 (47.1)	1.27 (0.96–1.68)	0.090	1.28 (0.97–1.68)	0.086
		TT	97 (19.4)	77 (15.3)	1.54 (1.06–2.22)	0.022	1.56 (1.08–2.25)	0.018
	Dominant	CC	155 (31.1)	189 (37.6)	1		1	
		TT-TC	344 (68.9)	314 (62.4)	1.34 (1.03–1.74)	0.030	1.35 (1.04–1.75)	0.027
		Recessive	TC-CC	402 (80.6)	426 (84.7)	1		1
	TT	97 (19.4)	77 (15.3)	1.34 (0.96–1.86)	0.085	1.35 (0.97–1.88)	0.073	
	Additive	–	–	–	1.25 (1.04–1.49)	0.016	1.25 (1.05–1.50)	0.013
	CHD: coronary heart disease; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.							
Adjust OR (95%CI) were calculated by logistic regression analysis with adjustments for age and gender.								
<i>p</i> < 0.05 indicates statistical significance.								

Subgroup analysis of NPAS4 polymorphisms and CHD risk

When stratifying by gender, we found that rs117186164 was significantly correlated with an increased risk of CHD in the allele (G vs. A: OR = 1.85, 95% CI: 1.09–3.16, $P = 0.022$), dominant (GG-GA vs. AA: OR = 1.81, 95% CI: 1.04–3.14, $P = 0.036$), and additive model (OR = 1.85, 95% CI: 1.09–3.15, $P = 0.024$) after adjusted with age in the males (Table 4). The rs4466842 was significantly correlated with an increased risk of CHD in the allele (T vs. C: OR = 1.37, 95% CI: 1.09–1.71, $P = 0.006$), codominant (TC vs. CC: OR = 1.42, 95% CI: 1.00–2.02, $P = 0.048$; TT vs. CC: OR = 1.83, 95% CI: 1.16–2.89, $P = 0.009$) dominant (TT-TC vs. CC: OR = 1.53, 95% CI: 1.10–2.12, $P = 0.012$), and additive model (OR = 1.36, 95% CI: 1.09–1.70, $P = 0.006$) after adjusted with age in the males (Table 4). However, no association was found between SNPs in *NPAS4* and CHD risk in females.

Table 4
Association of rs117186164 and rs4466842 with CHD risk stratified by gender

SNP-ID	Model	Genotype	Male				Female			
			Case (%)	Control (%)	OR (95% CI)	P	Case (%)	Control (%)	OR (95% CI)	P
rs117186164	Allele	A	597 (93.9)	624 (96.6)	1		342 (94.5)	341 (93.7)	1	
		G	39 (6.1)	22 (3.4)	1.85 (1.09–3.16)	0.022	20 (5.5)	23 (6.3)	0.87 (0.47–1.61)	0.651
	Codominant	AA	281 (88.4)	301 (93.2)	1		164 (90.6)	160 (87.9)	1	
		GA	35 (11)	22 (6.8)	1.70 (0.97–2.97)	0.063	14 (7.7)	21 (11.5)	0.66 (0.32–1.34)	0.244
		GG	2 (0.6)	0 (0)	–	–	3 (1.7)	1 (0.5)	3.03 (0.31–29.58)	0.341
	Dominant	AA	281 (88.4)	301 (93.2)	1		164 (90.6)	160 (87.9)	1	
		GG-GA	37 (11.6)	22 (6.8)	1.81 (1.04–3.14)	0.036	17 (9.4)	22 (12.1)	0.76 (0.39–1.49)	0.424
	Recessive	GA-AA	316 (99.4)	323 (100)	–		178 (98.3)	181 (99.5)	1	
		GG	2 (0.6)	0 (0)	–	–	3 (1.7)	1 (0.5)	3.17 (0.32–30.91)	0.321
	Additive	–	–	–	1.85 (1.09–3.15)	0.024	–	–	0.89 (0.50–1.59)	0.695
rs4466842	Allele	C	344 (54.1)	396 (61.7)	1		213 (58.8)	219 (60.2)	1	
		T	292 (45.9)	246 (38.3)	1.37 (1.09–1.71)	0.006	149 (41.2)	145 (39.8)	1.06 (0.79–1.42)	0.716
	Codominant	CC	94 (29.6)	125 (38.9)	1		61 (33.7)	64 (35.2)	1	
		TC	156 (49.1)	146 (45.5)	1.42 (1.00–2.02)	0.048	91 (50.3)	91 (50)	1.06 (0.67–1.66)	0.819
		TT	68 (21.4)	50 (15.6)	1.83 (1.16–2.89)	0.009	29 (16)	27 (14.8)	1.14 (0.61–2.15)	0.683
	Dominant	CC	94 (29.6)	125 (38.9)	1		61 (33.7)	64 (35.2)	1	

CHD: coronary heart disease; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval. OR (95%CI) were calculated by logistic regression analysis with adjustments for age. $P < 0.05$ indicates statistical significance.

SNP-ID	Model	Genotype	Male				Female			
			Case (%)	Control (%)	OR (95% CI)	<i>P</i>	Case (%)	Control (%)	OR (95% CI)	<i>P</i>
		TT-TC	224 (70.4)	196 (61.1)	1.53 (1.10–2.12)	0.012	120 (66.3)	118 (64.8)	1.07 (0.70–1.66)	0.747
	Recessive	TC-CC	250 (78.6)	271 (84.4)	1		152 (84)	155 (85.2)	1	
		TT	68 (21.4)	50 (15.6)	1.49 (1.00–2.24)	0.053	29 (16)	27 (14.8)	1.11 (0.62–1.96)	0.731
	Additive	–	–	–	1.36 (1.09–1.70)	0.006	–	–	1.07 (0.79–1.44)	0.683
CHD: coronary heart disease; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval. OR (95%CI) were calculated by logistic regression analysis with adjustments for age. <i>P</i> < 0.05 indicates statistical significance.										

The subgroup analysis was conducted based on mean age revealed that rs12785321 was significantly correlated with an increased risk of CHD in the allele (A vs. C: OR = 1.40, 95% CI: 1.06–1.84, *P* = 0.016), codominant (AC vs. CC: OR = 1.65, 95% CI: 1.05–2.58, *P* = 0.031; AA vs. CC: OR = 1.94, 95% CI: 1.09–3.44, *P* = 0.024), dominant (AA-AC vs. CC: OR = 1.72, 95% CI: 1.13–2.64, *P* = 0.012), and additive model (OR = 1.42, 95% CI: 1.07–1.89, *P* = 0.014) after adjusted with gender and age in the age ≤ 60 years old population (Table 5). The rs4466842 was also found to be significantly correlated with an increased risk of CHD in the allele (OR = 1.48, 95% CI: 1.12–1.94, *P* = 0.005), codominant (TT vs. CC: OR = 1.81, 95% CI: 1.03–3.19, *P* = 0.040), and additive model (OR = 1.34, 95% CI: 1.02–1.78, *P* = 0.038) after adjusted with gender and age in the age ≤ 60 years old population (Table 5). These results suggested that gender and age are important factors that influence the risk of CHD.

Table 5
Association of rs12785321 and rs4466842 with CHD risk stratified by age

SNP-ID	Model	Genotype	Age > 60 years old				Age ≤ 60 years old			
			Case (%)	Control (%)	OR (95% CI)	P	Case (%)	Control (%)	OR (95% CI)	P
rs12785321	Allele	C	316 (58.5)	350 (58.5)	1		238 (52)	236 (60.2)	1	
		A	224 (41.5)	248 (41.5)	1.00 (0.79–1.27)	0.997	220 (48)	156 (39.8)	1.40 (1.06–1.84)	0.016
	Codominant	CC	92 (34.1)	102 (34.1)	1		64 (27.9)	73 (37.2)	1	
		AC	132 (48.9)	146 (48.8)	1.02 (0.69–1.50)	0.932	110 (48)	90 (45.9)	1.65 (1.05–2.58)	0.031
		AA	46 (17)	51 (17.1)	1.26 (0.74–2.13)	0.396	55 (24)	33 (16.8)	1.94 (1.09–3.44)	0.024
	Dominant	CC	92 (34.1)	102 (34.1)	1		64 (27.9)	73 (37.2)	1	
		AA-AC	178 (65.9)	197 (65.9)	1.07 (0.74–1.54)	0.713	165 (72.1)	123 (62.8)	1.72 (1.13–2.64)	0.012
	Recessive	AC-CC	224 (83)	248 (82.9)	1		174 (76)	163 (83.2)	1	
		AA	46 (17)	51 (17.1)	1.24 (0.77–2.01)	0.370	55 (24)	33 (16.8)	1.44 (0.87–2.40)	0.156
	Additive	–	–	–	1.10 (0.85–1.42)	0.465	–	–	1.42 (1.07–1.89)	0.014
rs4466842	Allele	C	318 (58.9)	372 (60.8)	1		239 (52.2)	243 (61.7)	1	
		T	222 (41.1)	240 (39.2)	1.08 (0.85–1.37)	0.512	219 (47.8)	151 (38.3)	1.48 (1.12–1.94)	0.005
	Codominant	CC	94 (34.8)	111 (36.3)	1		61 (26.6)	78 (39.6)	1	
		TC	130 (48.1)	150 (49)	1.14 (0.77–1.69)	0.515	117 (51.1)	87 (44.2)	1.34 (0.85–2.10)	0.210
		TT	46 (17)	45 (14.7)	1.04 (0.62–1.75)	0.887	51 (22.3)	32 (16.2)	1.81 (1.03–3.19)	0.040
	Dominant	CC	94 (34.8)	111 (36.3)	1		61 (26.6)	78 (39.6)	1	

CHD: coronary heart disease; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval. OR (95%CI) were calculated by logistic regression analysis with adjustments for age. $P < 0.05$ indicates statistical significance.

SNP-ID	Model	Genotype	Age > 60 years old				Age ≤ 60 years old			
			Case (%)	Control (%)	OR (95% CI)	P	Case (%)	Control (%)	OR (95% CI)	P
		TT-TC	176 (65.2)	195 (63.7)	1.11 (0.77–1.62)	0.574	168 (73.4)	119 (60.4)	1.46 (0.96–2.24)	0.079
	Recessive	TC-CC	224 (83)	261 (85.3)	1		178 (77.7)	165 (83.8)	1	
		TT	46 (17)	45 (14.7)	0.96 (0.60–1.53)	0.867	51 (22.3)	32 (16.2)	1.52 (0.92–2.51)	0.098
	Additive	–	–	–	1.04 (0.81–1.34)	0.770	–	–	1.34 (1.02–1.78)	0.038
CHD: coronary heart disease; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval. OR (95%CI) were calculated by logistic regression analysis with adjustments for age. $P < 0.05$ indicates statistical significance.										

LD and haplotype analysis

Linkage analysis indicated that the five SNPs in *NPAS4* clustered two haplotype blocks, block 1: rs117957381 and rs12785321; block 2: rs117186164 and rs4466842, as shown in Fig. 1. Haplotype analysis results found that the haplotype AC (rs117186164 and rs4466842) was correlated with an increased CHD risk before and after adjusting by age and gender (OR = 1.25, 95% CI: 1.04–1.49, $P = 0.016$; adjusted OR = 1.25, 95% CI: 1.05–1.50, $P = 0.013$) (Table 6).

Table 6
Association between haplotypes in *NPAS4* and CHD risk

SNPs-ID	Haplotype	Case-Fre	Control-Fre	OR (95% CI)	p	adjust OR (95% CI)	p
rs117957381 rs12785321	AA	0.444	0.408	1.16 (0.97–1.38)	0.109	1.17 (0.98–1.39)	0.090
	GC	0.065	0.060	1.10 (0.77–1.57)	0.614	1.09 (0.76–1.56)	0.653
	AC	0.509	0.468	1.18 (0.99–1.41)	0.066	1.19 (0.99–1.42)	0.057
rs117186164 rs4466842	GT	0.059	0.045	1.32 (0.90–1.94)	0.162	1.33 (0.90–1.96)	0.147
	AT	0.383	0.344	1.18 (0.98–1.42)	0.073	1.19 (0.99–1.42)	0.066
	AC	0.442	0.389	1.25 (1.04–1.49)	0.016	1.25 (1.05–1.50)	0.013
CHD: coronary heart disease; SNP: Single nucleotide polymorphism; Fre: frequency; OR: odds ratio; 95% CI: 95% confidence interval							
Adjust OR and 95% CI were calculated using logistic regression adjusted with age and gender.							
$P < 0.05$ indicates statistical significance.							

Discussion

This case-control study explored the relationship between SNPs in *NPAS4* and susceptibility to CHD in the Chinese Han population. The results demonstrated that the rs4466842 was correlated with an increased risk of CHD in overall, males and age ≤ 60 years old population. The rs117186164 was related to an increased risk of CHD in the male. Moreover, rs12785321 was significantly correlated with an increased risk of CHD in the age ≤ 60 years old population. The haplotype AC in *NPAS4* was significantly correlated with an increased risk of CHD. Notably, there was significant difference in the distribution of some clinical indicators level in patients between the case and control groups.

NPAS4 is a member of the bHLH-PAS transcription factor family, which is involved in a wide range of physiologic and developmental events.(21) *NPAS4* as an activity dependent transcription factor which is responsible for gearing the expression of target genes involved in neuro-transmission. Previous study revealed the neuro-modulatory role of *NPAS4* in crucial pathways involved in neuronal survival and neural signalling hemostasis.(22) The *NPAS4* plays a neuroprotective role in ischaemic stroke by limiting progressive neurodegeneration and neuro-inflammation.(23) *NPAS4* is expressed in endothelial cells, regulates VE-cadherin expression and regulates sprouting angiogenesis.(24) *NPAS4* is a critical regulator of experience-dependent, structural, and functional plasticity at MF-CA3 synapses during contextual memory formation.(25) However, no study is known about the role of *NPAS4* in the development of CHD. This study is the first to explore the association between *NPAS4* polymorphisms and CHD risk. The results indicated that the rs4466842 in *NPAS4* was associated with an increased risk of CHD; rs117186164 and rs12785321 were found to be significantly associated with increased CHD risk only in the male and the age ≤ 60 years old population, respectively. These findings suggested that age and gender are important factors that influence the risk of CHD.

This study is the first to explore the association between *NPAS4* polymorphisms and CHD risk in the Chinese Han population. There are some limitations of our study should be mentioned. First, the study population is only the Chinese Han population and the sample size of this study is relatively small, thus the results are needed to confirm. Second, this study did not take into consideration the confounding factors such as smoking, drinking, hypertension, diabetes for CHD risk, as well as the interaction with *NPAS4* polymorphisms. Third, this study only investigated the association between five SNPs in *NPAS4* and CHD risk, there may be other SNPs in *NPAS4* that are associated with CHD risk but were not assessed for their potential associations. Finally, this study did not elucidate the specific mechanism of the *NPAS4* polymorphisms affect the development of CHD.

Conclusion

In conclusion, our results indicated that polymorphisms (rs117186164, rs12785321, and rs4466842) in *NPAS4* were significantly correlated with increased the risk of CHD in the Chinese Han population. However, this is the first study to explore and unclear whether those findings will be reproduced in other populations and/or different ethnicities. Therefore, further detailed association studies in larger samples are required to confirm the relationship of polymorphisms in *NPAS4* and risk of CHD. And functional studies are required to determine the effects of SNP in the gene expression or molecular pathways in the development of CHD.

Abbreviations

BASO
basophilic absolute value
CHB
Chinese Beijing Han population
CHD
coronary heart disease
CIs
confidence intervals

EDTA
ethylene diamine tetraacetic acid
EO
eosinophils absolute value
HDL-C
high-density lipoprotein cholesterol
HGB
hemoglobin
HWE
Hardy-Weinberg equilibrium
LD
linkage disequilibrium
LDL-C
low-density lipoprotein cholesterol
LYMP
lymphocyte absolute value
MAF
minor allele frequency
MCH
mean corpuscular hemoglobin
MCV
erythrocyte mean corpuscular volume
NEUT
neutrophil absolute value
ORs
odds ratios
RBC
red blood cell
RDW
red blood cell distribution width
RDW-CV
red blood cell distribution width coefficient of variation
SNP
single-nucleotide polymorphism
WBC
white blood cell

Declarations

Ethics approval and consent to participate

This study has been performed with the approval of the ethics committee of the First Affiliated Hospital of Xi'an Jiaotong (No.0846), and conducted in accordance with the standards of the Declaration of Helsinki. We obtained the written informed consent from each participant included in the study.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

None.

Authors' contributions

Gang Tian contributed to the design of the study and performed the statistical analysis. Yuping Yan was responsible for manuscript preparation. Xiangli Yin and Jingjie Li performed the experiments. Xiaoxi Liu and Yao Sun contributed to collect the data. Jiamin Wu and Zichao Xiong were responsible for checking the data. All authors were responsible for drafting the manuscript, read and approved the final version.

Acknowledgments

We thanks for the all individuals for their participation. We are grateful to the hospital staffs for collecting blood samples and clinical information.

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Figures

Figure 1

Haplotype block map for the five SNPs in NPAS4 Standard color frame is used to show LD pattern. Two blocks in the figure showed higher LD. The value in the each cell is the value of D' (multiplied by 100). Bright red represents very strong LD. pink represents intermediate LD. pink represents intermediate LD. White represents no linkage.