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Interleukin-4 and interleukin-4 receptor alpha polymorphisms in atopic dermatitis: A case-control study

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ABSTRACT

Background: Several lines of evidence suggest that the interleukin-4 (IL-4) is involved in the development of atopic diseases.

Objective: To determine whether IL-4 and IL-4Rα polymorphisms are associated with susceptibility to dermatitis in Egyptian children, and to evaluate their effects on serum immunoglobulin E (IgE) and serum IL-4 levels.

Methods: We genotyped three groups of children, consisting of 50 atopic dermatitis (AD) children, 50 non-AD children, and equal number of healthy controls, for IL-4 (–590C/T) and IL-4Rα (I50V) genes polymorphisms using PCR-RFLP assay. Total serum IgE and serum IL-4 levels were detected by ELISA.

Results: There was a non significant association of IL-4 -590C/T polymorphism in children with non- AD or those with AD when compared with the controls (P= 0.8, 0.4 respectively). We identified a significant association between IL-4Rα I50V polymorphism and dermatitis susceptibility in AD (p= 0.002), whereas no such association was observed in non-AD group (p=0.7). Evidence of gene interactions between both polymorphisms was found. Furthermore, there was no relation between each polymorphism and serum IL-4 level (p>0.05 for each) while homozygosity for the risk alleles of IL-4 -590 C/T and IL-4Rα I50V were significantly associated with increased total IgE levels in all studied groups.

Conclusion: In Egyptian children, the IL-4Rα (I50V) may play a role in susceptibility to AD. In addition, gene-gene interaction between the IL-4 -590T and the IL-4Rα G allele significantly increases an individual's susceptibility to AD. Both polymorphisms may be involved in the control of IgE production.

KEYWORDS

Atopic dermatitis;
Interleukin-4;
IL-4Rα;
Single nucleotide polymorphism;
Gene-gene interaction.

INTRODUCTION

Eczema is a chronic inflammatory skin disorder characterized by pruritic chronic lesions. Two subsets of eczema have been described: 70-80 % of eczematous patients present atopic form, with elevated serum

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immunoglobulin E (IgE) and allergic sensitization, whereas 20-30% of patients exhibit nonatopic form, with normal serum IgE levels and lack allergen-specific sensitization[1]. Some immunological differences between atopic dermatitis (AD) and the non atopic type have been observed in the cytokine pattern of peripheral blood and in the skin lesions[1].

Interleukin -4 (IL-4) is central to the development of the T helper 2 (Th2) phenotypes that induce immunoglobulin ε isotype switching, secretion of IgE, regulation of Fcε receptor and vascular cell adhesion molecule-1 (VCAM-1) expressions, and promote transmigration of effector cells[2]. IL-4 is a glycoprotein encoded by the IL-4 gene which has been localized to chromosomal region 5q31-33[3]. In 1995, Rosenwasser et al.[4] first described a functional promoter polymorphism –590C/T in the IL-4 gene, which was associated with elevated IgE levels in asthmatic individuals. It has been reported that polymorphisms within the promoter region of IL-4 gene seems to correlate with enhanced IL-4 activity, secondary to modification of IL-4 gene transcription. The T allele of –590C/T SNP was associated with an increased IL-4 gene expression in vitro[5].

The effect of IL-4 is mediated by a heterodimeric receptor composed of the IL-4Rα chain and either the common γ chain or the IL-13Rα subunit[6]. The coding gene of IL-4Rα has been localized to the short arm of chromosome 16 (16p12.1). Many studies have reported a polymorphic site resulting in the substitution of Ile for Val (I50V) (+4679A/G) in the extracellular domain[7,8]. It is believed that the Ile alleles, which strengthen signals through the IL4R, predispose the host immune system to Th2 cytokines[9].

Several studies analyzed the relation between IL-4 -590C/T and IL-4Rα I50V gene polymorphisms and atopy or asthma[10-13] but fewer analyzed the link between these polymorphisms and eczema. The results were inconsistent and the cause of inconsistency may be from ethnic related differences. In the present study, we first determine whether the IL-4 promoter polymorphism -590C/T and the IL-4Rα gene polymorphism (I50V) were associated with eczema in Egyptian children. We also study their effects on IL-4 and IgE levels.

SUBJECTS AND METHODS

The study was conducted by the Biochemistry Department, National Research Centre, Dokki, Giza and Medical Biochemistry and Dermatology departments at the faculty of Medicine of Zagazig University in Egypt. All patients were Egyptian children, residing in Zagazig, and unrelated to each other. The total studied sample consisted of age and sex- matched 150 examinees, divided into three different groups as follows: 50 healthy controls (8.9± 3.4 years, 23 female/ 27 male), 50 non-AD children (10.2± 3.8 years, 25female/ 25 male) and 50 children with AD (8.8±4.2 years, 24 female/ 26 male). All the patients did not receive any antihistamines, systemic or topical corticosteroids during the period of 3 weeks before clinical evaluation and were subjected to skin prick test. All subjects did not have manifestations of other dermatological or obvious medical, autoimmune or infectious diseases.

Atopy was diagnosed on the basis of positive skin prick test result in the form of 3mm or greater mean wheal diameter than the negative control against common allergens, or elevated specific IgE, clinical examination, and clinical sheets (symptoms). Atopic dermatitis children fulfilled the Hanifin and Rajka diagnostic criteria in the study[14]. Non-AD diseases were defined by a negative skin prick test result or negative specific IgE responses to common allergens. The control group had no previous clinical history of allergic diseases and had negative skin response to common allergen. Written informed consent was obtained from all parents' participants before blood sample collection.

Determination of serum IL-4

Serum IL-4 was measured by sandwich enzyme-linked immunosorbent assay (ELISA) (Koma Biotech Inc Human IL-4 kit; Seoul, Korea), with the minimum detectable dose established as 0.1 pg/mL.

Total IgE measurements

Total serum IgE levels were also measured by a solid phase enzyme-linked immunosorbent assay (ELISA) (BioCheck, Inc, Vintage Park Drive, Foster City), with the minimum detectable concentration of IgE estimated as 5.0 IU/ml.

IL4 -590C/T and IL-4Rα I50V genotyping

Genomic DNA was extracted from 200µl whole

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blood using Biospin whole blood genomic DNA extraction kit (Bioer Technology, Binjiang, Hanchuan, Hubei, China). Participants were genotyped for the promoter polymorphism of IL-4 -590C/T and a coding region polymorphism of IL-4Rα (I50V) in genomic DNA by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) according to[15]. PCR was performed using PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts. USA). Each 25 µL of PCR mixture contained 4.5 µL of H2O, 5 µL of genomic DNA, 1.5 µL of each primer (BioBasic Inc., Ontario, Canada), and Taq PCR master mix kit (12.5µL) (Qiagen GmbH, Hilden, Germany). The sequence of the primers and PCR cycling conditions were performed for all samples in TABLE 1.

TABLE 1 : Standard PCR conditions used in genotyping IL4 -590C/T and IL-4Rα I50V SNPs

Polymorphic site	Primer sequence	PCR condition	Restriction enzyme and fragment
IL4 C-590T	Forward, TAAACTTGGGAGAACATGGT Reverse, TGGGGAAAGATAGAGTAATA	30 s at 95°C, 30 s at 50°C, 45s at 72°C.	AvaII, C, 177+19 T, 195
IL-4Rα (I50V)	Forward, GGCAGGTGTGAGGAGCATCC Reverse, GCCTCCGTTGTTCTCAGGTA	30 s at 95°C, 30 s at 60°C, 45s at 72°C.	RsaI, A, 273 G, 254

Statistical analysis

The results for continuous variables are expressed as means ± SD. The means of the groups were compared by ANOVA-test. The statistical significances of differences in frequencies of variants between the groups were tested using the chi-square (x2) test. In addition, odds ratios (ORs) and 95% CIs were calculated as a measure of the association of the IL-4 and IL-4Rα alleles with dermatitis. A difference was considered significant if p was < 0.05. Data were analyzed using SPSS software version 11 (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Genotyping of IL-4 and IL–4Rα polymorphisms

The genotype and allele frequencies of the IL-4 and IL-4R polymorphisms in AD patients, non-AD patients and controls are shown in TABLE 2 & Figure 1. For IL-4 -590 C/T SNP, there was no significant association between genotypes or allele frequencies of the IL-4 polymorphism in the AD and non-AD groups compared to the control group [odds ratio and 95% CI for the T allele: 0.6 (0.3 – 1.4), p= 0.2 in atopic patients, 0.8 (0.4 – 4.65), p= 0.5 in non-atopic patients]. (Figure 1) For IL–4Rα I50V SNP, our results showed significant differences between AD group and controls regarding the genotype and allele distributions of IL-4Rα polymorphism while there were no significant differences in allele or genotype frequencies between non-AD children and controls for this polymorphism [odds ratio and 95% CI for the G allele: 4.3 (1.9 – 9.7), p< 0.001 in AD children, 0.7 (0.3 - 1.6), p=0.4 in non-AD children]. (Figure 2)

TABLE 2 : Genotype and allele frequencies of IL-4 -590 C/T and IL-4Rα I50V polymorphisms in studied groups

	Controls n (%)	Non – AD group		AD group	
		n (%)	P*	n (%)	P*
IL–4 (-590 C/T)					
Genotypes					
CC	40 (80%)	38 (76%)	0.8	34 (68%)	0.37
CT	9 (18%)	10 (20%)		15 (30%)	
TT	1 (2%)	2 (4%)		1 (2%)	
Alleles					
C	89 (89%)	86 (86%)	0.52	84 (84%)	0.30
T	11 (11%)	14 (14%)		16 (16%)	
IL–4Rα (I50V)					
Genotypes					
AA	42 (84%)	39 (78%)	0.7	26 (52%)	0.002
AG	7 (14%)	9 (18%)		18 (36%)	
GG	1 (2%)	2 (4%)		6(12%)	
Alleles					
A	91 (91%)	87 (87%)	0.4	71 (71%)	<0.001
G	9 (9%)	13 (13%)		29 (29%)	

*** As compared with controls**

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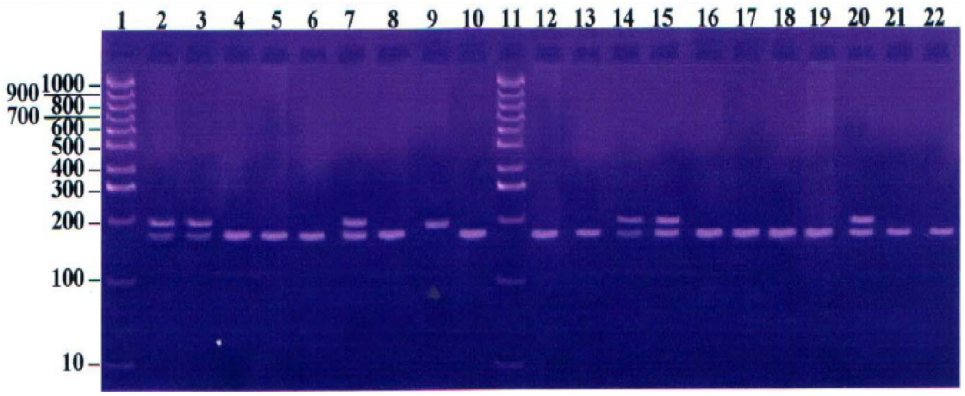

Figure 1 : Representative agarose gel electrophoresis, findings of C-590T polymorphism of IL-4 gene in studied groups. DNA marker (100 bp), (Lanes 1 11), homozygous TT (polymorphic) (lane 9, one band at 195 bp), heterozygous CT (Lanes 2 3 7 14 15 20, two bands at 177bp and 195 bp), homozygous CC (wild type) (lanes 4 5 6 8 10 12 13 16 17 18 19 21 22, ane band at 177 bp).

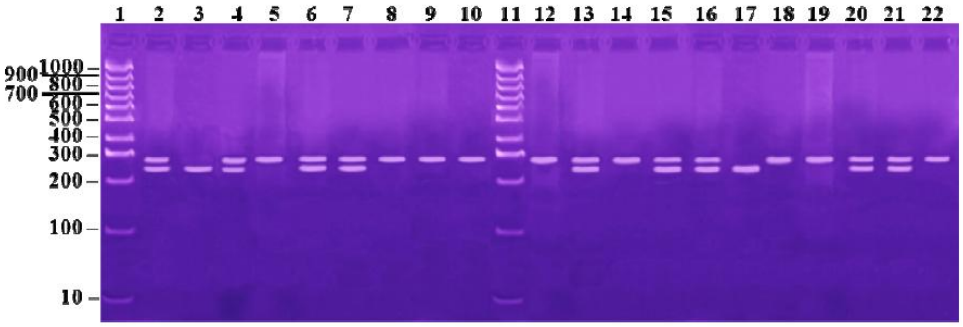

Figure 2 : Representative agarose gel electrophoresis, findings of Iso50Val polymorphism of IL-4Rα gene in studied groups. DNA marker (100 bp), (Lanes 1 11), homozygous GG (polymorphic) (lanes 3 17, one band at 254 bp), heterozygous AG (Lanes 2 4 6 7 13 15 16 20 21, two bands at 273 bp and 254 bp), homozygous AA (wild type) (lanes 5 8 9 10 12 14 18 19 22, ane band at 273 bp).

Serum IL-4 and IgE levels in all groups studied

Level of IL-4 had no significant difference between AD children; non-AD children and control group (P = 0.87) while levels of IgE were significantly increased in atopic patients compared to non AD (p< 0.001) and control groups (p< 0.001) (TABLE 3).

TABLE 3 : Serum interleukin 4 and immunoglobulin E levels in all studied groups

	Controls	Non-AD group	AD group	P
IL-4, Pg/ml	461.5 (103.8)	471.9 (105.1)	469.9 (108.6)	0.87
IgE, IU/ml	59.65 (12.7)	79.99 (16.2)	322.7 (53.4)	<0.001

Data are expressed as means (SD)

Association between measured parameters and IL-4, IL-4Rα polymorphisms

Serum levels of total IgE were significantly increased in IL-4 genotype TT than in CC in all groups studied (p< 0.001 for control and non-AD groups, p= 0.01 for AD group). Moreover, there was significant difference of the mean value of serum total IgE level among different allelic variants of IL-4Rα in control, non-AD and AD groups (P =0.005, 0.03 and 0.007 respectively). Also, there was a significant increase in serum level of total IgE towards homozygous GG than homozygous AA. On the other hand, there was no effect of IL-4, IL-4Rα polymorphisms on the level of IL-4 in all groups studied (TABLES 4, 5).

TABLE 4 : Association between IL-4 (-590 C/T) polymorphism and measured parameters

Groups	Measurements	CC	CT	TT	P
Controls	Serum IL-4	462.3 (104)	458.7 (113.3)	455 (0.0)	0.99
	Serum IgE	56.4 (8.1)	70.5 (19.1)	90 (0.0)	< 0.001
Non-AD Group	Serum ll-4	473.1 (106.8)	440.7 (87.7)	603.9 (69)	0.13
	Serum IgE	75.8 (14.5)	89.2 (12)	113.4 (4.5)	< 0.001
AD group	Serum ll-4	464.1 (107)	471.8 (111)	636.2 (0.0)	0.3
	Serum IgE	310.6 (48.3)	344.4 (53.9)	420 (0.0)	0.01

Data are expressed as means (SD)

TABLE 5 : Association between IL-4Rα (I50V) polymorphism and measured parameters

Groups	Measurements	AA	AG	GG	P
Controls	Serum IL-4	460.6 (100)	444.2 (122.5)	622.3 (0.0)	0.27
	Serum IgE	57.6 (11.2)	67.4 (13.8)	90.9 (0.0)	0.005
Non-AD Group	Serum ll-4	472.6 (106)	467.9 (102.1)	475.6 (161.99)	0. 99
	Serum IgE	77.7 (15.3)	83.9 (14.9)	106.9 (18.9)	0.03
AD group	Serum ll-4	457.6 (110.7)	485.8 (111.4)	475.5 (101)	0.7
	Serum IgE	310.7 (48.8)	320.4 (47.3)	383.7 (55.6)	0.007

Data are expressed as means (SD)

Interaction between IL4 and IL4Rα genes

To investigate an interaction between IL-4 and IL-4Rα genes, we studied dermatitis susceptibility in relation to various combinations of genotypes. We found that association of C→T and A→G substitutions of the IL-4 gene and IL-4Rα gene respectively was significant in both non-AD and AD groups ($p<0.001$& $p=0.004$ respectively). Patients who were carriers of both the T allele of IL4 -590C/T and the G allele of IL-4Rα had an increased risk of AD ($p< 0.001$).

DISCUSSION

Because the aberrant cytokine expression could be important in the pathogenesis of AD, functional relevant cytokine gene polymorphisms might affect cytokine production and therefore could determine disease susceptibility of AD[16,17]. Functional polymorphisms in the IL-4 gene may elevate IL-4 levels and there by influence the IL-4 dependant events which determine disease progression[5,8].

However, our study showed a non significant association of both TT genotype and T allele (mutant variant) frequencies at -590 of IL-4 promoter gene in children with non-AD and those with AD when compared with controls, and thus indicating that this SNP had no relationship with the development of both types of dermatitis in Egyptian children.

This finding was in agreement with Elliot et al.[18] who detected that the -590 C/T IL-4 promoter polymorphism separately is not associated with atopic eczema in childhood. This study indicated that this SNP separately lack power to detect linkage whereas the haplotype (4 alleles) is sufficiently polymorphic to detect a suggestive linkage. Tanaka et al.[19] determined that IL-4 -590 C/T genotypes was not significantly associated with either total patients with atopic eczema or atopic eczema patients who had normal IgE productivity. Recently, Stavric et al.[20] found that IL-4 -590 SPN did not show association with AD in macedonian children.

On the other hand, Novak et al.[21] reported that the frequency of the IL-4 polymorphism -590C/T tended to be higher in extrinsic atopic dermatitis than in intrinsic atopic dermatitis in children. He et al.[22] suggested that IL-4 -590T alleles are risk factor for the development of AD and atopy at 24 months of age.

Concerning IL4Rα I50V, we found that a significant association of both GG genotype and G allele (mutant variant) frequencies in children with AD but not in those with non-AD.

Recently, Tanaka et al.[23] showed that I50V allele seems to be involved in AD in children. On the other hand, another study determined that the IL-4Rα I50V genotypes were not significantly associated with either total patients with atopic eczema or atopic eczema patients who had normal IgE productivity[18].

Potential explanation for the differences between our results and others may be attributed to differences in the genetic variation in IL-4 and IL-4Rα, or in gene-gene or gene-environment interactions. For example, the T allele of IL-4 -590 C/T frequency is 70% in Japanese controls[24] 26% in Australian controls[25], 27% in white controls in the United Kingdom[26] while in our Egyptian sample was 11%.

Because these genes signal through a common pathway, gene-gene interactions may modify the effect of SNPs in the IL-4/IL-4R pathway and evidence of epistasis has been reported in asthma and atopy[27,28]. When we analyzed both polymorphisms simultaneously, patients who were carriers of both the T allele of IL-4 -590C/T and the G allele of IL-4Rα had an increased risk of AD.

Our study showed that serum level of IL-4 had no significant difference between AD children; non AD children and control group. Moreover, there was no effect of IL-4 and IL-4Rα genotypes on IL-4 serum level and this may attributed to that IL-4 levels in blood were not only related to the gene basis but also to other factors as cytokine inhibitors and used medications.

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Jeong et al.[29] showed that the levels of IL-4 were not different in the lesional skin of AD and non-atopic form. Many studies showed that serum level of IL-4 was significantly higher in AD patients than normal individuals[30,31] furthermore; the patients with atopic eczema had higher serum levels of IL-4 than non atopic form[1,31,32].

The differences between the current study and others may attributed to that AD is a biphasic disease where the initial phase is predominated by Th2 cytokines (including IL-4) that later switches to a more chronic Th1-dominated eczematous phase[21,33] and all these studies including ours do not stratify AD according to either acute or chronic.

In the current study, homozygosity for the risk alleles of IL-4 -590 C/T and IL-4Rα I50V were significantly associated with increased total IgE levels in all studied groups. These are going in hand with Rosenwasser et al.[4] and De Guia and Ramos[34] who reported a significant relationship between IL-4 -590 TT genotype and high IgE levels. Many studies revealed that AD patients with high IgE level were associated with IL-4Rα Ile50[23,35]. On the other hand, others reported that there is no statistically signiûcant association between total serum IgE levels and this variation in Kuwaiti arabs and Spanish populations respectively[36,37]. Liu et al.[38] found that IL-4 -590 C/T and IL-4Rα I50V did not influence total serum IgE levels in german multicenter atopy study (MAS) children. Also, Yang et al[39] showed that the IL-4 promoter (-590) polymorphism was not significantly associated with elevation of total IgE levels.

Many mechanisms were evidenced to explain our results that IL-4 -590 C/T affect IgE levels but not associated with AD; this promoter polymorphism might be in linkage disequilibrium with a true causal variant, it might influence total serum IgE levels rather than influence the development of atopic disorders, or it might be a modifier gene that affects disease severity. On the other hand, our results agreed well with the assumption that IL-4Rα I50V plays a role in the production of IgE in AD patients. There are a few limitations of our study. Identifying significant associations of genetic variants with diseases as atopic dermatitis may need a larger sample size. A replication sample is lacking to further confirm this association.

However, the present study fulfills most of the criteria of a good genetic association study[40]. In conclusion, the IL-4Rα (I50V) may play a role in susceptibility to AD in Egyptian children. In addition, gene-gene interaction between the IL-4 -590 T and the IL-4Rα G allele significantly increases an individual's susceptibility to AD. Both polymorphisms may be involved in the control of IgE production.

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