

RBPJ polymorphisms associated with cerebral infarction diseases in Chinese Han population

A Clinical Trial/Experimental Study (CONSORT Compliant)

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Abstract

Trial design: Cerebral small vessel diseases (CSVDs) are a group of brain pathological processes involving cerebral small arteries, brain venules, and capillaries. The recombination signal-binding protein Jκ (RBPJ) is implicated in the pathogenesis of these diseases but its actual roles need confirmation. The aim of this work was to evaluate variations in *RBPJ* gene for their possible associations with the disease.

Methods: The *RBPJ* gene was sequenced for 400 patients with cerebral infarction disease and 600 normal controls. The statistical analyses and Hardy–Weinberg equilibrium tests of the patients and control populations were conducted using the SPSS software (version 19.0) and Plink (version 1.9), Haploview software, and online software SNPSpD.

Results: We characterized variants rs2871198, rs1397731, rs3822223, rs2077777, rs2270226, and rs2788861 within or near the *RBPJ* gene. The genetic heterozygosity of rs2871198, rs1397731, rs3822223, rs2077777, and rs2270226 was very high. Statistical analysis showed that the variants rs2270226 and rs2077777 in the gene were associated with the risk of cerebral infarction diseases in the Chinese Han population.

Conclusions: rs2270226 and rs2077777 in the *RBPJ* gene were associated with the risk of cerebral infarction diseases in the Chinese Han population.

Abbreviations: CSVDs = cerebral small vessel diseases, LD = linkage-disequilibrium, RBPJ = recombination signal-binding protein Jκ, SNPs = single nucleotide polymorphism, VCI = cognitive impairment.

Keywords: cerebral infarction disease, cerebral small-vessel disease, notch signaling pathway, RBPJ

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QZ, JZ, and HL contributed equally to this work.

Patient consent obtained.

Ethics approval was taken from the Ethics Committee of Harbin Medical University.

The authors have declared that no competing interests exist.

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1. Introduction

Cerebral small vessel diseases (CSVDs), with many types including cerebral infarction, are a group of prevalent brain pathological processes involving cerebral small arteries, brain venules, and capillaries.^[1,2] These diseases are mainly divided into age-related cerebral small vessel disease, hypertension-related cerebral small vessel disease and cerebral amyloid angiopathy, and are often associated with other mental disorders, such as stroke, cognitive impairment (VCI), and vascular dementia.^[3–6]

Cerebral infarction is among the most common CSVDs, characterized by severe hypoxic ischemic tissue necrosis in the brain, often leading to repeated paralysis, sensory disability, language barrier, dementia, and other long-term functional disabilities with resultant low living quality.^[7–9] Clinically, a variety of therapies have been applied to treat the disease,^[10,11] but few are effective.^[12,13]

Insights into risk and etiologic factors would help develop novel strategies for the treatment of cerebral infarction diseases. The risk factors so far documented include smoking, obesity, dyslipidemia, high blood pressure, diabetes and aging,^[14,15] and the pathogenesis involves neuronal cell apoptosis.^[16,17] Blockage of the brain blood vessels is the direct cause of the disease, resulting from embolism or thrombosis and leading to cerebral ischemia,^[18] which may induce immune responses and inflammation.^[15,19–23]

The genetic etiology of the cerebral infarction disease involves the Notch signaling pathway,^[15,24,25] which is highly conserved in evolution and regulates the expression of many genes involved in cell proliferation or differentiation.^[26–28] The Notch signaling

Table 1**PCR primers used for *RBPJ* gene sequence analysis.**

Exon	Forward primer	Reverse primer	Size	Tm
1	AGCAGTTTAAACATACCAA	AATCCATAACCACATCTA	389	44.6
2	TACTTAGGGCATGTTGTT	GAGGGAAAGGAAGTTTGA	391	45.1
3	GCGTAGTAATGATGAAAG	GAACCCAGTAATAGAAGC	268	44.8
4	CCCgATTTCGTCCCTGTG	TTCCAATGTCAGCAGCGT	547	49.8
5	GGTTTTATCAGCTATCCCTT	TTTCCCAGAAATGTCCTT	529	48.8
6	TCCTTCTTTTGCTCCCTC	CCATCTCGGACTGTGAAT	402	48.1
7	TCACAGCAGTGGGGAGCC	AACAGCACTGATTACTAACATA	440	46.3
8	G TTCAGGGAAGAGGGTAA	GCTTGCATGCTGAGGAT	542	47.2
9	CTGCTGTCTATGGGGTTT	TCTGGCAAGTACGCTCCT	540	49.4
10	AAAATTAGGAGGAGCGTAC	TGCAGAAATGCTGGGAC	525	48.0
11	TTCTTCTCGGCACTGAT	GTTTTCCAAGAAACCAC	527	50.7

pathway also affects the proliferation of stem/progenitor cells and supports the central and peripheral nervous systems.^[29] In a previous study, we found that variants of the *Notch3* gene, combined with aging, are associated with the risk of cerebral infarction diseases.^[15]

In the process of Notch pathway functioning, the recombination signal-binding protein $J\kappa$ (*RBPJ*) mediates the signaling from the Notch receptors.^[30,31] *RBPJ* is a DNA binding regulatory factor, which recognizes the consensus sequence “C(T)GTGGGAA” and regulates many factors such as CSL transcription factors family.^[32] *RBPJ* is also associated with some corepressors and represses the Notch transcription,^[33] and the SNP rs874040 in *RBPJ* skews memory T cells toward a proinflammatory phenotype involving the notch signaling pathway.^[34] Based on these findings, we hypothesize that the *RBPJ* may play important roles in the pathogenesis of cerebral infarction diseases.

In the present study, we investigated variants rs2270226 and rs2077777 in the *RBPJ* gene for their associations with the risk of cerebral infarction disease in the Chinese Han population and demonstrated the association of the central repressor domain and 5'UTR with the functions of *RBPJ*. These findings provide novel insights into the roles of *RBPJ* gene in etiology and pathogenesis of the cerebral infarction diseases.

2. Materials and methods

2.1. Study population

A total of 400 patients with cerebral infarction disease and 600 normal controls were collected at the Department of Neurology and Medical Examination Center of the Daqing Oilfield General Hospital, Daqing, China. We performed the experiments in accordance with relevant guidelines and regulations and obtained a written informed consent from each participant. The Ethics Committee of HMU approved this work, which is consistent with the 1975 Declaration of Helsinki.

In the clinical examination, the medical histories and clinical features of the enrolled participants were recorded in detail. The participants also received physical, MRI, and other neurological

system examinations. The detailed information of the cerebral infarction patients and normal agedness controls were shown in the previous study.^[15]

2.2. DNA analysis

Genomic DNA of all the participants was extracted using standard protocols.^[35,36] The transcribed regions and splicing sites of the *RBPJ* gene were amplified by PCR with the primers shown in Table 1. The PCR products were sequenced for mutational analysis as previously described.^[37]

2.3. SNP genotyping analysis

The variations within or near the *RBPJ* gene were determined for the 400 cerebral infarction patients and 600 normal controls. The DNA regions were amplified and the PCR products were sequenced to determine the genotypes; 2 researchers conducted the measurements independently. Overall cerebral infarction disease genetics correlation analysis was also conducted.

2.4. Statistical analysis

Statistical analyses and Hardy–Weinberg equilibrium tests of the patients and control populations were conducted using the SPSS software (version 19.0)^[38] and Plink (version 1.9) as previously reported.^[39,40] The experiment-wide significance threshold, matrix of mpirwise linkage-disequilibrium (LD) correlation for the markers and haplotype diagram of LD structure were calculated using the online software SNPSpD and Haploview software as previously reported.^[41]

3. Results

3.1. SNP gene analyses

We sequenced the transcribed regions and splicing sites of the *RBPJ* gene to test the hypothesis that germline common genetic variants in the gene may confer the susceptibility to cerebral infarction diseases. We characterized variants rs2871198,



Figure 1. Schematic diagrams of rs2871198, rs1397731, rs3822223, rs2077777, rs2270226, and rs2788861 within or near the *RBPJ* gene.

Table 2

The genotype and allele frequency of rs2871198, rs1397731, rs3822223, rs2077777, and rs2270226 variants in 400 Chinese Han cerebral infarction and 600 normal agedness controls.

Variations	Group	Genotype frequency (%)			Allele frequency (%)		
rs2871198	Genotype		G/G	G/A	A/A	G	A
	CID	400	158 (39.5)	188 (47.0)	54 (13.5)	504 (63.0)	296 (37.0)
	Control	600	208 (34.7)	300 (50.0)	92 (15.3)	716 (59.7)	484 (40.3)
rs1397731	Genotype		C/C	C/T	T/T	C	T
	CID	400	331 (82.8)	64 (16.0)	5 (1.3)	726 (90.8)	74 (9.3)
	Control	600	490 (81.7)	106 (17.7)	4 (0.7)	1086 (90.5)	114 (9.5)
rs3822223	Genotype		C/C	C/A	A/A	C	A
	CID	400	148 (37.0)	179 (44.8)	73 (18.3)	475 (59.4)	325 (40.6)
	Control	600	246 (41.0)	266 (44.3)	88 (14.7)	758 (63.2)	442 (36.8)
rs2077777	Genotype		G/G	G/C	C/C	G	C
	CID	400	200 (50.0)	151 (37.8)	49 (12.3)	551 (68.9)	249 (31.1)
	Control	600	319 (53.2)	243 (40.5)	38 (6.3)	881 (73.4)	319 (26.6)
rs2270226	Genotype		T/T	T/C	C/C	T	C
	CID	400	85 (21.3)	170 (42.5)	145 (36.3)	340 (42.5)	460 (57.5)
	Control	600	91 (15.2)	326 (54.3)	183 (30.5)	508 (42.3)	692 (57.7)

CID=cerebral infarction diseases.

Table 3

SNP rs2270226 and rs2077777 variants within the *RBPJ* gene were associated with risk of cerebral infarction in Chinese Han populations.

Title	Statistical types	Pearson Chi-square				Risk		
		Value	Min count*	df	Asymp. sig. (2-sided)	Value	95%CI-low	95%CI-up
rs2871198	Genotype	2.527	58.40	2	0.283	–	–	–
	Allele	2.242	312.00	1	0.134	1.151	0.957	1.384
rs1397731	Genotype	1.334	3.60	2	0.513	–	–	–
	Allele	0.035	75.20	1	0.851	1.030	0.757	1.400
rs3822223	Genotype	2.898	64.40	2	0.235	–	–	–
	Allele	2.919	306.80	1	0.088	0.852	0.709	1.024
rs2077777	Genotype	10.581	34.80	2	0.005 [‡]	–	–	–
	Allele	4.869	227.20	1	0.027 [†]	0.801	0.658	0.976
rs2270226	Genotype	14.241	70.40	2	0.001 [‡]	–	–	–
	Allele	0.005	339.20	1	0.941	1.007	0.840	1.207

SNPs=single nucleotide polymorphism.

* The minimum expected count.

[†] Statistically significant, $P < .05$.

[‡] Statistically significant, $P < .005$.

rs1397731, rs3822223, rs2077777, rs2270226, and rs2788861 within or near the *RBPJ* gene (Fig. 1). Analysis of these SNPs showed that the genetic heterozygosity of rs2871198, rs1397731, rs3822223, rs2077777, and rs2270226 was very high, whereas that of rs2788861 was very low and was excluded from further analysis.

3.2. Polymorphism-disease association analyses

We conducted analyses on the SNPs to inspect their possible associations with the diseases and found that

the variants rs2270226 and rs2077777 in the gene were associated with the risk of cerebral infarction in the Chinese Han population (Tables 2 and 3). We conducted the Hardy-Weinberg equilibrium test for the study population groups, and it was in line with equilibrium (Table 4).

Experiment-wide significance threshold required to keep Type I error rate at 5% of rs2871198, rs1397731, rs3822223, rs2077777, and rs2270226 was 0.013. The Haploview software was used to conduct LD analysis of the

Table 4

Hardy-Weinberg equilibrium test for the study population groups.

SNPs	Genotype Homo/Hetero/Homozygote	H-W equilibrium Testing		
		O (HET)	E (HET)	P
Rs2871198	146/488/366	0.488	0.4758	.4646
Rs1397731	99/170/821	0.170	0.1703	.8540
Rs3822223	161/445/394	0.445	0.4729	.0615
Rs2077777	87/394/519	0.394	0.4067	.3132
Rs2270226	176/496/328	0.496	0.4884	.6507

SNPs=single nucleotide polymorphism.



Figure 2. LD analysis of the variants rs2871198, rs1397731, rs3822223, rs2077777, and rs2270226 in the *RBPJ* gene. The LD plots were generated using the Haploview software v4.2. (A) Data analysis between CID patients and controls from the present study; (B) data from HapMap CHB of variants in *RBPJ* gene. The data from the HapMap CHB and this work were consistent. No numeric in the cube, the value was 100. CID=cerebral infarction diseases, LD=linkage-disequilibrium.

variants rs2871198, rs1397731, rs3822223, rs2077777, and rs2270226, and the results were consistent with the data from the HapMap CHB population (Fig. 2). The genotype frequencies in the disease and control groups were further analyzed by 3 genetic models, including trend, dominant, and recessive models, in addition to chi-square tests, and all results indicated that the variants rs2077777 and rs2270226 were associated with the risk of cerebral infarction diseases (Table 5).

Table 5

SNP rs2270226 and rs2077777 within RBPJ gene associated with the risk of cerebral infarction diseases.

SNPs	Value	Trend model	Dominant model	Recessive model
rs2077777	ChiSQ	4.722 *	0.964	10.580 *
	P	.0298 *	.3262	.0011 *
rs2270226	ChiSQ	0.006	3.600	6.124 *
	P	.9406	.0578	.0133 *

SNPs=single nucleotide polymorphism.

* Statistically significant.

4. Discussion

In the present work, we validated the associations of *RBPJ* variants rs2270226 and rs2077777 with the risk of cerebral infarction diseases. Certain diseases or genetic factors can change the characteristics of the cerebral vascular systems and contribute to cerebral infarction or other CSVDs.^[18,42] In the blood vessel development, the Notch signaling pathway regulates the expression of many genes involved in the cell proliferation, differentiation, apoptosis, and cell fate decision,^[26] and coordinates arterial differentiation of endothelial cells.^[43–46] Targeted mutations of the Notch target genes could result in embryonic lethality due to severe defects in the angiogenic vascular remodeling.^[44,47] *RBPJ* is a transcriptional regulator, through which the Notch receptor regulates the downstream target genes when the Notch receptors bind with ligands.^[48,49] So, the DNA-binding protein *RBPJ* plays a central role in the Notch signaling pathway.^[50] In this work, we found that rs2270226 and rs2077777 in the *RBPJ* gene were associated with the risk of cerebral infarction disease, possibly via affecting the downstream target genes of the Notch signaling pathway.

As a member of the CSL (CBF-1, Suppressor of Hairless, Lag-2), *RBPJ* is a major downstream sequence-specific transcriptional repressor in the Notch signaling pathway.^[51] By recruiting distinct protein complexes to the promoter, the *RBPJ* functions on the target genes.^[52] The replication and transcription activators in the Notch signaling pathway activate the promoters by binding to the repression domain of *RBPJ*.^[53] It has been reported that mutations in the *RBPJ* binding sites suppressed the expression of the replication and transcription activators.^[54] The rs2077777 and rs2270226 in the *RBPJ* gene may affect the interactions between *RBPJ* and the activators.

Via the central repressor domain (aa 179–361 in *RBPJ* protein), the *RBPJ* protein recruits corepressor proteins and histone deacetylases, thus inhibiting gene expression.^[55] In the Notch signaling pathway, Notch proteins interact with their ligands, triggering a cascade of proteolytic reactions and ultimately liberating a fragment of their cytosolic tail also named Notch intracellular domain.^[56] When the fragments are transported to the nucleus, they interact with *RBPJ* by binding to the *RBPJ* central repressor domain.^[32,57] The rs2270226 variant is located near the central repressor domain of the *RBPJ* within the aa 50–100 region, consistent with the postulated roles of the central repressor domain for the functions of the *RBPJ*.

Through different regions or domains, *RBPJ* interacts with the Notch intracellular region, activates the downstream target genes transcription and regulates cell differentiation,^[53,57] such as the central repressor domain of *RBPJ* interacting with the RAM of Notch intracellular region.^[53] On the other hand, the N- and C-terminal regions of *RBPJ* bind to the Notch intracellular region ankyrin repeat; when mutated, the N- and C-terminal regions are defective in transcriptional activation.^[57] Analysis of rs2077777 located near the 5'UTR of the *RBPJ* gene further demonstrated the important roles of 5'UTR of the gene for its functions.

5. Conclusion

We validated the associations of *RBPJ* variants rs2270226 and rs2077777 with the risk of cerebral infarction diseases in the Chinese Han population and demonstrated the roles of central repressor domain and 5'UTR for the functions of the *RBPJ*.

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