Polymorphisms of interleukin 4 and interleukin 4 receptor genes and bronchial asthma risk among Egyptian children

Afaf Elsaid1, Rasha MS Shoaib2

1Biochemistry Section, Mansoura University Children's Hospital, Mansoura, Egypt.

2Food and Dairy Sciences and Technology Department, Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt.

Introduction

Bronchial asthma is a complex chronic airway disease characterized by bronchial hyperresponsiveness, inflammation, and reversible obstruction. It affects about 300 million persons all over the world. The pathogenesis of asthma involves the interaction of many cytokines such as interleukin 4 (IL4), IL5, and IL13. IL4 is the key cytokine that regulates the inflammatory cascade in asthma, right from the chemotaxis of eosinophils to the differentiation of naive T helper cells to Th2 cells. IL4 plays important roles in the modulation of the type 2 immune response, isotype switching from IgM/IgG to IgE by B lymphocytes, and in the mast cell recruitment. In patients with asthma, IL4 induces the vascular cell adhesion molecule-1 on the vascular endothelium, inhibits eosinophil apoptosis, and stimulates eosinophilicinflammatory reactions. Moreover, higher IL4 levels have been detected in bronchial smooth muscle cells of individuals with asthma. The role of IL4 in airway remodelling is also proved through its triggering effects on the mucus-producing cells and fibroblasts. The *IL4* gene (OMIM#: 147780) has been extensively studied for the possible involvement in asthma susceptibility among different populations, and it is proposed to have a fundamental role in the development and persistence of asthma through the modulation of serum IL4 levels. The *IL4* gene is located on the long arm of chromosome 5 (5q31.1), a region that has been associated with asthma and atopy. The IL4 variable number of tandem repeat (VNTR) polymorphism, which is located on the intron 3 of the *IL4* gene, is characterized by a wild A1-allele (three repeats), a less frequent A2-allele (two repeats) and a rarer A3-allele (four repeats). Despite *IL4 intron 3 VNTR* polymorphism (rs79071878) has been comprehensively investigated in the literature, only two studies have investigated its role in asthma. IL4 exerts its effects through binding to the alpha chain of the IL4 receptors (IL4R). When the IL4 binds to IL4R, the signal transducer and activator of transcription 6 (STAT6) is activated with subsequent higher expression of IL4-sensitive genes. The IL4R α-subunit is encoded by the *IL4R* gene, mapped on chromosome 16p12.1 (OMIM#: 147781). Several SNPs have been identified in the *IL4R* coding region. Among these polymorphisms, *IL4R c.1902 A>G p. (Q576R)* encodes *A-to-G* transition at the nucleotide 1902 causing an alteration from glutamine to arginine at the codon 576 in the IL4R cytoplasmic domain. Several studies previously assessed the *IL4R (A1902G)* polymorphism in atopic patients of different ethnic groups with conflicting results. Some authors confirmed the association between the *IL4R (A1902G)* variant and atopy, while others found no evidence of such association.

Methodology

Study participants

This preliminary case-control study included a convenience sample of 200 children, 100 children with bronchial asthma (64 males and 36 females), and 100 healthy controls (62 males and 38 females) of the same ethnicity.

Clinical evaluation and skin tests

We retrieved clinical data about the study participants through direct interviews and from patients' files. These data included age, gender, the duration of disease, the degree of asthma severity, clinical phenotypes (cough, wheezes, and dyspnea) and associated allergic rhinitis and atopic dermatitis.

Sample collection and analysis

Five milliliters of peripheral venous blood was withdrawn from all study participants using plastic disposable syringes under a complete aseptic technique. Each sample was divided into two tubes; 3 mL blood was collected without an anticoagulant and centrifuged for 15 min at 5000 RPM for serum IL4 and IgE levels assay, and 2 mL blood was placed in a test tube containing EDTA for DNA extraction. Genomic DNA extraction was carried out from 200 µL blood using the Biospin Whole Blood Genomic DNA Extraction Kit (BioFlux, Biospin series-BSC06M1, China).

Genotyping of the IL4 VNTR (rs79071878) polymorphism by PCR method

The genotyping of the VNTR (70-bp repeat) polymorphism in intron 3 of the IL4 gene was carried out by PCR method described by the Buchs method. The sequences of oligonucleotide primers used for the IL4 VNTR variant genotyping were 5′-GTA AAT AGG CTG AAA GGG GGA AA-3′ for the forward primer and 5′-CAT CTT TTC CTC CCC TGT ATC TT-3′ for the reverse primer. Thermo-cycling steps involved 2 min of denaturation at 95 ◦C, then 40 cycles of denaturation at 95 ◦C for 1 min, an annealing step at 56 ◦C for 1 min, and extension at 72 ◦C for 30 sec. A final extension was carried out at 72 ◦C for 5 min. PCR products were electrophoresed on 2% agarose gel, and visualized using ethidium bromide under ultraviolet illumination. The A1-allele (three repeats) was detected at 342 bp, while A2-allele (two repeats) was found at 272 bp. Heterozygotes generated two fragments of 342 bp and 272 bp.

Genotyping of the IL4R (A1902G) polymorphism by PCR-RFLP method

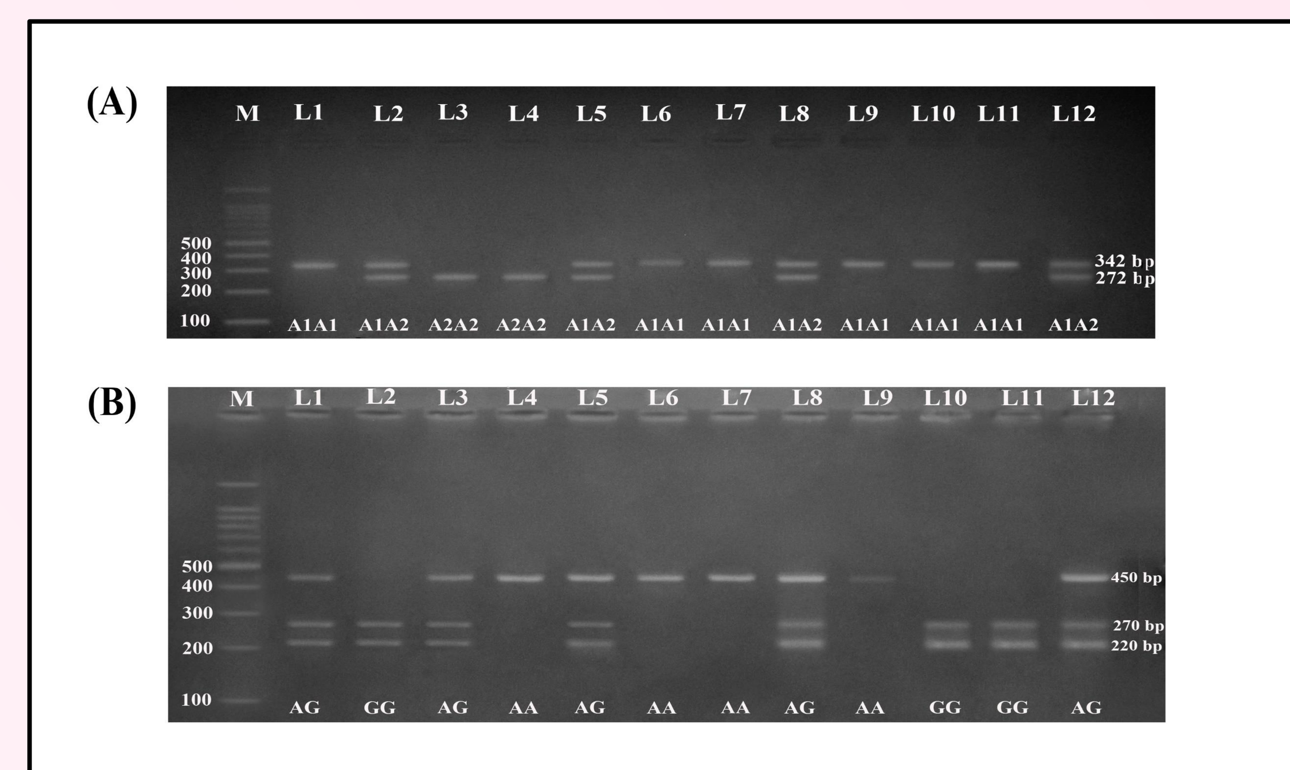
Genotyping of the IL4R (A1902G) polymorphism was carried out by PCR-RFLP method according to a previously published assay. The sequences of oilgonucleotide primers used for the IL4R (A1902G) variant genotyping were 5′-CCC CCA CCA CCA GTG GCT ACC-3′ for the forward primer and 5′ -CCA GGA ATG AGG TCT TGG AA-3′ for the reverse primer. Thermo-cycling steps involved 5 min of denaturation at 94 ◦C, then 30 denaturation cycles at 94 ◦C for 1 min, an annealing step at 55 ◦C for 1 min, and extension at 72 ◦C for 1 min. A final extension was carried out at 72◦ for 10 min. PCR products, before enzymatic digestion, were shown as 450 bp fragments by gel electrophoresis. PCR products were digested for 4 h at 37 ◦C with 1U of the MspI restriction enzyme (Catalog Number: R0106S, New England Biolabs, Boston, Massachusetts, USA). The PCR products were electrophoresed using 2% agarose gel, stained with ethidium bromide and visualized under ultraviolet illumination. The A allele remained undigested at 450 bp, while the G-allele produced two bands at 270 bp and 220 bp. Heterozygotes generated three fragments of 450, 270, and 220 bp.

Results

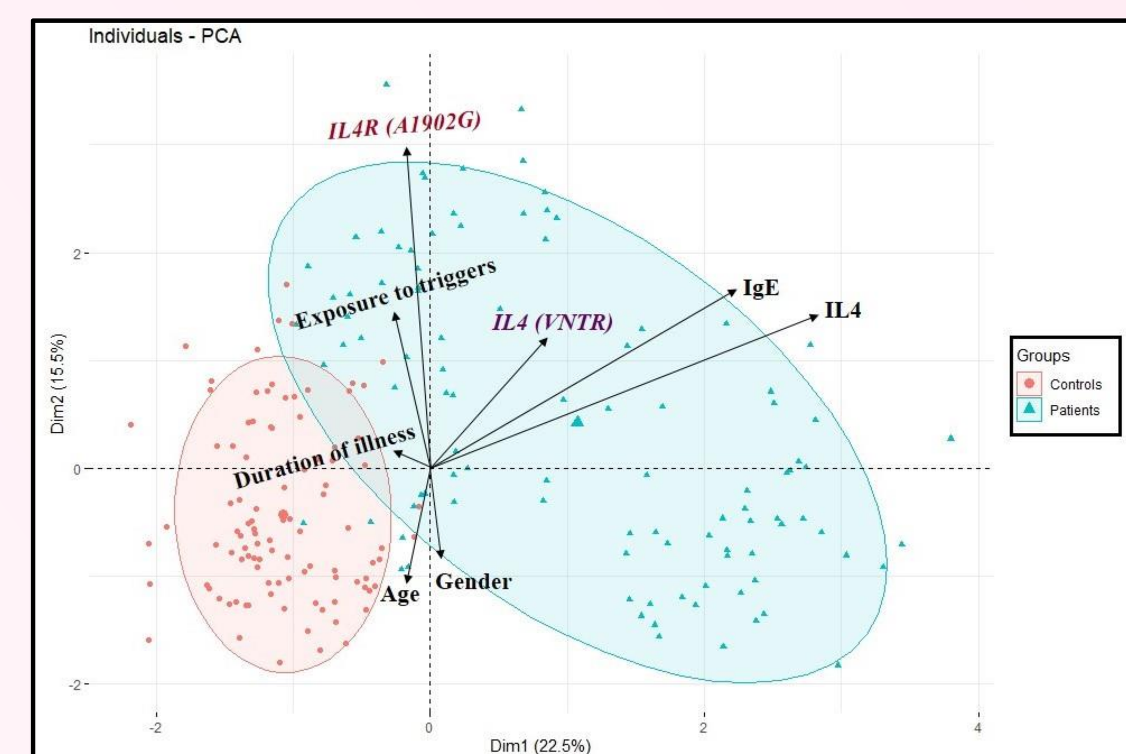
The frequencies of (A1A2+A2A2) genotypes and A2-allele of the IL4 VNTR variant were significantly higher among asthmatic patients than controls (p = 0.01, OR = 2.34, 95% CI = 1.24-4.44; p = 0.01, OR = 2.27, 95% CI = 1.29-3.99, respectively). The frequencies of (AG+GG) genotypes and G-allele of the IL4R (A1902G) variant were significantly higher among asthmatic patients than controls (p = 0.003, OR = 2.52, 95% CI = 1.39-4.58; p = 0.002, OR = 2.25, 95% CI = 1.35-3.76, respectively). There was a significant association between (A1A2+A2A2) genotypes of the IL4 VNTR variant and high serum IL4 level among asthmatic patients (p <0.001). The (AG+GG) genotypes of the IL4R (A1902G) variant were significantly associated with exposure to triggers, atopic dermatitis and higher serum IgE level in asthmatic patients (p = 0.02, 0.04 and 0.01, respectively).

Gene polymorphisms	Asthma (n=100) n (%)	Controls (n=100) n (%)	Statistics		OR (95%CI)	*p*-value
IL4 VNTR (rs79071878)						
A1A1	63 (63%)	80 (80%)	Recessive	A2A2 vs. A1A1+A1A2	5.21 (0.59-45.43)	0.21
A1A2	32 (32%)	19 (19%)	Dominant	A1A2 + A2A2 vs. A1A1	**2.34 (1.24-4.44)**	**0.01***
A2A2	5 (5%)	1 (1%)	Heterozygote	A1A2 vs. A1A1	**2.14 (1.11-4.12)**	**0.02***
A1	158 (79%)	179 (89.5)	Homozygote	A2A2 vs. A1A1	6.35 (0.72-55.73)	0.09
A2	42 (21%)	21 (10.5)	Allelic	A2 vs. A1	**2.27 (1.29-3.99)**	**0.01***
HWE	**χ^2= 0.126, *p* = 0.72**	**χ^2= 0.012, *p* = 0.91**				
IL4R (A1902G) (rs1801275)						
AA	53 (53 %)	74 (74%)	Recessive	GG vs. AA+AG	5.21 (0.59-45.43)	0.21
AG	42 (42%)	25 (25%)	Dominant	AG+GG vs. AA	**2.52 (1.39-4.58)**	**0.003***
GG	5 (5%)	1 (1%)	Heterozygote	AG vs. AA	**2.35 (1.28-4.31)**	**0.01***
A	148 (74%)	173 (86.5%)	Homozygote	GG vs. AA	6.98 (0.79-61.49)	0.09
G	52 (26%)	27 (13.5%)	Allelic	G vs. A	**2.25 (1.35-3.76)**	**0.002***
HWE	**χ^2= 0.84, *p* = 0.36**	**χ^2= 0.49, *p* = 0.48**				

CI: confidence interval; OR: odds ratio; *$p \leq 0.05$ is significant.



(A) IL4 VNTR (rs79071878) polymorphism using PCR for asthmatic patients and controls. Lane M: DNA size marker (100–1000 bp). Lanes 1, 6, 7, 9, 10 and 11 (three repeats genotype appeared at 342 bp), lanes 3, and 4 (two repeats genotype which had 272 bp fragment), lanes 2, 5, 8, and 12 (heterozygous- genotype A1A2 was found with 342 bp and 272 bp fragments). **(B)** IL-4R (A1902G) polymorphism using PCR-RFLP after enzymatic digestion of amplified DNA by the MspI restriction enzyme for asthmatic patients and controls. The lanes (4,6,7,9) showed homozygous wild-type asthmatic patient (AA), lanes (1,3,5,8,12) showed heterozygousgenotype asthmatic patient (AG) and lanes (2,10,11) showed homozygous mutant asthmatic patient (GG). The lane (M) was the DNA ladder marker.



Principal component analysis (PCA) for data evaluation. The multivariate analysis showed the clustering of the study population into two classes (patients and controls). Dimension 1 (Dim 1) and dimension 2 (Dim 2) accounted for 22.5% and 15.5% of the variability, respectively. The demographic, clinical, and biochemical data were represented as arrows; with the longer ones indicating more impact on the separation.

Conclusion

IL4 VNTR and *IL4R (A1902G)* polymorphisms could be considered as risk factors for occurrence of bronchial asthma among Egyptian children. The A1A2 and A2A2 genotypes of the *IL4 VNTR* variant are associated with higher serum IL4 levels, while AG and GG genotypes of *IL4R (A1902G)* variant are associated with higher serum IgE levels, exposure to triggers and atopic dermatitis.