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Original Article

Correlation of macrophage inflammatory protein-1α single gene polymorphisms with the susceptibility to pigeon breeder's lung in chinese uygur population

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Abstract: Objective: To investigate the correlation of macrophage inflammatory protein-1α (MIP-1α) gene single nucleotide polymorphisms (SNP) with the susceptibility to pigeon breeder's lung (PBL) in Chinese Uygur population. Methods: A total of 92 Uygur from Xinjiang, China were enrolled in the study. Among them, there were 32 patients with PBL, 30 negative controls with history of exposure to pigeons and 30 normal controls without pigeons contact. SNP genotyping for 24 SNPs of MIP-1α were performed. Results: Genotype distribution of MIP-1α SNPs rs1049191, rs1049195, rs3210166, rs1130374 and rs5029407 were significantly different among the three groups ($P<0.05$). Conclusion: MIP-1α SNPs rs1049191, rs1049195, rs3210166, rs1130374 and rs5029407 might have correlation with the susceptibility to pigeon breeder's lung in Chinese Uygur population.

Keywords: Pigeon breeder's lung, macrophage inflammatory protein 1α, single nucleotide polymorphism, susceptibility, uygur

Introduction

Pigeon breeder's lung (PBL) is one form of extrinsic allergic alveolitis (EAA) or hypersensitivity pneumonitis (HP) in which the repeated inhalation of dispersed antigens provokes a hypersensitivity reaction in the lungs of sensitized people [1]. Multiple pro-inflammatory, anti-inflammatory and other cytokines are involved in the process of the disease. In our previous study, the level of bronchoalveolar lavage fluid (BALF) and serum IL-10 decreased in Uygur patients with PBL while the level of IL-2 and TNF-α increased [2].

Macrophage inflammatory protein-1α (MIP-1α) is a member of the C-C chemokine family and induces chemotaxis of monocytes-macrophages, eosinophils, basophils, T cells (especially CD8+ memory cells), NK cells and B cells [3, 4]. In addition to chemotaxis, chemokines can influence differentiation of lymphocytes into either Th1 or Th2 cells, with MIP-1a promoting Th1 cell development [5-7]. MIP-1α plays an important role in the development of HP through attracting inflammatory cells (activated CD8+ T lymphocytes and neutrophils, respectively) into the airways of patients with HP [8]. Singh *et al.*'s study suggested that MIP-1α may be associated with susceptibility or resistance to pulmonary tuberculosis [9]. As Liang *et al.*'s report, the polymorphisms of MIP-1α was strongly associated with a higher risk of ulcerative colitis [10].

In this study, to investigate if MIP-1α is an important factor in the occurrence of PBL, 24 SNPs of MIP-1α were tested. And we found that there were 5 MIP-1α SNPs might have correlation with the susceptibility to PBL in Chinese Uygur population.

Materials and methods

Subjects

A total of 92 subjects enrolled were from Kashi Prefecture, China. PBL group (n=32, 23 males, 9 females): PBL patients with history of exposure to pigeons and positive serum antibody were diagnosed with Richerson HP diagnostic criteria. The average age of PBL group was 53.31±12.41 years and the average duration of

Table 1. The sequences of MIP-1α SNPs PCR primers and single-base extension (SBE) primers

rs number	PCR primers	SBE primers
rs1063340	F: ACGTTGGATGACTCGGTTGTCACCAGACAC	gggcGAGTTCCCCTGTCCCCTCCCC
	R: ACGTTGGATGTGTTTGCTCTGAGAGTTCCC	
rs1049191	F: ACGTTGGATGACTGTGGGACTCTTCTTAAC	agCACAAACACACTGTGAAATC
	R: ACGTTGGATGGCAAACAATCACAAACACAC	
rs1049195	F: ACGTTGGATGCCACTCGGTTGTCACCAGA	GTTCCCCTGTCCCCTCC
	R: ACGTTGGATGTTGCTCTGAGAGTTCCCCTG	
rs3210166	F: ACGTTGGATGTGTTTGCTCTGAGAGTTCCC	CGGTTGTCACCAGACACG
	R: ACGTTGGATGAGCCACTCGGTTGTCACCA	
rs1130374	F: ACGTTGGATGTTTGAGACGAGCAGCCAGTG	cccgcCTGGCACTTACATGACAC
	R: ACGTTGGATGTCTCTGCCCCAACCCTGAC	
rs5029407	F: ACGTTGGATGTCCAGAAGCTTCGAGGCCCA	tCTCCCCACTGGGCCCAC
	R: ACGTTGGATGTCACACGCATGTTCCCAAGG	
rs1049199	F: ACGTTGGATGCAGCCACTCGGTTGTCAC	CCTCCCCCTTCCCTCACAC
	R: ACGTTGGATGTGTTTGCTCTGAGAGTTCCC	
rs369972356	F: AACGTTGGATGAGTGGAGACCTGCATGATTC	aTGCTGACACTCGAGCCCACATTC
	R: ACGTTGGATGCAGTGGTCAGTCCTTTCTTG	
rs190805628	F: ACGTTGGATGAGTTGCTGCTGACACGCCGA	GGGAGGTGTAGCTGAAG
	R: ACGTTGGATGCTCGTCTCAAAGTAGTCAGC	
rs41409645	F: ACGTTGGATGCTGCCTATAAAGAGGAGAGC	ctACCACTGTCTGCTGCCC
	R: ACGTTGGATGAAAGGACTGACCACTGTCTG	
rs201437905	F: ACGTTGGATGAGGTCGCTGACATATTTC	cCTGACCCCAGTGAGGAGT
	R: ACGTTGGATGTTCTCCACAGCTTCCTAACC	
rs200498715	F: ACGTTGGATGCAGTGGTCAGTCCTTTCTTG	ATTCTGAGCAGGTGACGGAA
	R: ACGTTGGATGAGTGGAGACCTGCATGATTC	
rs8070375	F: ACGTTGGATGACTGTGGGACTCTTCTTAAC	CACTGTGAAATCGAAAATAAAT
	R: ACGTTGGATGCACAAACACACTGTGAAATCG	
rs372813049	F: ACGTTGGATGGACTGACAAATGTGTATCGG	ACGTTGGATGTTTATTATTTCCCCAGGCCG
	R: ACGTTGGATGTTTATTATTTCCCCAGGCCG	
rs374335429	F: ACGTTGGATGACTCACGTGATGCAGAGAAC	TCTCCACTGCTGCCCTT
	R: ACGTTGGATGTGCTCAGAATCATGCAGGTC	
rs3210157	F: ACGTTGGATGGCTGACATATTTCTGGACCC	cCTTCCTAACCAAGCGAAGC
	R: ACGTTGGATGCCCGGTGTCATCTTCCTAAC	
rs146554516	F: ACGTTGGATGACTGGCTGCTCGTCTCAAAG	ctCAGATTCCACAGAATTTCA
	R: ACGTTGGATGTGCTGCTTCAGCTACACCTC	
rs373020815	F: ACGTTGGATGATGATTCTGAGCAGGTGACG	tcttcCTTGGCTCTGCTGACACT
	R: ACGTTGGATGAGACAGTGGTCAGTCCTTTC	
rs372080858	F: ACGTTGGATGTTCTTCTGAGCTGTGACTCG	cacttAAGCAGCAGGCGGTCGGC
	R: ACGTTGGATGGTGTAGCTGAAGCAGCAGG	
rs141200748	F: ACGTTGGATGCGGGAGGTGTAGCTGAAG	CTGCTGACACGCCGACC
	R: ACGTTGGATGTTCTTCTGAGCTGTGACTCG	
rs111443598	F: ACGTTGGATGAGCCACCAGACTGACAAATG	ccGCCGATCACAGCCCTGAA
	R: ACGTTGGATGTTTATTATTTCCCCAGGCCG	
rs373603935	F: ACGTTGGATGTCTGTGCTGACCCCAGTGA	ggCGCTGGGCCTCGAAGCTTCT
	R: ACGTTGGATGGAGGTCGCTGGGCCTCGAA	
rs41360951	F: ACGTTGGATGCACAAACACACTGTGAAATC	GGACTCTTCTTAACTTAAATTTT
	R: ACGTTGGATGACTGTGGGACTCTTCTTAAC	
rs199548661	F: ACGTTGGATGACTCACGTGATGCAGAGAAC	ACCTGCTCAGAATCATGCAGGTCTCC
	R: ACGTTGGATGTCACCTGCTCAGAATCATGC	

Table 2. The general characteristics of PBL group, negative control group and normal control group

	PBL group (N=32)	Negative control group (N=30)	Normal control group (N=30)
Gender (Male/Female)	25/7	27/3	16/14
Age (year)	53.31±12.41	53.27±14.22	53.13±11.03
History of exposure to pigeons (month)	19.53±11.28	21.60±14.26	-
Contacting tobacco (case)	3	28	12
MIP-1α (pg/mL)	51.97±9.88*	40.28±8.46*	36.45±8.22

*P<0.05 vs normal control group.

exposure to pigeons was 19.53±11.28 months. Negative control group (n=30, 27 males, 3 females): subjects with history of exposure to pigeons (more than 1 year) but without PBL. The average age of negative control group was 53.27±14.22 years and the average duration of exposure to pigeons was 21.60±14.26 months. Normal control group (n=30, 16 males, 14 females): healthy people without history of exposure to pigeons or PBL. The average age of negative control group was 53.13±11.03 years. People met the following criteria were excluded from the study: 1) with idiopathic interstitial pneumonia (IIP), chronic bronchitis, acute respiratory distress syndrome (ARDS), pneumonia, rheumatoid immune system disease, autoimmune disease (AID) or malignant tumor; 2) with other allergic diseases or other pulmonary diffuse lesions; 3) bred other kinds of birds or bred pigeons less than 1 year. All subjects gave their informed consent, and the local ethics committee approved the study.

DNA extraction

DNA extraction from blood samples was performed with Wizard Genomic DNA purification Kit (Promega, Madison, USA). DNA samples with adjusted concentration of 50 ng/μl were stored at -20°C.

Primer design

24 SNPs were randomly selected from the promoter and exon regions of the MIP-1α gene. The primers were designed using Sequenom MassARRAY Assay Design 3.1 software. The primers were synthesized in the Capitalbio Biotech Co., Ltd, Beijing, China. The primer sequences were shown in **Table 1**.

SNP genotyping

The Sequenom MassARRAY iPLEX (Sequenom, San Diego, California, USA) was used for SNP genotyping following manufacturernufacturer manufacturerfacturerrer nterstitial pneumonia (IIP), chronic U Taq polymerase, 5 ng genome DNA, 2.5 pmol of each primer and 2.5 mmol dNTP under the following conditions: initial denaturation at 94°C for 4 min; 45 cycles of 94°C for 20 s, 56°C for 30 s, 72°C for 1 min; and a final extension at 72°C for 3 min. And then add 0.3 U shrimp alkaline phosphatase (SAP) to wipe out the residual dNTP under the following conditions: 37°C for 40 min and 85°C for 5 min. The single-base extension (SBE) reaction was performed with 5.4 pmol primer, 50 turer dNTP/ddNTP mixture and 0.5 U Thermo Sequenase DNA polymerase under the following conditions: initial denaturation at 94°C for 2 min; 40 cycles of 94°C for 5s, 50°C for 5 s, 72°C for 5 s; and a final extension at 72°C for 3min. The DNA products purified with resins were detected by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). The results of the genotyping were analyzed with TYPER 4.0 software (Sequenom, San Diego, California, USA).

Statistics analysis

SPSS 17.0 was used for statistical analysis. Numerical data were expressed as mean ± standard deviation (SD) ($\overline{x}\pm s$), and the statistical differences among different groups were assessed by one-way ANOVA if data followed a normal distribution and had an equal variance or rank sum test for non-normal distribution. Enumeration data were expressed as frequency or constituent ratio. Each group was tested for conformity to Hardy-Weinberg equilibrium using the χ^2 test, between observed and expected numbers. The alleles between two groups were compared using Fisher test. $P<0.05$ indicated a significant difference, $P<0.01$ indicated that there was a very significant difference.

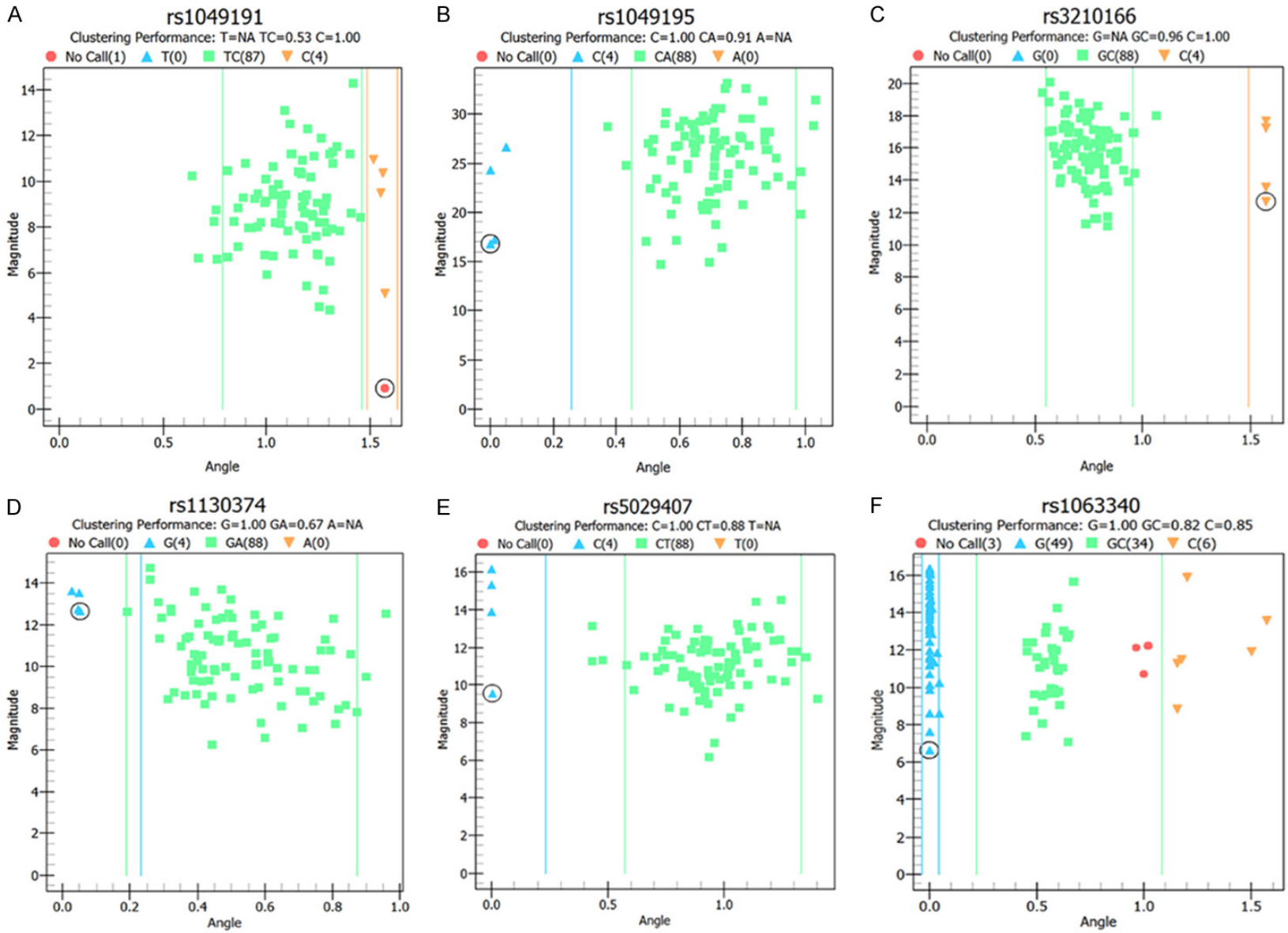

Figure 1. MALDI-TOF MS spectrum of the SNPs which were found to have polymorphism. A: MALDI-TOF MS spectrum of MIP-1α SNP rs1049191; B: MALDI-TOF MS spectrum of MIP-1α SNP rs1049195; C: MALDI-TOF MS spectrum of MIP-1α SNP rs3210166; D: MALDI-TOF MS spectrum of MIP-1α SNP rs1130374; E: MALDI-TOF MS spectrum of MIP-1α SNP rs5029407; F: MALDI-TOF MS spectrum of MIP-1α SNP rs1063340.

Results

General characteristics

A total of 92 Uygur from Xinjiang, China were enrolled in the study. Among them, there were 32 patients with PBL, 30 negative controls with history of exposure to pigeons and 30 normal controls without pigeons contact. As shown in **Table 2**, there were not significant differences among the three groups in number, gender and age. There were no significant differences between PBL and negative control groups in the history of exposure to pigeons.

There were 3 patients in PBL group, 16 subjects in negative control group and 8 in normal control group smoking. The smokers were all male. There were 12 subjects in negative control group and 4 in normal control group exposing to the secondhand smoke for a long time. The number of subjects contacting tobacco was significantly different among the three groups. Compare with the negative control group, there were much fewer pigeon breeders with PBL contacting tobacco at the same time. It suggested that contacting tobacco might have protective effect on the occurrence of PBL for pigeon breeders.

Compared with normal control group, people in PBL group and negative control group had significantly higher concentration of MIP-1α. However there were no significant differences between PBL and negative control groups. It suggested that MIP-1α might play an important role in the occurrence of PBL.

Genetic equilibrium test

MIP-1α SNPs rs1049191, rs1049195, rs3210166, rs1130374 and rs5029407 were not in Hardy-Weinberg equilibrium in PBL group. It suggested that the genotypes of MIP-1α SNPs rs1049191, rs1049195, rs3210166, rs1130374 and rs5029407 were skewed in the PBL patients. That might be because those SNPs had correlation with the susceptibility to PBL.

MIP-1α SNPs in the subject population

As the result, there were 2 genotypes TT and CC of MIP-1α SNP rs1049191 (**Figure 1A**), CC and CA of rs1049195 (**Figure 1B**), GC and CC of rs3210166 (**Figure 1C**), GG and GA of rs1130374 (**Figure 1D**), CC and CT of rs5029407 (**Figure 1E**), and 3 genotypes GG, GC and CC of rs1063340 (**Figure 1F**) in Chinese Uygur. As shown in **Table 3**, there were significant differences between the distribution frequencies of the 2 genotypes of MIP-1α SNPs rs1049191 (P=0.010), rs1049195 (P=0.032), rs3210166 (P=0.032), rs1130374 (P=0.032) and rs5029407 (P=0.032) respectively. However there were no significant differences between the distribution frequencies of the 2 alleles of those SNPs.

Discussion

HP is a pulmonary disease with symptoms of dyspnoea and cough resulting from the inhalation of an antigen contained in certain organic dusts to which the patient has been previously sensitized [11]. As a most common type of HP, PBL is caused by the exposure to pigeon antigens (feces or feathers) [12]. The clinical presentation of HP can be divided into acute and chronic forms. The acute form is characterized by an influenza-like manifestation and spontaneously improves after cessation of antigen exposure. The chronic form can be classified into two subgroups: the one is presented as chronic form and the other is a subgroup that gradually develops chronic form after repeated acute episodes. Chronic HP is often progressive, irreversible, and results in lung fibrosis, despite avoidance from the antigen exposure [13-16].

Uygur live in south Xinjiang have the habit of feeding pigeons. Some pigeon breeders with symptoms of recurrent cough and asthma were misdiagnosed as asthma, trachitis, pneumonia, etc. Those patients always can't get efficient treatment before developing into pulmonary fibrosis technology, pulmonary heart disease or respiratory failure and irreversible cardiopulmonary function damages already occurred.

There were only 5-15% people who highly exposed to pigeons found to be PBL [17, 18]. It suggested that environmental and genetic factors played important roles in the occurrence of PBL. In our study, there were 3 smokers in PBL group, while there were 28 people contacting tobacco (including smokers and people exposing to the secondhand smoke for a long time) in negative control group. Since 80-95% population of HP patients were non-smokers, smoking

Table 3. MIP-1α SNPs genotype and allele frequency distribution among the three groups

rs number	Genotype/Allele	PBL group [n (%)] (N=32)	Negative control group [n (%)] (N=30)	Normal control group [n (%)] (N=30)	*P*
rs1063340	GG	16 (50)	15 (50)	18 (60)	0.901
	GC	14 (43.75)	12 (40)	10 (33.3)	
	CC	2 (6.25)	3 (10)	2 (6.7)	
	G	46 (71.875)	42 (70)	44 (73.3)	0.937
	C	18 (28.125)	18 (30)	16 (26.7)	
rs1049191	TT	0 (0)	0 (0)	0 (0)	0.010
	TC	27 (84.375)	30 (100)	30 (100)	
	CC	5 (15.625)	0 (0))	0 (0)	
	T	27 (42.187)	30 (50)	30 (50)	0.616
	C	37 (57.813)	30 (50)	30 (50)	
rs1049195	CC	4 (12.5)	0 (0)	0 (0)	0.032
	CA	28 (87.5)	30 (100)	30 (100)	
	AA	0 (0)	0 (0)	0 (0)	
	C	36 (56.25)	30 (50)	30 (50)	0.726
	A	28 (43.75)	30 (50)	30 (50)	
rs3210166	GG	0 (0)	0 (0)	0 (0)	0.032
	GC	28 (87.5)	30 (100)	30 (100)	
	CC	4 (12.5)	0 (0)	0 (0)	
	G	36 (56.25)	30 (50)	30 (50)	0.726
	C	28 (43.75)	30 (50)	30 (50)	
rs1130374	GG	4 (12.5)	0 (0)	0 (0)	0.032
	GA	28 (87.5)	30 (100)	30 (100)	
	AA	0 (0)	0 (0)	0 (0)	
	G	36 (56.25)	30 (50)	30 (50)	0.762
	A	28 (43.75)	30 (50)	30 (50)	
rs5029407	CC	4 (12.5)	0 (0)	0 (0)	0.032
	CT	28 (87.5)	30 (100)	30 (100)	
	TT	0 (0)	0 (0)	0 (0)	
	C	36 (56.25)	30 (50)	30 (50)	0.726
	T	28 (43.75)	30 (50)	30 (50)	
rs1049199	CC	32 (100)	30 (100)	30 (100)	-
rs369972356	GG	32 (100)	30 (100)	30 (100)	-
rs190805628	CC	32 (100)	30 (100)	30 (100)	-
rs41409645	CC	32 (100)	30 (100)	30 (100)	-
rs201437905	CC	32 (100)	30 (100)	30 (100)	-
rs200498715	TT	32 (100)	30 (100)	30 (100)	-
rs8070375	TT	32 (100)	30 (100)	30 (100)	-
rs372813049	AA	32 (100)	30 (100)	30 (100)	-
rs3210157	CC	32 (100)	30 (100)	30 (100)	-
rs146554516	AA	32 (100)	30 (100)	30 (100)	-
rs373020815	GG	32 (100)	30 (100)	30 (100)	-
rs372080858	GG	32 (100)	30 (100)	30 (100)	-
rs141200748	CC	32 (100)	30 (100)	30 (100)	-
rs111443598	CC	32 (100)	30 (100)	30 (100)	-
rs373603935	GG	32 (100)	30 (100)	30 (100)	-
rs41360951	AA	32 (100)	30 (100)	30 (100)	-
rs199548661	TT	32 (100)	30 (100)	30 (100)	-

may prevent the occurrence of HP [19]. It might be because of the function of macrophages [20].

The hypersensitivity response in PBL patients is a type III immunologic reaction that produces blood precipitin antibodies. Furthermore, granuloma had formed in lung biopsies in PBL patients, and the activation of T lymphocytes may also indicate cell-mediated type IV hypersensitivity [13, 21].

However the interactions of the immune cells involved in the pathogenesis of HP are not fully understood as yet. In our study, the level of MIP-1α in serum increased significantly in PBL patients. MIP-1α induces the chemotaxis of macrophages and can recruit macrophages to the antigens [22]. Immune complexes are formed when the inhaled soluble antigens combine with IgG and they activate the macrophages through the activating pathway of complements. And then macrophages release diverse chemotactic factors including MIP-1α. MIP-1α can induce CD4+ Th0 cells into Th1 cells resulting in a large number of activated macrophages [23, 24]. At last lung damages can arise from the Th1/Th2 imbalance induced by the cascade inflammatory responses.

As our results, MIP-1α SNPs rs1049191, rs1049195, rs3210166, rs1130374 and rs5029407 might have an association with the susceptibility of PBL in Chinese Uygur population. That would be verified in further studies of a bigger sample size. Those SNPs might have influence to the expression or activity of MIP-1α. And the further studies are needed to clarify the pathogenesis of PBL related to the SNPs of MIP-1α.

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Disclosure of conflict of interest

None.

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References

[1] Bourke SJ, Banham SW, Carter R, Lynch P, Boyd G. Longitudinal course of extrinsic allergic alveolitis in pigeon breeders. Thorax 1989; 44: 415-8.

[2] Wang W, Wu C, Yang X. Cytokines level in Urgur pigeon breeder's lung disease patients. Chin Gen Pract 2014; 17: 3837-9.

[3] Schall TJ, Bacon K, Camp RD, Kaspari JW, Goeddel DV. Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. J Exp Med 1993; 177: 1821-6.

[4] Taub DD, Conlon K, Lloyd AR, Oppenheim JJ, Kelvin DJ. Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1 alpha and MIP-1 beta. Science 1993; 260: 355-8.

[5] Karpus WJ, Kennedy KJ. MIP-1alpha and MCP-1 differentially regulate acute and relapsing autoimmune encephalomyelitis as well as Th1/Th2 lymphocyte differentiation. J Leukoc Biol 1997; 62: 681-7.

[6] Karpus WJ, Lukacs NW, Kennedy KJ, Smith WS, Hurst SD, Barrett TA. Differential CC chemokine-induced enhancement of T helper cell cytokine production. J Immunol 1997; 158: 4129-36.

[7] Lukacs NW, Chensue SW, Karpus WJ, Lincoln P, Keefer C, Strieter RM, Kunkel SL. C-C chemokines differentially alter interleukin-4 production from lymphocytes. Am J Pathol 1997; 150: 1861-8.

[8] Denis M. Proinflammatory cytokines in hypersensitivity pneumonitis. Am J Respir Crit Care Med 1995; 151: 164-9.

[9] Singh B, Chitra J, Selvaraj P. CCL2, CCL3 and CCL4 gene polymorphisms in pulmonary tuberculosis patients of South India. Int J Immunogenet 2014; 41: 98-104.

[10] Liang WD, Yang JF, Yan J, Jin J, Li KS, Li JS, Bi YT. Association of combined polymorphisms in MIP-1alpha and ApoE genes with the susceptibility of inflammatory bowel disease. Zhonghua Yi Xue Za Zhi 2011; 91: 1250-3.

[11] Kumar R, Singh M. Bird fancier's lung: clinical-radiological presentation in 15 cases. Pneumonol Alergol Pol 2015; 83: 39-44.

[12] Camarena A, Aquino-Galvez A, Falfán-Valencia R, Sánchez G, Montaño M, Ramos C, Juárez A, García-de-Alba C, Granados J, Selman M. PSMB8 (LMP7) but not PSMB9 (LMP2) gene polymorphisms are associated to pigeon breeder's hypersensitivity pneumonitis. Respir Med 2010; 104: 889-94.

[13] Bourke SJ, Dalphin JC, Boyd G, McSharry C, Baldwin CI, Calvert JE. Hypersensitivity pneumonitis: current concepts. Eur Respir J Suppl 2001; 32: 81s-92s.

[14] Yoshizawa Y, Ohtani Y, Hayakawa H, Sato A, Suga M, Ando M. Chronic hypersensitivity pneumonitis in Japan: a nationwide epidemiologic survey. J Allergy Clin Immunol 1999; 103: 315-20.
[15] Ohtani Y, Saiki S, Sumi Y, Inase N, Miyake S, Costabel U, Yoshizawa Y. Clinical features of recurrent and insidious chronic bird fancier's lung. Ann Allergy Asthma Immunol 2003; 90: 604-10.
[16] Kondoh K, Usui Y, Ohtani Y, Inase N, Miyake S, Yoshizawa Y. Proinflammatory and anti-inflammatory cytokine gene polymorphisms in hypersensitivity pneumonitis. J Med Dent Sci 2006; 53: 75-83.
[17] Carrillo T, Rodriguez de Castro F, Cuevas M, Diaz F, Cabrera P. Effect of cigarette smoking on the humoral immune response in pigeon fanciers. Allergy 1991; 46: 241-4.
[18] Thomeer M, Demedts M, Vandeurzen K; VRGT Working Group on Interstitial Lung Diseases. Registration of interstitial lung diseases by 20 centres of respiratory medicine in Flanders. Acta Clin Belg 2001; 56: 163-72.
[19] Uzun O, Yilmaz DK, Sunter AT, Erkan L. The prevalence of "pigeon breeder's disease" in a Turkish city and review of the literature. Lung 2011; 189: 243-50.
[20] Sung NY, Yang MS, Song DS, Byun EB, Kim JK, Park JH, Song BS, Lee JW, Park SH, Park HJ, Byun MW, Byun EH, Kim JH. The procyanidin trimer C1 induces macrophage activation via NF-kappaB and MAPK pathways, leading to Th1 polarization in murine splenocytes. Eur J Pharmacol 2013; 714: 218-28.
[21] Baldwin CI, Todd A, Bourke S, Allen A, Calvert JE. Pigeon fanciers' lung: effects of smoking on serum and salivary antibody responses to pigeon antigens. Clin Exp Immunol 1998; 113: 166-72.
[22] Schuyler M, Gott K, French V. The Role of MIP-1? in Experimental Hypersensitivity Pneumonitis. Lung 2004; 182: 135-49.
[23] Patel AM, Ryu JH, Reed CE. Hypersensitivity pneumonitis: current concepts and future questions. J Allergy Clin Immunol 2001; 108: 661-70.
[24] Schuyler M, Gott K, Cherne A. Mediators of hypersensitivity pneumonitis. J Lab Clin Med 2000; 136: 29-38.