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The association between IFN- γ and IL-4 genetic polymorphisms and childhood susceptibility to bronchial asthma

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ABSTRACT

The present study aims to investigate the association between the genetic polymorphisms of interferon (IFN)- γ and interleukin (IL)-4 with childhood susceptibility to asthma and the levels of IFN- γ , IL-4, and immunoglobulin (Ig) E among asthmatic children. A total of 100 asthmatic children and 122 control children were enrolled in the present study. The genotypes of the IFN- γ gene at the -179G/T locus and the IL-4 gene at the -33C/T and -589C/T loci were detected using polymerase chain reaction with restriction fragment length polymorphism. The IFN- γ gene at the $+874\text{A/T}$ locus and the IFN- γ CA repeats were tested using allele-specific and capillary electrophoresis, respectively, whereas the IFN- γ , IL-4, and total IgE levels were measured using enzyme-linked immunosorbent assays. The 100 asthmatic children and the 122 control children were all GG homozygous in the -179 locus of the IFN- γ gene, which shows that the IFN- γ gene is not mutated at the -179 locus. No significant differences were found in terms of genotypic and allelic frequency distribution in the IFN- γ gene or the CA repeat at the $+874\text{A/T}$ locus between the asthmatic children and the control ($P>0.05$). An association was found between the polymorphism of the IFN- γ gene at $+874\text{A/T}$ and IFN- γ levels. IFN- γ expression was lower among patients with the AA genotype than those with the AT genotype ($P<0.05$); the genotypic and allelic frequency distributions of the IL-4 gene at -33C/T and -589C/T were significantly different between the asthmatic children and the control ($P<0.05$). The levels of IL-4 and IgE among children with TT genotype at the -33 and -589 loci were higher than those with the CT genotype, but only the polymorphism at -33C/T was associated with IL-4 levels ($P<0.05$). The polymorphisms of the IFN- γ gene at $+874\text{A/T}$ or the CA repeats are not correlated with susceptibility to asthma. Thus, the polymorphism at $+874\text{A/T}$ is correlated with IFN- γ level. The TT genotypes of the IL-4 gene at the -33 and -589 loci are associated with asthma susceptibility in children, and polymorphism at the -33 locus may be associated with IL-4 level.

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1. Introduction

Bronchial asthma is one of the most common chronic respiratory diseases in children, but its pathogenesis has not been fully elucidated until now. Asthma is a chronic inflammatory disease of the airways, and its main immunologic pathogenesis is an imbalance in help T cell (Th) 1/Th2 (Kaminuma et al., 2009). Decreased Th1 cytokine secretion and increased Th2 cytokine secretion both directly and indirectly increase immunoglobulin (Ig) E synthesis, thereby inducing

the degranulation of mast cells and eosinophils and causing airway hyperreactivity and inflammation (Mazzarella et al., 2000; Yazdanbakhsh et al., 2002). Given that interferon (IFN)- γ and interleukin (IL)-4 are the signature cytokines of Th1 and Th2 cells, respectively, they both play important roles in the pathogenesis of (Shirai et al., 2003; Steikne and Borish, 2001). At present, the alleles in the gene control region and the promoter region of each cytokine are believed to be different. Allelic polymorphism may affect the synthesis of cytokines, which make the immunologic pathogenesis of asthma more complex (Movahedi et al., 2008). Although IFN- γ and IL-4 are the predisposing genes of asthma (Malerba and Pignatti, 2005), the correlation between polymorphisms of IFN- γ and IL-4 and bronchial asthma in children, which considers the balance between Th1 and Th2 have not been reported. Genetic polymorphism exhibits significant differences because of ethnic diversity and regional variability. Thus, the polymorphisms of IFN- γ and IL-4 among asthmatic children in the Guangdong Pearl River Delta region were analyzed to investigate their association between childhood susceptibility to asthma and the levels of IFN- γ , IL-4, and total IgE.

Abbreviation: IFN- γ , interferon- γ ; IL-4, interleukin-4; Ig, immunoglobulin; Th, help T cell; EDTA, ethylene diamine tetraacetic acid; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR, polymerase chain reaction; AS-PCR, allele-specific polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; ANOVA, analysis of variance; SPSS, statistical package for social science; OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus; CREB, c-AMP response element binding protein.

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2. Material and methods

2.1. Subjects

A total of 100 asthmatic children (51 males and 49 females) between 2 and 13 years old (6.57 ± 2.76 years) and 122 non-asthmatic children (70 males and 52 females) between 3 and 14 years old (7.46 ± 2.94) were enrolled in the present case–control study. The children in the asthmatic group were patients in the Pediatric Asthma Outpatient Department of the Second Affiliated Hospital of Sun Yat-sen University from March 2009 to February 2010 in the Guangdong Pearl River Delta region. The control group included healthy children who received health examinations in the pediatric care outpatient clinic, and those children with surgical trauma and bone fracture, among others. All controls had no family history of asthma, typical history of anaphylactic diseases (including eczema, allergic rhinitis, and atopic dermatitis), or history of recent infections. The two groups of children were not significantly different in terms of gender or age distribution ($P > 0.05$). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the Sun Yat-sen memorial Hospital, Sun Yat-sen University. Written informed consent was obtained from all participants.

2.2. DNA extraction

Up to 2 mL of blood from the peripheral vein was drawn from each subject; Ethylene diamine tetraacetic acid (EDTA) was used as anticoagulant. Then, 300 μ L of the whole blood was stored for genomic DNA extraction. The remainder was centrifuged to separate the plasma, and then subpackaged and cryopreserved for the detection of IL-4, IFN- γ , and total IgE. Genomic DNA was extracted from 300 μ L of whole blood using a genomic DNA extraction kit; the concentration and purity of the DNA were detected through UV-2450 spectrophotometry, whereas its integrity was detected using agarose gel electrophoresis. The extracted DNA was then preserved in a refrigerator at -20°C .

2.3. Detection of the polymorphisms

Polymorphisms of the IFN- γ gene at the -179G/T locus and the IL-4 gene at the -33C/T and -589C/T loci were detected using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The PCR primers, annealing temperature, PCR product sizes, and restriction enzymes used for each gene locus are listed in Table 1 (Kamali-Sarvestani et al., 2007; Qi et al., 2005; Takabayashi et al., 1999). The PCR amplification was conducted using the following program: predenaturation at 95°C for 4 min, denaturation at 95°C for 40 s, annealing for 40 s (see the temperature settings in Table 1), and extension at 72°C for 40 s; 35 cycles at 72°C for 8 min, and then preservation at 4°C . The restriction enzyme digestion was performed in a 20 μ L enzyme reaction system at 37°C (Ava II) and 55°C (Bsm AI) for 6 h. For analyses of the restriction enzyme products and identification of genotypes, different electrophoretic

Table 2

Genotype of each locus and length of DNA fragment after restriction enzyme digestion.

Gene	Polymorphism	PCR product	PCR-RFLP genotype: fragment	Gel electrophoresis
IFN-	-179G/T	272 bp	GG:(164+108)bp GT:(272+164+108)bp TT:272 bp	3% agarose gel
IL-4	-33C/T	150 bp	CC:(135+15)bp CT:(135+97+38+15)bp	8% native polyacrylamide gel
IL-4	-589C/T	195 bp	TT:(97+38+15)bp CC:(177+18)bp CT:(195+177+18)bp TT:195 bp	8% native polyacrylamide gel

methods were determined according to the fragment size of the enzyme products, such as agarose gel electrophoresis and native polyacrylamide gel electrophoresis. The genotypes were determined by observing the electrophoretic gel images using DNA markers for reference. The corresponding relationship between fragment length and genotype after restriction enzyme digestion at each gene locus is shown in Table 2.

Polymorphisms of the IFN- γ gene at $+874\text{A/T}$ were detected using allele-specific polymerase chain reaction (AS-PCR). The allele-specific primers were designed and synthesized as previously described using the following primers (Hussein et al., 2009): the upstream primer T 5'-TTCTTACAACACAAAATCAAATCT-3'; the upstream primer A, 5'-TTCTTACAACACAAAATCAAACA-3'; the universal downstream primer 5'-TCAACAAAG CTGATACTCCA-3'; the upstream and downstream β -actin primers (internal reference) were 5'-CTA-CATG AGCTGCGTGTGG-3' and 5'-AAGGAAGGCTGGAAGAGTGC-3'. Specific fragments were amplified using PCR into two reaction tubes (A and T), thereby producing two parallel PCR processes. The PCR conditions were as follows: 10 cycles of predenaturation at 95°C for 4 min, denaturation at 95°C for 15 s, annealing at 62°C for 50 s, extension at 72°C for 40 s. This was followed by 20 cycles of denaturation at 95°C for 20 s, annealing at 56°C for 50 s, extension at 72°C for 50 s, with final extension at 72°C for 8 min, and then preservation at 4°C . The PCR products and genotypes were analyzed and were determined using 1.5% agarose gel electrophoresis after the PCR reaction. The size β -actin internal reference was 500 bp. If the target bands (264 bp) in the IFN- γ $+874$ locus were amplified in both tubes, then the AT genotype would be determined. If the target bands were only amplified in the tube containing upstream primer A, then AA would be determined. In contrast, if the target bands were only found in the tube containing upstream primer T, then TT would be determined.

Polymorphism of the IFN- γ CA repeats was detected using capillary electrophoresis. The target fragments were amplified by PCR. The primers were as follows: 5'-GCTGTCATAATAATATTCAGAC-3' (upstream primer) and 5'-CGAGCTTTAAAGATAGTTCC-3' (downstream primer) (Nakao et al., 2001), in which the 5'-end of the upstream primer was labeled with 6-carboxyfluorescein. The PCR reaction conditions were as follows: predenaturation for 4 min at 95°C , followed by 35 cycles of denaturation for 40 s at 95°C , annealing for 40 s at 52°C , with a 40 s extension step at 72°C . A final exposure was performed at 72°C for 8 min. The products were preserved at 4°C . Proper amounts of the PCR-amplified products were collected for capillary electrophoresis on an ABI 3730XL, whereas the internal standard method was used for molecular weight determination. Electrophoretograms were analyzed for DNA fragment length using the Gene Marker V1.6 software. Fragment lengths reaching 122 bp were counted as 12 CA repetitions, 124 bp as 13 repetitions, and so on. One waveform in the electrophoretograms represented a homozygote, and two waveforms represented a heterozygote, which eventually determined the genotype.

Table 1

PCR primer sequences, annealing temperature and restriction enzymes.

Gene	Polymorphism	Primer sequence (5' $\rightarrow$ 3')	Annealing temperature ($^\circ\text{C}$)	Restriction enzymes
IFN- γ	-179G/T	F: AATGATCAATGTGCTTTGTG ^[1] R: TTAAGATGAGATGGTGACAG	57	AvaII
IL-4	-33C/T	F: CTCATTTCCTCCGTTTCAGC ^[2] R: GAAGCAGTTGGGAGGTGAGA	58	BsmAI
IL-4	-589C/T	F: TAAACTTGGGAGAACATGGT ^[3] R: TGGGGAAGATAGAGTAATA	48	AvaII

2.4. Enzyme-linked immunosorbent assay (ELISA)

The levels of IFN- γ , IL-4, and total IgE were measured by ELISA according to the manufacturer's instructions.

2.5. Statistical analyses

The measurement data with a normal distribution of the measurement data is expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). The means of the two groups were compared using an independent sample t-test, and those among multiple groups were compared using analysis of variance (ANOVA). The measurement data with an abnormal distribution were expressed in terms of medians and were tested using the Mann–Whitney rank test. The enumeration data are expressed as rates or ratios and they were analyzed by χ^2 test or Fisher's exact test. All data were analyzed using the statistical package for social science (SPSS) 11.5 software.

3. Results

3.1. Genotype detection at different loci

No mutant IFN- γ gene was found at -179 . All subjects exhibited the GG genotype, and the genotyping results of the restriction enzyme products are shown in Fig. 1A. The AA, AT, and TT genotypes were detected at IFN- γ +874, and the genotyping results are shown in Fig. 1B. Detection of the IFN- γ CA repeat sequence through capillary electrophoresis shows that the CA repeat sequence was repeated 11–18 times, which indicates the presence of 8 alleles in the Guangdong Pearl River Delta region, specifically, CA₁₁–CA₁₈ (Fig. 1C). The CC, CT, and TT genotypes were detected at the -33 and -589 loci of IL-4 through PCR and electrophoresis, which are shown in Figs. 2A and B. The genotypic and allelic frequency distributions at each locus in the asthmatic group and the control group are shown in Table 3. The test for the Hardy–Weinberg equilibrium shows that the genotypic frequencies of IFN- γ at the different loci (874A/T and the CA repeat sequence) and those of the IL-4 gene (-33 C/T and -589 C/T) in the control group were in line with the Hardy–Weinberg equilibrium (all $P > 0.05$), which indicates good data representation.

3.2. Associations of IFN- γ polymorphisms at +874 and the CA repeats with asthma

The allelic frequencies of the AA, AT, and TT genotypes in IFN- γ at +874 in the asthmatic group and the control group were 65.0%, 33.0%, and 2.0% and 61.5%, 35.2%, and 3.3%, which show no significant differences ($\chi^2 = 0.522$, $P > 0.05$). The allelic frequencies of A and T in the asthmatic group were 81.5% and 18.5%, which corresponds to 79.1% and 20.9% in the control group and display no significant differences ($\chi^2 = 0.399$, $P > 0.05$).

The genotypic frequencies of the CA₁₂CA₁₂, CA₁₂CA₁₇, and CA₁₇CA₁₇ of the CA repeats of IFN- γ in the asthma group were 1.1%, 28.7%, and 70.2%, and those in the control group were 1.7%, 30.6%, and 67.8%, which show no significant differences ($\chi^2 = 0.239$, $P > 0.05$). There were no significant differences in the allelic CA₁₂ or CA₁₇ frequency between groups (15.4% and 84.6% in the asthma group compared with 16.9% and 83.1% in the control group) ($\chi^2 = 0.179$, $P > 0.05$).

3.3. Association of IL-4 polymorphisms at -33 and -589 with asthma

The frequencies of the CC, CT, and TT genotypes of IL-4 at -33 in the asthma group and the control group were 1.0% and 2.5%, 23.0% and 40.2%, and 76.0% and 57.4%, respectively, which shows significant differences ($\chi^2 = 8.539$, $P < 0.05$). The allelic C and T frequencies were 12.5% and 22.5% and 87.5% and 77.5% in the two groups, which shows

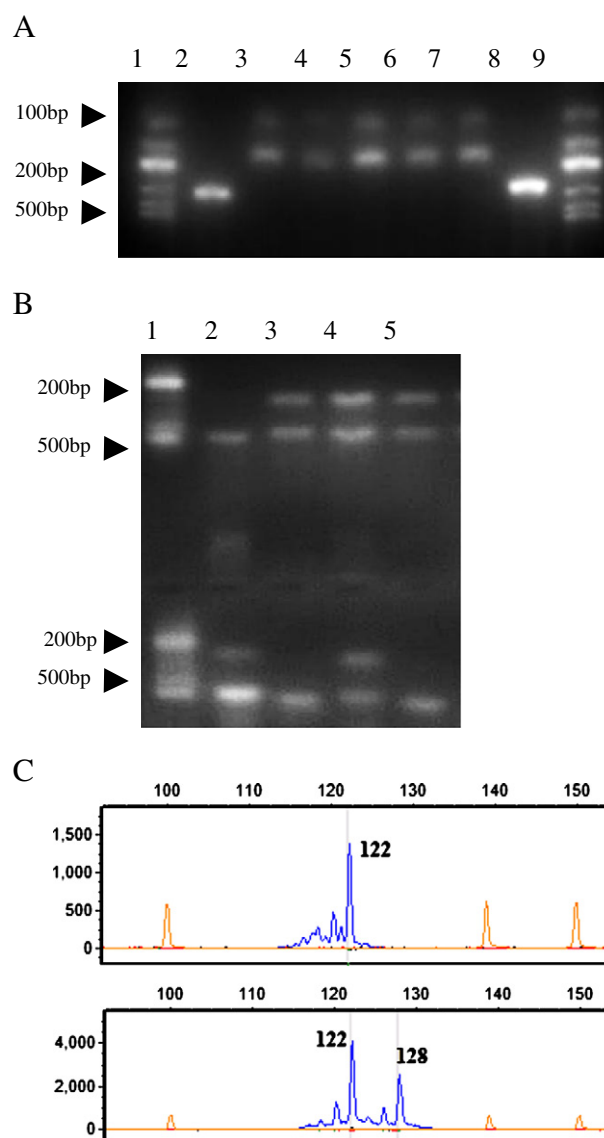


Fig. 1. A. IFN- γ gene at the -179 locus by PCR-RFLF. Lanes 1 and 9: DNA Maker; Lanes 2 and 8: PCR product; Lane 3–7: GG genotype. B. IFN- γ gene at the +874A/T locus by AS-PCR. Lane 1: DNA Maker; Lane 2: TT genotype; Lanes 3 and 5: AA genotype; Lane 4: AT genotype. C. The IFN- γ gene at the CA repeat by capillary electrophoresis. CA₁₂CA₁₂ genotype, 122 bp; CA₁₂CA₁₅ genotype, 122 bp and 128 bp.

significant differences ($\chi^2 = 7.502$, $P < 0.01$) with odds ratio (OR) C/T = 0.49 [95% confidence interval (CI) = 0.29–0.82] and OR T/C = 2.04 (95% CI = 1.22–3.41).

The CC, CT, and TT genotypic frequencies at -589 in the asthma group were 1.0%, 19.0%, and 80.0%, whereas those in the control group were 3.3%, 35.2%, and 61.5%, which show significant differences ($\chi^2 = 9.161$, $P < 0.05$). The C and T allelic frequencies in the asthma group were 10.5% and 89.5%, whereas those in the control group were 20.9% and 79.1%, which shows significant differences ($\chi^2 = 8.752$, $P < 0.01$) with OR C/T = 0.44 (95% CI = 0.26–0.77) and OR T/C = 2.25 (95% CI = 1.30–3.89).

3.4. Levels of IFN- γ , IL-4, and total IgE

The IFN- γ levels in the asthma group (pg/mL) ($M = 17.61$, $P_{25} = 8.68$, $P_{75} = 32.64$) were significantly lower than those in the control group ($M = 43.45$, $P_{25} = 32.02$, $P_{75} = 67.41$) ($P < 0.01$). The IL-4 levels (pg/mL) ($M = 26.46$, $P_{25} = 12.49$, $P_{75} = 45.35$) in the asthma group were significantly higher ($M = 11.42$, $P_{25} = 7.36$,

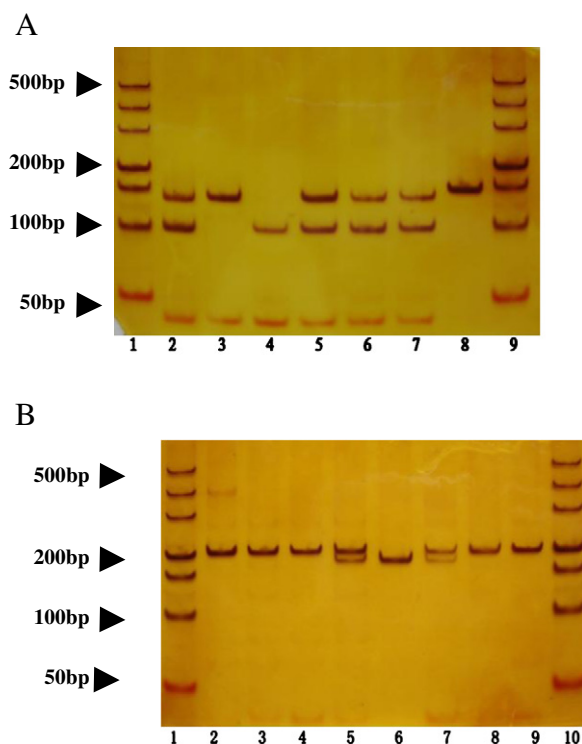


Fig. 2. A. The IL-4 gene at the -33C/T locus by PCR-RFLP. 1, 9: DNA Maker; 8: PCR product; 2, 5, 6, 7: CT genotype; 3: CC genotype; 4: TT genotype. The 15 bp fragment is not displayed because it migrated rapidly on the native polyacrylamide gel. B. The result of IL-4 gene at the -589C/T locus by PCR-RFLP. 1, 10: DNA Maker; 2: PCR product; 3, 4, 8, 9: TT genotype; 5, 7: CT genotype; 6: CC genotype. The 18 bp fragment was dim because it migrated rapidly on the native polyacrylamide gel.

Table 3

The genotype and allele frequencies of IFN- γ and IL-4 at loci among asthmatic patients and control subjects.

Gene	Polymorphism	Genotype/ Allele	AS patients n = 100 (%)	Control group n = 122 (%)	P value	OR (95%CI)
IFN- γ	+874A/T	AA	65 (65.0)	75 (61.5)	0.770	–
		AT	33 (33.0)	43 (35.2)	–	–
		TT	2 (2.0)	4 (3.3)	–	–
		A	163 (81.5)	193 (79.1)	0.528	1.116 (0.73–1.87)
		T	37 (18.5)	51 (20.9)	0.528	0.086 (0.54–1.38)
IFN- γ	CA _(n)	CA ₁₂ CA ₁₂	1 (1.1)	2 (1.7) ^a	0.887	–
		CA ₁₂ CA ₁₂	27 (28.7)	37 (30.6)	–	–
		CA ₁₂ CA ₁₂	66 (70.2)	82 (67.8)	–	–
		CA ₁₂	29 (15.4)	41 (16.9)	0.673	0.089 (0.53–1.50)
		CA ₁₂	159 (84.6)	201 (83.1)	0.673	1.112 (0.67–1.88)
IL-4	–33C/T	CC	1 (1.0)	3 (2.5)	0.014	–
		CT	23 (23.0)	49 (40.2)	–	–
		TT	76 (76.0)	70 (57.4)	–	–
		C	25 (12.5)	55 (22.5)	0.006	0.049 (0.29–0.82)
		T	175 (87.5)	189 (77.5)	0.006	2.204 (1.22–3.41)
IL-4	–589C/T	CC	1 (1.0)	4 (3.3)	0.010	–
		CT	19 (19.0)	43 (35.2)	–	–
		TT	80 (80.0)	75 (61.5)	–	–
		C	21 (10.5)	51 (20.9)	0.003	0.044 (0.26–0.77)
		T	179 (89.5)	193 (79.1)	0.003	2.225 (1.30–3.89)

AS patients: asthma patients; OR: odds ratio; 95% CI: 95% confidence interval.

^a Only 94 asthmatic patients and 121 control subjects were available for CA repeat polymorphism.

$P_{75} = 17.21$ ($P < 0.01$); the total IgE levels in the asthmatic group (IU/mL) ($M = 49.17$, $P_{25} = 16.14$, $P_{75} = 117.20$) were higher than those in the control group ($M = 35.42$, $P_{25} = 18.55$, $P_{75} = 62.24$) ($P < 0.05$). Considering that the TT frequency was rather low at the +874 locus, only the frequencies of AA and AT were compared between the two groups to determine the IFN- γ levels (Table 4). The levels of IFN- γ were lower in the AA genotype than those in AT ($P < 0.05$). Given that CA₁₂ homozygotes are rare, only the CA₁₂ heterozygotes (CA₁₂CA₁₂) and the non-homozygotes (CA₁₂CA₁₂) were compared between the two groups to determine the IFN- γ levels. Although the levels of CA₁₂CA₁₂ of IFN- γ were higher than those of CA₁₂CA₁₂, no significant differences were observed ($P > 0.05$). The three genotypes were found at both the –33 and the –589 loci; however, only the CT and TT genotypes were compared to determine the levels of IL-4 and total IgE because of the low genotypic frequency of CC (Table 5). In addition, the levels of IL-4 and total IgE among the children with the TT genotype at both the –33 and the –589 loci were higher than those with CT; only the IL-4 levels at –33 in TT were significantly higher than those in CT ($P < 0.05$).

4. Discussion

Asthma is a complex polygenetic disease with a clear genetic predisposition (Gerritsen, 2002; Le Souëf, 2009). Bream et al. (2002) found a polymorphic locus, namely, –179G/T, in the IFN- γ gene promoter region while testing an American population. This locus, combined with a transcriptional activator protein-1 analog after gene mutation, upregulates IFN- γ gene transcription with TNF- α induction and increases IFN- γ expression. Cabantous et al. (2005) found that IFN- γ expression among those with the T allele at –179G/T locus was increased in cerebral malaria in children from the Mali Bamako region, which enhanced the control of the malarial infection and

reduced the incidence of cerebral malaria. Research conducted among the Han population in northern China showed that polymorphism at this locus is correlated with hepatitis B virus (HBV) infection (Qi et al., 2005) and that the T allele at this locus has an aberration rate of 5.71%. However, no report has been conducted in southern China until now. Furthermore, this locus is located in the promoter region and can affect IFN- γ gene transcription through binding with transcription factor, which further affects IFN- γ expression. However, no report has been released yet regarding the association between this locus and asthma. Based on these facts, the –179 locus among asthmatic children and among the control children in the Pearl Delta of Guangdong was first investigated in the present study, and the results show no mutant genotypes at this locus. This indicates that the –179 locus in the IFN- γ gene may not be correlated with asthma susceptibility, considering that the genotype of all subjects were homozygous GG.

Hussein et al. (2009) studied 75 patients with atopic diseases (specifically asthma, atopic dermatitis, and allergic rhinitis) in Egypt. They found that polymorphism at +874A/T is significantly associated with atopic diseases, and there were significant differences in IgE levels, increases in eosinophil counts, and decreases in IFN- γ levels between the AA group and the AT group. Scholars in China

Table 4

The levels of IFN- γ at +874A/T locus and CA repeat polymorphisms in different genotypes.

Polymorphism	Genotype	IFN- γ (pg/ml)	P value
+874A/T	AA	20.59 (9.06, 35.19)	<0.05
	AT	33.59 (19.07, 49.78)	
CA(n)	CA ₁₂ CA ₁₂	20.59 (8.73, 36.36)	>0.05
	CA ₁₂ CA ₁₂	18.86 (8.56, 31.61)	

Data are presented as interquartile rang: median (25% quartile, 75% quartile).

Table 5

The levels of IL-4 and total IgE at –33C/T and –589C/T polymorphisms in different genotypes.

Polymorphism	Genotype	IL-4 (pg/mL)	P value	Total IgE (IU/mL)	P value
–33C/T	CT	21.62 (10.42, 43.54)	<0.05	49.17 (18.54, 117.2)	>0.05
	TT	35.50 (21.96, 48.90)		54.95 (13.96, 141.40)	
–589C/T	CT	21.31 (6.75, 42.93)	>0.05	43.96 (15.11, 112.9)	>0.05
	TT	31.76 (0.92, 46.90)		49.71 (16.02, 115.6)	

Data are presented as interquartile rang: median (25% quartile, 75% quartile).

found that polymorphisms at this locus are associated with asthma susceptibility among adults in Chongqing, China (Li et al., 2007). In the present study, the frequency distribution of the AA, AT, and TT genotypes at +874 was not significantly different between the asthmatic group and the control group ($P>0.05$). Comparison of the IFN- γ levels among different genotypes shows that the IFN- γ gene expression in the AA group is significantly lower than that in the AT group ($P<0.05$), which indicates a correlation between the polymorphism at 874 and IFN- γ expression. Whether the correlation affects the severity and prognosis of asthma still needs further study.

The CA repeat sequence is located in the first intron of IFN- γ . In the Guangdong Pearl River Delta region, the results show that the CA repeat sequence was repeated 11–18 times (i.e., alleles CA₁₁–CA₁₈), as determined by capillary electrophoresis. This was different from other reports wherein 12–18 repetitions were found among the Taiwanese population (Wang et al., 2006) and 10–18 times among Indians (Nagarkatti et al., 2002). In the present study, the genotypic frequency distributions of CA₁₂CA₁₂, CA₁₂CA₁₂ and CA₁₂CA₁₂ were compared with those in the asthmatic group and the control group and no correlation was found between the frequency and asthma susceptibility. This observation is consistent with the results by Kumar and Ghosh (2008), but is contradictory with the results obtained from the Taiwan population (Wang et al., 2006) and that in India (Nagarkatti et al., 2002) wherein the correlation between genotypic frequency and asthma was reported. The disagreement might be related to the differences in ethnicity, region, and research methods among the different studies. In the present study, the IFN- γ levels among the different genotypes were also compared and the results show no statistically significant difference in IFN- γ levels in CA₁₂CA₁₂ and CA₁₂CA₁₂ between the two groups ($P>0.05$).

IL-4–33C/T is located at the –33 bp locus upstream of the start codon ATG of the IL-4 gene, which is also at the 33 bp locus downstream of the transcription initiator (designated as +33C/T). Gervaziev et al. (2006) reported that the polymorphism at this locus is correlated with asthma susceptibility and with the levels of IL-4 and total IgE in the Russian population. Scholars in China reported that polymorphism at this locus is associated with asthma susceptibility and serum total IgE levels (Wang et al., 2009a, 2009b). Genetic mutation at this locus changes the stability of its binding with CREB (c-AMP response element binding protein), thereby affecting IL-4 gene transcription (Gervaziev et al., 2006) or through linkage disequilibrium at the –589 and –33 loci. However, there are some contradictory findings: research on 13 polymorphic loci in Taiwan showed that the –33C/T locus is not associated with asthma (Wang et al., 2009a, 2009b). In the present study, the genotypic and allelic frequency distributions at the –33 locus in the asthmatic group and the control group were significantly different. The T allele is associated with asthma susceptibility and it has a 2.04 times higher risk of asthma than the C allele. Meanwhile, the IL-4 level of the TT genotype at the –33 locus was higher than that of the CT genotype, but comparison of the total IgE between the different genotypes did not show such a correlation. The results suggest a close relationship

between gene polymorphism at the –33 locus and asthma susceptibility, and that the T allele is correlated with an increased risk of asthma, affecting the initiation and development of asthma by influencing IL-4 expression.

Iranian scholars reported that the T allele at –589 of IL-4 is correlated with asthma susceptibility (Kamali-Sarvestani et al., 2007). Russian scholars reported that this locus is correlated with IL-4 levels and the total IgE of asthmatic patients (Gervaziev et al., 2006). In addition, a study conducted on a Taiwanese population found that polymorphism at this locus is closely related with asthma susceptibility and airway hyperresponsiveness (severity), but it has no correlation with total IgE level (Chiang et al., 2007). In the present study, the genotypic and allelic frequency distribution at the –589 locus in the asthmatic group and the control group are significantly different; the T allele is an asthma susceptibility gene and it has 2.25 times higher correlation with the risk of asthma than the C allele. Further correlation analysis of the polymorphism at –589 and the levels of IL-4 and total IgE show no correlation between them. Asthma is a complex disease, and apart from the many immunocytes and their cytokines that cause this disease (Bottema et al., 2010; Karwot et al., 2008; Liang et al., 2010; Xu et al., 2010), other factors affect IL-4 and total IgE levels.

The study of asthma-related gene polymorphism is still in the initial phase. With the development of molecular genetics and molecular biological techniques, the study of asthma-related gene polymorphism is a promising strategy for the further exploration of the pathogenesis of asthma, confirmation of asthma susceptible individuals, and gene therapy for asthma.

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