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Short Communication

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 +874A/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 +874A/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 +874A/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 +874A/T or the CA repeats are not correlated with susceptibility to asthma. Thus, the polymorphism at +874A/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.

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) l/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 Thl 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 μL 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 μL 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 μL 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 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.

Table 1
PCR primer sequences, annealing temperature and restriction enzymes.

Gene	Polymorphism	Primer sequence (5′→3′)	Annealing temperature (°C)	Restriction enzymes
IFN-γ	−179G/T	F: AATGATCAATGTGCTTTGTG [1] R:TTAAGATGAGATGGTGACAG	57	AvaII
IL-4	−33C/T	F:CTCATTTTCCCTCGGTTTCAGC[2] R:GAAGCAGTTGGGAGGTGAGA	58	BsmAI
IL-4	−589C/T	F:TAAACTTGGGAGAACATGGT[3] R:TGGGGAAAGATAGAGTAATA	48	AvaII

Table 2
Genotype of each locus and length of DNA fragment after restriction enzyme digestion.

Gene	Polymorphism	PCR product	PCR-RFLPgenotype: 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 TT:(97 + 38 + 15)bp	8% native polyacrylamide gel
IL-4	−589C/T	195 bp	CC:(177 + 18)bp CT:(195 + 177 + 18)bp TT:195 bp	8% native polyacrylamide gel

Polymorphisms of the IFN-γ gene at +874A/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′-CTACAATG 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′-CGAGCTTTAAAAGATAGTTCC-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.