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Gene Polymorphism of Interleukin-4, Interleukin-4 Receptor and STAT6 in Children with Atopic Dermatitis in Taif, Saudi Arabia

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ABSTRACT

Aim of study: This work was performed to evaluate the level of IL-4, and to clarify the role of IL-4 gene polymorphism at position cytosine –590-to-thyamine (C-590T), IL-4Rα gene polymorphism at position adenine +4679-to-guanine (A+4679G) [isoleucine-50-valine (I50V)] and STAT6 gene polymorphism at position guanine 2964-to-adenine (G2964A) in Saudi children with non-atopic dermatitis (non-AD) and atopic dermatitis (AD) to identify their role in the pathogenesis of these diseases.

Subjects and methods: This study included 150 children: 50 healthy children as controls, 50 with non-AD, and 50 with AD. They were subjected to full clinical examination, complete blood picture, skin prick test, and determination of serum interleukin-4 (IL-4) and total immunoglobulin-E (IgE) levels. Detection of interleukin-4 gene (C-590T), interleukin-4 receptor alpha gene (A+4679G) (I50V), and STAT6 gene (G2964A) polymorphisms were performed by PCR-based restriction fragment length polymorphism (PCR-RFLP).

Results: There was a significant ($P < 0.01$) association between genotype and allele frequencies of IL-4Rα (A+4679G) (I50V) polymorphism in the AD group (but not non-AD group). Moreover, there was a significant association between genotype and allele frequencies of the STAT6 (G2964A) polymorphism in the non-AD ($P < 0.05$) and AD ($P < 0.01$) groups. On the other hand, there was no significant association between genotype and allele frequencies of the (C-590T) polymorphism in the non-AD group and AD group. There was a significant ($P < 0.001$) higher total IgE level in patients compared to the controls. Moreover, the mean values of total IgE were significantly different among the different allelic variants of (C-590T), (I50V), (G2964A) polymorphisms of IL-4, IL-4Rα, and STAT6 genes, respectively, in all the studied groups. On the other hand, there was no significant difference of serum IL-4 levels among all the studied patients, or among the different allelic variants of (C-590T), (I50V), (G2964A) polymorphisms of IL-4, IL-4Rα, and STAT6 genes, respectively.

Conclusion: IL-4Rα gene (I50V) and STAT6 gene (G2964) polymorphisms may play a role in development of eczema; however, the IL-4 gene polymorphism (C-590T) had no relationship with susceptibility to the disease among Saudi children.

KEYWORDS

IL-4Rα; interleukin-4 (IL-4); single nucleotide polymorphism (SNP) atopic dermatitis (AD); STAT6

Introduction

Dermatitis, a chronic inflammatory skin disease, can be categorized into the atopic dermatitis (AD) and non-AD types. AD type shows high total serum IgE levels and presence of specific IgE for allergens, whereas the non-AD type exhibits normal total IgE values and absence of specific IgE (Tokura, 2010). Some immunological differences between the AD and non-AD types have been observed in the cytokine pattern and in skin lesions (Novak & Bieber, 2003). AD is a complex multifactorial disease with a strong genetic predisposition. Some genetic linkages have been demonstrated in and around genes of which the products are involved in the pathophysiology of atopy (Jahn-Schmid et al., 2011). Single nucleotide polymorphisms (SNPs) located within enhancer, promoter or other regulatory sequences of cytokine genes have been documented (Zahran et al., 2013; Zhu et al., 2013). IL-4 is a glycoprotein encoded by the IL-4 gene located in chromosomal region 5q31-33 (Namkung et al., 2011). It is required for development of the T helper 2 (Th2) and the induction of immunoglobulin ϵ isotype switching, IgE secretion and effector cell transmigration. In atopic patients, Th2 phenotype predominates, leading to increased production of interleukin-4 (IL-4) and IL-13 and class-switching in B lymphocytes to synthesize IgE antibodies (Lu et al., 2011; Namkung et al., 2011). The promoter area of the IL4 gene exhibits a number of polymorphisms. The -590 C/T is one of the mostly characterized polymorphisms (Nie et al., 2013). It has been hypothesized that polymorphisms within the promoter region of IL-4 gene can modify IL-4 gene transcription and enhance IL-4 activity (Lu et al., 2011; Nie et al., 2013). The IL-4 receptor is a heterodimer that is composed of the IL-4R α chain and either the common γ chain or the IL-13R α subunit (Tanaka et al., 2012). The gene coding for IL-4R α is located on the short arm of chromosome 16p12.1. The IL-4R α I50V polymorphism, due to an A to G transition, results in a change of amino acid from isoleucine to valine. Its extracellular domain site is believed to play an important role in atopy and has been associated with enhanced IgE synthesis (Mitsuyasu et al., 1998). Polymorphisms of cytokines and their receptor genes might influence the occurrence and course of AD, but the results are contradictory (Kiyohara et al., 2008). STAT6 (signal transducer and activator of transcription) is a central molecule in the signal transduction pathway used by IL-4 in IgE isotype switching and Th2 cytokines production (Kabesch et al., 2006). The STAT6 gene is localized on chromosome 12q13.3-q14.1. STAT6 2964 G/A polymorphism is located in the 3'UTR region of the STAT6 gene at chromosome 12q13.3-q14 and has been reported to be associated with allergic diseases (Zhu et al., 2013). Another common polymorphism is located in intron 2 of STAT6 gene; 2892 C/T conferred risk for asthma and elevated serum IgE levels in various ethnic groups (Kabesch et al., 2006; Zhu et al., 2013). The relation between polymorphisms in IL-4 pathway genes and atopy has been evaluated in many studies (Hussein et al., 2010, 2011, 2012, 2014; Zahran et al., 2013; Zhu et al., 2013). However, few of them analyzed polymorphisms in dermatitis. Moreover, the results were mostly inconsistent in these studies due to ethnic differences. In the present study, we examined the association of IL-4, IL-4R α , and STAT6 gene polymorphisms with IL-4 and IgE levels in dermatitis in Saudi children.

Subjects and methods

Subjects

The studied children included three different age- and sex-matched children: 50 non-AD, 50 AD patients, and 50 healthy children as controls. All the subjects were males and their age ranged between 7 and 10 years. All the subjects did not receive any antihistamines or corticosteroids during the period of 3 weeks before enrollment in the study and were subjected to skin prick test. All patients and controls were clinically evaluated. AD patients were diagnosed by clinical examination, a positive skin prick test or elevated specific IgE. The children fulfilled the Hanifin and Rajka diagnostic criteria (Hanifin & Rajka, 1980). Non-AD patients were diagnosed by a negative skin prick test result, or negative specific IgE responses to the allergens. The control group had no history of allergic diseases and had negative skin response to allergens. Stool and urine analyses, and complete blood picture were performed to exclude parasitic infestations. A written informed consent was obtained from parents of the selected children before participating in the study. This study was approved by the ethical committee of Taif University before its beginning.

Blood sampling

Six mL of blood were obtained from every participant under complete aseptic conditions and divided into two portions: 1.5 mL of whole blood was collected in sterile EDTA-containing tubes for DNA extraction. 4.5 mL was left for 30–60 minutes then centrifuged at 3000 rpm for 10 minutes. Serum samples were separated into another set of tubes and kept frozen at -20°C for the determination of total IgE, and IL-4.

Laboratory methods

Determination of serum total IgE: total serum IgE was measured by ELISA kits (BioCheck Inc., Vintage Park Drive, Foster City).

- (a) Determination of human serum IL-4: serum IL-4 was measured by sandwich ELISA kits (Koma Biotech Inc., Seoul, Korea).
- (b) Detection of IL-4 gene polymorphism at (C-590T), IL-4Ra gene polymorphism at (A+4679G) [I50V], and STAT6 gene polymorphism at (G2964A): detection was performed by PCR-based restriction fragment length polymorphism (PCR-RFLP). All the reagents were highly purified analytical PCR-materials. All the tubes, tips, and pipettes used for DNA extraction were DNase, RNase free purchased from Gentra (Minneapolis, USA). The sequence of the primers and PCR cycling conditions are shown in Table 1. The digested PCR products were electrophoresed on ethidium bromide-containing 3% agarose gels to separate the fragments, and then the gel was visualized under UV transilluminator with 100 base pair ladder (Qiagen GmbH, Hilden, Germany) and photographed.

Table 1. Primer sequences and PCR cycling conditions used in genotyping of IL4, IL-4Rα, and STAT6 SNPs.

Polymorphic site	Primer sequence	PCR condition	Restriction enzyme	Fragment
IL-4 -590 C/T	Forward, TAAACTTGGGAGAACATGGT	30 s at 95°C, 30 s at 50°C, 45 s at 72°C.	<i>Bme18 I</i>	C, 177 + 19
	Reverse, TGGGGAAAGATAGAGTAATA			T, 195
IL-4Rα/G+4679 (I50V)	Forward, GGCAGGTGTGAGGAGCATCC	30 s at 95°C, 30 s at 60°C, 45 s at 72°C.	RsaI	A, 273
	Reverse, GCCTCCGTTGTTCTCAGGTA			G, 254 + 19
STAT6 2964 G/A	Forward, GAAGTTCAGGCTCTGAGAGAC	45 s at 95°C, 45 s at 55°C, 45 s at 72°C.	BsaHI	G, 74 + 19
	Reverse, CCATCACCTCAGAGAGC			A, 93

- *DNA extraction*: whole blood genomic DNA extraction kit (Bioer Technology, Binjiang, Hanchuan, Hubei, China) was used.
- *Amplification*: It was carried out using thermal cycler PTC-100 machine (MJ Research, Inc., Water town, Mass., USA).
- *PCR-based restriction fragment length polymorphism (PCR-RFLP)*: it was performed according to Namkung et al. (2011).

- (a) *Detection of IL-4 gene polymorphism at (C-590T)*: detection of (-590 C/T) polymorphism in IL-4 gene by PCR was done by amplification of the region containing this polymorphism (De Guia & Ramos, 2010). The amplified PCR products were digested by restriction endonuclease enzyme “*Bme18 I*” restriction enzyme (SibEnzyme Ltd., Russia).
- (b) *Detection of IL-4Rα gene polymorphism at (A+4679G) [I50V]*: detection of IL-4Rα gene polymorphism at (A+4679G) [I50V] was performed by PCR amplification of the region containing this polymorphism (Hussein et al., 2014). The amplified PCR products were digested by restriction endonuclease enzyme “*RsaI*” restriction enzyme (New England BioLabs Inc., Boston, MA).
- (c) *Detection of G/A2964 polymorphism in the regulatory 3'-untranslated region (3'-UTR) of the STAT6 gene*: A G → A transition polymorphism at position 2964 in the 3'-UTR of STAT6 was identified by PCR-RFLP-based on the amplification-created restriction site (ACRS) method according to Amoli et al. (2000). New England BioLabs Inc., Boston, MA.

Statistical analysis

The Statistical Package for Social Science (SPSS) program (release 7.5.1; SPSS Inc., Chicago, IL, USA) was used for data analysis. The results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) and chi-square (χ^2) tests were performed at 5% level of significance.

Results

Serum levels of total IgE and IL-4

Table 2 shows that there was no significant difference regarding the serum level of IL-4 between the different study groups. On the other hand, the total IgE level was significantly ($P < 0.001$) higher among AD patients as compared with non-AD patients or the control group.

Genotype frequencies of IL-4, IL-4Ra, and STAT6 gene polymorphisms

Representative agarose gel electrophoresis (Figures 1–3) shows C-590T polymorphism of IL-4 gene, A/G+4679 (150V) polymorphism of IL-4Ra gene, and G2964A polymorphism of STAT6 gene, respectively. The genotype and allele frequencies of the IL-4, IL-4Ra, and STAT6 gene polymorphisms in AD and non-AD, and the control groups are shown in Table 3. Regarding IL-4 –590 C/TSNP polymorphism, there was no significant association between the genotype and allele frequencies in the AD or the non-AD patients as compared with the controls. For IL-4RaA/G+4679 (150V) SNP polymorphism, there was significant differences between AD patients and the control group in the genotype and allele ($p < 0.01$ and $p < 0.001$) distributions. On the other hand, no significant differences were detected in the genotype or allele frequencies between children with non-AD and the controls. Concerning STAT6 2964 G/A, there was significant association between genotype and allele

Table 2. Serum levels of total IgE and IL-4 in the studied groups.

The studied parameter	Control group (n = 50)	Non-atopic dermatitis group (n = 50)	Atopic dermatitis group (n = 50)	F-value (t-value)
	Mean ± SD	Mean ± SD	Mean ± SD	
Serum IL-4 (pg/ml)	463.8 ± 98.2	473.1 ± 101.2	475.8 ± 104.2	0.15 ($p > 0.05$)
Serum IgE (IU/ml)	62.1 ± 11.9	80.7 ± 16.9	335.4 ± 51.8	912.9 ($p < 0.001$)

ANOVA test was performed at 5% level of significance.

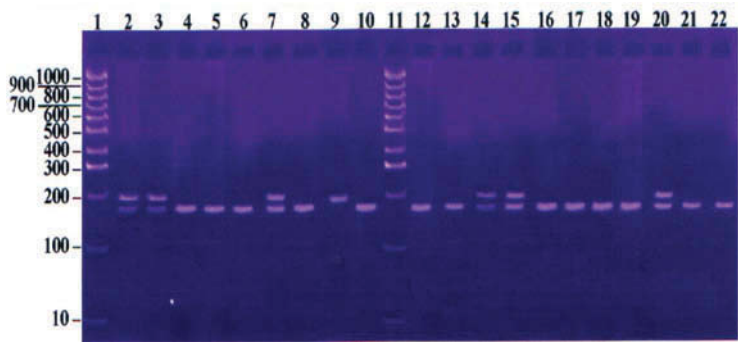


Figure 1. Representative agarose gel electrophoresis shows C-590T polymorphism of IL-4 gene. DNA marker (100 bp), (lanes 1 11), homozygous TT (polymorphic) (lane 9, one band at 195 bp), heterozygous CT (lanes 2 3 7 14 15 20, two bands at 177 bp and 195 bp), homozygous CC (wild type) (lanes 4 5 6 8 10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

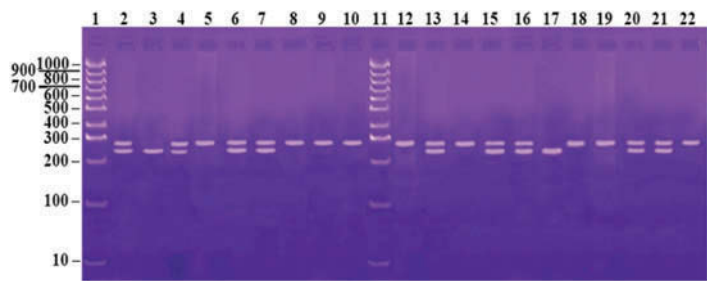


Figure 2. Representative agarose gel electrophoresis shows A/G+4679 (I50V) polymorphism of IL-4Rα gene. DNA marker (100 bp), (lanes 1 11), homozygous GG (polymorphic) (lanes 3 17, one band at 254 bp), heterozygous AG (lanes 2 4 6 7 13 15 16 20 21, two bands at 273 bp and 254 bp), homozygous AA (wild type) (lanes 5 8 9 10 12 14 18 19 22, and band at 273 bp).

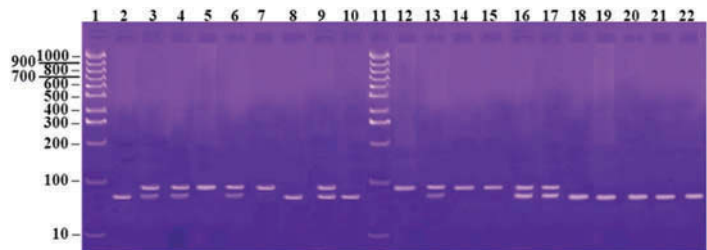


Figure 3. Representative agarose gel electrophoresis shows G2964A polymorphism of STAT6 gene. DNA marker (100 bp), (lanes 1 11), homozygous AA (mutant) (lanes 5 7 12 14 15, one band at 93 bp), heterozygous GA (lanes 3 4 6 9 13 16 17, two bands at 93 bp and 74 bp), homozygous GG (wild type) (lanes 2 8 10 18 19 20 21 22, and band at 73 bp).

Table 3. Genotype and allele frequencies of IL-4 –590 C/T, IL-4RαA+4679G (I50V) and STAT6 2964 G/A polymorphisms in the studied groups.

The studied parameter	The studied genotypes & alleles	Controls	Non-atopic dermatitis group		Atopic dermatitis group	
		<i>n</i> (%)	<i>n</i> (%)	<i>p</i> -value	<i>n</i> (%)	<i>p</i> -value
IL-4 –590 C/T	Genotypes	50 (100%)	50 (100%)	>0.05	50 (100%)	>0.05
	CC	38 (76%)	36 (72%)		32 (64%)	
	CT	10 (20%)	11 (22%)		16 (32%)	
	TT	2 (4%)	3 (6%)	>0.05	2 (4%)	>0.05
	Alleles	100 (100%)	100 (100%)		100 (100%)	
	C	86 (86%)	83 (83%)		80 (80%)	
IL-4RαA/G+4679 (I50V)	Genotypes	50 (100%)	50 (100%)	>0.05	50 (100%)	<0.01
	AA	40 (80%)	38 (76%)		23 (46%)	
	AG	8 (16%)	10 (20%)		20 (40%)	
	GG	2 (4%)	2 (4%)	>0.05	7 (14%)	<0.001
	Alleles	100 (100%)	100 (100%)		100 (100%)	
	A	88 (88%)	86 (86%)		66 (66%)	
STAT6 2964 G/A	Genotypes	50 (100%)	50 (100%)	<0.05	50 (100%)	<0.01
	GG	37 (74%)	25 (50%)		24 (48%)	
	GA	12 (24%)	20 (40%)		16 (32%)	
	AA	1 (2%)	5 (10%)	<0.01	10 (20%)	<0.001
	Alleles	100 (100%)	100 (100%)		100 (100%)	
	G	86 (86%)	70 (70%)		64 (64%)	
	A	14 (14%)	30 (30%)		36 (36%)	

Chi-square (χ^2) tests at 5% level of significance.

frequencies of the STAT6 polymorphism in both AD ($p < 0.01$ and $p < 0.001$) and non-AD patients ($p < 0.05$ and $p < 0.01$), respectively, as compared with the controls.

Serum level of IL-4 in relation to genotype polymorphisms

Table 4 shows that there was no significant effect of IL-4 gene (C-590T), IL-4R α gene (A+4679G) [I50V], or STAT6 gene (G2964A) polymorphisms on the serum levels of IL-4 in the three studied groups.

Serum levels of total IgE in relation to genotype polymorphisms

Table 5 shows that serum levels of the total IgE were significantly elevated among persons with the TT genotype than those with the CC genotype (IL-4 -590 C/T SNP polymorphism) in all the studied groups [(AD ($p < 0.01$, non-AD ($p < 0.001$) and the controls ($p < 0.001$)). Also, there was a relation between IgE level and IL-4R α /G+4679 (I50V) SNP polymorphism. The total IgE levels were significantly elevated among persons with the GG genotype than those with the AA genotype in the three studied groups [(AD ($p < 0.01$, non-AD ($p < 0.05$) and the controls ($p < 0.01$)). Furthermore, there was an association between IgE level and STAT6 2964 G/A SNP polymorphism. The total IgE levels were significantly ($p < 0.01$) elevated among persons with the AA genotype than those with the GG genotype in the three studied groups.

Discussion

This 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 both non-AD and AD as compared with controls, indicating that this SNP had no relationship with the development of both types of dermatitis in Saudi children. The (C-590T) IL-4 promoter polymorphism separately was not associated with AD in Australian (Elliott et al., 2001)

Table 4. Serum interleukin-4 (IL4) (pg/ml) in relation to the studied genotype polymorphisms in the studied groups.

The studied polymorphism	Genotype	Controls	Non-atopic dermatitis group	Atopic dermatitis group
		<i>n</i> (%)		
		Mean ± SD	Mean ± SD	Mean ± SD
IL-4 −590 C/T	CC	460.3 ± 102.3	472.9 ± 104.3	465.7 ± 106.9
	CT	461.8 ± 112.9	439.6 ± 86.9	474.3 ± 109.6
	TT	453.4 ± 57.2	601.5 ± 65.8	631.4 ± 46.5
	<i>F</i> -value (<i>p</i> -value)	<i>F</i> = 0.052 (<i>p</i> > 0.05)	<i>F</i> = 2.62 (<i>p</i> > 0.05)	<i>F</i> = 1.62 (<i>p</i> > 0.05)
IL-4Rα/G+4679 (I50V)	AA	462.1 ± 1002.5	476.2 ± 105.7	459.4 ± 108.9
	AG	442.9 ± 124.7	468.6 ± 101.6	487 ± 108.5
	GG	625.8 ± 65.6	476.9 ± 160.8	473.8 ± 103.1
	<i>F</i> -value (<i>p</i> -value)	<i>F</i> = 1.52 (<i>p</i> > 0.05)	<i>F</i> = 0.016 (<i>p</i> > 0.05)	<i>F</i> = 0.67 (<i>p</i> > 0.05)
STAT6 2964 G/A	GG	456.2 ± 102.8	468.4 ± 90.2	460.9 ± 104.9
	GA	485.6 ± 99.7	505.7 ± 106.7	464.9 ± 101.2
	AA	329.1 ± 37.3	394.4 ± 102.9	492.7 ± 130.5
	<i>F</i> -value (<i>p</i> -value)	<i>F</i> = 1.23 (<i>p</i> > 0.05)	<i>F</i> = 1.89 (<i>p</i> > 0.05)	<i>F</i> = 0.47 (<i>p</i> > 0.05)

ANOVA test was performed at 5% level of significance.

Table 5. Serum levels of total IgE (IU/ml) in relation to the studied genotype polymorphisms in the studied groups.

The studied polymorphism	Genotype	Controls	Non-atopic dermatitis group	Atopic dermatitis group
		<i>n</i> (%)		
		Mean ± SD	Mean ± SD	Mean ± SD
IL-4 -590 C/T	CC	55.9 ± 7.6	74.8 ± 13.6	319.8 ± 47.9
	CT	71.1 ± 18.1	87.3 ± 11.2	344.7 ± 52.1
	TT	91.3 ± 3.6	112.5 ± 4.6	418.2 ± 43.2
	<i>F</i> -value (<i>p</i> -value)	<i>F</i> = 10.3 (<i>p</i> < 0.001)	<i>F</i> = 9.4 (<i>p</i> < 0.001)	<i>F</i> = 4.5 (<i>p</i> < 0.01)
IL-4RaA/G+4679 (I50V)	AA	56.2 ± 10.9	76.9 ± 15.7	312.1 ± 46.9
	AG	67.9 ± 12.6	85.2 ± 14.2	322.1 ± 46.9
	GG	91.2 ± 14.8	105.2 ± 17.4	386.2 ± 54.3
	<i>F</i> -value (<i>p</i> -value)	<i>F</i> = 5.8 (<i>p</i> < 0.01)	<i>F</i> = 3.7 (<i>p</i> < 0.05)	<i>F</i> = 5.7 (<i>p</i> < 0.01)
STAT6 2964 G/A	GG	57.9 ± 10.8	77.2 ± 13.6	297.9 ± 37.2
	GA	65.1 ± 11.8	78.9 ± 12.9	339.7 ± 54.8
	AA	102.2 ± 43.1	102.8 ± 13.9	360.2 ± 51.8
	<i>F</i> -value (<i>p</i> -value)	<i>F</i> = 5.8 (<i>p</i> < 0.01)	<i>F</i> = 7.4 (<i>p</i> < 0.01)	<i>F</i> = 6.5 (<i>p</i> < 0.01)

ANOVA test was performed at 5% level of significance.

and Macedonian (Stavric et al., 2012) children. Similar results were reported in adult asthmatic Kuwaiti Arabs (Hijazi & Haider, 2002), and in Korean (Lee et al., 2004), Japanese (Takabayashi et al., 2000), Italian (Rigoli et al., 2004), Jordanian (Attab et al., 2008), Chinese (Li et al., 2009), and Caucasian (Liu et al., 2012) children. On the other hand, a significant association between IL-4 (C/T-590) gene polymorphism AD was reported in Czech children (Kayserova et al., 2012). Also, IL-4-589C/T polymorphism was demonstrated as a risk factor for asthma (Li et al., 2008; Nie et al., 2013). These differences may be attributed to racial differences, interaction of -590C/T polymorphism with another gene, or the existence of another polymorphism (Nie et al., 2013). In our study, there was a significant association of both GG genotype and G allele (50V) (mutant variant) frequencies of IL-4Ra gene (A→G+4679) in children with AD but not in those with non-AD, indicating that the G allele of (I50V) polymorphism acts as an atopy susceptibility gene. SNPs in this cytokine receptor alters an amino acid in the extracellular domain of the receptor (isoleucine → valine at 50 [Ile50Val]), which in turn leads to an increased IL-4-mediated downstream activation of STAT6 and an increased IgE production in B cells (Yadav et al., 2012). Tanaka et al. (2012) demonstrated that (Ile50Val) allele seems to be involved in AD in Japanese children. Moreover, Ile50Val was strongly and specifically associated with atopic or extrinsic asthma and cedar pollionsis in a Japanese population (Nakamura et al., 2003). In contrast, no significant difference was found in the genotypes and allele frequency of (I50V) polymorphism between asthmatic subjects and controls in Han nationality (Tang et al., 2006) and Chinese population (Zhang et al., 2007). Moreover, no significant association of Ile50Val polymorphism with allergic rhinitis was reported by Lu et al. (2011; Yadav et al., 2012). In the current study, we demonstrated that there was a significant association between occurrence of eczema and STAT6 G2964A polymorphism in Saudi children, indicating that STAT6 gene may play an important role in its pathogenesis in these children. An association between G2964A polymorphism and mild atopic asthma was reported in a Japanese population (Gao et al., 2000) and childhood nut allergy in the United Kingdom (Amoli et al., 2002). In contrast, other studies showed no association between the 2964A variant of the STAT6 gene and allergic rhinitis in Korean population (Kim et al., 2004) or asthma in Chinese population

(Ding et al., 2010). No association was reported in Japanese (Shao et al., 2004), British (Gao et al., 2000), and Caucasian (Duetsch et al., 2002) populations. These findings suggest that there are marked differences not only between different ethnic groups but also within one ethnic group, indicating genetic heterogeneity of allergic diseases. Our study showed that the serum level of IL-4 had no significant difference between children with non-AD, AD and controls. This result indicates that IL-4 does not play an important role in the pathogenesis of both types of dermatitis in Saudi children and that other biological processes probably are involved in their development. IL-4 levels were not different between AD and non-AD patients in skin lesions (Tazawa et al., 2004) and serum (Park et al., 2006). Also, Nurse et al. (1997) demonstrated normal IL-4 release by peripheral blood mononuclear cells (PBMCs) from children with atopic asthma. On the other hand, high serum levels of IL-4 were reported in patients with AD (Novak & Bieber, 2003). Ricci et al. (2008) showed that all children affected by eczema had higher IL-4 serum level compared to normal controls. These results may indicate that immunological differences may exist in peripheral blood of patients affected by atopic and non-atopic eczema; in particular, Th2 cells seem to detect a key role in the pathogenesis of atopic form, while non-atopic form seems to be characterized by Th1/Th2 mechanisms. Salem et al. (2010) showed that IL-4 was significantly higher in acute compared to chronic AD patients. The cause of differences in the results of our study and other studies may be attributed to the fact 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 (Ricci et al., 2008). All these studies including ours did not classify AD according to disease duration. In an effort to determine the functional relevance of the three polymorphisms, associations of the genotypes-590 in IL-4, +4679 in IL-4R α , and 2964 in STAT6 with IL-4 serum levels were examined in this study. Our results showed no effect of these polymorphisms on IL-4 serum level. These findings may imply that IL-4 levels in blood were not only related to the gene basis but also to other factors as cytokine inhibitors (Vidyarani et al., 2006). In our study, we found a significant higher level of the total serum IgE in AD patients, which indicates that serum total IgE may be used as an indicator of this disease. Similar findings were reported in AD by Lin et al. (2011). The role of IgE in type I allergy was confirmed by many investigators (Jahn-Schmid et al., 2011; Schmidt-Weber, 2012). Our results showed that IL-4 -590 C/T polymorphism had an effect on IgE levels but was not associated with AD. Many mechanisms may explain this finding: 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 (De Guia & Ramos, 2010). In the current study, we also examined the association of the genotypes at C-590T, I50V, and G2964A in IL-4, IL-4R α , and STAT6 genes, respectively, with IgE serum levels. For IL-4 promoter gene polymorphism, significant differences among different allelic variants were detected in the three studied groups. Moreover, there was a significant increase in serum level of total IgE towards homozygous TT than homozygous CC in all studied groups. These findings correlate with that reported by De Guia and Ramos (2010) and Lu et al. (2011). However, there was no association between total serum IgE levels and this variation in Kuwaiti Arabs (Hijazi & Haider, 2002), Spanish populations (Torres-Galván et al., 2001), and German children (Liu et al., 2003). Although IgE synthesis is dependent on IL-4, IL-13 is the dominant IgE-inducing cytokine in

situations where IL-4 is either absent or present at low levels (Tazawa et al., 2004). In this study, IL-4Rα coding region polymorphism was associated with a significant increased total IgE levels towards homozygous GG than homozygous AA in all the studied groups. It was previously reported that IL-4Rα Ile50 was associated with high IgE level in AD (Tanaka et al., 2012) and hay fever (Nieters et al., 2004). However, some studies found no association between Ile50Val polymorphism and total serum IgE level in Japanese patients and attributed their finding to environmental factors (Noguchi et al., 1999). Furthermore, Liu et al. (2003) demonstrated that the genotype Ile/Ile was not related to serum IgE levels in children with atopic diseases. Other predisposing genes which control IgE production in patients with atopic eczema may exist and need to be clarified. Regarding STAT6 gene polymorphism, our study demonstrated significant increase in the levels of serum IgE in homozygous AA compared to homozygous GG genotype. This result indicates that genetic variants in the STAT6 gene significantly contribute to the regulation of serum IgE levels. In agreement with our result, other studies indicated that STAT6 3'-UTR polymorphisms significantly contributed to elevated serum IgE levels (Duetsch et al., 2002). However, no significant association between the 2964A variant and serum IgE levels was detected in Korean allergic rhinitis patients, and Finnish asthmatic families (Pykäläinen et al., 2005). In conclusion, our results suggested that the IL-4 gene polymorphism (C-590T) had no relationship with the development of both types of dermatitis, while the IL-4Rα gene polymorphism (I50V) and STAT6 gene polymorphism (G2964) may play a role in susceptibility to eczema in Saudi children.

Declaration of interest

All the authors declare that there is no conflict of interest to this paper.

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