

Association between genes encoding components of the IL-10/IL-10R receptor pathway and asthma in children

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Background: Asthma is a chronic inflammation of the airways associated with recurrent symptoms that range from mild to debilitating. Interleukin-10 (IL-10) is a cytokine that displays pleiotropic effects in asthma and allergy.

Objective: To determine whether polymorphisms of IL-10/IL-10R pathway contribute to asthma susceptibility in Egyptian children.

Methods: The IL-10 (−1082G/A), IL-10R1 (G330R), and signal transducer and activator of transcription 3 (STAT3) rs2293452 single-nucleotide polymorphism (SNP) were genotyped in 110 atopic children with asthma, 110 non-atopic children with asthma, and 110 healthy children. Serum IL-10 and immunoglobulin E (IgE) levels were determined by enzyme-linked immunosorbent assay.

Results: A significant association was observed between the IL-10 polymorphism and asthma in both atopic ($P = .03$) and non-atopic asthma groups ($P = .04$). The genotype frequencies of IL-10R1 polymorphisms did not differ between all groups. We identified a significant association between STAT3 polymorphism and asthma susceptibility in atopic asthma ($P < .001$), whereas no such association was observed in the non-atopic asthma group ($P = .9$). No evidence of gene interactions was found.

Conclusion: Polymorphisms of IL-10 and STAT3 may be useful as a new DNA-based diagnostic biomarker for identifying high-risk children susceptible to asthma.

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INTRODUCTION

Asthma is a heterogeneous respiratory disease characterized by chronic inflammation of the airways associated with recurrent symptoms that range from mild to debilitating.¹ Many cytokines involved in inflammation and immunoregulation pathways have been associated with asthma.² Interleukin-10 (IL-10) is a cytokine that displays pleiotropic effects in asthma and allergy.³ Interleukin-10 may have a role in lessening allergic inflammation through inhibition of production of proinflammatory cytokines IL-1, IL-6, IL-8, tumor necrosis factor, and interferon δ .⁴ T cells engineered to secrete IL-10 greatly reduce the development of airway hyperresponsiveness and airway inflammation.⁵

Interleukin-10 produces its effects via a heterotetramer receptor that is composed of 2 of each of the receptor chains IL-10R1 and IL-10R2.⁶ The IL-10R1 chain mediates high-

affinity ligand binding and signal transduction, whereas the IL-10R2 subunit is required for signaling only.⁶ Activation of IL-10R leads to activation of Janus kinase (JAK)–signal transducer and activator of transcription (STAT). STAT3 is directly recruited to the IL-10R1 and becomes phosphorylated by receptor-associated JAK kinases.⁷ On phosphorylation, STAT3s dimerize and translocate to the nucleus, where they bind to STAT-binding elements in the promoters of various IL-10–responsive genes.⁷

Interleukin-10 as well as IL-10R and STAT3 genes are involved in a gene network in the IL-10/IL-10R signaling pathway. Interleukin-10 (−1082G/A) variant lies in a putative E-twenty-six-like transcriptional factor–binding site.⁸ The effect of IL-10 (−1082G/A) single-nucleotide polymorphism (SNP) on various asthma-associated phenotypes was studied in different populations, but conflicting results were seen.^{9,10} Only a few studies analyzed the relation between IL-10R and STAT3 polymorphisms and asthmatic phenotype.

Gasche et al¹¹ screened the IL-10R1 gene in European cohort and found a guanine-to-adenine transition at position 1112, causing a substitution of glycine (G) 330 to arginine (R). This polymorphism may act as a loss of function allele.¹¹ The IL-10R1 (G330R) (rs2229113) polymorphism was studied in several diseases.^{12,13} However, effects of IL-10R α (G330R) variant have not yet been assessed on asthma.

Of the known STAT genes, possibly STAT1, but certainly STAT6, has been associated with asthma.^{14,15} STAT6−/− mice had an impaired IL-4/IL-13 signaling, resulting in de-

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fective T-helper2 differentiation. In contrast, STAT3^{-/-} mice were more difficult to study because they were embryonically lethal.¹⁶

The IL-10/IL-10R signaling pathway may play a key role in pathogenesis of asthma; therefore, studying the components of this pathway is necessary to gain a more comprehensive genetic view of the susceptibility conferred by variants located in closely related genes. Accordingly, in this study we investigate whether IL-10, IL-10R, and STAT3 polymorphisms are associated with asthma in children.

METHODS

The study was conducted by the medical biochemistry and pediatric departments at the faculty of medicine of Zagazig University, Egypt.

Participants

The study comprised 220 patients (mean age, 8 years) and 110 healthy controls (mean age, 10.5 years). The patients were divided into 2 groups: those with atopic bronchial asthma ($n = 110$), and those with non-atopic bronchial asthma ($n = 110$).

None of the participants received antihistamine, systemic, or topical corticosteroids in the 3 weeks before clinical evaluation, and they all underwent skin prick testing.

The control group consisted of 110 children presented to the pediatric department, Zagazig Hospital–Zagazig University with other than respiratory or atopic complaints. Patients and controls belonged to the same ethnic group: African Caucasian. Characteristics of the participants are presented in Table 1.

Asthma phenotypes and bronchial responsiveness were determined by a pediatric physician. Following the American Thoracic Society guidelines,¹⁷ asthma was confirmed by a history of chest tightness and wheezing during the previous 12 months, a greater than 12% reversibility of forced expiratory volume (FEV_1) spontaneously or after β_2 -agonist inhalation, or a methacholine provocation test result with a provocative concentration of methacholine producing 20% reduction in the FEV_1 less than 16 mg/mL. Atopy was defined by a positive skin prick test (wheal diameter ≥ 3 mm) to at least 1 of 12 common aeroallergens. Non-atopy was defined by negative skin prick test to the 12 common aeroallergens. The non-atopic control subjects had no history of asthma or other allergic diseases, negative skin prick tests,

normal total immunoglobulin (Ig) E values (≤ 100 IU/mL), normal lung function tests, and no airway hyperresponsiveness ($PC_{20} > 25$ mg/mL). All of the patients and healthy individuals gave their written consent before blood sample collection. In addition to a full history and clinical examination, stool and urine analysis was performed in all cases to exclude factors that could influence measurements.

The study was approved by the ethical committee of the Faculty of Medicine, Zagazig University, and written informed consent was obtained from all participants.

Collection of Blood Samples

Six milliliters of blood was taken from each participant under complete aseptic conditions, and it was divided into 2 portions; 1.5 mL of whole blood was collected in sterile ethylenediaminetetra-acetic acid-containing tubes for DNA extraction, and the rest was left for 30 to 60 minutes for spontaneous clotting at room temperature and then centrifuged at 1,200 g for 10 minutes. Serum samples were separated into another set of tubes and kept frozen at -20°C for determination of total IgE and IL-10.

Determination of Serum IL-10

Serum IL-10 was measured by sandwich enzyme-linked immunosorbent assay (RayBio Human IL-10 kit; Norcross, Georgia), with the minimum detectable dose established as 1 pg/mL.

Total IgE Measurements

Total serum IgE levels were also measured by sandwich enzyme-linked immunosorbent assay (General Biologicals Corp, Hsin Chu, Taiwan), with assay sensitivity for total IgE of 5.0 IU/mL.

Genotyping

DNA extraction. Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit purchased from Promega (Madison, Wisconsin). Participants were genotyped for the IL-10 (−1082G/A) in genomic DNA by the amplification refractory mutation system–polymerase chain reaction (PCR).¹⁸ To assess the success of PCR amplification in both reactions, an internal control was amplified, using a pair of primers designed from the nucleotide sequence of the human growth hormone (HGH). The primer sequences used for amplification were as follows: allele-specific sense primer G 5'-TACTAAGGCTTCTTTGGGAG-3'; allele-specific sense primer A 5'-CTACTAAGGCTTCTTTGGGAA-3'; generic antisense primer of IL-10 5'-CAGCCCTTCCATTTTACTTTC-3'; control primer (HGH) sense 5'-GCCTTCCAACCATTC-CCTTA-3'; antisense 5'-TCA CGG ATTCT GTT GTG TTTC-3'.

Fragments were amplified in 50- μL reaction mixtures containing 10 μL genomic DNA, 30 μL 2 $\times$ Super Hot PCR Master Mix (from Bioron GmbH), and 1 μL of each allele-specific and control primers (BioBasic Inc, Toronto, Ontario, Canada) and 6 μL double distilled H_2O . Each reaction employed a generic antisense primer and 1 of the 2 allele-

Table 1. Clinical Profiles of Study Subjects

	Control group	Atopic asthma group	Non-atopic asthma group
Number of subjects	110	110	110
Age ^a	10.5 $\pm$ 2.3	8.6 $\pm$ 2.5	8.7 $\pm$ 3
FEV1 (%) ^a	95.5 $\pm$ 15	87.5 $\pm$ 16	91.8 $\pm$ 15.8
Log PC20 (mg/ml) ^a	1.38 $\pm$ 0.15	0.33 $\pm$ 0.53	0.54 $\pm$ 0.50

^a $P < .05$ for difference among the 3 groups.

specific sense primers. Amplification was performed using a PTC-100 thermal cycler (MJ Research, Inc, Watertown, Massachusetts) according to the following protocol: an initial denaturation at 95°C for 1 minute followed by 10 cycles (95°C for 15 seconds, 62°C for 50 seconds, and 72°C for 40 seconds), then 20 cycles (95°C for 20 seconds, 56°C for 50 seconds, and 72°C for 50 seconds). Amplification refractory mutation system-PCR for the IL-10 (−1082G/A) showed a 426-bp product for HGH as a control gene and a 600-bp product for −1082G allele or −1082A allele.

Participants were genotyped for the IL-10R1 (1112 OG/A) polymorphism by multiplex allele-specific PCR,¹¹ using 2 allele-specific primers A (sense) and B (antisense), which are specific to wild-type and variant alleles, respectively. External primers Q and P, which form a common product in all cases (515 bp), were used to provide a positive control. Although the wild-type-specific primer (A) in conjunction with Q produces 318-bp amplification products, the variant-specific primer (B), in conjunction with P, forms 248-bp products. The primer sequences used for the IL-10R1 SNP were as follows: P: 5′-CCCTTCATCTTCATCAGCCAGCGTC-3′; Q: 5′-TCAGGTAACCCTGGAATGCCACAG-3′; A: 5′-GCCGGGGGGGAGAACGCTGGGAAACG-3′ (sense); and B: 5′-GGGGGCGGGCCACAGGGGGCTCCCT-3′ (antisense).

Polymerase chain reaction was performed in a final volume of 25 μ L containing 5 μ L Genomic DNA, 1 μ L of each primer (BioBasic Inc), 12.5 μ L 2 $\times$ Super Hot PCR Master Mix, and 3.5 μ L DdH₂O.

The amplification was carried out according to the following protocol: 1 cycle of 95°C for 5 minutes, followed by 40 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 60 seconds, and a final extension step at 72°C for 7 minutes.

Participants were genotyped for rs2293452 SNP in intron 11 of STAT3 gene by PCR-based restriction fragment length polymorphism.¹⁹ Fragments were amplified in 25- μ L reaction mixtures containing 5 μ L genomic DNA, 1 μ L of each primer (BioBasic Inc), 12.5 μ L 2 $\times$ Super Hot PCR Master Mix, and 3.5 μ L DdH₂O. The primer sequences used for amplification were as follows: sense primer 5′-TCCCCTGTGATTCAGATCCC-3′ and antisense primer 5′-CATTC-CACATCTCTGCTCC-3′.

The thermocycling conditions consisted of an initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension step at 72°C for 5 minutes. The PCR products were digested with restriction enzyme, HpaII (Takara Bio Inc, Kyoto, Japan).

Statistical Analysis

Data were analyzed using SPSS software version 11 (SPSS Inc, Chicago, Illinois). Differences among variables in all the inferential statistics were considered significant at $P < .05$. The appropriate sample size and power of the study were determined using PAWE-3D (Laboratory of Statistical Genetics, New York, New York).²⁰ PAWE-3D calculations showed that

the sample size, together with the specified study design, allele frequencies, prevalence of disease, and allowable error rates, can give as high as 90% power and can detect variant allele frequency of at least 0.05 and genotype relative risk of ≥ 1.8 at 80% power. All SNPs were in Hardy-Weinberg equilibrium both in patients and controls.

RESULTS

Genotyping of IL-10, IL-10R1, and STAT3 Polymorphisms (Tables 2 and 3).

For IL-10 (−1082G/A) SNP, the A/A genotype was significantly more frequent in the atopic and non-atopic children than in the control group (38.2 vs 20.9% and 36.4 vs 20.9% respectively); similarly, the −1082A allele was more frequent in the atopic and non-atopic children than in the controls (odds ratio and 95% CI for the A allele: 1.7 [1.15–2.44] in atopic patients, 1.6 [1.11–2.35] in non-atopic patients).

For IL-10R1 (G330R) SNP, there was no significant association between genotypes or allele frequencies of the IL-10R polymorphism in the atopic asthma and non-atopic asthma groups compared with the control group (odds ratio and 95% CI for the A allele: 1.23 [0.9–1.9] in atopic patients, 1.14 [0.76–1.71] in non-atopic patients).

Our results showed significant differences between the atopic asthma group and controls regarding the genotype and allele distributions of STAT3 polymorphism, whereas there were no significant differences in allele or genotype frequencies between non-atopic asthma children and controls for this polymorphism (odds ratio and 95% CI for the C allele: 1.6

Table 2. Distribution Frequency of Genotypes and Alleles of IL-10, IL10R, and STAT3 Polymorphisms Between Atopic Patients and Controls

	Controls		Atopic asthma		P^a
	N	(%)	N	(%)	
IL-10					
G/G	33	(30)	24	(21.8)	.03
G/A	54	(49.1)	44	(40)	
A/A	23	(20.9)	42	(38.2)	
A allele	100	(45.5)	128	(58.2)	.01
G allele	120	(54.5)	92	(41.8)	
IL-10R					
G/G	59	(53.6)	51	(46.4)	.5
G/A	38	(34.6)	42	(38.2)	
A/A	13	(11.8)	17	(15.4)	
A allele	64	(29.1)	76	(34.5)	.3
G allele	156	(70.9)	144	(65.5)	
STAT3					
G/G	52	(47.3)	23	(20.9)	<.001
G/C	41	(37.3)	54	(49.1)	
C/C	17	(15.4)	33	(30)	
C allele	75	(34.1)	120	(54.5)	<.001
G allele	145	(65.9)	100	(45.5)	

^a Calculated by χ^2 test.

[1.09–2.37] in atopic asthmatic children, 1.06 [0.72–1.57] in non-atopic asthmatic children).

Serum IL-10 and IgE Levels in All Groups Studied

Level of IL-10 had no significant difference between atopic asthmatic children, non-atopic asthmatic children, and the control group (3.3 ± 1.7 pg/mL, 3.25 ± 1.92 pg/mL, and 3.16 ± 1.48 pg/mL, respectively), whereas levels of IgE were significantly increased in atopic patients compared with non-atopic asthma and control group (320.7 ± 71.09 IU/mL, 73.07 ± 19.3 IU/mL, and 70.1 ± 18.29 IU/mL, respectively).

Effect of IL-10, IL10, and STAT3 Gene Polymorphisms on Serum IgE (IU/mL) Levels in All Studied Groups

Serum levels of total IgE were significantly increased in IL-10 genotypes AA, GA than in GG in all groups studied ($P < .001$) (Table 4).

No effect of different IL-10R genotypes on serum IgE levels was found. Concerning STAT3 results, a more significant increase of serum level of IgE was seen in homozygous CC, heterozygous GC than in homozygous GG in the atopic asthma group ($P < .001$); however, different STAT3 genotypes were not associated with any differences in IgE levels in either the non-atopic asthma group ($P = .27$) or the control group ($P = .14$).

Effect of IL-10, IL10, and STAT3 Gene Polymorphisms on Serum IL-10 (IU/mL) Levels in All Studied Groups

No effect of IL-10, IL-10R, or STAT3 polymorphisms were seen on the level of IL-10 in any of the groups studied (Table 5).

Table 3. Distribution Frequency of Genotypes and Alleles of IL-10, IL10, and STAT3 Polymorphisms Between Non-atopic Patients and Controls

	Controls		Non-atopic asthma		P^a
	N	(%)	N	(%)	
IL-10					
G/G	33	(30)	24	(21.8)	.04
G/A	54	(49.1)	46	(41.8)	
A/A	23	(20.9)	40	(36.4)	
A allele	100	(45.5)	126	(57.3)	.01
G allele	120	(54.5)	94	(42.7)	
IL-10R					
G/G	59	(53.6)	54	(49)	.8
G/A	38	(34.6)	42	(38.2)	
A/A	13	(11.8)	14	(12.8)	
A allele	64	(29.1)	70	(31.8)	.7
G allele	156	(70.9)	150	(68.2)	
STAT3					
G/G	52	(47.3)	50	(45.5)	.9
G/C	41	(37.3)	42	(38.2)	
C/C	17	(15.4)	18	(16.3)	
C allele	75	(34.1)	78	(35.5)	.8
G allele	145	(65.9)	142	(64.5)	

^a Calculated by χ^2 test.

Table 4. Effect of IL-10, IL10, and STAT3 Gene Polymorphisms on Serum IgE (IU/mL) Levels in All Studied Groups

	11	12	22 ^a	P^b
IL-10				
Atopic asthma	230.9 $\pm$ 40.6	315.7 $\pm$ 31.2	374.9 $\pm$ 58.5	<.001
Non-atopic asthma	72.57 $\pm$ 14.3	82.5 $\pm$ 17.5	106.4 $\pm$ 5.9	<.001
Control	56 $\pm$ 16.3	76.4 $\pm$ 16.9	90.6 $\pm$ 3.6	<.001
IL-10R				
Atopic asthma	310.2 $\pm$ 32.5	308.4 $\pm$ 72.4	315.7 $\pm$ 68.6	.9
Non-atopic asthma	95.1 $\pm$ 17.9	97.2 $\pm$ 12.8	98.1 $\pm$ 14.3	.7
Control	66.9 $\pm$ 15.3	68.7 $\pm$ 21.4	69.9 $\pm$ 18.5	.8
STAT3				
Atopic asthma	269.5 $\pm$ 66.4	322.7 $\pm$ 57.7	387.5 $\pm$ 31.1	<.001
Non-atopic asthma	100.8 $\pm$ 9.4	103.9 $\pm$ 13.4	104.5 $\pm$ 4.8	.3
Control	85.7 $\pm$ 16.4	82.7 $\pm$ 13.9	90.9 $\pm$ 4.4	.2

^a The major alleles are referred to as allele 1 and the minor alleles as allele 2.

^b Calculated by ANOVA test.

Interaction Between IL-10, IL-10R, and STAT3 Genes

To investigate an interaction between IL-10, IL-10R, and STAT3 genes, we studied asthma susceptibility in relation to various combinations of genotypes. We found that the interaction of the IL-10, IL-10R, and STAT3 polymorphisms had no effect on asthma susceptibility (data not shown).

We also analyzed serum total IgE in relation to various combinations of genotypes. In atopic children with asthma, we found that the combination of mutant alleles (AAC) showed nonsignificant difference of the mean levels of IgE compared with those carrying the wild-type alleles (GGG) for IL-10, IL-10R, and STAT3 polymorphisms, respectively (260.5 ± 52.2 vs 253.8 ± 42.8 , $P = .35$).

DISCUSSION

The IL-10/IL10R signaling pathway is central to the inflammation leading to asthma. For this reason, polymorphisms of IL-10, IL-10R, and STAT3 may affect the gain-of-function of IL-10, IL-10R, and STAT3, thus altering the efficiency of the IL-10/IL-10R signaling pathway.

Interleukin-10 has been identified as an asthma susceptibility gene in a recent genome-wide association study (GWAS)²¹ and by several reviewers.^{22,23}

Much of the interest in IL-10 as a candidate gene in disease pathogenesis has focused on the promoter region as a way of explaining the variation in IL-10 levels by alterations in transcription. An interesting study by Chatterjee et al²⁴ showed that although the –1082A/G, –819C/T, and –592C/A polymorphisms are in close proximity to each other, only –1082 was associated with asthma susceptibility. In the

study by Seifart et al,²⁵ 5 SNPs were analyzed, and only the –1082A/G SNP was found to be associated with chronic obstructive pulmonary disease.

Our results showed that the –1082A/G SNP was associated with both atopic and non-atopic asthma in Egyptian children. Heterozygosity or homozygosity for the risk allele of the –1082A/G was significantly associated with increased total IgE but not IL-10 serum levels. Karjalainen et al⁹ suggested that eosinophil counts and serum IgE were differently regulated by IL-10 genotype in asthmatic and normal subjects. In a population-based association study of white asthmatic children, Lyon et al¹⁰ reported a suggestive association between the –1117A/G (–1082A/G in our report) and both IgE and FEV₁ as a percentage of predicted (FEV₁PP). Another study showed that the SNPs in the promoter region of IL-10 gene at –1082 had no impact on asthma susceptibility in adults with asthma.⁹ In the report of Burgess et al,²⁶ the rate of FEV₁ decline was studied with respect to 5 SNPs, and the –1082A/G was not found to be associated with FEV₁ decline in firefighters. From these results, we can hypothesize that the pathologic mechanism resulting in asthma from IL-10 (–1082) SNP affects lung function more strongly in young subjects than in older patients.

The association between –1082A/G SNP and increased total IgE may be attributable to –1028A, the low IL-10–producing allele^{27,28} affecting the release of IgE.⁹ Other studies demonstrated increased IL-10 production associated with the A allele compared with the G allele of –1028A/G.²⁹ Interleukin-10 may positively or negatively influence IgE by inhibiting the ϵ isotype switch through inhibition of IL-4 and IL-13 or by stimulating IgE release from B lymphocytes.³ Our demonstrated association between total IgE and the IL-10 polymorphism therefore cannot predict whether this polymorphism acts to promote or inhibit IL-10 production.

The functional receptor complex of IL-10 consists of 2 subunits, IL-10R1 and IL-10R2, which belong to the class II cytokine receptor family.⁶ The complexity of IL-10R signaling is further enhanced by the presence of SNPs in the coding sequence of IL-10R1. A G330A variant is located in the cytoplasmic domain of IL-10R1 close to the membrane and in a region that affects receptor stability.²⁷ Gruber et al²⁸ analyzed differential signaling of cytomegalovirus IL-10 (cmvIL-10), a viral homologs to human IL-10, through common variants of the IL-10 R 1. Gruber et al^[28] concluded that IL-10R1 G330A SNP impaired cytomegalovirus IL-10 signal transduction by a decrease in ligand-induced STAT3 phosphorylation.

Our results showed that the IL-10R1 G330A SNP was not significantly associated with neither atopic nor non-atopic asthma groups.

He et al²⁹ showed no association of polymorphisms of the gene encoding IL-10R1 (G330R one of them) with the rate of decline in, or value of, FEV₁ in smoking-induced chronic obstructive pulmonary disease. In fact, from various disease models, associations of IL-10R1 SNP were found only with progression of liver fibrosis, rheumatoid arthritis, systemic

Table 5. Effect of IL-10, IL10, and STAT3 Gene Polymorphisms on Serum IL-10 (pg/mL) Levels in All Studied Groups

	11	12	22 ^a	P ^b
IL-10				
Atopic asthma	3.6 ± 1.9	2.98 ± 1.9	3.4 ± 1.5	.3
Non-atopic asthma	4.7 ± 2.4	3.9 ± 1.5	3.7 ± 1.8	.1
Control	3.1 ± 1.4	3.12 ± 1.56	3.4 ± 1.5	.7
IL-10R				
Atopic asthma	3.02 ± 1.7	3.4 ± 1.8	3.9 ± 1.6	.17
Non-atopic asthma	4.1 ± 2.2	3.6 ± 1.5	3.1 ± 1.3	.15
Control	4.1 ± 1.9	3.5 ± 1.6	3.2 ± 1.2	.1
STAT3				
Atopic asthma	3.3 ± 1.6	3.4 ± 2.1	3.9 ± 1.3	.3
Non-atopic asthma	4.1 ± 1.8	3.7 ± 1.4	4.3 ± 2.1	.4
Control	3.1 ± 1.4	2.9 ± 1.9	2.7 ± 1.1	.6

^a The major alleles are referred to as allele 1 and the minor alleles as allele 2.

^b Calculated by ANOVA test.

lupus, and schizophrenia.^{12,13,30} In a recent study, we studied another common variant of IL-10R1, S138G, in atopic patients.³¹ S138G was in strong linkage disequilibrium with G330R.¹¹ We showed an association between S138G SNP and atopic asthma, but the sample size was too small. Simhan et al³² showed a significant association between cervical concentrations of IL-10 and SNPs in the IL-10RA gene in pregnant women in the context of cigarette smoking. However, our study showed no significant differences in the mean serum IL-10 levels between the allelic variants in different groups.

The gene STAT3 is induced by a wide array of cytokines, including IL6, IL-10, and IL13. STAT3 was implicated in asthma pathogenesis in a study that showed that STAT3-dependent pathways induced by IL13 in lung myofibroblasts were inhibited by the administration of the inhaled corticosteroid fluticasone.³³ Airway epithelial STAT3 seemed to be required for the allergic inflammation in a murine model of asthma.³⁴ STAT3 may play a role in the regulation of lung surfactant³⁵ and in the inflammatory response in lung fibrosis.³⁶

Several known asthma candidate genes, including STAT3, map to chromosome 17q21.³⁷ The first GWAS identified multiple markers on chromosome 17q21 reproducibly and strongly associated with childhood-onset asthma.³⁸ The findings were replicated in German and British cohorts.³⁸ A recent GWAS reported the strong association of 17q21 locus and pediatric asthma in whites.³⁹

Our results showed that the presence of C allele at rs2293152 SNP in STAT3 conferred susceptibility to atopic asthma but not to non-atopic asthma. In atopic children with asthma, serum IgE concentration was significantly higher in those with homozygous (CC) and heterozygous (GC) mutant STAT3 alleles compared with those with wild-type (GG), whereas these allelic effects on IgE were not observed in

non-atopic or in healthy children. These results suggested that STAT3 polymorphism was associated with lung function more in children with atopic asthma than in children with non-atopic asthma. Recent studies indicated that those mutations in STAT3 cause hyper-IgE syndrome. Hyper-IgE syndrome is a rare immunodeficiency syndrome characterized by extremely elevated levels of IgE in serum.^{40–42} Collectively, these data confirmed the state that polymorphisms in STAT3 gene may lead to overshooting IgE production.

An interesting study done by Litonjua et al⁴³ indicated that genetic variants in STAT3, independent of asthma treatment, are determinants of FEV₁ in both adults and children with asthma, however, this study unfortunately did not include total IgE as the main outcome. Conversely, Wjst et al⁴⁴ showed that no association of any STAT3 variant, including rs2293152, with asthma, allergic rhinitis, or eczema in German and Swedish populations. In addition, neither total and specific IgE nor any lung function parameter showed any significant association. A study of the 1958 British birth cohort did not find any of 19 STAT3 SNPs associated with log (IgE) values.³⁸ The cause of differences in the results of our study and these studies may be attributed to the fact that they were performed in different population groups.

Our study showed no effect of IL-10, IL10R, and STAT3 genotypes on IL-10 serum level, and this may be attributed to the fact that IL-10 levels in blood were not only related to the gene basis but also to other factors as cytokine inhibitors and use of medications.

The current study showed no evidence of genetic and functional interactions between IL-10, IL-10R1, and STAT3 genes. He et al²⁹ showed that no gene–gene interaction of IL-10 with IL-10R1 was found.

Positive or negative associations observed between these SNPs and asthma was not attributable to chance, because sample size was predetermined for the 3 polymorphisms to avoid type 1 error.⁴⁵ *P* values were subjected to Bonferroni's correction. Population stratification is another potential concern; thus, we included only white subjects in this study. Although population stratification may have produced false-positive results in the current study, this was addressed in part by other studies evaluating the role of these polymorphisms in their own populations, especially among whites.

We concluded that IL-10 and STAT3 polymorphisms are genetic determinants of asthma susceptibility in the Egyptian children. STAT3 polymorphism affects lung function in children with atopic asthma more than in children with non-atopic asthma. Finally, no effect of IL-10, IL-10R1, and STAT3 polymorphisms is seen on IL-10 serum levels.

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