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Do CRP gene variants and smoking elevate recurrent stroke risk in minor ischemic stroke patients?

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Abstract

Background Minor ischemic strokes, despite their initial mild symptoms, pose a significant risk of recurrence, potentially leading to severe disability. The role of C-reactive protein (CRP) gene variants in predicting the recurrent minor stroke, particularly their interaction with lifestyle factors such as smoking, remains unclear. We aimed to investigate the relationship of single-nucleotide polymorphisms (SNPs) in CRP gene with minor stroke recurrence, focusing on gene–environment interactions.

Methods A retrospective cohort study was conducted using data from the stroke registry at Linfen People's Hospital, including 2032 first-time minor stroke patients (NIHSS score ≤ 5) admitted within 7 days of symptom onset from January 2019 to December 2022. Follow-up assessments were conducted every 3 months for one year. Based on recurrence during follow-up, participants were classified into Recurrence and Non-recurrence group. Genomic DNA was extracted for genotyping four CRP gene SNPs: rs1130864, rs1800947, rs2808632, and rs3093059. Genetic associations with the stroke recurrence were analyzed using additive, dominant, recessive genetic and allelic models. Generalized Multifactor Dimensionality Reduction (GMDR) was employed to explore the complex interaction of these SNPs with smoking status. Multivariate logistic regression was used to estimate the strength of these associations.

Results Our study recruited 261 participants who experienced recurrent minor strokes and 264 age- and sex-matched controls without recurrence. The A allele of rs2808632 ($P = 0.002$) and C allele of rs3093059 ($P = 0.001$) were significantly associated with an increased risk of stroke recurrence. Participants with the combined genotypes rs2808632 CA + AA and rs3093059 TC + CC had a 1.325-fold higher risk of recurrence when compared to those with the genotypes rs2808632 CC and rs3093059 TT ($P = 0.003$). Additionally, among smokers, the rs3093059 TC + CC genotypes were associated with a 2.467-fold increased risk of recurrence compared to non-smokers with TT genotype ($P < 0.001$).

Conclusion The rs2808632 and rs3093059 polymorphisms independently and interactively contribute to an increased risk of recurrent minor stroke. Furthermore, the interaction between rs3093059 SNP and smoking status significantly influenced stroke recurrence, highlighting the importance of considering both genetic and environmental factors in predicting the risk of minor stroke recurrence.

Keywords C-reactive protein, Gene variants, Recurrent minor stroke, Smoking, Interaction

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Introduction

Stroke continues to be one of the leading causes of morbidity and mortality, with a substantial burden in China [1, 2]. Every year, nearly 3 million new stroke cases are reported in China, approximately 30% of which are classified as minor ischemic strokes (minor strokes) [3]. Defined by a National Institutes of Health Stroke Scale (NIHSS) score ≤ 5, minor strokes often present with mild symptoms but carry a significant risk of recurrence, which can lead to severe long-term consequences [4, 5]. Despite advancements in acute stroke management, the high recurrence rate among minor stroke patients remains a major concern, with studies showing up to 17.7% of patients experiencing recurrence within the first year [6]. This recurrence contributes significantly to increased disability and mortality, underscoring the need for a deeper understanding of the underlying risk factors [7].

Although hypertension, diabetes mellitus, and tobacco use are established risk factors for minor stroke, the role of genetics in stroke recurrence is an emerging area of research [8]. C-reactive protein (CRP), a key biomarker of systemic inflammation, is associated with cardiovascular diseases [9]. In particular, elevated CRP levels have been shown to be associate with both the occurrence of initial stroke and with its recurrence [10–12]. Several studies suggest that single-nucleotide polymorphisms (SNPs) within the CRP gene may modulate CRP expression and inflammatory responses, potentially affecting stroke outcomes [13–16]. However, the specific role of CRP gene variants in minor stroke recurrence remains underexplored, especially in the context of gene–environment interactions.

Although smoking is a well-established modifiable risk factor for minor stroke, contributing to mechanisms such as atherosclerosis and endothelial dysfunction [17], the interaction between smoking and genetic susceptibility—particularly CRP gene polymorphisms—has not been adequately explored. Although there have been studies linking smoking with recurrent stroke risk, most have not considered the potential modulating effects of genetic variants, such as those within the CRP gene [18–20]. Investigating how CRP gene variants interact with smoking could offer valuable insights into the combined effects of genetic and environmental factors on minor stroke recurrence.

In this study, we aim to bridge this knowledge gap by exploring the potential relationship between CRP gene polymorphisms and minor stroke recurrence, with a particular focus on the interaction between these genetic variants and smoking status. While CRP gene variants have been studied in the context of stroke, our study uniquely focuses on minor stroke patients in a Chinese cohort, where genetic susceptibility and environmental factors such as smoking may exert distinct influences. Furthermore, we apply the Generalized Multifactor Dimensionality Reduction (GMDR) algorithm to explore complex gene–environment interactions, an approach that has not been extensively utilized in this field. By examining these interactions, we hope to provide insights into the combined effects of genetics and lifestyle factors, which could inform more personalized and effective prevention strategies for recurrent minor strokes.

Methods

Study group

Our study was approved by the Ethics Committee of Linfen City People's Hospital, and all participants provided informed written consent.

This single-center, hospital-based stroke registry at Linfen People's Hospital was conducted from January 2019 to December 2022. Initially, we identified a cohort of 2032 patients experiencing their first minor stroke, defined by an NIH Stroke Scale (NIHSS) score ≤ 5 within 7 days of onset [21]. Diagnosis strictly followed the World Health Organization's criteria, which stipulate that symptoms must persist beyond 24 h or lead to death, excluding non-vascular causes [22]. All patients underwent a comprehensive radiographic assessment, including MRI or CT, to confirm ischemic stroke (IS) and rule out hemorrhagic or non-ischemic events. The TOAST classification system was meticulously applied to categorize IS patients, with a particular focus on the small-vessel occlusion (SVO) and large-artery atherosclerosis (LAA) subtypes [23]. Clinical information was collected via in-person interviews performed by professional neurologists at the time of admission, which included demographic information and medical history (hypertension, diabetes, coronary heart disease), and lifestyle factors (smoking and alcohol use). Blood samples were collected for biochemical analysis, including measurements of triglycerides, total cholesterol, LDL-C, and HDL-C levels.

Follow-up and outcome assessment

Regular follow-up assessments were conducted every three months, continuing until the recurrence of stroke or the study's completion in December 2023. Data were collected through standardized questionnaires during telephone interviews or clinical visits. Recurrent stroke was defined as a novel episode of cerebral infarction occurring at least 21 days after the initial minor stroke, characterized by the abrupt onset of neurological deficits indicative of ischemic pathology [24, 25]. Participants were categorized into two groups based on outcomes: (1) the Recurrence group, consisting of those who experienced a recurrent stroke within the first year of their

initial stroke, and (2) the Non-recurrence group, comprising those who did not experience a recurrent stroke during the study period.

Following a rigorous screening process, we selected a study population of 1516 participants from the registry cohort of 2032 patients experiencing first minor stroke (Fig. 1). Among these, 298 patients experienced recurrence, while 1148 did not. Ultimately, we collected 261 blood samples from individuals with a history of minor stroke recurrence (Recurrence group) by the end of the follow-up period. A control group was then selected from the remaining 1,148 patients who did not experience a recurrent stroke, resulting in a control group of 264 blood samples (Non-recurrence group).

Definition of smoking status

Smokers were defined as individuals who had smoked more than 400 cigarettes in their lifetime [26]. Participants were further categorized into two groups based on their smoking status at baseline: current smokers and non-smokers. Current smokers were defined as individuals who had smoked within the 6 months prior to the onset of minor stroke or continued smoking during the follow-up period [20]; Non-smokers included individuals who had never smoked or had quit smoking for at least 6 months prior to the onset of minor stroke and remained smoke-free throughout the follow-up period.

SNP selection

We identified tagging SNPs within the CRP gene using two criteria: (1) SNPs previously associated with IS, and (2) genotype data from the International HapMap Project. We accessed the HapMap website (http://hapmap.ncbi.nlm.nih.gov/) and downloaded SNP genotype data from the Han Chinese population in Beijing, China. The data were analyzed using Haploview 3.0 software (http://www.broad.mit.edu/mpg/haploview/) in HapMap format. Tagging SNPs were selected based on a minor allele frequency (MAF) > 0.05 and linkage disequilibrium ($r^2 > 0.8$). Finally, four specific SNPs within the CRP gene were chosen for this study: rs1130864, rs1800947, rs2808632, and rs3093059. The biological information analysis for locations, mutations, MAFs, molecular consequence, and protein effect of the four SNPs is shown in Table S1.

Primer design and genotyping

Fasting venous blood samples (2 mL) were obtained from each participant in the early morning and promptly stored at – 80 °C for subsequent analysis. Genomic DNA was meticulously extracted from peripheral white blood cells utilizing the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences), adhering to the manufacturer's protocol. Primers for PCR–RFLP genotyping were designed utilizing Primer 5.0 software (Premier Biosoft

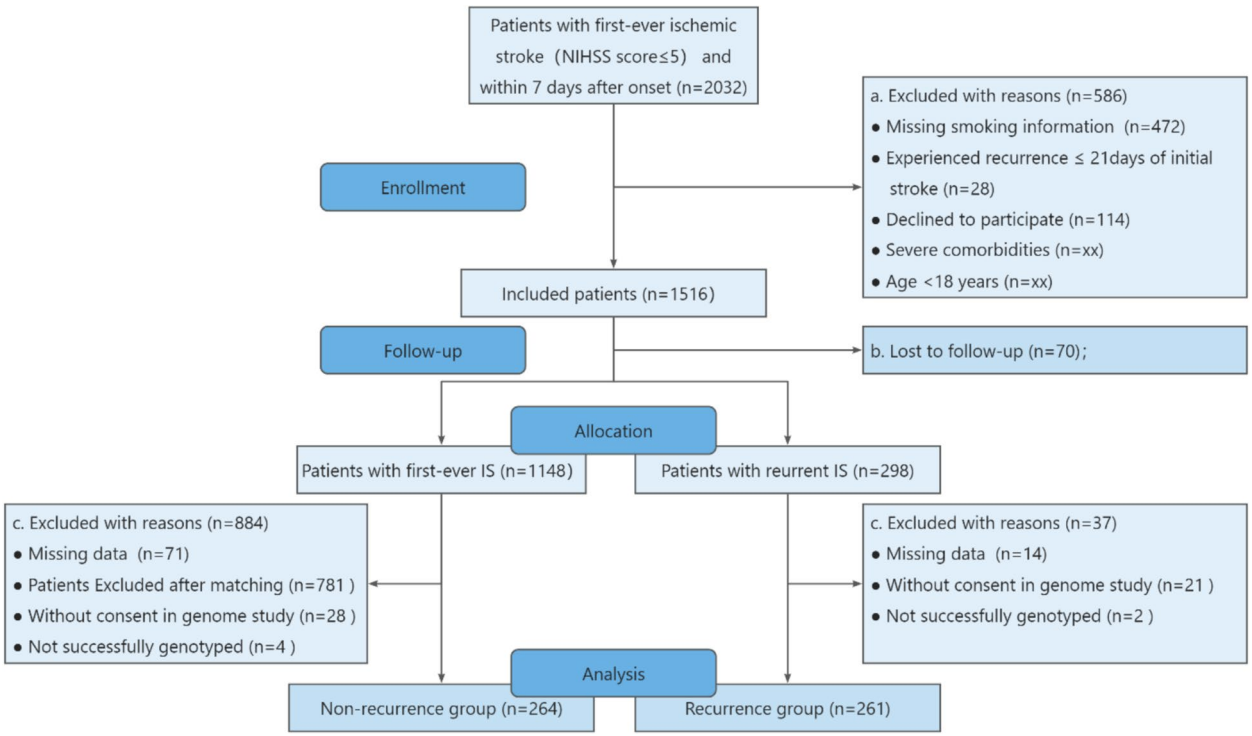

Fig. 1 Flowchart of participants selection

International). The PCR amplification was conducted in a 20-μL reaction mixture containing 10 ng of genomic DNA, 1.5 U of Taq DNA polymerase, 0.2 mmol/L of each dNTP, 10 μL of 2×PCR Master Mix, and 1 μL of each primer. The thermal cycling profile included an initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 90 s, and a final extension at 72 °C for 7 min. The PCR products were resolved on 3% agarose gels and visualized under ultraviolet light. Subsequently, the PCR amplicons were digested with 2 U of restriction enzymes (New England Biolabs) at 65 °C for 1 h, and the digested fragments were separated on a 3% agarose gel containing ethidium bromide to identify the genotypes, as detailed in Table 1.

Statistical analysis

Statistical analyses were performed with SPSS software (version 25.0) and GMDR software (version 0.9). The Shapiro–Wilk test was used to check the normality of data distribution of continuous variables, which are expressed as mean ± SD or median (interquartile range). Descriptive statistics for categorical data were expressed as counts (%), with comparisons made using Pearson's Chi-square and Fisher's exact test. Baseline demographics and clinical features were compared between the Recurrence and Non-recurrence groups. Quantitative variables with a normal distribution were compared by Student's *t*-test, while those without a normal distribution were analyzed using the Mann–Whitney *U* test. Hardy–Weinberg equilibrium (HWE) for the distribution of CRP SNPs In the Non-recurrence group was tested using Chi-square test. To better investigate the association between these SNPs and recurrent stroke risk, we employed three common genetic models: additive, dominant, and recessive. These models describe different ways in which genetic variants may influence disease risk (Table S2).

Multiple logistic regression analysis was conducted to evaluate the genotypes and combinations and calculated the odds ratios (OR) and 95% confidence intervals (CI). A Bonferroni correction was applied to the p-values from the multifactorial logistic regression to adjust for the multiple comparisons of four different SNPs in the same sample set (corrected $\alpha = 0.05/4 = 0.0125$). The GMDR method was employed to explore gene–gene and gene–environment interactions, identifying the best predictive model for minor stroke recurrence based on combinations of selected SNP genotypes. The optimal model was selected based on the highest testing balanced accuracy and cross-validation consistency (CVC). Significant interactions between SNPs and smoking status were visualized using interaction dendrograms and hierarchical graphs, generated with the MDR software (version 2.0). Interaction effects were quantified using entropy percentages, with the strongest synergistic interactions were highlighted. A two-tailed *P*-value of less than 0.05 was considered statistically significant. Post hoc power analysis was performed using G*Power software (version 3.1.9.2) with a significance level (α) set at 0.05, focusing on evaluating the statistical power for detecting associations between SNPs and recurrent stroke risk.

Results

Demographic characteristics

A total of 535 mild IS patients were screened and included in the study, and were subsequently categorized into two distinct groups according to the occurrence of recurrent stroke: the Recurrence group ($n = 261$) and the Nonrecurrence group ($n = 264$) (Fig. 1). The demographic and clinical characteristics of the participants are detailed in Table 2. The Recurrence group had higher rates of male prevalence, hypertension, diabetes, coronary heart disease, family history of stroke, smoking, and alcohol consumption ($P < 0.05$). However, no significant disparities were observed in terms of age and BMI between the two groups ($P > 0.05$).

Table 1 Primer sequences, restriction enzymes, and fragment lengths of SNPs in the CRP gene

SNP	Polymorphism	Primer	Restriction enzyme	Fragment length (bp)
rs1130864	C > T	F: 5′-GGCAGAAGCAAGCATCATCTT-3′ R: 5′-GGTCATGGGTGGGGAATTAAA-3′	HpyCH4III	C: 463 + 194 T: 657
rs1800947	G > C	F: 5′-GGCAGAAGCAAGCATCATCTT-3′ R: 5′-GGTCATGGGTGGGGAATTAAA-3′	Tsp45I	G: 114 + 543 C: 657
rs2808632	C > A	F: 5′-ACTCCCGTTTCCAATAAGTTCCT-3′ R: 5′-GCCATGCAGAACTGTGAGTCAA-3′	BfaI	C:178 + 170 A:348
rs3093059	T > C	F: 5′-GACTCCTGCCTGAAGCTTTACATAT-3′ R: 5′-TTCCCCTTCCTGTGTCCAAGT-3′	Tsp509I	T: 157 + 64 + 38 C: 221 + 38

Table 2 Baseline characteristics of patients with Recurrence group and Nonrecurrence group

Variables	Recurrence ($n=261$)	Nonrecurrence ($n=264$)	*P* values
Age (years)	66.93 ± 9.63	66.76 ± 6.51	0.817
Male, *N* (%)	151 (57.9)	147 (55.7)	0.615
BMI (kg/m^2)	24.67 ± 2.18	24.27 ± 2.95	0.083
Systolic BP (mmHg)	148.35 ± 23.91	135.94 ± 19.22	< 0.001
Diastolic BP (mmHg)	85.49 ± 12.44	80.40 ± 12.49	< 0.001
Lipids			
Total cholesterol (mmol/L)	5.27 ± 0.92	4.89 ± 1.22	< 0.001
Triglycerides (mmol/L)	1.63 ± 0.90	1.34 ± 0.70	< 0.001
HDL-cholesterol (mmol/L)	1.01 ± 0.41	1.12 ± 0.31	< 0.001
LDL-cholesterol (mmol/L)	3.48 ± 0.90	3.27 ± 0.85	0.006
Hypertension, *N* (%)	159 (60.9)	96 (36.4)	< 0.001
Diabetes, *N* (%)	65 (24.9)	37 (14.0)	0.002
Coronary heart disease, *N* (%)	75(28.7)	42 (15.9)	< 0.001
Family history of stroke, *N* (%)	65 (24.9)	33 (12.5)	< 0.001
Smoking, *N* (%)	104 (39.8)	64 (24.2)	< 0.001
Alcohol drinking, *N* (%)	70 (26.8)	44 (16.7)	0.005

Mean ± standard deviation values for quantitative variables and n (%) for qualitative variables

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. TC: total cholesterol; TG: triglyceride; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein

SNPs and recurrent minor stroke susceptibility

The genotype frequencies of CRP SNPs (rs1130864, rs1800947, rs2808632, and rs3093059) in the Nonrecurrence group were in accordance with HWE. Table 3 presents the association between CRP SNPs and minor stroke recurrence, assessed using multivariate logistic regression analysis. The CC genotype for rs1130864 and the GG genotype for rs1800947 were more frequently observed in both the Recurrence and Nonrecurrence groups. However, no statistically significant differences were found in the distribution of genotype or allele frequencies when comparing the two groups.

For rs2808632, a significant difference in genotype distribution was observed, with the AA genotype being more prevalent in the Recurrence group (OR 2.479, 95% CI 1.446–4.250, $P=0.001$). This association was further supported by both dominant and recessive models, which indicated a higher risk of minor stroke recurrence for AA genotype carriers (dominant: OR 1.582, 95% CI 1.081–2.314, $P=0.018$; recessive: OR 2.198, 95% CI 1.332–3.636, $P=0.002$). The A allele was also significantly more frequent in the Recurrence group compared to the Nonrecurrence group (OR 1.484, 95% CI 1.156–1.906, $P=0.002$). Similarly, rs3093059 was significantly associated with stroke recurrence, particularly for the CC genotype ($P=0.009$), and this association was consistent across both models (dominant: OR 1.571, 95% CI 1.113–2.217, $P=0.010$; recessive: OR 1.920, 95% CI 1.172–3.146, $P=0.014$). The C allele was significantly more frequent in the Recurrence group than in the Nonrecurrence group, indicating a potential association between the C allele and an increased risk of stroke recurrence (OR 1.556, 95% CI 1.201–2.018, $P=0.001$). However, after Bonferroni correction, only the additive and recessive models for rs2808632, and the additive model for rs3093059, remained significantly associated with an increased risk of minor stroke recurrence. These results were consistent following the correction, with the allele distributions of rs2808632 and rs3093059 continuing to show a significant association with stroke recurrence risk. Post hoc power analysis revealed 88% power to detect the association of rs2808632 and 93% power for rs3093059 with stroke recurrence, respectively.

GMDR analysis of SNP–SNP and gene–smoking interactions

The SNP–SNP and SNP–smoking interactions on minor stroke recurrence were analyzed using GMDR (Table 4). In the one-locus model, rs3093059 showed the highest attribution to predicting recurrence, with a testing balanced accuracy of 52.90% and a cross-validation consistency (CVC) of 7/10 ($P=0.0547$), indicating a modest association. The two-locus model, which considered the interaction between rs3093059 and rs2808632, the highest testing balanced accuracy at 58.65% achieved, with a perfect CVC score of 10/10 ($P=0.0010$), suggesting a significant synergistic effect between these two genetic variants in predicting stroke recurrence. Figure 2A illustrates the genotypes associated with high and low risks of stroke recurrence, as determined by the optimal two-locus model from the GMDR analysis, focusing on the interaction between rs2808632 and rs3093059. The genotype combinations most strongly associated with high recurrence risk were AA × CC, AA × TC, CA × CC, and CA × TC. In contrast, the genotypes indicating a lower risk of recurrence were CC × TC and CA × TT.

In the SNP–smoking interaction model, incorporating rs3093059 into the smoking model resulted in a slight decrease in testing balanced accuracy to 55.93%, while maintaining a high CVC of 7/10 ($P=0.0107$. This suggests a significant synergistic synergy between the genetic variant and smoking exposure as risk factors for recurrence. Figure 2B presents the risk genotypes identified in the optimal two-locus model, which includes the interaction between rs3093059 and smoking status, as determined by GMDR analysis. The genotypes associated

Table 3 Association of genotypes and alleles of CRP SNPs with minor stroke recurrence

SNPs	Genotypes and alleles	Recurrence *N* = 261	Nonrecurrence N = 264	OR (95%CI)	*P* value
rs1130864	Additive				
	CC	230 (88.1)	225 (85.2)	1.000 (ref)	
	CT	31 (11.9)	39 (14.8)	0.809 (0.469–1.396)	0.447
	Allele				
	C	491 (94.1)	489 (92.8)	1.000 (ref)	
	T	31 (5.9)	39 (7.2)	0.792 (0.486–1.290)	0.347
rs1800947	Additive				
	GG	228 (87.4)	237 (89.8)	1.000 (ref)	
	GC	33 (12.6)	27 (10.2)	1.331 (0.746–1.356)	0.334
	Allele				
	G	489 (93.7)	501 (94.9)	1.000 (ref)	
	C	33 (6.3)	27 (5.1)	1.252 (0.742–2.114)	0.399
rs2808632	Additive				
	CC	94 (36.0)	123 (46.6)	1.000 (ref)	
	CA	108 (41.4)	103 (39.0)	1.291 (0.851–1.960)	0.230
	AA	59 (22.6)	38 (14.4)	2.479 (1.446–4.250)	0.001*#
	Dominant				
	CC	94 (36.0)	123 (46.6)	1.00 (ref)	
	CA + AA	167 (64.0)	141 (53.4)	1.582(1.081–2.314)	0.018*
	Recessive				
	CC + CA	202 (77.4)	226 (85.6)	1.00 (ref)	
	AA	59 (22.6)	38 (14.4)	2.198 (1.332–3.636)	0.002*#
	Allele				
	C	296 (56.7)	348 (66.0)	1.00(ref)	
	A	226 (43.4)	179 (34.0)	1.484 (1.156–1.906)	0.002*#
rs3093059	Additive				
	TT	114 (43.7)	145 (54.9)	1.00(ref)	
	TC	97 (37.2)	90 (34.1)	1.435 (0.951–2.164)	0.085
	CC	50 (19.2)	29 (11.0)	2.117 (1.208–3.711)	0.009*#
	Dominant				
	TT	114 (43.7)	145 (54.9)	1.000 (ref)	
	TC + CC	147 (56.3)	119 (45.1)	1.605 (1.102–2.338)	0.014*
	Recessive				
	TT + TC	211 (80.8)	235 (89.0)	1.000 (ref)	
	CC	50 (19.2)	29 (11.0)	1.809 (1.604–3.077)	0.029*
	Allele				
	T	325 (62.3)	380 (72.0)	1.000 (ref)	
	C	197 (37.7)	148 (28.0)	1.556 (1.201–2.018)	0.001*#

The P value for additive, dominant and recessive models were calculated by multivariate logistic regression analysis with adjustment for age, sex, smoking, alcohol consumption, hypertension, diabetes, coronary heart disease and family history of stroke

* Significant according to ($P < 0.05$)

Significant according to Bonferroni's correction ($P < 0.0125$)

with the highest recurrence risk in the presence of smoking were CC × smoking and TC × smoking, whereas the genotypes linked to a reduced risk of recurrence under non-smoking conditions were TC × non-smoking and TT × non-smoking.

MDR analysis of SNP–SNP and gene–smoking interactions

We leveraged information gain theory to elucidate the complex interplay among these genetic variants. This approach facilitated the creation of an interaction dendrogram and hierarchical interaction graphs based on

Table 4 Multifactor dimensionality reduction results in the Recurrence group and Nonrecurrence group

Locus no	Best model	Training accuracy (%)	Testing accuracy (%)	Sign test (*P*)	CVC
Gene–gene interactions					
1	rs3093059	55.82	52.90	8 (0.0547)	7/10
2	rs3093059, rs2808632	60.10	58.65	10 (0.0010)	10/10
3	rs1800947, rs2808632, rs3093059	60.90	54.30	6 (0.3770)	6/10
4	rs1130864, rs1800947, rs2808632, rs3093059	61.88	54.88	8 (0.0547)	10/10
Gene–smoking interaction					
1	Smoking	57.80	57.90	9 (0.0107)	10/10
2	Smoking, rs3093059	60.75	55.93	9 (0.0107)	7/10
3	Smoking, rs3093059, rs2808632	62.22	56.10	8 (0.0547)	8/10
4	Smoking, rs1800947, rs2808632, rs3093059	64.12	54.87	6 (0.3770)	6/10
5	Smoking, rs1130864, rs1800947, rs2808632, rs3093059	66.39	56.52	7 (0.1719)	10/10

CVC: cross-validation consistency

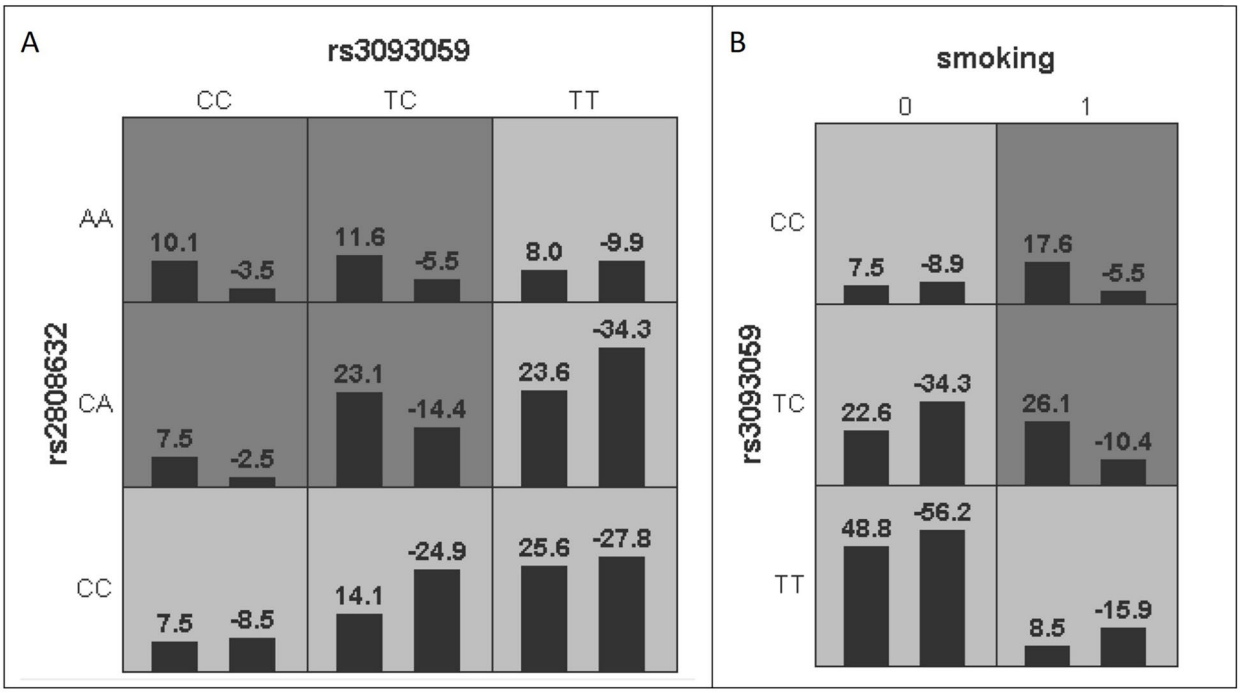

Fig. 2 GMDR for the distribution of high-risk and low-risk genotypes in the best two-factor model. The left bars correspond to positive interaction scores, whereas the right bars signify negative scores. Dark-shaded cells represent high-risk associations for IS recurrence, whereas light shading denotes low-risk associations. **A** The figure illustrates the interaction scores for two SNPs. **B** The figure illustrates the interaction scores for rs3093059 and smoking status (0 = no smoking, 1 = smoking history)

MDR analysis (Fig. 3). The interaction dendrogram showed that rs3093059 and smoking exhibited the strongest synergy, as indicated by the red line (Fig. 3A). The hierarchical interaction graphs demonstrated significant interactions between rs2808632 and rs3093059, with an interaction entropy of 1.06%, suggesting a positive interaction effect (Fig. 3B). Additionally, the interaction between rs3093059 and smoking status exhibited a notable entropy change of 1.42%, indicating a substantial impact on stroke recurrence. Notably, rs3093059 showed the strongest synergy with smoking status, as denoted by the red line, highlighting the potential influence of environmental factors such as smoking, in modulating genetic risk.

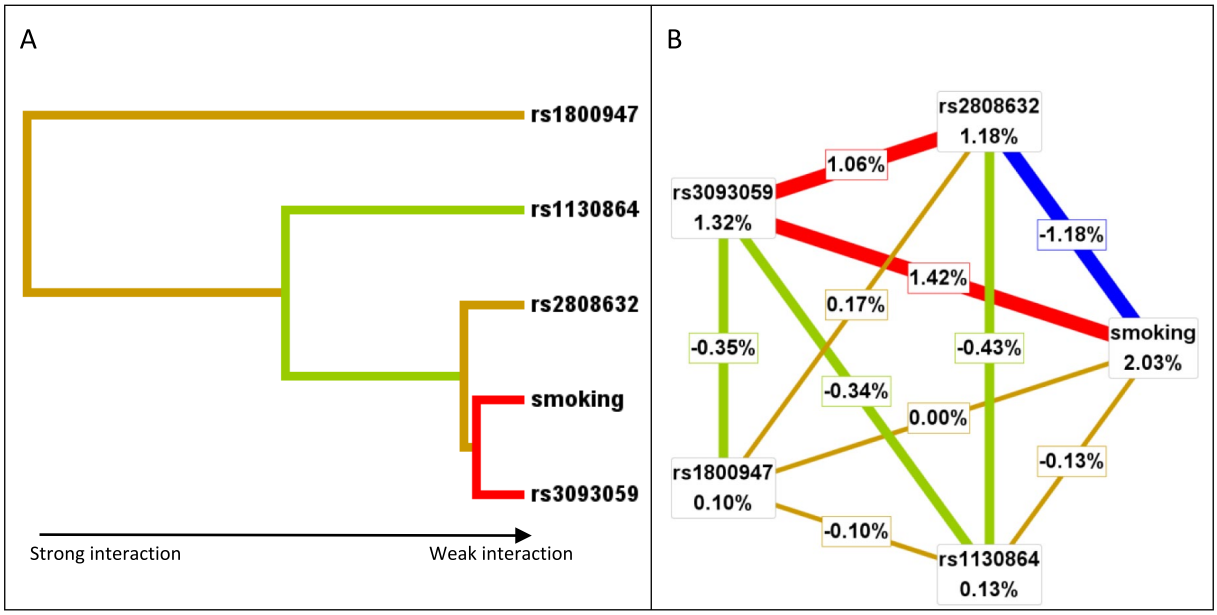

Fig. 3 Gene–environment interaction analysis diagram. **A** The interaction dendrogram provides a visual hierarchy of interactions, with the thickness of the red lines representing the strength of synergistic interactions, and thinner orange lines indicating weaker synergistic effects. The arrangement from left to right corresponds to increasing intensity of interaction. **B** The hierarchical interaction graph illustrates the interaction between SNPs and smoking status, where the percentage at the base of each SNP denotes its individual entropy percentage. The percentages on the connecting lines signify the interaction entropy percentage between paired SNPs and along with smoking status. A red line indicates a synergistic interaction with significant redundancy, while a blue line suggests a less pronounced redundancy interaction

Combined genetic variants and environmental factors in minor stroke recurrence

The logistic regression analysis in Table 5 reveals significant combined effects of rs3093059 and rs2808632 within CRP gene on minor stroke recurrence risk. Notably, the rs3093059 TC + CC genotypes, combined with the rs2808632 CA + AA genotypes, were associated with an increased risk of recurrence, with an adjusted OR of 2.325 (95% CI 1.341–4.032, $P = 0.003$), compared to individuals with the rs3093059 TT genotype and rs2808632 CC genotype.

Table 6 further explores the interaction between rs3093059 and smoking status. Smokers with the rs3093059 TC + CC genotypes exhibited a significantly higher risk of IS compared to never smokers with the TT genotype, with an adjusted OR of 3.467 (95% CI 2.040–5.923, $P < 0.001$).

Discussion

Our study focused on recurrent minor stroke, as stroke survivors were at an increased risk of subsequent strokes, which tended to be more fatal and disabling than the initial event [27, 28]. To the best of our knowledge, this was the first investigation to examine the relationship between four CRP gene SNPs and the 12-month risk of recurrent events in among patients with a history of minor stroke. Our findings revealed significant associations between specific CRP gene polymorphisms, particularly rs2808632 and rs3093059, and an increased susceptibility to recurrence. Moreover, an interplay

Table 5 Combined effects of rs3093059 and rs2808632 on the risk of minor stroke recurrence

rs3093059	rs2808632	Crude OR (95% CI)	Crude *P*	Adjusted OR (95% CI)	Adjusted *P*
TT	CC	1[a]		1[a]	
TC + CC	CC	0.777 (0.472–1.279)	0.321	0.865 (0.502–1.489)	0.600
TT	CA + AA	0.705 (0.411–1.208)	0.203	0.812 (0.451–1.460)	0.486
TC + CC	CA + AA	2.196 (1.326–3.638)	0.002	2.325 (1.341–4.032)	0.003

[a] The non-risk genotype for each genetic factor was used as the reference OR. The adjusted *P* value was calculated by multivariate logistic regression analysis with adjustment for sex, smoking, alcohol consumption, hypertension, diabetes, coronary heart disease and family history of stroke

Table 6 Association between CRP SNPs–environment interactions and minor stroke recurrence

rs3093059	Smoking	Crude OR (95% CI)	Crude *P*	Adjusted OR (95% CI)	Adjusted *P*
TT	No	1[a]		1[a]	
TC + CC	No	0.803 (0.525–1.231)	0.314	0.830 (0.525–1.313)	0.426
TT	Yes	0.619 (0.324–1.183)	0.147	0.537 (0.276–1.187)	0.134
TC + CC	Yes	3.167 (1.087–3.206)	< 0.001	3.467 (2.040–5.923)	< 0.001

[a] The non-risk genotype for each genetic factor was used as the reference OR. The adjusted *P* value was calculated by multivariate logistic regression analysis with adjustment for sex, alcohol consumption, hypertension, diabetes, coronary heart disease and Family history of stroke

between rs3093059 and smoking was identified, indicating that smoking amplified the genetic risk associated with this SNP. These findings emphasize the importance of considering both genetic and lifestyle elements in the management of minor stroke patients.

CRP is a key biomarker of systemic inflammation, and elevated CRP levels are associated with inflammation-related atherosclerosis and increased thrombogenic potential [29–31]. Several studies have shown that CRP levels correlate with the prognosis of IS [32–34]. It is worth noting that CRP SNPs are not only related to its serum level but also to inflammatory responses and the progression of atherosclerotic plaque, thus contributing to the occurrence of stroke [16, 35, 36]. rs2808632 and rs3093059, located in the 5′-upstream regulatory region of the CRP gene, may affect CRP gene transcription by altering transcription factor binding or promoter activity. This region contains enhancers and silencers that regulate gene expression, and SNPs here can influence the binding of inflammation-related transcription factors [37, 38]. Consequently, these variants may modulate CRP levels, thereby influencing systemic inflammation, a key factor in ischemic stroke pathogenesis [16].

While few studies have specifically examined the relationship between CRP gene polymorphisms and the likelihood of minor stroke, existing literature has identified a correlation between the rs3093059 polymorphism and adverse outcomes within three months for patients with large artery atherosclerosis IS [39]. This finding suggests a potential association between the rs3093059 CC genotype and the increased propensity for stroke recurrence. While limited research has focused on the role of rs2808632 in IS, this SNP is located in a critical region that is responsible for regulating the expression of CRP, a key mediator of inflammation. rs2808632 may influence CRP gene expression by modulating the interaction of transcription factors like NF-κB, which is central to inflammation [40]. Depending on the allele, these changes could either upregulate or downregulate CRP production, contributing to systemic inflammation, endothelial dysfunction, and atherosclerosis, thereby increasing the risk of recurrent stroke [41]. Although previous studies have linked rs2808632 to inflammatory diseases like rheumatoid arthritis [42], its direct relationship with stroke recurrence remains underexplored. In our study, minor stroke patients with the AA genotype of rs2808632 exhibited significantly higher risk of recurrence compared to those with the CC genotype, highlighting its potential as a genetic risk factor for stroke recurrence.

Our study also revealed the significant interaction between rs2808632 and rs3093059 within CRP gene. Patients with the AA genotype of rs2808632 and the CC genotype of rs3093059 had a markedly higher risk of minor stroke recurrence compared to other genotype combinations. This suggests a synergistic effect, where the presence of both high-risk genotypes exacerbates the likelihood of recurrence. This finding points to the critical role of genetic interactions in modulating stroke outcomes, and future studies should further explore the combined effects of these SNPs on stroke pathogenesis.

Smoking is a well-established risk factor for minor stroke, known to promote atherosclerosis and endothelial dysfunction [43, 44]—key mechanisms involved in the recurrence [15]. Previous studies have shown that patients who smoked at the time of their stroke or had a history of smoking are at a significantly higher risk of death or recurrent vascular events compared to those who have never smoked [25, 45, 46]. A multicenter hospital-based stroke registry in Japan found that current smokers (patients who smoked within 6 months prior to the onset of IS) had a higher risk of poor functional outcomes (1.29 [1.11–1.49]) compared to non-smokers among stroke patients [20]. A 10-year prospective cohort study of first-ever IS patients by Yao et al. found that higher daily cigarette consumption during the follow-up period was significantly associated with an increased risk of recurrence, with a hazard ratio (HR) of 1.027 (95% CI 1.003, 1.052) [46]. Similarly, Chen et al. also observed that continued smoking significantly increased the risk of stroke recurrence after the initial event, with a dose–response relationship between smoking intensity and recurrence risk [25].

In addition to its well-known vascular effects, smoking also exacerbates the inflammatory response and oxidative stress which further contribute to stroke recurrence

[47, 48]. These effects may be particularly pronounced when combined with genetic factors, such as CRP gene polymorphisms. The CRP gene plays a central role in the body's inflammatory response, and its genetic variants, especially rs3093059, may interact with smoking to amplify the inflammatory and oxidative processes that drive endothelial dysfunction. This gene–environment interaction could significantly increase the risk of recurrent ischemic events. By examining how smoking interacts with CRP gene variants, we aim to provide new insights into the complex interplay between genetic susceptibility and environmental exposures in stroke recurrence, potentially leading to more personalized risk predictions and preventive strategies for stroke patients.

In our study, smokers were defined as individuals who had smoked within 6 months prior to the onset of stroke or continued smoking during the follow-up period. This timeframe was chosen to capture the influence of recent smoking behavior on stroke recurrence, as smoking within this period is known to have a direct and immediate impact on vascular health [20]. Smoking during the months leading up to a stroke can significantly exacerbate systemic inflammation, oxidative stress, and endothelial dysfunction, all of which are critical mechanisms underlying stroke recurrence. Additionally, while many stroke patients attempt to quit smoking after their initial event, the adverse effects of smoking may persist even after cessation due to cumulative damage inflicted during the pre-stroke phase [25, 49]. By focusing on individuals who smoked within 6 months prior to stroke onset, we aimed to assess the short-term and residual effects of recent smoking behavior on recurrence risk, particularly in the context of genetic predispositions, such as CRP gene polymorphisms. This categorization allowed us to evaluate the combined impact of smoking and genetic factors on stroke outcomes.

This study provides valuable insights into the interaction between CRP gene polymorphisms and smoking status in minor stroke patients. However, several limitations should be considered. First, while the study provides strong evidence, the sample size may limit the statistical power to detect subtle associations, raising the possibility that some findings could be due to chance. Furthermore, although previous studies have indicated that both continuing smoking and smoking cessation after the first stroke are associated with a higher risk of recurrence compared to non-smokers [20, 25, 46], smoking status was assessed only at baseline in our study. This approach may have missed the potential impact of smoking during the follow-up period, where the interaction between smoking and CRP gene variants could be more pronounced. Additionally, the single-center design may limit the generalizability of our findings. To enhance the applicability of these results, future research should involve larger, multicenter cohorts. Longitudinal studies with continuous smoking status assessments would provide deeper insights into the long-term effects of smoking cessation and its interaction with genetic predisposition. Finally, exploring other gene–environment interactions, such as those related to diet and physical activity, could further elucidate the complex determinants of stroke outcomes.

Conclusion

Our study suggests that the rs2808632 and rs3093059 polymorphisms within CRP gene are key factors contributing to an increased risk of recurrent minor stroke, both independently and through their interaction. Additionally, we found that the interaction between the rs3093059 SNP and smoking status significantly influenced stroke recurrence, emphasizing the critical role of both genetic and environmental factors in predicting the risk of minor stroke recurrence.

Supplementary Information

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Supplementary Material 1.

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Author contributions

XL formulated and developed the research, secured financial support and ethical clearance, evaluated the findings, and authored the document in its entirety or partially. SS and ZS provided valuable assistance in the study design and contributed significantly to the preparation and review of the manuscript. WJ conducted data analysis and performed statistical analysis. LX was responsible for data collection and acquisition. MF and CL provided critical revisions to the manuscript and supervised the study. Manuscript preparation, editing, and review were collaboratively conducted by all authors.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Linfen City People's Hospital, and all participants provided informed written consent.

Competing interests
The authors declare no competing interests.

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