

## Association Between Single Nucleotide Polymorphisms of the Interleukin-4 Gene and Atopic Dermatitis

Mohammad Gharagozlou<sup>1</sup>, Nasrin Behniafard<sup>1,2</sup>, Ali Akbar Amirzargar<sup>3</sup>, Sima Hosseinverdi<sup>2</sup>, Soheila Sotoudeh<sup>1</sup>, Elham Farhadi<sup>4</sup>, Mojdeh Khaledi<sup>5</sup>, Zahra Aryan<sup>2</sup>, Zahra Gholizadeh Moghaddam<sup>1</sup>, Maryam Mahmoudi<sup>6</sup>, Asghar Aghamohammadi<sup>1,2</sup>, Nima Rezaei<sup>1,2,3,7</sup>

<sup>1</sup>Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; <sup>2</sup>Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran; <sup>3</sup>Molecular Immunology Research Center; and Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; <sup>4</sup>Hematology Department, School of Allied Medical Science, Iran University of Medical Sciences, Tehran, Iran; <sup>5</sup>Growth and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran; <sup>6</sup>School of Nutrition and Dietetics, Tehran University of Medical Sciences, Tehran, Iran; <sup>7</sup>Universal Scientific Education and Research Network (USERN), Teheran, Iran

### Corresponding author:

Nima Rezaei, MD, PhD  
Research Center for Immunodeficiencies  
Children's Medical Center Hospital  
Dr Qarib St  
Keshavarz Blvd  
Tehran 14194  
Iran  
[rezaei\\_nima@tums.ac.ir](mailto:rezaei_nima@tums.ac.ir)

Received: August 19, 2014

Accepted: May 14, 2015

**ABSTRACT** Atopic dermatitis (AD) is an inflammatory skin disease in which both genetic and environmental factors seem to be involved. Several studies investigated the association of certain genetic factors with AD in different ethnic groups, but conflicting data were obtained. This study was performed to check the possible association between single nucleotide polymorphisms (SNPs) of interleukin 4 (IL-4) and the IL-4 receptor  $\alpha$  chain (IL-4R $\alpha$ ) and AD in a group of Iranian patients. The allele and genotype frequencies of genes encoding for IL-4 and IL-4R $\alpha$  were investigated in 89 patients with AD in comparison with 139 healthy controls, using methods based on polymerase chain reaction sequence-specific primers. The most frequent alleles of IL-4 in patients were T at -1098 ( $P < 0.001$ , odds ratio (OR)=2.35), C at -590 ( $P < 0.001$ , OR=4.84) and C at -33 ( $P = 0.002$ , OR=2.08). The most frequent genotypes of IL-4 in patients were TT, CC, and CC at positions -1098 ( $P < 0.001$ , OR=3.59), -590 ( $P < 0.001$ , OR=31.25) and -33 ( $P < 0.001$ , OR=3.46), respectively. We found a significant lower frequency of GT at -1098 GT, TC at -590, and TC at -33 in patients. There were no statistically significant differences in the frequency of alleles and genotypes of IL-4R $\alpha$  gene at position +1902. A strong positive association was seen between TCC haplotype and AD (68% in patients vs. 23.4% in controls,  $P < 0.001$ , OR=8.91). We detected a significantly lower frequency of TTC, GCC, and TTT haplotypes ( $P < 0.001$ , OR=0.02,  $P < 0.001$ , OR=0.40,  $P < 0.001$ , OR=0.39, respectively) in patients compared to controls. A significant association between the polymorphisms of the IL-4 gene promoter at positions -1098, -590, and -33 and AD was detected in the Iranian population.

**KEY WORDS:** atopic dermatitis; polymorphism, single nucleotide; interleukin-4 gene

## INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disorder, affecting 15 to 30% of children and 2 to 10% of adults. It can also occur with other atopic diseases such as asthma and allergic rhinitis (1).

In terms of pathogenesis, genetic disturbances and alterations in the immune system with presence of environmental triggers are involved in AD. Immunologically, strong association was established between AD and high serum immunoglobulin E (IgE) levels. Dysregulation of type 2 helper T (Th2) and type 1 helper T cells (Th1) contributes in the pathogenesis of AD. Th2 derived cytokines including interleukin (IL) 4, 5, and 13 up-regulate IgE synthesis leading to allergic reactions, whereas cytokines of Th1 down-regulate the production of IgE (2). On this account, variations in the genes encoding cytokines may have a role in the development of AD. In this regard, several genes have been proposed, using candidate gene and genome-wide association studies. Recent evidence suggests that single nucleotide polymorphisms (SNPs) of cytokine genes affect their serum level. Association of such SNPs with AD has been examined in different ethnic groups (3). Such associations have also been shown between AD and SNPs of IL-6 (4), TNF- $\alpha$  (5), and IL-1 (6). Association between IL-4/IL-4 receptor  $\alpha$  chain (IL-4R $\alpha$ ) gene polymorphisms and several immunological diseases (7-12), including AD (13-16) has already been investigated with similar and contradictory results. In the present study, we aimed to conduct a case-control study to explore association between SNPs of IL-4 and IL-4R $\alpha$  genes and AD among the Iranian population.

## PATIENTS AND METHODS

### Subjects

Eighty nine children with AD who were referred to the Immunology Clinic of the Children's Medical Center Hospital, affiliated to the Tehran University of Medical Sciences, Tehran, Iran, were included in the study. The diagnosis of AD was made based on the standard criteria of Hanifin and Rajka (17). One hundred and thirty nine unrelated healthy subjects with no history of atopy were also selected as a control group.

This project was approved by the ethical committee of the Tehran University of Medical Sciences. Written informed consent was obtained from patients' parents or their guardians.

### Genotyping

DNA samples were extracted from blood samples. Polymerase chain reaction with sequence-specific

primers (PCR-SSP assay Kit, Heidelberg University, Germany) was used for cytokine genotyping. Gene amplification was performed using Tedane Flexigene thermal cyclor (Roche): initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 10 seconds, annealing plus extension at 65°C for 1 minute (10 cycles), denaturation at 94°C for 10 seconds, annealing at 61°C for 50 seconds, and extension at 72°C for 30 seconds (20 cycles). The PCR products were read on 2% agarose gel electrophoresis. The frequencies of alleles, genotypes, and haplotypes of the IL-4 gene at positions -1098, -590, and -33 and of the IL-4R $\alpha$  gene at position +1902 were counted.

### Statistical analysis

SPSS statistical software (IBM, New York, NY, USA) was used for data analysis. Allele frequencies were determined by direct gene counting. Frequencies of alleles, genotypes, and haplotypes were compared between patients and controls, using the chi-square test. The odds ratios were calculated with 95% confidence intervals. Statistical significance was set at *P* value of <0.05.

## RESULTS

### Patient characteristics

This study was performed on 89 patients (52 men and 37 women) with mean age of  $2.2 \pm 1.2$  years. Scoring AD (SCORAD) index was measured for all the cases, indicating severe and moderate AD in 37 and 52 cases, respectively. A family history of atopy was found in 80.9% of patients. Mean eosinophil count was  $269/\text{mm}^3$ , and median total serum IgE was 33 IU/mL. Skin prick test was performed in 60 patients, which was positive in 33 patients (to at least one allergen); the results are shown in Table 1.

**Table 1.** The results of skin prick test in 60 enrolled patients with atopic dermatitis

Allergen	Frequency (%)
Mite	5 (8.33)
Wheat	4 (6.66)
Fish	4 (6.66)
Tomato	2 (3.33)
Walnut	5 (8.33)
Hazelnut	3 (5.00)
Cow's milk	11 (18.33)
Egg	16 (26.66)
Soybean	11 (18.33)
Peanut	1 (1.66)

### Alleles and genotypes frequencies

The results of allele and genotype frequencies are shown in Table 2. The most frequent alleles of IL-4 in patients were T at -1098 ( $P < 0.001$ , odds ratio (OR)=2.35), C at -590 ( $P < 0.001$ , OR=4.84) and C at -33 ( $P = 0.002$ , OR=2.08). The most frequent genotypes of IL-4 in patients were TT, CC, and CC at positions -1098 ( $P < 0.001$ , OR=3.59), -590 ( $P < 0.001$ , OR=31.25), and -33 ( $P < 0.001$ , OR=3.46), respectively. We found a significantly lower frequency of GT at -1098 GT, TC at -590, and TC at -33 in our patients. There were no statistically significant differences in the frequency of alleles and genotypes of the IL-4R $\alpha$  gene at position +1902.

### Haplotype frequencies

The results of haplotype analysis are shown in Table 3. A strong positive association was seen between the TCC haplotype and AD (68% in patients vs. 23.4% in controls,  $P < 0.001$ , OR=8.91). We detected a significantly lower frequency of TTC, GCC, and TTT haplotypes ( $P < 0.001$ , OR=0.02;  $P < 0.001$ , OR=0.40;  $P < 0.001$ , OR=0.39, respectively) in patients in comparison to controls.

### DISCUSSION

AD results from interaction between genetic and environmental factors. To date, loss of the function mutation in the filaggrin gene (FLG) is believed to be the strongest genetic alteration that causes epider-

mal barrier dysfunction, increasing predisposition to AD. Apart from that, recent evidence suggests that immune imbalance has a prominent influence in the manifestation of AD. It is well known that IL-4 promotes IgE synthesis through both isotype switching in B cells and inhibition of interferon gamma (IFN $\gamma$ ) production. Furthermore, high levels of IL-4 have been observed in acute skin lesions of patients with AD. Increased production of Th2 cytokines IL-4 could also impair the function of the epidermal barrier. It has been shown that IL-4 causes the under-expression of filaggrin and calcium-binding A11 proteins by keratinocytes. On the other hand, IL-4 increases the presence and function of the serine protease kallikrein 7 of keratinocytes, leading to increased desquamation (18-20).

It has been documented that SNPs in the genes encoding such cytokines could affect their production. Polymorphisms in genes encoding IL-4 and IL-4R $\alpha$  (located on chromosomes 5q31 and 16p12, respectively) may be associated with total serum level of IgE. The IL-4R $\alpha$  chain is a component of the IL-4 receptor and required for signal transduction of IL-4. Several studies showed association between SNPs of the IL-4 and IL-4R $\alpha$  genes and AD, with conflicting outcomes (21); however, demographic characteristics of these studies were different.

In the present study, we investigated AD for association with the IL-4 gene promoter at -1098, -590, and -33 and the IL4R $\alpha$  gene at +1902 positions. We detected an over-expression of the T allele at position

**Table 2.** Interleukin-4 (IL-4) and IL4RA gene polymorphisms in patients with atopic dermatitis and controls

Cytokine	Position	Alleles/ Genotypes	Control (n=139) No. (%)	Atopic Dermatitis (n=89) No. (%)	P value	Odds ratios (95% confidence interval)
IL-4	-1098	G	84 (30.2)	28 (15.7)	<.001	<b>0.43 (0.26-0.70)</b>
		T	194 (69.8)	150 (84.3)	<.001	<b>2.35 (1.42-3.90)</b>
		GG	1 (0.7)	2 (2.2)	0.563	3.14 (0.22-88.72)
		GT	82 (59.0)	24 (27.0)	<.001	<b>0.25 (0.14-0.47)</b>
		TT	56 (40.3)	63 (70.8)	<.001	<b>3.59 (2.03-6.34)</b>
	-590	C	149 (53.6)	151 (84.8)	<.001	<b>4.84 (3.01-7.76)</b>
		T	129 (46.4)	27 (15.2)	<.001	<b>0.20 (0.12-0.33)</b>
		CC	10 (7.2)	63 (70.8)	<.001	<b>31.25 (14.19-68.81)</b>
		TC	129 (92.8)	25 (28.1)	<.001	<b>0.03 (0.01-0.07)</b>
		TT	0	1 (1.1)	0.393	-
	-33	C	200 (71.9)	150 (84.30)	.002	<b>2.08 (1.29-3.37)</b>
		T	78 (28.1)	28 (15.70)	.003	<b>0.47 (0.28-0.78)</b>
		CC	61 (43.9)	65 (73.00)	<.001	<b>3.46 (1.96-6.15)</b>
		TC	78 (56.1)	20 (22.5)	<.001	<b>0.22 (0.12-0.42)</b>
		TT	0	4 (4.5)	<b>0.023</b>	-
IL-4R $\alpha$	+1902	A	242 (87.7)	147 (82.6)	0.130	0.66 (0.39-1.12)
		G	34 (12.3)	31 (17.4)	0.184	1.48 (0.85-2.59)
		AA	106 (76.8)	61 (68.5)	0.168	0.65 (0.36-1.19)
		GA	30 (21.7)	25 (28.1)	0.377	1.38 (0.72-2.67)
		GG	2 (1.5)	3 (3.4)	0.385	2.34 (0.31-20.50)

**Table 3.** Interleukin-4 (IL-4) haplotype polymorphisms in patients with atopic dermatitis and controls

Haplotype	Controls (n=139)	Atopic Dermatitis (n=89)	P value	Odds ratio (95% confidence interval)
	No. (%)	No. (%)		
TTC	51 (18.3)	1 (0.6)	<.001	<b>0.02 (0.00-0.17)</b>
GCC	83 (30.0)	26 (14.6)	<.001	<b>0.40 (0.24-0.66)</b>
TTT	76 (27.3)	23 (12.9)	<.001	<b>0.39 (0.23-0.67)</b>
TCC	65 (23.4)	121 (68.0)	<.001	<b>8.91 (5.88-13.50)</b>
TCT	2 (0.7)	5 (2.8)	0.117	3.94 (0.67-29.65)
GTT	1 (0.3)	0	1.00	0.00 (0.00-26.85)
GCT	0	0	-	-
GTC	0	2 (1.1)	0.153	-

-1098 in patients with AD in comparison to controls, while the presence of the G allele was decreased at the same position indicating the protective role of this allele against AD. A significant increase in the frequency of the -1098/TT genotype was also found. Similarly, Stavric *et al.* (13) found the protective role of the G allele at this position in Macedonian pediatric patients.

At position -590, we found an over-expression of the C allele and of the CC genotype in patients with AD, whereas the frequencies of the T allele and TC genotype were lower in patients with AD than controls. It has been shown that the presence of the CC genotype at this position is associated with low production of IL-4 (22). Therefore, decreased level of IL-4 could be expected in our patients. Contrary to our data, Kawashima *et al.* (16) reported that an over-expression of the -590/T allele increased the risk of AD in Japanese patients. Rosenwasser *et al.* (23) associated over expression of the -590/T allele with increased IgE levels in an American population. deGuia *et al.* (14) showed a positive association between the TT genotype of IL-4 -590 and high IgE levels. Interestingly, Elliott *et al.* found no association between -590 C/T polymorphisms and AD in a cohort of Australian patients (24). Notably, they suggest there may be linkage between -590C/-34C haplotypes and AD.

Our data also showed significant positive association between AD and the C allele and CC genotype of IL-4 at position -33. In contrast, Stavric *et al.* stated that the C allele of IL-4 -33 has a protective role against AD. Although some authors found association between SNPs of IL-4R $\alpha$  (25), our study failed to show significant allelic and genotypic association between IL4R $\alpha$  +1902 A/G and AD. A similar finding was found in the studies conducted by Stavric *et al.* and Kayserova *et al.* (26); however, Kayserova *et al.* showed significant differences between IL-4R $\alpha$  +1902 A/G and positivity of tree pollen-specific IgE in the AD group that may be a predictive factor in the development of respiratory allergy. A gain-of-function mutation in IL-4R $\alpha$

at position +1902 was associated with atopy (27). A significant increase was also seen in the frequency of TCC haplotype of IL-4 in patients with AD. Inconsistently, Stavric *et al.* found no association between the TCC haplotype and AD. They found a significant increase in the frequency of TTT haplotype in patients with AD, while our result was the opposite.

## CONCLUSION

It is of note that such incompatibilities in different studies may be due to racial differences as well as sample sizes. In conclusion, we have identified genotypes that make the patients susceptible to AD, including TT at position-1098, CC at position -590, and CC at position -33. Moreover, the TCC haplotype had a role in development of AD. This is the first Iranian study showing association between AD and IL-4 polymorphisms. However, further observations with larger sample sizes of patients with AD are required to support the results of this study.

## ACKNOWLEDGEMENTS

This study was supported by a grant from the Tehran University of Medical Sciences and Health Services (89-04-80-12136).

## References

1. Kapoor R, Menon C, Hoffstad O, Bilker W, Leclerc P, Margolis DJ. The prevalence of atopic triad in children with physician-confirmed atopic dermatitis. *J Am Acad Dermatol* 2008;58:68-73.
2. Bieber T. Atopic dermatitis. *N Engl J Med* 2008;358:1483-94.
3. Kiyohara C, Tanaka K, Miyake Y. Genetic susceptibility to atopic dermatitis. *Allergol Int* 2008;57:39-56.
4. Gharagozlou M, Farhadi E, Khaledi M, Behniafard N, Sotoudeh S, Salari R, *et al.* Association between the interleukin 6 genotype at position -174 and

- atopic dermatitis. *J Investig Allergol Clin Immunol* 2013;23:89-93.
- Behniafard N, Gharagozlou M, Farhadi E, Khaledi M, Sotoudeh S, Darabi B, *et al.* TNF-alpha single nucleotide polymorphisms in atopic dermatitis. *Eur Cytokine Netw* 2012;23:163-5.
  - Behniafard N, Gharagozlou M, Sotoudeh S, Farhadi E, Khaledi M, Moghaddam ZG, *et al.* Association of single nucleotide polymorphisms of interleukin-1 family with atopic dermatitis. *Allergol Immunopathol (Madr)* 2014;42:212-5.
  - Barkhordari E, Rezaei N, Mahmoudi M, Larki P, Ahmadi-Ashtiani HR, Ansari-pour B, *et al.* T-helper 1, T-helper 2, and T-regulatory cytokines gene polymorphisms in irritable bowel syndrome. *Inflammation* 2010;33:281-6.
  - Movahedi M, Amirzargar AA, Nasiri R, Hirbod-Mobarakeh A, Farhadi E, Tavakol M, *et al.* Gene polymorphisms of Interleukin-4 in allergic rhinitis and its association with clinical phenotypes. *Am J Otolaryngol* 2013;34:676-81.
  - Amirzargar AA, Movahedi M, Rezaei N, Moradi B, Dorkhosh S, Mahloji M, *et al.* Polymorphisms in IL4 and iLARA confer susceptibility to asthma. *J Investig Allergol Clin Immunol* 2009;19:433-8.
  - Mahmoudi MJ, Hedayat M, Taghvaei M, Nematipour E, Farhadi E, Esfahanian N, *et al.* Association of interleukin-4 gene polymorphisms with ischemic heart failure. *Cardiol J* 2014;21:24-8.
  - Rezaei N, Aghamohammadi A, Mahmoudi M, Shakiba Y, Kardar GA, Moradi B, *et al.* Association of IL-4 and IL-10 gene promoter polymorphisms with common variable immunodeficiency. *Immunobiology* 2010;215:81-7.
  - Shahram F, Nikoopour E, Rezaei N, Saeedfar K, Ziaei N, Davatchi F, *et al.* Association of interleukin-2, interleukin-4 and transforming growth factor-beta gene polymorphisms with Behcet's disease. *Clin Exp Rheumatol* 2011;29:S28-31.
  - Stavric K, Peova S, Trajkov D, Spiroski M. Gene polymorphisms of 22 cytokines in Macedonian children with atopic dermatitis. *Iran J Allergy Asthma Immunol* 2012;11:37-50.
  - de Guia RM, Ramos JD. The -590C/TIL4 single-nucleotide polymorphism as a genetic factor of atopic allergy. *Int J Mol Epidemiol Genet* 2010;1:67-73.
  - Novak N, Kruse S, Kraft S, Geiger E, Klüken H, Fimmers R, *et al.* Dichotomic nature of atopic dermatitis reflected by combined analysis of monocyte immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin-13 receptor gene: the dichotomy of extrinsic and intrinsic atopic dermatitis. *J Invest Dermatol* 2002;119:870-5.
  - Kawashima T, Noguchi E, Arinami T, Yamakawa-Kobayashi K, Nakagawa H, Otsuka F, *et al.* Linkage and association of an interleukin 4 gene polymorphism with atopic dermatitis in Japanese families. *J Med Genet* 1998;35:502-4.
  - Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;(Suppl 92):44-7.
  - Eyerich K, Novak N. Immunology of atopic eczema: overcoming the Th1/Th2 paradigm. *Allergy* 2013;68:974-82.
  - Vercelli D, Jabara HH, Lauener RP, Geha RS. IL-4 inhibits the synthesis of IFN-gamma and induces the synthesis of IgE in human mixed lymphocyte cultures. *J Immunol* 1990;144:570-3.
  - Tang M, Kemp A, Varigos G. IL-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 1993;92:120-4.
  - Ring J, Möhrenschrager M, Weidinger S. Molecular genetics of atopic eczema. *Chem Immunol Allergy* 2012;96:24-9.
  - Amirzargar AA, Rezaei N, Jabbari H, Danesh AA, Khosravi F, Hajabdolbaghi M, *et al.* Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. *Eur Cytokine Netw* 2006;17:84-9.
  - Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klinnert M, *et al.* Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy* 1995;2:74-8;discussion 95-6.
  - Elliott K, Fitzpatrick E, Hill D, Brown J, Adams S, Chee P, *et al.* The -590C/T and -34C/T interleukin-4 promoter polymorphisms are not associated with atopic eczema in childhood. *J Allergy Clin Immunol* 2001;108:285-7.
  - Miyake Y, Tanaka K, Arakawa M. Case-control study of eczema in relation to IL4Ra genetic polymorphisms in Japanese women: The Kyushu Okinawa Maternal and Child Health Study. *Scand J Immunol* 2013;77:413-8.
  - Kayserova J, Sismova K, Zentsova-Jaresova I, Katinina S, Vernerova E, Polouckova A, *et al.* A prospective study in children with a severe form of atopic dermatitis: clinical outcome in relation to cytokine gene polymorphisms. *J Investig Allergol Clin Immunol* 2012;22:92-101.
  - Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. *N Engl J Med* 1997;337:1720-5.