

IL-4 Receptor α Polymorphisms May Be a Susceptible Factor for Work-Related Respiratory Symptoms in Bakery Workers

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Purpose: The IL-4 and IL-4 receptor α (IL-4R α) genes are the key candidate genes for atopy and asthma susceptibility. Exposure to wheat flour can cause IgE sensitization and respiratory symptoms in bakery workers. Therefore, we hypothesized that IL-4 and IL-4R α single nucleotide polymorphisms (SNPs) may be involved in the pathogenic mechanism of baker's asthma. **Methods:** Clinical and genetic data from 373 bakery workers were analyzed. A survey questionnaire, spirometry, and skin prick tests with wheat flour were performed. Serum-specific IgE, IgG1, and IgG4 to wheat flour were determined using ELISA. Five candidate IL-4 (-729 T>G, 589 T>C, and 33 T>C) and IL-4R α (Ile75Val A>G and Gln576Arg A>G) SNPs were genotyped and analyzed. **Results:** Workers with the G allele of IL-4R α Ile75Val A>G had a significantly higher prevalence of work-related lower respiratory symptoms than those with the AA genotype ($P=0.004$, 16.0% vs. 2.9%). In the skin prick test, workers with the AA genotype of IL-4R α Gln576Arg A>G had a significantly higher positive rate to wheat flour ($P=0.015$, 8.2% vs. 1.1%) than those with AG/GG genotype. No significant associations were found in the three genetic polymorphisms of IL-4. For the predicted probabilities, workers with the AA genotype of Gln576Arg A>G had a higher prevalence of IgG1 and IgG4 in response to wheat flour, according to increased exposure intensity ($P=0.001$ for IgG1 and $P=0.003$ for IgG4). **Conclusions:** These findings suggest that the IL-4R α Ile75Val and Gln576Arg polymorphisms may be associated with work-related respiratory symptom development.

Key Words: Genetic polymorphism; IL-4 Receptor α ; IL-4; baker's asthma; work-related symptom

INTRODUCTION

Interleukin-4 (IL-4), one of the most important mediators of allergic inflammation, is produced by Th2 cells and mast cells.¹ IL-4 and IL-13 are the key cytokines that induce IgE production, and promote differentiation of Th0 cells into Th2 cells.² Therefore, there have been several reports demonstrating that IL-4^{3,4} and IL-13^{5,6} genetic polymorphisms are associated with the development of asthma and atopy. IL-4 and IL-13 are both located at chromosome 16, bind to their receptors, and share the α chain of the IL-4 receptor (IL-4R α) to activate STAT6 and induce Th2 differentiation. They also lead to airway inflammation, airway hyper-responsiveness, mucus production, atopy susceptibility.^{7,8}

Baker's asthma is the most prevalent type of occupational asthma worldwide.⁹ Although the pathogenic mechanism of baker's asthma is not fully understood, the IgE-mediated response is the major pathogenic mechanism because exposure to wheat flour

can induce IgE sensitization and respiratory symptoms, leading to the development of baker's asthma.¹⁰ However, a previous study suggested that the production of serum-specific IgG4 in response to wheat flour was associated with work-related respiratory symptoms in exposed workers,¹¹ in which genetic polymorphisms of a few candidate genes, such as adrenergic receptor β 2 (ADRB2) or TLR4,¹² were associated with the development of work-related respiratory symptoms and/or specific IgG4 production in highly exposed workers.¹³

In the present study, we analyzed genetic polymorphisms of

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IL-4 and IL-4R α in association with clinical and immunological findings to further understand the pathogenic mechanism of baker's asthma in bakery workers.

MATERIALS AND METHODS

Study population

Among 392 bakery workers identified in a previous study,¹¹ 373 workers participated in this study. All subjects agreed to provide whole blood samples for genetic analyses, and they completed a respiratory questionnaire regarding whether they had experienced work-related upper- or lower-respiratory symptoms. The workers who had rhinitis symptoms such as runny nose, sneezing and congestion, which were aggravated during work and relieved after work or during holiday periods, were defined as having work-related upper-respiratory symptoms. And workers who had asthma-like symptoms such as shortness of breath, wheezing, which were also aggravated or relieved according to working periods, were defined as having work-related lower-respiratory symptoms. To define atopy status, skin prick tests (SPTs) to common inhalant allergens and serum total IgE levels were measured (ImmunoCAP system, Phadia AB, Uppsala, Sweden). SPTs for wheat flour were also performed. Serum-specific IgE, IgG1, and IgG4 in response to wheat flour were detected using enzyme-linked immunosorbent assays (ELISA), as described previously.¹⁴ The exposure environment for all subjects was classified using a device that measured individual dust exposure density as described previously.¹¹ The Ajou University Institute Review Board reviewed and approved the study protocols. Informed consent was obtained from each participant.

Genotyping

Genomic DNA was prepared from whole blood samples us-

ing a Puregene DNA purification kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. We selected three SNPs of IL-4 (-729 T>G [rs2243248], -589 T>C [rs2243250], and -33 T>C [rs2070874]) and two SNPs of IL-4R α (Ile75Val A>G [rs1805010] and Gln576Arg A>G [rs1801275]). They were genotyped using a single-base extension method and the SNaPshot ddNTP primer extension kit (Applied Biosystems, Foster City, CA, USA). Genotyping of Gln576Arg A>G for IL-4R α was screened using the Taqman assay (Assay ID, C_2351160_20; Applied Biosystems). The primer sequences used for IL-4 and IL-4R α SNP genotyping are listed in Table 1. The results were analyzed using the ABI Prism GeneScan and Genotyper software (Applied Biosystems).

Statistical analyses

All data are expressed as the mean \pm standard deviation of the

Table 2. Clinical characteristics of the subjects (n=373)

	Mean \pm SD, n
Age (years)	34.99 \pm 7.73
Gender (Male)	210 (56.3%)
Working period (years)	3.97 \pm 3.48
Atopy	127 (34.6%)
Smokers	145 (41.9%)
Upper respiratory work-related symptoms	63 (16.9%)
Lower respiratory work-related symptoms	51 (13.7%)
FEV1(L)	3.18 \pm 0.74
Log total IgE (kU/L)	4.40 \pm 1.47
Presence of specific IgE to wheat flour	24 (6.4%)
Positive SPT response to wheat flour	23 (6.2%)

SPT, Skin prick test.

Table 1. Primer sequences for SNP genotyping

Gene	rsNumber	Methods	Primer sequence	
IL-4	rs2243248	SNaPshot assay	Forward	GAGTGGTGGGGTCCTTAC
			Reverse	TGTTGCCTAGGCTCATCT
			Genotyping	CTGATTGTAAAGTTGGTAAGAC
	rs2243250	SNaPshot assay	Forward	AAACTAGGCCTCACCTGATACG
			Reverse	TGCATAGAGGCAGAATAACAGG
			Genotyping	AGACTCTCCTACCCAGCACTGGGG
	rs2070874	SNaPshot assay	Forward	TATATATAGAGATATCTTTGTGTCAGCATTGC
			Reverse	TTGGGAGGTGAGACCCAT
			Genotyping	TGTCGATTGTCAGTGACAATGTGAG
IL-4R α	rs1805010	SNaPshot assay	Forward	CTACAGGTGACCAGCCTAAC
			Reverse	GTGGTCTACTGCGACCAG
			Genotyping	ACCCCGCGCCTCCGTTGTTCTCAGGGA
	rs1801275	Taqman assay	Assay ID	C_2351160_20*

*Taqman assays were performed using a predesigned primer sequence according to the manufacturer's protocol.

mean. SPSS software (version 11.05; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Significant departures of the genotype frequency from Hardy-Weinberg equilibrium at the polymorphic site were tested by χ^2 analysis. The clinical parameters of subjects were compared to each genotype using Pearson's χ^2 test and Student's *t*-test. Predictive probability was calculated for the presence of serum-specific IgG subclasses in response to wheat flour in subjects with the IL-4R α Gln576Arg A>G polymorphism using logistic regression.¹⁵ All data were adjusted by age, sex, atopy status, and exposure density using

logistic regression.

RESULTS

Subject characteristics and clinical findings

Table 2 summarizes the demographic and clinical findings of the study participants. The mean age was 34.9 ± 7.73 years, and 56.3% of the subjects were male. They had worked in the bakery industry for an average of 3.97 ± 3.48 years. A total of 127 (34.6%) were atopics, and smoking prevalence was 41.9%. Among the

Table 3. Associations between clinical and immunologic parameters and IL-4 (A) and IL-4R α (B) genotypes

Parameters	IL-4 -729 T>G			IL-4 589 T>C			IL-4 33 T>C		
	TT	GT+GG	P	TT	CT+CC	P	TT	CT+CC	P
Age	35.12 \pm 7.71	34.64 \pm 7.63	0.672	35.46 \pm 7.83	34.23 \pm 7.53	0.142	35.49 \pm 7.83	34.16 \pm 7.51	0.11
Gender (male)	177 (57.3%)	28 (50%)	0.312	132 (55.9%)	76 (56.3%)	0.946	132 (55.7%)	76 (56.7%)	0.849
Working period (yr)	4.04 \pm 3.54	3.77 \pm 3.21	0.602	4.14 \pm 3.55	3.68 \pm 3.38	0.23	4.16 \pm 3.55	3.65 \pm 3.37	0.184
Atopy	109 (35.9%)	15 (27.3%)	0.218	81 (34.8%)	45 (34.1%)	0.897	81 (34.6%)	45 (34.4%)	0.959
Smoking status	65 (59.1%)	15 (68.2%)	0.426	57 (60.6%)	24 (60%)	0.945	57 (60.6%)	24 (60%)	0.945
Work-related Symptoms									
Upper-respiratory	106 (34.3%)	15 (26.8%)	0.271	78 (33.1%)	44 (32.6%)	0.928	79 (33.3%)	43 (32.1%)	0.806
Lower-respiratory	42 (13.6%)	6 (10.7%)	0.558	34 (14.4%)	17 (12.6%)	0.625	34 (14.3%)	17 (12.7%)	0.656
FEV1(L)	3.2 \pm 0.76	3.05 \pm 0.67	0.164	3.18 \pm 0.75	3.18 \pm 0.73	0.979	3.17 \pm 0.75	3.18 \pm 0.73	0.895
Log total IgE (kU/L)	4.44 \pm 1.47	4.07 \pm 1.32	0.081	4.42 \pm 1.52	4.33 \pm 1.36	0.584	4.42 \pm 1.52	4.33 \pm 1.37	0.546
Positive skin prick test to wheat flour	21 (6.9%)	1 (1.8%)	0.149	16 (6.8%)	7 (5.3%)	0.561	16 (6.8%)	7 (5.3%)	0.58
Specific IgE to wheat flour	21 (6.8%)	2 (3.6%)	0.361	18 (7.6%)	6 (4.4%)	0.231	18 (7.6%)	6 (4.5%)	0.241
Specific IgG1 to wheat flour	59 (19.2%)	16 (28.6%)	0.109	46 (19.6%)	30 (22.2%)	0.544	46 (19.5%)	30 (22.4%)	0.507
Specific IgG4 to wheat flour	38 (12.3%)	13 (23.2%)	0.031	30 (12.8%)	22 (16.3%)	0.347	30 (12.7%)	22 (16.4%)	0.324

Parameters	IL-4R α Ile75Val A>G			IL-4R α Gln576Arg A>G		
	AA	AG+GG	P	AA	AG+GG	P
Age	34.99 \pm 8.02	35.02 \pm 7.71	0.973	34.9 \pm 7.8	35.5 \pm 7.7	0.545
Gender (male)	39 (56.5%)	167 (55.7%)	0.897	148 (54.2%)	56 (60.2%)	0.314
Working period (yr)	3.91 \pm 3.37	3.96 \pm 3.51	0.905	3.8 \pm 3.4	4.3 \pm 3.7	0.282
Atopy	21 (31.4%)	105 (35.7%)	0.407	101 (37.8%)	25 (26.9%)	0.057
Smoking status	16 (57.1%)	64 (61.0%)	0.714	56 (57.7%)	23 (67.6%)	0.309
Work-related Symptoms						
Upper-respiratory	20 (29%)	100 (33.3%)	0.487	98 (35.9%)	23 (24.7%)	0.048
Lower-respiratory	2 (2.9%)	48 (16%)	0.004	34 (12.5%)	17 (18.3%)	0.161
FEV1 (L)	3.24 \pm 0.7	3.16 \pm 0.75	0.395	3.16 \pm 0.75	3.22 \pm 0.71	0.453
Log total IgE (kU/L)	4.54 \pm 1.67	4.36 \pm 1.42	0.361	4.5 \pm 1.4	4.1 \pm 1.5	0.052
Positive skin prick test to wheat flour	2 (2.9%)	21 (7.1%)	0.195	22 (8.2%)	1 (1.1%)	0.015
Specific IgE to wheat flour	4 (5.8%)	20 (6.7%)	0.792	19 (7%)	5 (5.4%)	0.594
Specific IgG1 to wheat flour	17 (24.6%)	58 (19.4%)	0.33	58 (21.3%)	18 (19.4%)	0.686
Specific IgG4 to wheat flour	9 (13%)	42 (14%)	0.828	37 (13.6%)	15 (16.1%)	0.547

study subjects, 63 (16.9%) had suffered from work-related upper-respiratory symptoms, whereas 51 (13.7%) had experienced work-related lower-respiratory symptoms. The mean lung function of the subjects was 3.18 ± 0.74 L (forced expiratory volume in 1 s, FEV1), and the mean total IgE level was 4.40 ± 1.47 kU/L. The prevalence of serum-specific IgE antibody in response to wheat flour was 6.4%, and 6.2% of participants had positive SPT results for wheat flour.

Associations between genetic polymorphisms and clinical parameters

The genetic association of each SNP with the clinical parameters is shown in Table 3. No significant associations were found between the three IL-4 SNPs (-729 T>G, 589 T>C, 33 T>C) and any of the clinical parameters tested, as shown in Table 3A. However, significant associations were observed with the two IL-4R α SNPs (Ile75Val A>G and Gln576Arg A>G), as shown in Table 3B. Subjects with the G allele of IL-4R α Ile75Val A>G had a significantly higher prevalence of work-related lower-respiratory symptoms ($P=0.004$). Subjects with the mutant A allele at Gln576Arg A>G of IL-4R α had a significantly higher prevalence of work-related upper-respiratory symptoms ($P=0.048$). In addition, subjects with the AA genotype showed a significantly higher positive SPT rate in response to wheat flour ($P=0.015$).

Predictive probabilities for serum-specific IgE or IgG subclasses in response to wheat flour

As shown in Table 3B, no significant associations were found in the presence of serum-specific IgE, IgG1, and IgG4 antibodies in response to wheat flour according to the genotype of two IL-4R α SNPs. The predicted probabilities for the presence of serum-specific antibodies in response to wheat flour were analyzed according to the exposure intensity (minimal and intermediate to high) using a logistic regression model as shown in Figure A and B. Subjects with the AA genotype of IL-4R α Gln576Arg A>G

had a significantly higher prevalence of serum-specific IgG1 and IgG4 antibodies in response to wheat flour upon increasing exposure density (Figure, $P=0.001$ and $P=0.003$ for IgG1 and IgG4, respectively), whereas the specific IgG1 response tended to increase in subjects with the G allele upon increasing exposure density to wheat flour ($P=0.043$, Figure A). No significant differences were observed in the serum-specific IgE response according to each genotype of IL-4R α and IL-4.

DISCUSSION

Various studies have investigated the potential associations between IL-4R α polymorphisms in asthma and allergic disease, with some conflicting results. Most studies revealed that variants of IL-4R α SNPs contribute to increased risk of asthma and allergic disease along or in combination with other genes. In child¹⁶⁻¹⁸ and adult^{19,20} asthmatics, there have been several reports on the positive association between IL-4R α polymorphisms (Ile50Val, rs1805010; Arg551Gln, Q576R, Gln576Arg, rs1801275; Glu375Ala, rs1805011; and Ser411Leu, rs1805013) and asthma susceptibility. In patients with atopic dermatitis, an IL-4R α polymorphism (Ile50Val, rs1805010) is also associated with increased susceptibility.²¹ Furthermore, using a meta-analysis, Loza et al.²² revealed that a variant allele of the Q576R (rs1801275) IL-4R α polymorphism is associated with a significant risk of atopic asthma. Based on these previous results of IL-4R α polymorphisms, we selected two candidate SNPs, Ile50Val (rs1805010), and Arg551Gln (rs1801275), which have been found to have a significant association with asthma susceptibility. In the present study, we also found that these SNPs are associated with the development of work-related symptoms and positive SPTs in response to wheat flour. These findings suggest that IL-4R α polymorphisms may contribute to the development of baker's asthma in wheat-exposed workers.

The genetic polymorphism of the Ile75Val SNP has previously

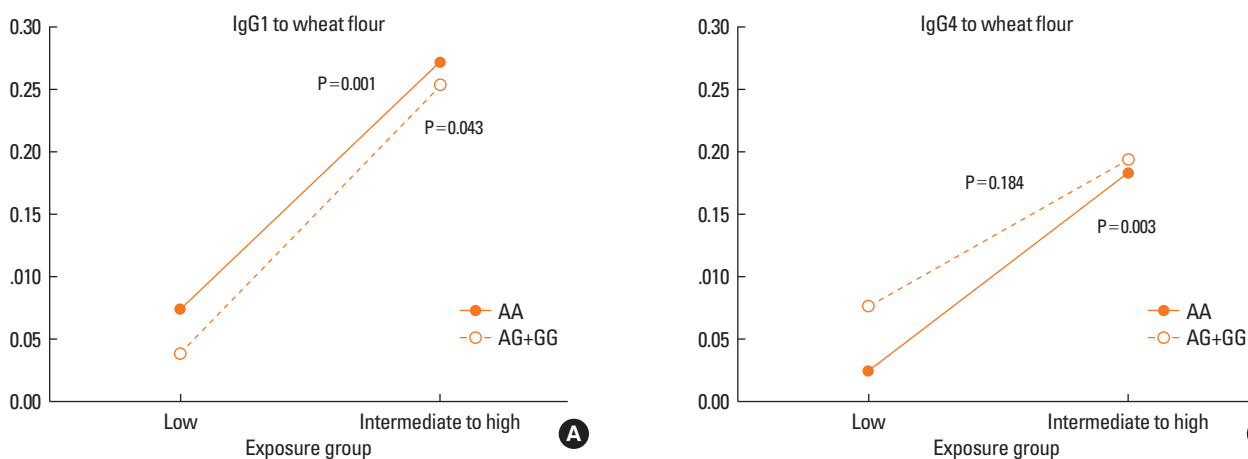


Figure. Predictive probabilities for the Gln576Arg G>A polymorphism in IL-4R α and the presence of serum-specific IgG1 (A) and IgG4 (B) antibodies in response to wheat flour related to exposure density.

been reported to be associated with asthma susceptibility in a Chinese pediatric asthma population.¹⁸ Another study showed that variants of the Ile75Val SNP are associated with asthma susceptibility through gene-gene interactions in a cohort of Taiwanese school children.¹⁷ In addition, the Ile75Val polymorphism may be associated with an increased risk of β -lactam allergy in atopic women.²³ These previous data are comparable to our findings because they show that variants of the Gln576Arg (rs1801275) polymorphism of IL-4R α are related to higher asthma and allergic disease susceptibility.^{16,18} In the present study, the Ile75Val polymorphism is associated with a higher prevalence of work-related respiratory symptoms in exposed bakery workers (Table 3B, $P=0.004$). A possible mechanism is that the Gln576Arg polymorphism may promote asthma development by augmenting IL-4R α -dependent signaling, which has been demonstrated by several experimental²⁴ and human studies.²⁵ Regarding the genetic polymorphism of the IL-4 Gln576Arg polymorphism, the Q576 allele was significantly prevalent in patients with penicillin allergy, and was associated with increased serum-specific IgE levels in response to penicillin.²⁶ Other studies have demonstrated that variants of the Gln576Arg polymorphism are associated with lower total IgE levels in healthy adults²⁷ and family members with atopic children,²⁸ confirmed by *in vitro* analyses. In the present study, the GG genotype at the Gln576Arg polymorphism was significantly associated with a positive SPT in response to wheat flour, although the association between the Gln576Arg polymorphism and serum-specific IgE levels in response to wheat flour did not reach statistical significance. Considering that the IgE-mediated response is a major mechanism in the pathogenesis of baker's asthma, and that the results of the SPTs conducted in the present study in response to wheat were correlated with serum-specific IgE in response to wheat flour in our previous study,¹¹ the genetic polymorphism at IL-4R α Gln576Arg A>G may be a genetic risk factor that increases IgE sensitization to wheat in exposed workers, and could thus contribute to work-related respiratory symptoms and baker's asthma.

The IL-4R α polymorphism at Gln576Arg A>G had a significantly higher prevalence of serum-specific IgG1 and IgG4 in response to wheat, upon increasing exposure intensity. Our previous study showed that serum-specific IgG subclasses in response to wheat flour were associated with work-related respiratory symptoms.¹¹ Moreover, a β -adrenergic receptor genetic polymorphism was associated with specific IgG production and the development of work-related respiratory symptoms in one aspect of gene-environment interaction.¹³ The current study provides more evidence showing a possible gene-environment association in the development of baker's asthma. A few studies have suggested a possible gene-environment interaction in the development of occupational asthma.⁹ The HLA class II molecules, which are involved in the presentation of processed antigens to T lymphocytes, might have a critical role in these asso-

ciations. Therefore, most genetic susceptibility studies in occupational asthma have focused on low-molecular-weight antigens.^{29,30} The genetic polymorphisms of IL-4, IL-13, and IL-4R α have been found to be associated with a susceptibility to isocyanate-induced occupational asthma.³¹ However, this is the first study to investigate a gene to environmental interaction with exposure of high-molecular-weight antigens in an occupational exposure setting. The previous studies showed significant associations between several candidate genes, including β 2-adrenergic receptor,¹³ TLR4,¹² and IL-1832 in association with work related symptoms or immunologic findings in bakery workers. In the present study, we found significant associations of IL-4 and IL-4R α genetic polymorphism with work-related respiratory symptoms and positive SPTs to wheat in a single cohort of bakery workers, which may provide a possible clue of gene to environmental association in bakery workers.

In conclusion, our findings suggest that two genetic polymorphisms (Ile75Val and Gln576Arg G>A) of IL-4R α may be associated with work-related respiratory symptoms and serum-specific antibody production in bakery workers, which may contribute to the development of bakers' asthma.

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