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The association of genetic variants in chemokine genes with the risk of psoriasis vulgaris in Chinese population

A case–control study

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Abstract

This study aimed to investigate the influence of polymorphisms in chemokine genes, including *MCP1*, *CCR2*, and *CCR5* with psoriasis vulgaris (PV) risk in a Chinese population.

The genotyping of studied polymorphisms through polymerase chain reaction (PCR) and sequencing was conducted in 142 PV patients and 147 healthy controls. The genotype distribution of the polymorphisms in the control group was checked to determine whether it conformed to Hardy–Weinberg equilibrium (HWE). The genotype and allele frequencies were compared between PV patients and the healthy controls using Chi-square test. Odds ratio (OR) with 95% confidence interval (95% CI) was calculated to assess the relative risk of PV related to genetic variants.

CCR2 rs1799864 polymorphism was associated with significantly elevated risk of PV (AA+AG vs GG: OR = 1.62, 95% CI = 1.02–2.59; A vs G: OR = 1.48, 95% CI = 1.02–2.16). In the meanwhile, *CCR5* rs1800024 polymorphism also exhibited significant differences in genotype and allele distribution ($P < .05$), demonstrating its promoting effect on the risk of PV under heterozygous model (OR = 1.73, 95% CI = 1.06–2.82), dominance model (OR = 1.83, 95% CI = 1.14–2.94), and allele model (OR = 1.68, 95% CI = 1.13–2.48).

CCR2 rs1799864 and *CCR5* rs1800024 polymorphisms may function as independent risk factors for PV in Chinese population.

Abbreviations: AGE = agarose gel electrophoresis, CCL2 = chemokine (C-C motif) ligand 2, CCR2 = C-C chemokine receptor type 2, CI = confidence interval, HWE = Hardy–Weinberg equilibrium, MCP1 = monocyte chemoattractant protein 1, OR = odds ratio, PCR = polymerase chain reaction, PV = psoriasis vulgaris.

Keywords: *CCR2*, *CCR5*, chemokine genes, *MCP1*, polymorphisms, psoriasis vulgaris

1. Introduction

Psoriasis is a common chronic inflammatory skin disease with high recurrence and characterized by red, slightly elevated scaly papules or plaques.[1] It affects 0.3% to 3% of the global population, and shows higher prevalence in American, Canadian, and European than in other populations.[2,3] In clinic, psoriasis is classified into 4 types according to features: psoriasis vulgaris (PV), psoriasis arfhropathica, generalized pustular, and erythrodermic psoriasis.[4] Among them, PV is the most common type, accounting for approximately 95% of total psoriasis cases.[5] Although PV does not threaten its patients' lives, it seriously affects their life quality.[6] The pathogenesis of PV is still unclear, but genetic and environmental factors have been considered to participate in this process.[7–9] So far, a number of genes have been identified to be involved in this disease, though they could not completely explain all of the PV cases.

Chemokines are a group of cytokines controlling cells orient immigration, which is involved in immune response of the body, and realize their functions through chemokine receptors. The interaction between chemokines and receptors can regulate the orient immigration of various immune cells in recycle system, tissues, and organs so as to maintain the healthy microenvironment of the body.[10] Monocyte chemoattractant protein 1 (MCP1), also called chemokine (C-C motif) ligand 2 (CCL2), is a member of CC chemokine family and is encoded by *MCP1* located on chromosome 17q11.2–21.1.[11] It plays an important role in inflammatory response.[12] As the receptor of MCP-1, CCR2 participates in the conducting function of MCP1.[13] CCR5 is also an important chemokine receptor on the surface of white blood cells and involved in immune system and inflammatory reaction,[14] with CCL3, CCL4, CCL5, and CCL8 chemokines as its ligands.[15] According to previous

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Table 1

PCR primers sequences of polymorphisms.

Polymorphisms	Position		Primer sequences	Annealing temperature
MCP1 rs1024611	Promoter	For.	5'GGGAACTTCCAAAGCTGCCT3'	58°C
		Rev.	5'AGCTTTGCTGGCTGAGTGTT3'	
CCR2 rs1799864	Exon2	For.	5'TGCGGTGTTTGTGTTGTGTGGTCA3'	60°C
		Rev.	5'AGATGGCCAGGTTGAGCAGGT3'	
CCR5 rs1800024	Intron2	For.	5'TGTCTCCGTTCGGGTTGA3'	58°C
		Rev.	5'GTGGGGTCAGACGTCCAAAA3'	

reports, some functional polymorphisms in chemokine and chemokine receptors are correlated to the dysregulation of chemokine system, which are involved in some inflammation and other diseases.[16–18]

Therefore, in the present study, we selected 3 common functional polymorphisms (*MCP1* rs1024611, *CCR2* rs1799864, and *CCR5* rs1800024) to explore their associations with the risk of PV development in a Chinese Han population.

2. Materials and methods

2.1. The participants

In this study, we adopted a case–control design and enrolled a total of 289 people from August 2014 to January 2016, including 142 patients with PV and 147 healthy people as the controls. PV patients were diagnosed by at least 2 attending physicians in the Southwest Hospital, Third Military Medical University according to their clinical features, based on the diagnosis criteria of PV. Patients with other immune diseases would be excluded. In the case group, there were 88 males and 54 females with the initial age of 14 to 58 years. The healthy controls were selected from the physical examination center of the same hospital, consisting of 79 males and 68 females between 17 and 63 years old, and had no family history of PV. Necessarily, the controls were frequency-matched with the cases by age and gender.

The study subjects were all Chinese Han people without blood correction in Chongqing region. This study had obtained the approval of the Research Ethics Committee of Southwest Hospital, Third Military Medical University, and the objective of this study was informed to the participants and their families. Meanwhile, written informed consents were signed by each participant.

2.2. Blood sample collecting

Two milliliter peripheral venous blood was collected in the early morning from every subject with 12 hours empty stomach, and put into blood collection tube with anticoagulant EDTA2Na. The blood samples were stored at -80°C for next step.

2.3. Genotyping

First, blood genomic DNA was extracted using the methods of the conventional phenol chloroform extracting and ethanol precipitation. The quality and concentration of DNA were detected by 1.0% agarose gel electrophoresis (AGE) and NanoDrop 2000c NanoVue Plus. The genotyping for our studied polymorphisms was conducted via polymerase chain reaction (PCR) and sequencing. PCR primers were designed using Primer Premier 5.0 software based on the sequence information of chemokine genes in NCBI website, and synthesized by Shanghai Sangon Biotech Co., Ltd. The detailed information of PCR primers is listed in Table 1. PCR reaction was conducted in a volume of 25.0 μL system, containing 10 ng genomic DNA, 12.5 μL PCR Mix, 0.5 μL of each primer, and ddH_2O adding to designed volume. PCR procedure was completed in specific annealing temperature (see Table 1) for every polymorphism. Amplification products were detected for their quality and concentration using 1.0% AGE.

The eligible PCR products were sent to Sangon Biotech (Sangon, Shanghai, China) for sequencing so as to determine the detailed genotype of every polymorphisms in all subjects.

2.4. Statistical analysis

The genotype frequency of every polymorphism was obtained by counting. Genotype distribution of these polymorphisms in the control group was tested with Chi-square test to determine whether it was in line with Hardy–Weinberg equilibrium (HWE). The genotype and allele frequencies were compared between the case and control groups using Chi-square test, too. The risk of PV was evaluated via calculating odds ratio (OR) with the corresponding 95% confidence interval (95% CI). The data analyses were completed with PASW Statistics 18.0 software. The data were expressed by $\bar{x}+s$ or % in this study and $P < .05$ was considered as statistically significant difference.

3. Results

3.1. Basic characteristics of the subjects in the case and control groups

The detailed clinical information about our studied subjects in the 2 groups is summarized in Table 2. The mean age of PV patients in this study was 35.6 ± 11.3 years and 34.6 ± 10.7 years in the controls, which showed no significant difference between the 2 groups. The mean of the initial age in PV patients was 27.4 ± 9.5 years. The gender distribution in PV patients was 64 males and

Table 2

The detailed characteristics of subjects.

	Index	PV patients	The controls	*P*
	Total subjects, n	142	147	
Age/y	The range	14–73	17–69	
	Mean age	35.6 ± 11.3	34.6 ± 10.7	.43
	Mean initial age	27.4 ± 9.5		
	Gender (male/female)	88/54	79/68	.16
	Family history/%	38/26.8		
	Disease duration/y	6.7 ± 5.1		

PV = psoriasis vulgaris.

Table 3

The genotype frequencies of polymorphisms in chemokine genes in the case and control groups.

Polymorphism	Genotype/%			Allele/%	
MCP1 rs1024611	GG	AG	AA	G	A
Case, n = 142	58/40.84	70/49.30	14/9.86	186/65.49	98/34.51
Control, n = 147	47/31.97	79/53.74	21/14.29	173/58.84	121/41.16
P_{HWE}	0.184				
CCR2 rs1799864	GG	AG	AA	G	A
Case, n = 142	68/47.89	65/45.77	9/6.34	201/70.77	83/29.23
Control, n = 147	88/59.86	54/36.74	5/3.40	230/78.23	64/21.77
P_{HWE}	0.341				
CCR5 rs1800024	CC	CT	TT	C	T
Case, n = 142	72/50.70	61/42.96	9/6.34	205/72.18	79/27.82
Control, n = 147	96/65.31	47/31.97	4/2.72	239/81.29	55/18.71
P_{HWE}	0.535				

HWE = Hardy–Weinberg equilibrium.

78 females, and 57 males and 90 females in the healthy controls, without obvious difference between the 2 ($P = .28$). In PV patients, more than a quarter had the family history of PV and the mean disease duration was 6.7 ± 5.1 years.

3.2. HWE test

The genotype distribution in the control group was detected, and the results showed fine conformity to HWE for the rs1024611, rs1799864 and rs1800024 polymorphismss in the control group ($P = 0.184$, 0.341 and 0.535 respectively). So this study population was representative for typical Mendelian population, and the data are showed in Table 3.

3.3. The genotype distribution of the polymorphisms in chemokine genes in the 2 groups and their associations with PV risk

The detailed genotype frequencies of every polymorphism in chemokine genes are listed in Table 3 and the association of these polymorphisms with PV risk is summarized in Table 4. No significant difference in genotype or allele distribution was observed for *MCP1* rs1024611 polymorphism between the case and control groups. The genotype (GG, AG, and AA) frequencies of *CCR2* rs1799864 polymorphism were 47.89%, 45.77%, 6.34% in the case group and 59.86%, rs36.74%, 3.40% in the control group, respectively. The allele frequencies in 2 groups were 70.77% and 78.23% for G allele, and 29.23% and 21.77% for A allele. *CCR2* rs1799864 polymorphism showed significant association with the increased risk of PV in dominance model (OR = 1.62, 95% CI = 1.02–2.59) and allele model (OR = 1.48, 95% CI = 1.02–2.16). Similarly, the genotype and allele distributions of *CCR5* rs1800024 polymorphism were both detected to have a significant difference between the 2 group, showing an increasing effect on the risk of PV under the models of heterozygote, dominance, and allele (OR = 1.73, 95% CI = 1.06–2.82; OR = 1.83, 95% CI = 1.14–2.94; OR = 1.68, 95% CI = 1.13–2.48).

4. Discussion

In the current study, we investigated the influence of 3 common polymorphisms in chemokine relative genes on the risk of PV development in a Chinese Han population. In basic characteristics of the study subjects, no significant difference was observed in gender and age between PV patients and the controls. More than a quarter of PV patients in this study had the family history of PV, which suggested that the occurrence of PV had a genetic predisposition. This conclusion has been previously reported in several studies. For example, Harden et al[19] revealed a number of immune genes and relative encoded pathways associated with PV susceptibility to manifest the genetic predispositions of psoriasis.

In our data analyses, the polymorphism rs1024611 in *MCP1* did not show any association with PV susceptibility in genotype or allele. However, in the study by Zablotna et al,[20] *MCP1* rs1024611 polymorphism was found to be significantly associated with the increased risk of PV in northern Poland. Such difference may result from the distribution difference of the polymorphism, different ethnicity, and sample size. For *CCR2*

Table 4

The association of polymorphisms in chemokine genes with psoriasis vulgaris risk.

	Homozygous model	Heterozygous model	Dominance model	Allele model
MCP1 rs1024611				
OR (95% CI)	0.54 (0.25–1.18)	0.72 (0.44–1.19)	0.68 (0.42–1.10)	0.75 (0.54–1.06)
χ^2/P	2.44/.12	1.68/.20	2.46/.12	2.71/.10
CCR2 rs1799864				
OR (95% CI)	2.33 (0.75–7.27)	1.56 (0.96–2.52)	1.62 (1.02–2.59)	1.48 (1.02–2.16)
χ^2/P	2.22/.14	3.29/.07	4.17/.04	4.24/.04
CCR5 rs1800024				
OR (95% CI)	3.00 (0.89–10.13)	1.73 (1.06–2.82)	1.83 (1.14–2.94)	1.68 (1.13–2.48)
χ^2/P	3.40/.07	4.89/.03	6.33/.01	6.73/.01

CI = confidence interval, OR = odds ratio.

rs1799864 polymorphism, the carriage of minor allele A was significantly related to higher risk of PV occurrence, functioning as the risk factor for the disease. But Soto-Sánchez et al[21] reported that *CCR2* rs1799864 polymorphism did not contribute to the risk of PV in Spanish Caucasians. The inconsistent results may be caused by different races and sample size. *CCR5* rs1800024 polymorphism was also correlated to the elevated risk of PV development in Chinese Han population, and this is the first time to explore its association with PV susceptibility.

MCP1, *CCR2*, and *CCR5* all belong to chemokine relative genes. Chemokines combined with relative receptors could exert chemotactic effect on various cells, such as lymphocytes, monocytes, neutrophils, and dendritic cells, which are involved in multiple immune reaction and inflammatory injury. CC-chemokine is the largest one in chemokine family and is found to participate in the inflammation-immune mechanism of psoriasis. As an important chemokine, MCP1 is mainly responsible for the recruitment of monocytes and T-lymphocytes.[22] Lembo et al[23] conducted a case–control study on psoriasis and found that serum MCP1 level in psoriasis patients was significantly higher than that in healthy controls and exhibited positive association with TNF-α level. As MCP1 receptor, CCR2 could regulate the infiltration of monocytes and macrophages in PV patients after binding to MCP1.[24] Furthermore, CCR5 mRNA level in the dermis of PV patients' skin was obviously higher than that in epidermis. IFN-γ+ iNKT cells have been reported to show high expression of CCR5 in PV skin, and its migration and invasion to PV skin are dependent on CCL5/CCR5 chemotaxis.[25]

Reportedly, rs1024611 is located on the promoter region of *MCP1* causing the substitution of G/A and associated with upregulated expression of MCP1.[26] This polymorphism has been reported to participate in various diseases, such as gestational diabetes mellitus, age-related macular degeneration, and cardiovascular disease. *CCR2* rs1799864 polymorphism is a nonsynonymous mutation in exon2 region leading to the alteration of Val/Ile amino acid, which causes increased stability and expression of CCR2A isoform on cell surface.[27] Rs1800024 in *CCR5* is located on intron2 region and tightly linked to *CCR2* rs1799864 for predicting HIV transmission.[28] Unfortunately, these polymorphisms are rarely studied for their association with PV development.

In conclusion, the polymorphisms rs1799864 in *CCR2* and rs1800024 in *CCR5* were significantly associated with the occurrence risk of PV in Chinese population. However, the detailed mechanisms underlying the functional roles of rs1799864 and rs1800024 in PV incidence are still unknown, and further explorations are needed. In this study, some limitations should not be neglected, especially small sample size and single population. Therefore, in the future, more works are needed to verify our findings and illustrate the detailed mechanism of these involved polymorphisms based on larger sample size and multiple populations.

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