

Original article

Relationships between specific serum IgE, cytokines and polymorphisms in the IL-4, IL-4R α in patients with penicillins allergy

Background: Excessive production of interleukin (IL)-4, IL-13 and interferon (IFN)- γ is thought to be important in the development of allergic disease and atopy. Several investigators have linked the IL-4 and IL-4R genes to allergic disease and atopy. The aim of this study is to further explore the mechanism of penicillins allergy and evaluate the possible role of the IL-4 C-589T and IL-4R α Q576R polymorphisms in modulating the allergic responses to penicillins.

Methods: Radioallergosorbent test (RAST) was used to detect eight kinds of specific immunoglobulin E (IgE) to penicillins in serum. Serum levels of IL-4, IL-13 and IFN- γ were measured by using enzyme-linked immunosorbent assay (ELISA). The IL-4 C-589T and IL-4R α Q576R polymorphisms were genotyped by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

Results: Compared with control subjects, there were significantly higher levels of IL-4, IL-13 and IFN- γ in allergic patients with positive specific IgE ($P < 0.01$), and the lower levels of IL-4 and IFN- γ were observed in allergic patients with negative specific IgE ($P < 0.05$). We found a growing trend of IL-4 and IL-13 levels with the kind increasing of positive specific IgE, and even there were significant correlations between the three kinds of cytokines and many kinds of specific IgE ($P < 0.05$). The IL-4R α *Q576 allele was significantly increased in patients with penicillins allergy compared with control subjects ($P < 0.01$). Furthermore, the allele was strongly associated with increased serum-specific benzylpenicilloyl (BPO)-, phenoxymethylpenicillanyl (PVA)- or ampicillanyl (APA)-IgE levels in patients with positive specific IgE ($P < 0.05$).

Conclusions: These data suggest that IL-4, IL-13 and IFN- γ play an important roles in penicillins allergy. The IL-4R α Q576R polymorphism may involve in the development of penicillins allergy, and through modulating specific serum IgE levels.

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The mechanisms involved in allergic reactions to penicillins can be antibody-dependent or T-cell mediated. A role for T cells has been inferred by analogy to contact dermatitis and some immunohistological data. T cells take a central role in organizing the immune defence and are practically involved in all types of immune reactions either by orchestrating the type of immune response or as effectors themselves (1). Serum levels of immunoglobulin E (IgE) are frequently elevated in patients with penicillins allergy. Classically, IgE production is strongly influenced by cytokines produced by activated T cells, and among the cytokines, interleukin (IL)-4, IL-13 and interferon (IFN)- γ have important roles. The IL-4 acts on B cells as an isotype switch factor for the induction of IgE synthesis. The IL-13 is closely related to IL-4, and the

two factors share many biological and immunoregulatory functions including stimulation of IgE synthesis (2). On the contrary, IFN- γ inhibits IgE production induced by IL-4.

Biological variability in the allergic and inflammatory response, and a clear recognition of the importance of IgE and various cytokines in this response, has stimulated analyses of candidate genes for genetic variability in structure and function. Recently, a number of allergic disease and atopy susceptibility genes have been identified (3). Some of these, such as those located in chromosome 5 and in the human leucocyte antigen (HLA) region, regulate the production of IgE, whereas others, such as Fc ϵ I β , mediate IgE effector functions. Previously, we reported the association between penicillins allergy and

the E237G variant of the $Fc\epsilon RI\beta$ gene (4), however, cytokines genes are particularly attractive candidates because cytokine dysregulation is a characteristic of allergic and asthmatic inflammation. Chromosome 5 contains numerous candidate genes that may potentially play a role in inflammation associated with allergic disease, such as cytokines, growth factors and growth factor receptors. In particular, IL-4 seems to be an attractive gene candidate on the basis of its key role in IgE production and in the induction of inflammation. Marsh et al. (5) suggested that IL-4 or a nearby gene in chromosome 5 regulates IgE production in a nonantigen-specific fashion. A single nucleotide (C to T at -589 bp relative to the open reading frame) polymorphism in the IL-4 promoter region has been associated with elevated total serum IgE levels (6). Additionally, the gene for IL-4R α is another ideal candidate susceptibility gene for allergic disease and atopy because of: (i) its pivotal role in both IL-4 and IL-13 signalling, (ii) its key role in allergic inflammation by promoting IgE production and Th2 cell development and (iii) its location on chromosome 16 which has been linked to specific IgE. An amino acid substitution in IL-4R α (glutamin $\rightarrow$ arginine) at position 576 of the protein was found to be associated with atopy (7). From these data we suggest that the IL4 C-589T and IL-4R α Q576R polymorphisms may influence penicillins allergy.

The cytokine pattern in penicillins allergy has been extensively studied, but different studies have conflicting findings. Kerdine et al. (8) suggested that Th1 or Th2 differentiation in primary immune responses to penicillin may be driven by the kinetics and the level of IL-4 production rather than by the level of IFN- γ . However, it has been reported that PenG-stimulated peripheral blood mononuclear cells (PBMC) show a heterogenous cytokine pattern and clones secrete high amounts of IFN- γ (9). Lately, Brugnolo et al. (10) believed that cytokines produced by Th2 cells played an important role in all β -lactams-induced adverse drug reaction, even when late clinical manifestations occurred and an IgE-mediated mechanism was apparently indemonstrable. Most of these studies have been mainly focused on the comparison between patients with penicillins allergy and healthy individuals. Little is known about the relation between the cytokine profile and the clinical manifestation or specific IgE levels.

In the present study, we examined the levels of IL-4, IL-13 and IFN- γ in the sera of patients with penicillins allergy, and investigated the relationships between specific IgE, cytokine and patient's clinical manifestation. Additionally, to evaluate the possible role of the IL-4 and IL-4R α polymorphisms in modulating the allergic response to penicillins, we performed an association study between specific IgE levels, cytokines and the polymorphisms (IL-4 C-589T, IL-4R α Q576R) in patients with penicillins allergy as well as a random group from the general population.

Materials and methods

Subjects

The study was conducted in 245 patients with penicillins allergy (123 male and 122 female; age 5–80 years, mean age 23.08 ± 10.83) and 101 control subjects (75 male and 26 female; age 16–60 years, mean age 22.95 ± 6.82) who were recruited from the first affiliated hospital of Zhengzhou University. Among them, patients with penicillin G (PG), ampicillin (AMP), amoxycillin (AX) allergy were 222, 14, 9, respectively. A questionnaire was used which included a full history related to allergic reaction and disease.

The study included patients with positive skin test and patients with typical clinical symptoms after penicillins administration when skin test was negative (it was divided into patients with immediate reactions (IR) and with nonimmediate reactions (NIR) according to the time interval between drug intake and appearance of clinical manifestation). Skin test was performed with PG on the inner side of the forearms by intradermal testing. Penicillin G sodium salt diluted in saline solution until reaching a concentration of 200 U/ml as reagent. Approximately 0.1 ml (20 U) of reagent was injected for intradermal testing. The diameter of wheal was measured after 20 min and the reaction was considered positive when the diameter was >5 mm with a surrounding area of erythema. When skin test was negative, patients with typical clinical symptoms after penicillins administration were also enrolled in the study. A reaction was considered immediate when symptoms appeared within a maximum of 1 h after drug intake, and these reactions included: anaphylactic shock, urticaria, pruritus, dyspnoea, angioedema. Nonimmediate reactions were those occurring more than 1 h after drug intake, and the symptoms of these reactions mainly included maculopapular. Control subjects were selected according to have no evidence of any personal or family history of drug allergy and atopy (atopic dermatitis, allergic rhinitis or other allergic disease). All control subjects showed negative skin test and no specific IgE reactions to penicillins by radioallergosorbent test (RAST). Informed consent was obtained from all participants in this study and the experiment was carried out under approval from the Ethics Committee, University of Zhengzhou. Blood samples were drawn from each subjects at the time of the clinical manifestations and/or at the time of positive skin test. After clotting for 2 h at room temperature the sera were separated and stored at -30°C until the *in vitro* tests were performed.

Radioallergosorbent test

Eight kinds of specific IgE to penicillins, which included benzylpenicilloyl-polylysine (BPO-PLL), phenoxomethylpenicilloyl-polylysine (PVO-PLL), ampicilloyl-polylysine (APO-PLL), amoxicilloyl-polylysine (AXO-PLL), benzylpenicillanyl-polylysine (BPA-PLL), phenoxomethylpenicillanyl-polylysine (PVA-PLL), ampicillanyl-polylysine (APA-PLL), amoxicillanyl-polylysine (AXA-PLL), were detected as previously described (11). The positive criterion of eight kinds of specific IgE referred to our previous report (4).

Enzyme-linked immunosorbent assay

Serum levels of IL-4, IL-13 and IFN- γ were measured using the enzyme-linked immunosorbent assay (ELISA) kit purchased from Trinity Biotech PIC (Bray, Ireland) according to the manufacturer's protocol.

Genotyping

DNA from 156 patients with penicillins allergy and 88 control subjects was extracted from peripheral blood by standard phenol-chloroform extraction followed by ethanol precipitation (12). The subjects were genotyped for IL-4 C-589T and IL-4R α Q576R polymorphisms by means of polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

The IL-4 C-589T and IL-4R α Q576R polymorphisms were amplified with the following primers: 5'-CAC TAA ACT TGG GAG AAC ATG GT-3' and 5'-TGG GGA AAG ATA GAG TAA TA-3', and 5'-GCC CCC ACC AGT GGC TAC C-3' and 5'-GAG GTC TTG GAA AGG CTT ATA C-3', respectively. The PCR was performed with reagents composed of 0.25 μ M primers, 200 μ M dNTPs, 1.5 mM MgCl₂, 2.5 μ l 10X buffer, 100–200 ng DNA and 1 U *Taq* DNA polymerase in a 25 μ l reaction solution. The programme was accomplished by 38 cycles at 95°C for 30 s, 48°C for 30 s (IL-4 C-589T) or 58°C for 30 s (IL-4R α Q576R), 72°C for 50 s, and a final 8 min extension at 72°C. The PCR product for IL-4 C-589T was 195 bp in length and was digested with 2 U of *Ava*II restriction enzyme, while that for IL-4R α Q576R was 204 bp in length and was digested with 5 U of *Hap*II restriction enzyme. Digested fragments were separated on a 3% agarose gel. The results of genotypes were shown in Figs 1 and 2.

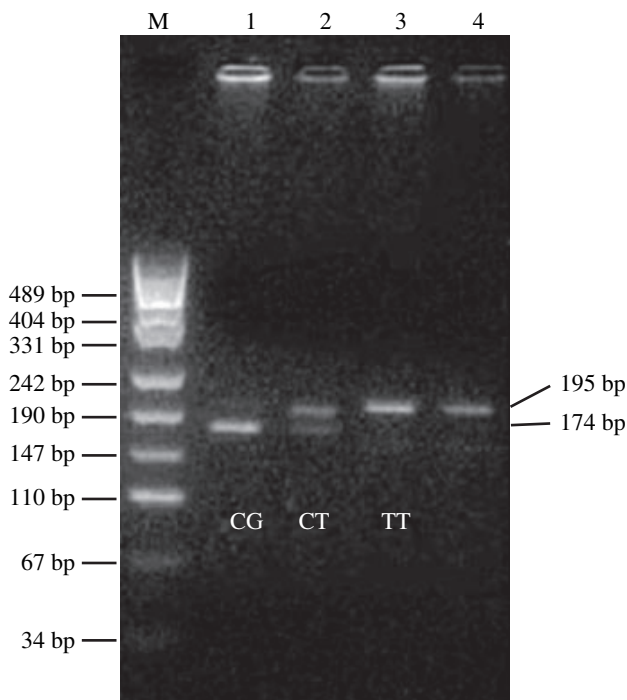


Figure 1. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis with *Ava*II for the interleukin (IL)-4 C-589T polymorphism of IL-4 gene. The size of the PCR product was 195 bp and digested fragments derived from the C-589 allele were 174 and 21 bp. PCR products from the T-589 allele were not cut with *Ava*II. Lanes 1–3: digested PCR products from a C-589 homozygote (lane 1), a C/T-589 heterozygote (lane 2) and a T-589 homozygote (lane 3). Lane 4: an undigested control.

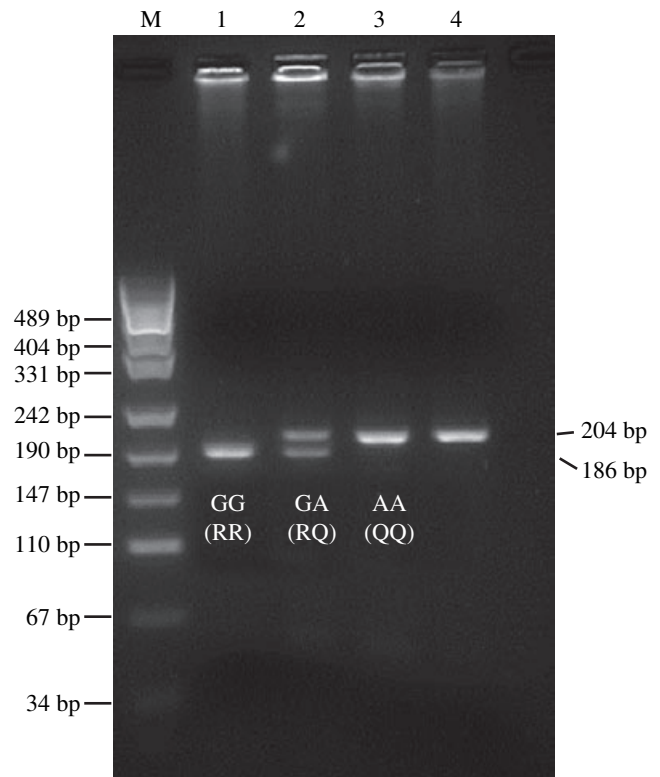


Figure 2. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis with *Hap*II for the interleukin (IL)-4R α Q576R polymorphism.

GG GA AA, genotype of IL-4R α Q576R according to single nucleotide polymorphism;

RR RQ QQ, genotype of IL-4R α Q576R according to single nucleotide polymorphism causing amino acid substitutions in the mature protein.

The size of the PCR product was 204 bp and digested fragments derived from the G576 allele were 186 and 18 bp. PCR products from the A576 allele were not cut with *Hap*II. Lanes 1–3: digested PCR products from a G576 homozygote (lane 1), a G/A576 heterozygote (lane 2) and a A576 homozygote (lane 3). Lane 4: an undigested control.

Statistical analysis

Comparisons for statistical significance were performed using Mann–Whitney *U*-test (two-tailed *P*-value) and Kruskal–Wallis *H* test. Correlation coefficients and statistical significance were determined using Spearman test. The chi-square test or Fisher's exact test were used to test for significant association between penicillin allergy and alleles or genotypes. A *P*-value of < 0.05 was considered statistically significant.

Results

Serum IgE levels

Table 1 showed that the clinical data and results of antipenicillins antibodies in 245 patients. The positive rate of specific IgE in 245 patients was 57.55% (141).

Table 1. The clinical data and results of antipenicillins antibodies in 245 patients with penicillins allergy

Group	Cases	Positive cases (%)
Age		
<18	22	13 (59.09)
18–59	216	126 (58.33)
≥60	7	2 (28.57)
Gender		
Male	123	79 (64.23)*
Female	122	62 (50.82)
All patients		
IR	71	42 (59.15)
NIR	64	30 (46.88)
Positive skin test	110	69 (62.73)
Total	245	141 (57.55)

* $P < 0.05$ vs female group.

IR, immediate reactions; NIR, nonimmediate reactions.

There was no statistical difference between patients with IR, those with NIR, and those with positive skin test. The same was true between different age (<18, 18–59, ≥60 years). However, the positive rate of specific IgE to the male was significantly higher than that of specific IgE to the female ($P < 0.05$). But there was no difference in the positive rate of skin test between male and female group (data not shown).

IL-4, IL-13 and IFN- γ levels

Association between cytokine and penicillins allergy. There were no significant differences in levels of IL-4, IL-13 and IFN- γ between all allergic patients tested and control subjects (Table 2). However, compared with control subjects, there were significantly higher levels of IL-4, IL-13 and IFN- γ in allergic patients with positive specific IgE ($P < 0.01$), and the lower levels of IL-4 and IFN- γ were observed in allergic patients with negative specific IgE ($P < 0.05$; Table 2). The result showed that the increase of IL-4, IL-13 and IFN- γ levels may be associated with

Table 2. IL-4, IL-13, IFN- γ levels in the sera of allergic patients and control subjects

Group	IL-4 level		IL-13 level		IFN- γ level	
	n	Median (ng/l)	n	Median (ng/l)	n	Median (ng/l)
Control	62	38.50	60	1.71	60	8.59
A + B	143	28.00			141	7.80
A	88	100.00**	63	10.05**	86	19.59**
B	55	7.00**			50	1.35*
IR	57	26.00	33	4.50**	55	13.15*
NIR	54	25.00	30	7.52**	54	13.89

* $P < 0.05$, ** $P < 0.01$ vs control.

A, allergic patients with positive specific IgE to penicillins; B, allergic patients with negative specific IgE to penicillins; A and B, all allergic patients; IR, immediate reactions; NIR, nonimmediate reactions; IgE, immunoglobulin E; IL, interleukin; IFN, interferon.

allergic reaction of positive specific IgE, and the decrease of them was associated with that of negative specific IgE.

Association between cytokine and the kind of specific IgE. According to the kind of specific IgE detected, the allergic patients with positive IgE were divided into groups with 1, 2, 3 or more positive specific IgE. Patients with two kinds of positive specific IgE had significantly higher levels of IL-4 and IL-13 than patients with one kind of positive specific IgE ($P < 0.01$), and the same result occurred between patients with three or more kinds and those with two kinds of positive specific IgE ($P < 0.01$) (data not shown). We found a growing trend of IL-4 and IL-13 levels with the kind increasing of positive specific IgE.

Association between cytokine and the clinical manifestation. According to allergic types, patients were divided into two groups: group with IR and with NIR. There was no significant differences in IL-4, IL-13 and IFN- γ levels between the two groups (Table 2).

Correlation between cytokine and specific IgE. Among patients with positive specific IgE, there was a significant correlation between IL-4 and IL-13 ($r = 0.948$; $P < 0.01$), but not between either IL-4 or IL-13 and IFN- γ ($r = 0.002$, $r = 0.142$; $P > 0.05$) (data not shown). As shown in Table 3, significant correlations existed between the three kinds of cytokines and many kinds of specific IgE.

IL-4 C-589T and IL-4R α Q576R polymorphisms

Association between penicillins allergy and polymorphism. The IL-4R α *Q576 allele was present in a significantly higher proportion of allergic patients with NIR and with PG than control subjects ($P < 0.01$). Furthermore, we demonstrated that the IL-4R α *Q576 allele was associated with PG reaction with positive IgE (Table 4). We found no significant differences in the distribution of the IL-4 C-589T genotype between any of the study groups and control group (data not shown).

Association between specific IgE and polymorphism. Among patients with positive specific IgE, significantly different distributions of IL-4R α Q576R genotype were observed between patients with positive BPO-, PVA- or APA-IgE and control subjects ($P < 0.05$) (Table 5). The similar result of IL-4R α Q576R was present between positive and negative PVA-IgE patients ($P < 0.01$) (Tables 6 and 7). However, we found no significant differences in the distribution of IL-4 C-589T genotype between any of the groups above and control group (data not shown).

Association between cytokine and polymorphism. We assessed the relationships between the levels of IL-4, IL-13 and IFN- γ , and IL-4 C-589T and IL-4R α Q576R

Table 3. The correlation between IL-4, IL-13, IFN- γ and specific IgE in serum of allergic patients with positive specific IgE

	IL-13	IL-4	IFN- γ
BPO			
Correlation coefficient	0.148	0.205	0.185
Significance (two-tailed)	0.248	0.055	0.088
<i>n</i>	63	88	86
PVO			
Correlation coefficient	0.395**	0.335**	0.089
Significance (two-tailed)	0.001	0.001	0.414
<i>n</i>	63	88	86
APO			
Correlation coefficient	0.226	0.110	0.131
Significance (two-tailed)	0.075	0.307	0.231
<i>n</i>	63	88	86
AXO			
Correlation coefficient	-0.045	0.075	0.023
Significance (two-tailed)	0.728	0.488	0.835
<i>n</i>	63	88	86
BPA			
Correlation coefficient	0.249*	0.215*	0.198
Significance (two-tailed)	0.049	0.045	0.068
<i>n</i>	63	88	86
PVA			
Correlation coefficient	-0.021	-0.025	0.259*
Significance (two-tailed)	0.871	0.816	0.016
<i>n</i>	63	88	86
APA			
Correlation coefficient	0.271*	0.323*	0.040
Significance (two-tailed)	0.032	0.002	0.717
<i>n</i>	63	88	86
AXA			
Correlation coefficient	0.603**	0.612**	0.148
Significance (two-tailed)	0.000	0.000	0.174
<i>n</i>	63	88	86
Total			
Correlation coefficient	0.335**	0.605**	0.252*
Significance (two-tailed)	0.001	0.000	0.023
<i>n</i>	63	88	86

* $P < 0.05$, ** $P < 0.01$.

IL, interleukin; IFN, interferon; BPO, benzylpenicilloyl; PVO, phenoxomethylpenicilloyl; APO, ampicilloyl; AXO, amoxicilloyl; BPA, benzylpenicillanyl; PVA, phenoxomethylpenicillanyl; APA, ampicillanyl; AXA, amoxicillanyl.

alleles. There were no significant associations of IL-4 C-589T and IL-4R α Q576R alleles with the level of IL-4, IL-13 and IFN- γ in patients with penicillins allergy (data not shown).

Discussion

In this study, we showed the relationships between specific IgE, the levels of IL-4, IL-13 and IFN- γ , and the polymorphisms of IL-4 C-589T and IL-4R α Q576R in patients with penicillins allergy. To our knowledge, this is the first study of patients with penicillins allergy to show associations of IgE, cytokine and polymorphism at molecular level.

Table 4. Genotype and allele distribution of IL-4R α Q576R polymorphism in allergic patients and control subjects

IL-4R α Q576R	<i>n</i>	Genotype			Allele	
		AA (QQ) (%)	GA (RQ) (%)	GG (RR) (%)	A (Q) (%)	G (R) (%)
Control	87	52 (60)	32 (37)	3 (3)	136 (78)	38 (22)
A	98	77** (79)	19 (19)	2 (2)	173 (88)	23 (12)
B	58	43 (74)	12 (21)	3 (5)	98 (84)	18 (16)
A + B	156	120** (77)	31 (20)	5 (3)	271 (87)	41 (13)
Allergic type						
IR	68	50 (74)	16 (24)	2 (3)	116 (85)	20 (15)
NIR	55	43* (78)	10 (18)	2 (4)	96 (87)	14 (13)
Allergic drug						
Penicillin G	125	97** (78)	24 (19)	4 (3)	218 (87)	32 (13)
Positive IgE	84	66** (79)	16** (19)	2 (2)	148 (88)	20 (12)
Amoxycillin	13	8 (62)	4 (31)	1 (8)	20 (77)	6 (23)
Ampicillin	8	7 (88)	1 (13)	0	15 (94)	1 (6)

* $P < 0.05$, ** $P < 0.01$ vs control.

A, allergic patients with positive specific IgE to penicillins; B, allergic patients with negative specific IgE to penicillins; A + B, all allergic patients; IR, immediate reactions; NIR, nonimmediate reactions; IgE, immunoglobulin E; IL, interleukin.

AA GA GG, genotype of IL-4R α Q576R according to single nucleotide polymorphism; QQ RQ RR, genotype of IL-4R α Q576R according to single nucleotide polymorphism causing amino acid substitutions in the mature protein;

A G, allele of IL-4R α Q576R according to single nucleotide polymorphism;

Q R, allele of IL-4R α Q576R according to single nucleotide polymorphism causing amino acid substitutions in the mature protein;

AA (QQ), homozygosity for the wild-type allele; GA (RQ), heterozygosity; GG (RR), homozygosity for the mutant allele.

Table 5. Genotype and allele distribution of IL-4R α Q576R polymorphism in patients with positive specific IgE and control subjects

Group	<i>n</i>	Genotype frequency			Allele frequency	
		AA (QQ) (%)	GA (RQ) (%)	GG (RR) (%)	A (Q) (%)	G (R) (%)
Control	87	52 (60)	32 (37)	3 (3)	136 (78)	38 (22)
BPO(+)	35	28* (80)	7 (20)	0 (0)	63 (90)	7 (10)
PVO(+)	31	20 (65)	11 (35)	0 (0)	51 (82)	11 (18)
APO(+)	25	19 (76)	5 (20)	1 (4)	43 (86)	7 (14)
AXO(+)	19	13 (68)	6 (32)	0 (0)	32 (84)	6 (16)
BPA(+)	38	27 (71)	11 (29)	0 (0)	65 (86)	11 (14)
PVA(+)	35	33** (94)	2 (5.7)	0 (0)	68 (97)	2 (3)
APA(+)	14	13* (93)	1 (7.1)	0 (0)	27 (96)	1 (4)
AXA(+)	35	25 (71)	9 (26)	1 (3)	59 (84)	11 (16)

* $P < 0.05$, ** $P < 0.01$ vs control.

IL, interleukin; BPO, benzylpenicilloyl; PVO, phenoxomethylpenicilloyl; APO, ampicilloyl; AXO, amoxicilloyl; BPA, benzylpenicillanyl; PVA, phenoxomethylpenicillanyl; APA, ampicillanyl; AXA, amoxicillanyl; AA (QQ), homozygosity for the wild-type allele; GA (RQ), heterozygosity; GG (RR), homozygosity for the mutant allele; IgE, immunoglobulin E.

The function Th1/Th2 dichotomy plays an important role in the allergic phenomena induced by penicillins, in particular with a prevalence of Th2 responses in immediate hypersensitivity reaction and of Th1 responses in delayed hypersensitivity reaction. As part of the Th1/Th2 paradigm the selective differentiation of either subset takes place during T-cell priming, with the contribution of several factors, e.g. antigen dose, cytokine environ-

Table 6. Genotype and allele distribution of IL-4RαQ576R polymorphism in patients with negative specific IgE

Group	n	Genotype frequency (%)			Allele frequency (%)	
		AA (QQ) (%)	GA (RQ) (%)	GG (RR) (%)	A (Q) (%)	G (R) (%)
BPO(–)	63	49 (78)	12 (19)	2 (3)	110 (87)	16 (13)
PVO(–)	67	57 (85)	8 (12)	2 (3)	122 (91)	12 (9)
APQ(–)	73	58 (79)	14 (19)	1 (1)	130 (89)	16 (11)
AXO(–)	79	64 (81)	13 (16)	2 (3)	141 (89)	17 (11)
BPA(–)	60	50 (83)	8 (13)	2 (3)	108 (90)	12 (10)
PVA(–)	63	44** (70)	17 (27)	2 (3)	105 (83)	21 (17)
APA(–)	84	64 (76)	18 (21)	2 (2)	146 (87)	22 (13)
AXA(–)	63	52 (83)	10 (16)	1 (2)	114 (90)	12 (10)

***P* < 0.01, vs PVA(+).

IL, interleukin; BPO, benzylpenicilloyl; PVO, phenoxomethylpenicilloyl; APQ, ampicilloyl; AXO, amoxicilloyl; BPA, benzylpenicillanyl; PVA, phenoxomethylpenicillanyl; APA, ampicillanyl; AXA, amoxicillanyl; AA (QQ), homozygosity for the wild-type allele; GA (RQ), heterozygosity; GG (RR), homozygosity for the mutant allele.

Table 7. The results of IL-4RαQ576R polymorphism between patients with positive specific IgE and control subjects as well as patients with positive and negative specific IgE

	IL-4RαQ576R	
	χ ² -value	<i>P</i> -value
BPO(+) vs		
Control	4.525*	0.033
BPO(–)		NS
PVO(+) vs		
Control		NS
PVO(–)		NS
APQ(+) vs		
Control		NS
APQ(–)		NS
AXO(+) vs		
Control		NS
AXO(–)		NS
BPA(+) vs		
Control		NS
BPA(–)		NS
PVA(+) vs		
Control	14.072**	0.000
PVA(–)	7.985**	0.005
APA(+) vs		
Control	5.755*	0.016
APA(–)		NS
AXA(+) vs		
Control		NS
AXA(–)		NS

P* < 0.05, *P* < 0.01.

NS, nonsignificant; IL, interleukin; BPO, benzylpenicilloyl; PVO, phenoxomethylpenicilloyl; APQ, ampicilloyl; AXO, amoxicilloyl; BPA, benzylpenicillanyl; PVA, phenoxomethylpenicillanyl; APA, ampicillanyl; AXA, amoxicillanyl; IgE, immunoglobulin E.

ment, and the nature and activation status of antigen-presenting cells (13). We demonstrated that there was a striking higher level of IL-4, IL-13 and IFN-γ in patients with positive specific IgE compared with control subjects,

which was consistent with our findings of previous study (not publication). Moreover, comparisons of absolute levels of individual cytokines can be misleading, as immunological effects depend on the relative concentrations of interacting cytokines. By comparing the IL-4/IFN-γ cytokine ratios, we could show that the Th1/Th2 balance in the patients with positive IgE was significantly skewed towards a Th2-like response. As for higher level of IFN-γ, it is likely that IFN-γ shift the immune system towards Th1 and thus should protect before IgE production. However, there was a significantly lower level of IL-4, IL-13 and IFN-γ in patients with negative specific IgE compared with control subjects. This indicates a complex status in patients with negative specific IgE. Two causes of the RAST results are false-negative may result from the detection of circulating IgE, which underestimates the amount of cell-bound antibody, and the possible concealment of allergenic determinants following covalent coupling of the drug to the carrier solid phase. Additionally, detection of IgE antibodies in the skin or serum depends on the time interval between the reaction and the evaluation (14). Therefore, patients with a history of penicillins allergy may have negative IgE responses.

We found no associations between IL-4, IL-13 or IFN-γ levels and the clinical manifestation. This suggests that the clinical manifestation in penicillins allergy may not be directly attributed to altered cytokine levels. We also showed a correlation between IL-4 and IL-13 levels in patients with positive specific IgE, but the level of IFN-γ did not correlate with either IL-4 or IL-13. This indicates that different cells might be producing the Th1- and Th2-type cytokines. New data have also shown that the transcription of the genes coding for the Th2 cytokines is regulated differently from the transcription of the IFN-γ gene (15, 16). Taken together, it is suggested that IL-4, IL-13 and IFN-γ may play crucial roles in penicillins allergy, supporting the Th1/Th2 imbalance hypothesis.

Both IL-4 and IL-13 are T cell-secreted molecules known to participate in IgE synthesis in association with class switching to IgE by human B cells. Our results confirmed that there were significant correlation between IL-4 and IL-13, and the same results occurred between the three kinds of cytokines and many kinds of specific IgE. Furthermore, we found a growing trend of IL-4, IL-13 levels with the kind increase of positive specific IgE.

Another major goal in this study is to understand the role of IL-4 C-589T and IL-4RαQ576R genetic polymorphisms in penicillins allergy. The results showed that there was an association between penicillins allergy and IL-4Rα*Q576 allele, and the allele related to the expression of many kinds of specific IgE. However, we could not confirm the association of IL-4 C-589T polymorphism with penicillins allergy. This result, along with the previously published data, make the possibility explanation for the association found between IL-4RαQ576R polymorphism and penicillins allergy. First, it is possible that this polymorphism is

directly involved in penicillins allergy. The polymorphism has been shown to affect receptor signalling (7). The PBMCs from individuals who had the *576R isoform of the IL-4 receptor were shown to have higher levels of IL-4-induced CD23 expression. The enhanced IL-4 receptor signalling associated with the *576R isoform may have relevance to the severity of asthma. The second possible explanation is that this polymorphism is not involved causally in contributing to penicillins allergy, but is in linkage disequilibrium with a locus in the IL-4R α gene or a nearby gene in chromosome 16p.

Linkage studies first pointed out to the cytokine-rich 5q31 region as a location for a gene involved in IgE regulation. We found no association with a previously reported polymorphism in the promoter region of the IL-4 gene (IL-4-589) and IgE levels. An association between IL-4 C-589T and penicillins allergy had not been observed. A possible explanation for the results may be that IL-4 C-589T is only a marker for the polymorphisms in 5q31. In this case, different degrees of linkage disequilibrium between the polymorphisms in IL-4 C-589T and nearby gene may explain the different findings. It is also possible that IL-4 C-589T may influence transcription, but this influence may be affected by gene–gene interactions, which may be different in different populations.

In conclusion, these findings suggest that IL-4, IL-13 and IFN- γ play important roles and IL-4R α Q576R polymorphism may involve in the development of penicillins allergy. As the pool of candidate genes for atopy and allergic disease continues to grow, it is imperative that the functional consequences of these genes are examined (17). Candidate genes that have no functional consequences can be effectively eliminated from the pool. Once the field of candidate gene is narrowed to those genes, which confer changes in immune function, we will better understand allergic mechanism and be able to target therapies. Studies to examine the functional consequences of the IL-4R α polymorphisms are needed to conduct in penicillins allergy.

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