Int J Clin Exp Pathol 2016;9(4):4729-4737
www.ijcep.com /ISSN:1936-2625/IJCEP0022974

Original Article

Correlation of IL-10 and IL-2 single gene polymorphisms with the susceptibility to pigeon breeder's lung in Chinese Uygur population

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Received December 30, 2015; Accepted March 17, 2016; Epub April 1, 2016; Published April 15, 2016

Abstract: Objective: To investigate the correlation of interleukin (IL)-10 and IL-2 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 16 SNPs of IL-10 and 4 SNPs of IL-2 were performed. Results: Genotypes and alleles of 16 IL-10 SNPs were not significantly different between the three groups. GT genotype of IL-2 rs2069762 had significant association with the susceptibility to PBL. However, alleles of IL-2 SNPs were not significantly different between the three groups (*P*=0.719). Conclusion: Chinese Uygur pigeon breeders with GT genotype of IL-2 rs2069762 had more risk to have PBL compared with those with GG and TT genotype (OR 16.545, 95% CI 1.814-150.930, *P*=0.002; OR 5.673, 95% CI 1.611-19.981, *P*=0.005).

Keywords: Pigeon breeder's lung, IL-10, IL-2, polymorphism, 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].

Interleukin (IL)-10, a major immunosuppressive cytokine, helps T cells promote immune tolerance and plays an important anti-inflammatory role in the development of allergic disease [2-5]. IL2 is important for the proliferation of activated T lymphocytes and thereby for the activation of the phagocytes to destroy uptaken material (Th1-activation) and for the B-lymphocytes to produce antibodies (Th2-activation). Other important functions are the central role of IL2 in enhancing AICD (activation-induced cell death) of T lymphocytes, and thereby the elimination of self-reactive cells [6], and the termination of lymphocyte response by inducing the production of suppressive T cells [7]. Due to this central position in the regulation of the immune response, IL2 is an obvious candidate gene for allergic disease [8].

In 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 increased [9]. To investigate the genetics mechanism of PBL, single nucleotide polymorphism (SNP) genotyping for IL-10 and IL-2 were performed in Chinese Uygur population and the correlation of IL-10 and IL-2 SNPs with the susceptibility to PBL was analyzed.

Experimental procedures

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 Ricberson HP diagnostic criteria. The average age of PBL group was 53.31±12.41 years and the average duration of exposure to pigeons was 19.53±11.28. Neg-

Table 1. The sequences of IL-10 and IL-2 SNPs PCR primers and SBE primers

Gene	rs number	PCR primers	SBE primers
IL-10			
	rs1800896	F: ACGTTGGATGATTCCATGGAGGCTGGATAG	CCTATCCCTACTTCCCC
		R: ACGTTGGATGGACAACACTACTAAGGCTTC	
	rs3024500	F: ACGTTGGATGGAGTGGGTGAATGAATTCTG	ggGAGAAAGGAAGCCAGCTACCCCC
		R: ACGTTGGATGTCTCTGATCAGGTTTGGAGC	
	rs1800871	F: ACGTTGGATGATGCTAGTCAGGTAGTGCTC	AACTGAGGCACAGAGAT
		R: ACGTTGGATGGGTGTACCCTTGTACAGGTG	
	rs1800872	F: ACGTTGGATGTCCTCAAAGTTCCCAAGCAG	gGAGACTGGCTTCCTACAG
		R: ACGTTGGATGAAAGGAGCCTGGAACACATC	
	rs1800893	F: ACGTTGGATGCCTGCCATTCCAGTTTAGAC	gGTTTAGACTGTAACTGGGAGGAACA
		R: ACGTTGGATGCTGACTATAGAGTGGCAGG	
	rs1800894	F: ACGTTGGATGTCACCTGTACAAGGGTACA	taACCTGTACAAGGGTACACCAGTGC
		R: ACGTTGGATGTGGAGATGGTGTACAGTAGG	
	rs117106652	F: ACGTTGGATGAGACAAGAGTCAACTGACAC	GTCAACTGACACCAGAAC
		R: ACGTTGGATGGTCTGTCTTCATAGCAGATT	
	rs142726516	F: ACGTTGGATGTAGAAGCCTACATGACAATG	cAGCCTACATGACAATGAAGATA
		R: ACGTTGGATGCCAGATCCGATTTTGGAGAC	
	rs3024489	F: ACGTTGGATGAGTGACGTGGACAAATTGCC	caAAATAATTGGGTCCCCC
		R: ACGTTGGATGAGGAGAAGTCTTGGGTATTC	
	rs145922845	F: ACGTTGGATGAAAGAAGGCATGCACAGCTC	accGCCTGGTCCTCCTGACT
		R: ACGTTGGATGTGCAGCTGTTCTCAGACTGG	
	rs5743623	F: ACGTTGGATGGGTTTCTACATTGACACTCC	tttAGAGTTGGGAAGAGACA
		R: ACGTTGGATGCTGTGTGACCTATGGATCAG	
	rs5743625	F: ACGTTGGATGTTTAGGATGGGCTACCTCTC	aaggAAAAAAAGTTGATTTCCTGG
		R: ACGTTGGATGCCTCTGCGCACAGAACAGC	
	rs146520891	F: ACGTTGGATGCTATTTAGTCCCCAGAAAGG	ccATCTCTCTGCACAGCTCCAAG
		R: ACGTTGGATGACTCACTCATGGCTTTGTAG	
	rs149143243	F: ACGTTGGATGCCAAGCTGAGAACCAAGACC	cccctAGAACCAAGACCCAGACATC
		R: ACGTTGGATGTACTTACACAGCGCCGTAG	
	rs150423829	F: ACGTTGGATGGATCTGCTACTTACACAGCG	taAGCCTGAGGGTCTTCAGGT
		R: ACGTTGGATGACATCAAGGCGCATGTGAAC	
	rs41432052	F: ACGTTGGATGAAATCTGCTATGAAGACAG	gGCTATGAAGACAGACAAAC
		R: ACGTTGGATGTGGTTCTAATAGAACTCAG	
IL-2			
	rs2069762	F: ACGTTGGATGCCACCACAATATGCTATTCAC	ATTCACATGTTCAGTGTAGTTTTA
		R: ACGTTGGATGTTTCCTCCTTTCTTTAAGGG	
	rs146566026	F: ACGTTGGATGGAAATATACTTACATTAATTCC	ggTACTTACATTAATTCCATTCAAAAT
		R: ACGTTGGATGGAAAACACAGCTACAACTGG	
	rs200621841	F: ACGTTGGATGCTTGATAATTAAGTGCTTCCC	ctacTTAAGTGCTTCCCACTTAAAACA
		R: ACGTTGGATGAGGTAGCAAACCATACATTC	
	rs146270985	F: ACGTTGGATGATTATTCTAGGCCACAGAAC	gTTCTAGGCCACAGAACTGAAACAT
		R: ACGTTGGATGTCCTCCAGAGGTTTGAGTTC	

IL, interleukin; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; SBE, single-base extension.

ative control group (n=30, 27 males, 3 females): subjects with history of exposure to pigeons 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. Normal control group (n=30, 16 males, 14 females): subjects without history of exposure to pigeons or PBL. The average age of

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 (year)	19.53±11.28	21.60±14.26	-

PBL, pigeon breeder's lung.

negative control group was 53.13±11.03 years. All subjects gave their informed consent, and the Ethics Committee of People's Hospital of Xinjiang Uygur Autonomous Region has 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

SNP screening of IL-10 and IL-2 was conducted using the UCSC database (URL: http://genome.ucsc.edu/). 16 IL-10 SNPs and 4 IL-2 SNPs were randomly selected from the promoter region of the genes. 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 manufacturer's instruction. Target genes were amplified in a mixture containing 0.1 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 μmol 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 5 s, 50°C for 5 s, 72°C for 5 s; and a final extension at 72°C for 3 min. 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) ($\bar{x}\pm s$), 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.

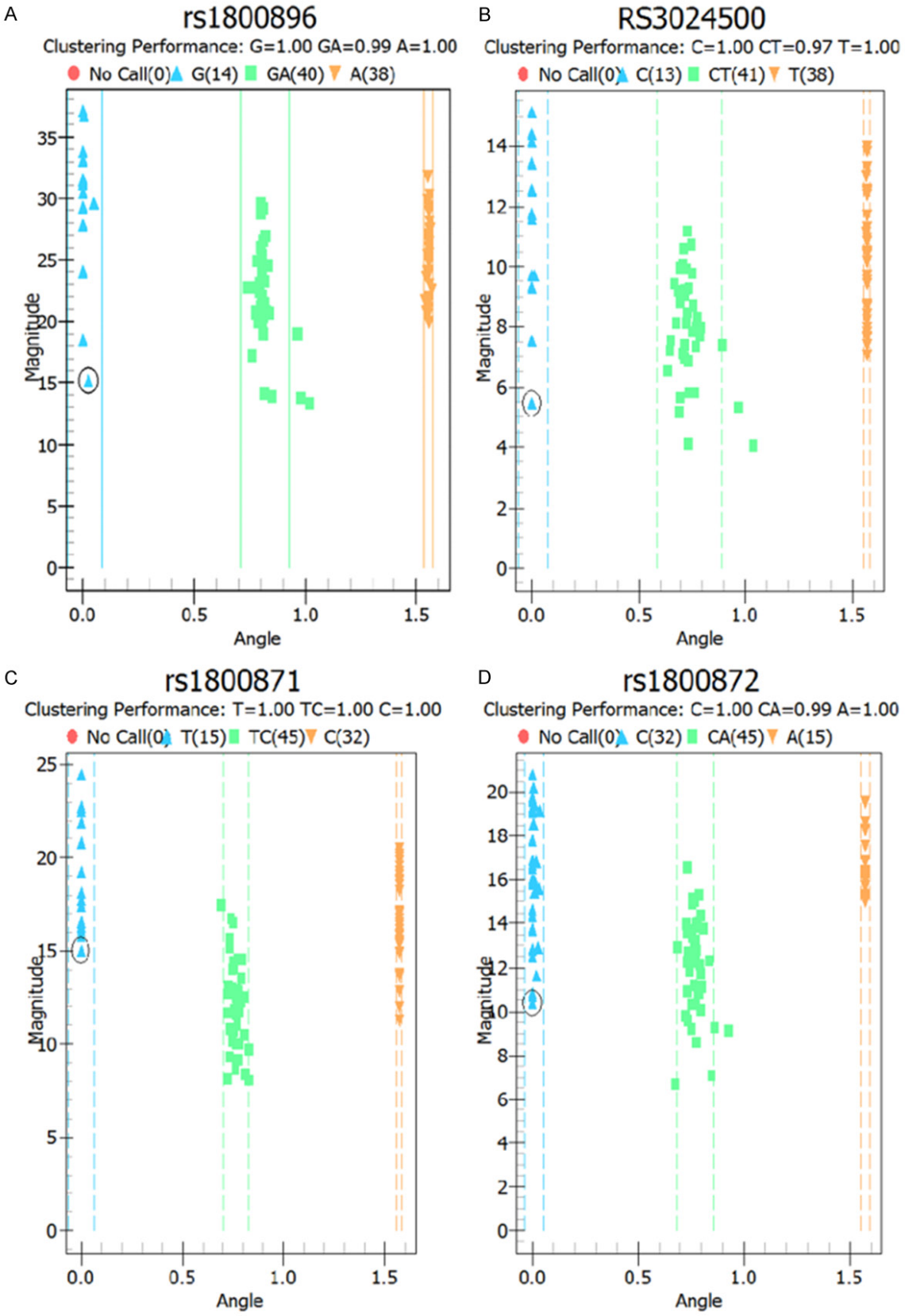
Results

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.

Genetic equilibrium test

IL-10 rs1800896, rs1800893, rs1800894, rs3024500, rs1800871 and rs1800872 were in


A
rs1800896
Clustering Performance: G=1.00 GA=0.99 A=1.00
No Call(0) G(14) GA(40) A(38)
Magnitude
Angle
B
RS3024500
Clustering Performance: C=1.00 CT=0.97 T=1.00
No Call(0) C(13) CT(41) T(38)
Magnitude
Angle
C
rs1800871
Clustering Performance: T=1.00 TC=1.00 C=1.00
No Call(0) T(15) TC(45) C(32)
Magnitude
Angle
D
rs1800872
Clustering Performance: C=1.00 CA=0.99 A=1.00
No Call(0) C(32) CA(45) A(15)
Magnitude
Angle

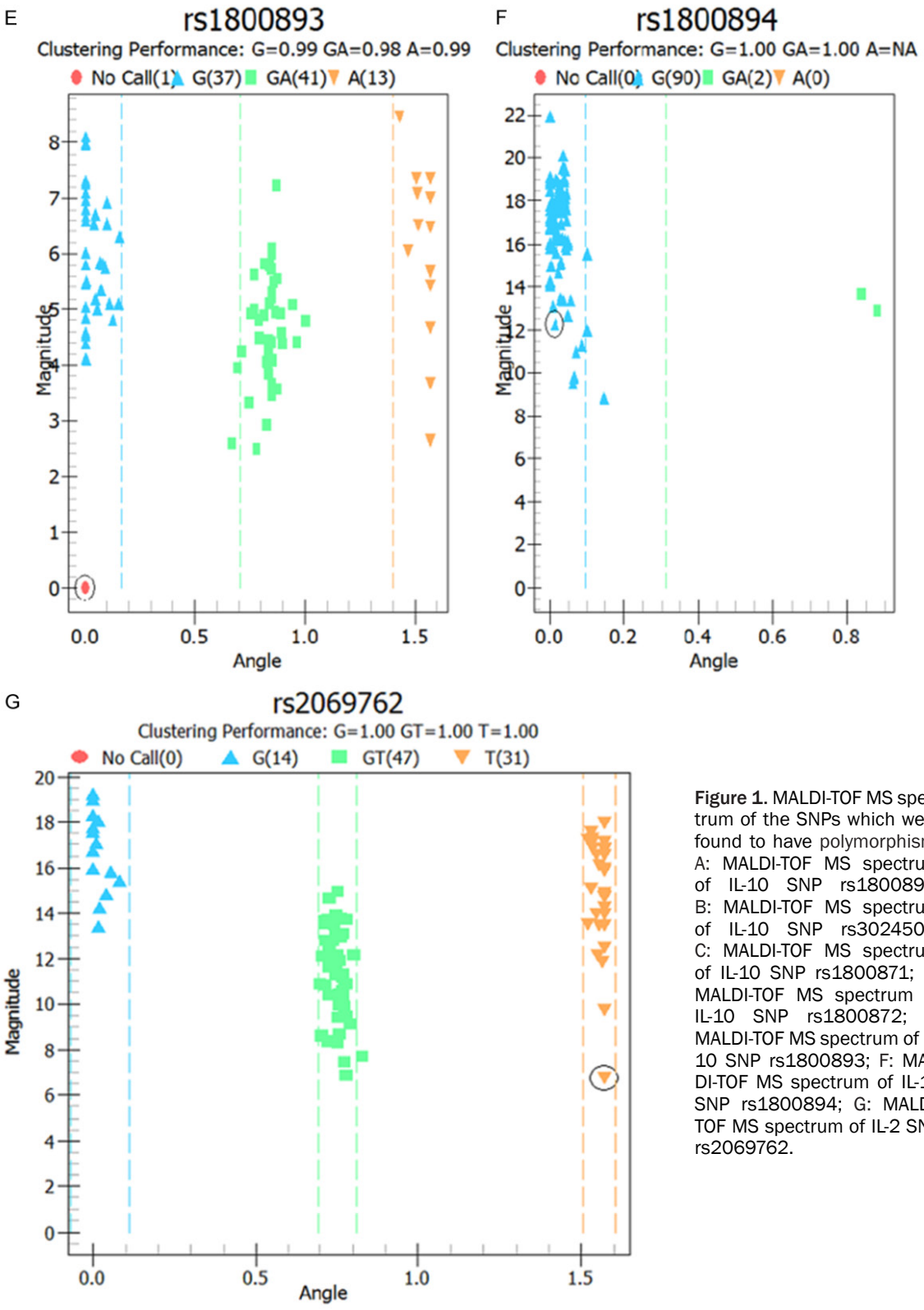


Figure 1. MALDI-TOF MS spectrum of the SNPs which were found to have polymorphism. A: MALDI-TOF MS spectrum of IL-10 SNP rs1800896; B: MALDI-TOF MS spectrum of IL-10 SNP rs3024500; C: MALDI-TOF MS spectrum of IL-10 SNP rs1800871; D: MALDI-TOF MS spectrum of IL-10 SNP rs1800872; E: MALDI-TOF MS spectrum of IL-10 SNP rs1800893; F: MALDI-TOF MS spectrum of IL-10 SNP rs1800894; G: MALDI-TOF MS spectrum of IL-2 SNP rs2069762.

Hardy-Weinberg equilibrium. No significant differences in allele frequencies for the 6 SNPs were seen among the three groups. IL-2 rs2069762 was not in Hardy-Weinberg equilib-

Table 3. IL-10, IL-2 SNPs genotype and allele frequency distribution among the three groups

Gene	rs number	Genotype/ Allele	PBL group [n (%)] (n=32)	Negative control group [n (%)] (n=30)	Normal control group [n (%)] (n=30)	χ^2	*P*
IL-10							
	rs1800896	GG	6 (18.75)	6 (20)	2 (6.667)	6.591	0.157
		GA	10 (31.25)	16 (53.333)	14 (46.667)		
		AA	16 (50)	8 (26.667)	14 (46.667)		
		G	22 (34.375)	28 (46.667)	18 (30)	3.784	0.158
		A	42 (65.625)	32 (53.333)	42 (70)		
	rs3024500	CC	5 (15.625)	6 (20)	2 (6.667)	5.753	0.219
		CT	11 (34.375)	16 (53.333)	14 (46.667)		
		TT	16 (50)	8 (26.667)	14 (46.667)		
		C	21 (32.813)	28 (46.667)	18 (30)	4.051	0.134
		T	43 (67.187)	32 (53.333)	42 (70)		
	rs1800871	TT	6 (18.75)	3 (10)	6 (20)	2.406	0.667
		TC	16 (50)	17 (56.667)	12 (40)		
		CC	10 (31.25)	10 (33.333)	12 (40)		
		T	28 (43.75)	23 (38.333)	24 (40)	0.412	0.832
		C	36 (56.25)	37 (61.667)	36 (60)		
	rs1800872	CC	10 (31.25)	10 (33.333)	12 (40)	2.406	0.667
		CA	16 (50)	17 (56.667)	12 (40)		
		AA	6 (18.75)	3 (10)	6 (20)		
		C	36 (56.25)	37 (61.667)	36 (60)	0.412	0.832
		A	28 (43.75)	23 (38.333)	24 (40)		
	rs1800893	GG	16 (50)	8 (26.667)	13 (43.333)	4.474	0.337
		AG	11 (34.375)	16 (53.333)	14 (46.667)		
		AA	5 (15.625)	6 (20)	3 (10)		
		G	43 (67.187)	32 (53.333)	40 (66.667)	3.129	0.218
		A	21 (32.813)	28 (46.667)	20 (33.333)		
	rs1800894	GG	31 (96.875)	29 (96.667)	30 (100)	1.224	1.000
		AG	1 (3.125)	1 (3.333)	0 (0)		
		AA	0 (0)	0 (0)	0 (0)		
		G	63 (98.438)	59 (98.333)	60 (100)	1.213	1.000
		A	1 (1.563)	1 (1.667)	0 (0)		
	rs117106652	AA	32 (100)	30 (100)	30 (100)	-	-
	rs142726516	GG	32 (100)	30 (100)	30 (100)	-	-
	rs3024489	GG	32 (100)	30 (100)	30 (100)	-	-
	rs145922845	CC	32 (100)	30 (100)	30 (100)	-	-
	rs5743623	CC	32 (100)	30 (100)	30 (100)	-	-
	rs5743625	CC	32 (100)	30 (100)	30 (100)	-	-
	rs146520891	TT	32 (100)	30 (100)	30 (100)	-	-
	rs149143243	TT	32 (100)	30 (100)	30 (100)	-	-
	rs150423829	TT	32 (100)	30 (100)	30 (100)	-	-
	rs41432052	TT	32 (100)	30 (100)	30 (100)	-	-
IL-2							
	rs2069762	GG	1 (3.125)	7 (23.333)	6 (20)	18.767	0.001
		GT	26 (81.25)	11 (36.667)	10 (33.333)		
		TT	5 (15.625)	12 (40)	14 (46.667)		
		G	28 (43.75)	25 (41.667)	22 (36.667)	0.686	0.719
		T	36 (56.25)	35 (58.333)	38 (63.333)		
	rs146566026	CC	32 (100)	30 (100)	30 (100)	-	-
	rs200621841	AA	32 (100)	30 (100)	30 (100)	-	-
	rs146270985	GG	32 (100)	30 (100)	30 (100)	-	-

IL, interleukin; SNP, single nucleotide polymorphism; PBL, pigeon breeder's lung.

Table 4. Associations between rs2069762 polymorphism and PBL

Genotype	PBL group [n (%)] (n=32)	Negative control group [n (%)] (n=30)	OR (95% CI)	*P*
TT	5	12	1.000	-
GG	1	7	0.343 (0.330-3.562)	0.624
GT	26	11	5.673 (1.611-19.981)	0.007
GG/GT	27	18	16.545 (1.814-150.930)	0.004

PBL, pigeon breeder's lung; OR, odds ratio; CI, confidence interval.

rium in PBL group. It suggested that IL-2 rs2069762 genotype was skewed in the PBL patients. That might be because the SNP had correlation with the susceptibility to PBL.

IL-10 and IL-2 SNPs in the subject population

There were 3 genotypes GG, GA and AA of IL-10 rs1800896, rs1800893 and rs1800894 (**Figure 1A**, **1E**, **1F**). There were 3 genotypes CC, CT and TT of IL-10 rs3024500 and rs1800871 (**Figure 1B**, **1C**). There were 3 genotypes GG, GT and TT of IL-2 rs2069762 (**Figure 1G**). As shown in **Table 3**, there were significant differences between the distribution frequencies of the 3 genotypes of IL-2 rs2069762 (χ^2=18.767, *P*=0.001). The GT genotype of IL-2 rs2069762 in PBL group increased significantly and GG, TT genotypes decreased significantly, respectively compared with negative control group (χ^2=13.250, *P*=0.001) and normal control group (χ^2=14.731, *P*=0.0001). However the allele G and T were not significantly (χ^2=0.686, *P*=0.719). People in negative control group and normal control group seemed to have similar genotype distribution (χ^2=0.338, *P*=0.894). As shown in **Table 4**, pigeon breeders with GT genotype of IL-2 rs2069762 had more risk to have PBL compared with those with GG and TT genotype (OR 16.545, 95% CI 1.814-150.930, *P*=0.002; OR 5.673, 95% CI 1.611-19.981, *P*=0.005).

Discussion

HP is a pulmonary disease with symptoms of dyspnea and cough resulting from the inhalation of an antigen contained in certain organic dusts to which the patient has been previously sensitized [10]. As a most common type of HP, PBL is caused by the exposure to pigeon antigens (feces or feathers) [11]. 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 into chronic form after repeated acute episodes. Chronic HP is often progressive, irreversible, and results in lung fibrosis, despite avoidance from the antigen exposure [12-15]. There were only 5-15% people who highly exposed to pigeons found to be PBL [16, 17]. It suggested that environmental and genetic factors played important roles in the occurrence of PBL.

In our research 84% subjects in PBL group and 90% in negative control group were males, much more than females. In PBL group 18.75% males smoked, however 54% males in negative control group smoked more than 5 years. It suggested that smoking might prevent the occurrence of PBL.

In previous research, levels of proinflammatory cytokines such as IL-2, IFN-γ and serum TNF-α increased in BALF of HP patients [18]. Compared with farmers without farmer's lung, a hypersensitivity pneumonitis induced by the inhalation of biologic dusts coming from hay dust or mold spores or other agricultural products, farmers with acute farmer's lung had more lymphocytes and higher expression of IL-2 in BALF and the level of soluble IL-2 receptor also increased in the serum [19]. In the previous study, low expression of IL-10 and high expression of IL-2 were found in Uygur patients with PBL [20].

Westendorp *et al*.'s study indicated that approximately 75% of the variation in IL-10 production appears genetically determined [20]. As SNP is the most common type of genetic variation among people, therefore SNP might have important influence on the variation in IL-10. The associations between IL-10 gene polymorphisms and many diseases such as PBL, lung cancer, IgA nephropathy and acute respiratory distress syndrome (ARDS) had been reported. IL-10 can inhibit the IL-2 production in T cells [21].

Hoffmann *et al*. found individuals with homozygous G allele of IL-2 SNP rs2069762 produced over 3 times the amount of IL-2 than their GT and TT counterparts [22]. Dakhama *et al*.'s research suggested that IL-2 plays a role in farmer's lung by providing a stimulus for the accumulation and persistence of lymphocytes [23]. In our study, we found only 1 PBL patient with GG genotype. We suspected GG genotype might have a mortal effect on PBL patients. That hypothesis still needs more reliable evidences.

In our study, IL-10 SNPs rs1800896, rs1800893, rs1800894, rs3024500, rs1800871, rs1800872 and IL-2 SNP rs2069762 were found to have polymorphism. Among those, the genotype distribution was significantly different only in IL-2 SNP rs2069762 (P=0.001). It suggested that IL-2 SNP rs2069762 might have an association with the susceptibility of PBL in Chinese Uygur population. As the result, Chinese Uygur pigeon breeders with GT genotype of IL-2 rs2069762 had more risk to have PBL compared with those with GG and TT genotype (OR 16.545, 95% CI 1.814-150.930, P= 0.002; OR 5.673, 95% CI 1.611-19.981, P= 0.005). However, the IL-10 SNPs had no correlations with the susceptibility to PBL. Further studies are needed to clarify the mechanisms of interaction between the SNP of IL-2 and PBL.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81260003).

Disclosure of conflict of interest

None.

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