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Correlation Between *IL-13*rs20541(A> G) Gene Polymorphism and Bronchial Asthma Among Iraqi Patients

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Abstract

Background: Bronchial asthma has a complicated genetic history. Changes in gene expression may be caused by gene polymorphism, cytokines play a central role. IL-13 is an interleukin that has been shown to play a role in the disease's immunopathogenesis. The current study investigated the relationship between rs20541 of the IL-13 gene and Bronchial asthma in Iraqi patients.
Methods: Seventy-five patient and fifty healthy individuals as a control. The DNA was extracted from blood samples. Detection of genotype IL-13SNP (rs20541) were achieved by RFLP-PCR.
Results: indicated a highly significant the levels of the IgE, and IL-13 in the patients compared to control at (p value≤ 0.01), (456.45±290.106 vs. 30.08±24.414), (59.5980±20.93750 vs.6.7034±4.10547) pg/ml respectively. Result shows no significant differences in the frequency distributions of IL-13 SNP (rs20541) for all genotypes in cases and controls. A protective role of asthma, (OR: 0.62; CI.95%: 0.23 - 1.6) and (OR 0.89; CI.95%:0.42 - 1.89) were observed for wild type homozygous and heterozygous genotype respectively. Whereas the AA genotype (42.7%) in cases and (34.0%) in control, that (OR:1.44; CI.95%:(0.66 - 3.07) mutant homozygous were risk factors of asthma among individuals. The genotypes of IL-13 rs20541 (GG, AG, AA) among patients and controls were significantly correlated with IgE and IL-13 results at (p≤ 0.05).
Conclusions: AA genotype in case and control mutant homozygous were risk factors of asthma among individuals. It's possible that this has a predisposing impact on the development of asthma.

Keywords: Bronchial Asthma, RFLP, IL-13, SNP.

Introduction

Asthma is a common respiratory disorder worldwide. It is a most common chronic inflammatory airway disease associated with cytokines which promote airway eosinophilia, mucus over production and immunoglobulin E (IgE) synthesis (1). Mast cells have long been thought to be effector cells in type I hypersensitivity caused by IgE, it is an immunoglobulin related to allergic and hypersensitivity reactions (2). This immunoglobulin fundamentally binds on high-affinity with IgE receptors on basophils and mast cells (3,4). Previous genetic study proved several important cytokine SNPs that were found to be

implicated in asthma (5). Many studies have found a link between IL-13vs20541 single nucleotide polymorphisms and asthma in adults and children, in the presence of infections, atopy, IgE levels, or asthma risk (6, 7). The goal of this study was to detect the relationship between asthma susceptibility in Iraqi bronchial patients and polymorphism *IL-13* rs20541(A> G).

Materials and Methods

Study Subjects

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Blood samples were taken from seventy-five asthmatic patients, whose age range from (14-65) years, the samples were collected during September 2020 to February2021 at from the Specialized Center of Allergy in Babylon city, Iraq. Fifty of healthy people as a control, with ages ranging from (10-50) years. After allowing the blood to clot, it was centrifuged, and the sera was collected and preserved at -20 °C. A consent form and questionnaire were filled for everyone. In Iraq, the Research Ethical Committee reviews scientific research that has received ethical approval from the Ministry of Health and the Ministry of Higher Education and Scientific Research.

Measurement of serum IL-13 and total IgE

IL-13 levels in the blood were measured using an enzyme-linked immunosorbent test according to the manufacturer's instructions (Human IL13 ELISA/ Biotechnolog-laboratory/ China). Total IgE serum levels were determined using the Vidas test (Total IgE / Vidas / Korea).

Extraction of Genomic DNA

Genomic DNA was extracted from whole blood samples of seventy-five asthmatic patients and. Fifty apparently healthy controls. The protocol of genomic DNA extraction supplied by (gSYNC DNA Extraction KIT/ Taiwan). The DNA samples purity and concentration measured by using Nano drop equipment. The 260/280 optical density ratio of pure DNA is less than 1.8.

Genotyping

The genotyping of *IL13* rs 20541 was done using the RFLP-PCR process. Two primers were selected (F: CTTCCGTGAGGACTGAATGAGACGGTC) and (R: GCAAATAATGAGCTTTCGAAGTTTCAGTGGA) (Macrogen /Korea) (8). Aliquots of amplified DNA products were digested with *Alu*I (New England Biolabs/England) to amplify a 245-bp fragment for allele detection. The PCR settings were 95 °C for 5 minutes, and the amplifications were performed in a GTC thermal cycler (Cleaver Scientific, UK). At 95 °C, 35 cycles were performed, each with a 45-second denaturation. 45 seconds annealing at 60 °C and 45 seconds extension at 72 °C. The final elongation cycle at 72 °C lasted 7 minutes. The PCR product IL-13 rs20541 were digested by restriction endonucleases *Alu*I. On a 3% (w/v) agarose gel, the PCR products were verified for predicted size.

Biostatistical Analysis

Differences in the analytical paraments were identified in the statistical analysis using the Statistical Program for Social Science (SPSS). To compare the percentages significantly, Chi-square tests were utilized (0.05 and 0.01 probability). In this investigation, the odds ratio and confidence intervals were calculated (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

Results

This study conducted on blood samples seventy -five patients and fifty from apparently healthy control blood samples. With regard to age, the age of asthma patients and control ranged from 14-60 year, with ages mean of 36.85±13.50 SD and 32.06±11.77 SD for case and control, respectively, and characterized as previously described (9).

Estimation of serum total IgE and IL-13

The results of this investigation revealed a highly significant ($p \leq 0.01$) increase in Total IgE levels in the serum of asthma patients compared to the control group, with IgE levels averaging (456.45 290.106 *vs.* 30.08 24.414) IU/ml, respectively. The result showed a highly significant elevation differences in the levels of the IL-13 in the bronchial asthmatic patients and control at ($p \leq 0.01$), (59.5980±20.93750 *vs.* 6.7034±4.10547) pg/ml, respectively (Table 1).

Table 1. Serum level of IgE and IL-13 in patients and controls with bronchial asthma.

	Controls (NO.50) (Mean±SD)	Patients (NO.75) (Mean±SD)	p- value
IgE (IU /ml)	30.08±24.414	456.45±290.106	0.0000 **
IL-13 (pg/ml)	6.7034±4.10547	59.5980±20.93750	0.0000 **

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**** High significance differences (p≤ 0.01)**

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Isolation of Genomic DNA

Genomic DNA was extracted from the blood samples (Fig. 1). DNA values in high purity samples ranged from 1.7 to 1.9 ng/L, approximating to 80-200 ng/L.

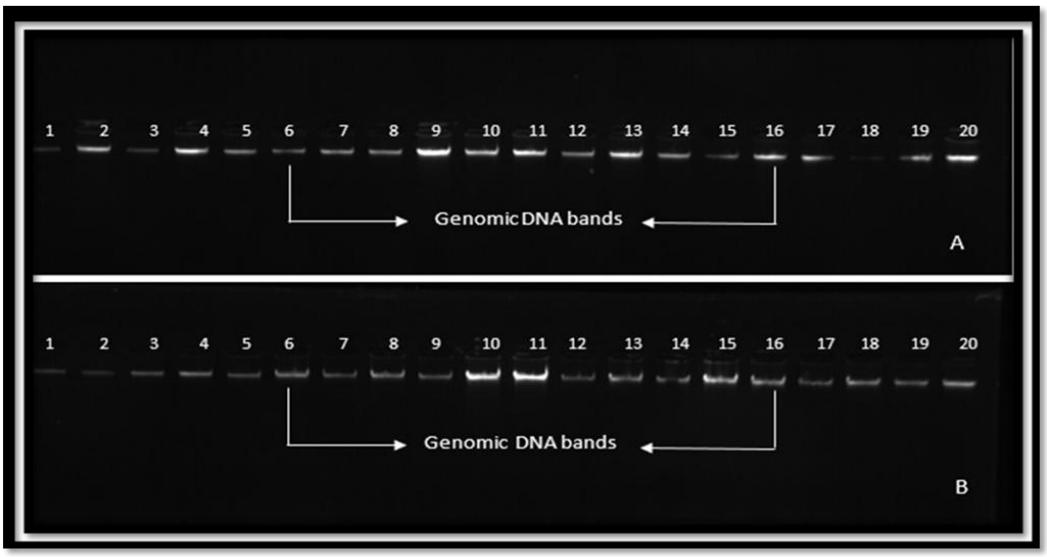

Fig. 1. Gel electrophoresis of genomic DNA extracted from blood samples of asthmatic, on 1% agarose at 70 volts for 40 minutes, A: patient, B: control.

Genotype of IL-13 (rs20541) Polymorphism

Analysis IL-13 polymorphism by RFLP-PCR

The result for genotype the rs 20541 (G/A) in the presence IL-13 rs 20541 polymorphisms by RFLP-PCR. The distribution of IL-13 rs 20541 genotype was distributed in groups based on the presence or absence of the polymorphism: GG wild homozygous contain restriction site for *NIa*IV have both 209 bp and 26 bp bands, the second genotype was AA mutant homozygous with have three band expected 178 bp, 31 bp, 26 bp. While the third group AG heterozygous exhibited four band 209 bp, 178 bp, 31 bp, 26 bp (Fig. 2).

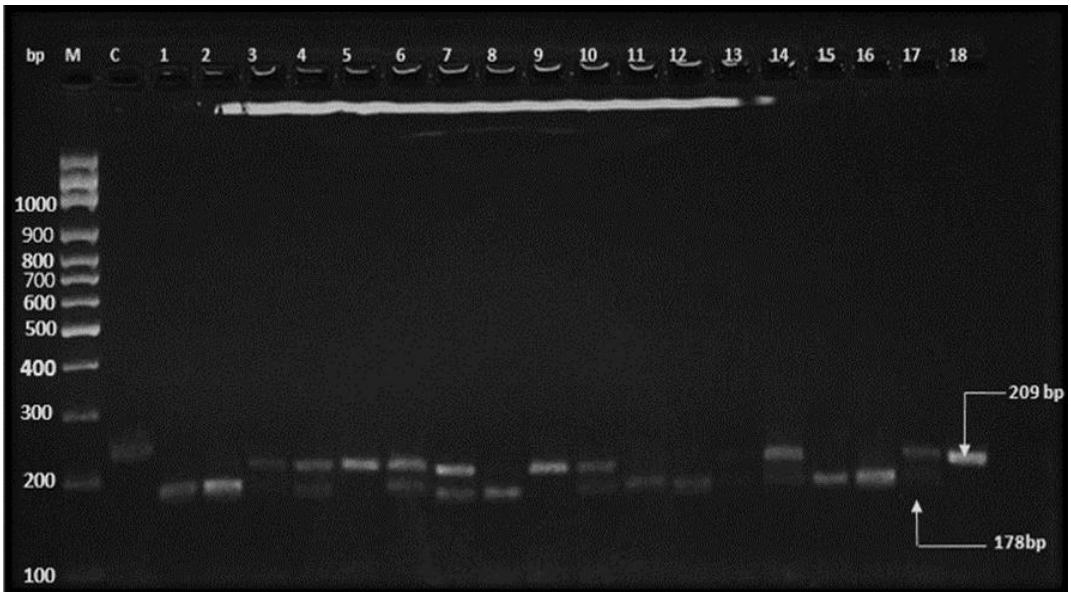

Fig. 2. RFLP -PCR product with restriction enzyme *NIaI*V1 for *IL-13* gene. On 3% agarose at 70 volts for 2hrs. Lane M,100-bp DNA marker. Lane C uncut gene. Lane (1-10) case. Lane (11-18) control. Lane (5,9,18): two band 209 bp+26 bp homozygous.

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Lane (1,2,8,11,12,15,16) 3 bands 178 bp + 31 bp + 26 bp mutant homozygous. Lane (3,4,6,7,10,13,14,17): 4 band 209bp+178 bp+31 bp+26 bp (31 bp and 26 bp bands cannot be visualized in agarose gel) heterozygous.

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Distribution of Genotype and Allele Frequency of the IL-13 SNP (rs20541) A> G

The distribution of the observed *IL-13* gene genotype and alleles frequencies in the case group was heterozygous AG34(45.0%), while 32(42.7%) for homozygous mutant AA genotype, and wide type GG 9 (12.0%) (Table 2).

Table 2. Distribution of genotype and allele frequency of *IL-13* SNP (rs20541) in case and control.

IL-13 SNP (rs20541)	Case No. (%)	Control No. (%)	Sig.	O. R	C.I (95%)
GG	9(12.0%)	9(18%)	0.34	0.62	(1.6 - 0.23)
AG	34(45.0%)	24(48.0%)	0.76	0.89	(1.89 - 0.42)
AA	32(42.7%)	17(34.0%)	0.33	1.44	(3.07 - 0.66)
Total No.	75	50	---	---	---
Allele	Frequency	Frequency			
G	34.7%	42%	---	----	----
A	65.3%	58%	-----	----	-----

Table 2 demonstrates that the frequency distributions of IL-13 SNP (rs20541) for all genotypes in case and control had no significant differences, the result show protective role of asthma, (OR: 0.62; CI 95%:(1.6 - 0.23) and (OR 0.89; CI 95%: - 0.42 (1.89) for wide type homozygous and heterozygous genotype respectively. Whereas the AA genotype (42.7%) in case and (34.0%) in control, that (OR:1.44; CI 95%:(0.66 - 3.07) mutant homozygous were risk factors of asthma among individuals. This result showed that G allele frequency was (34.7%) in asthmatic group and (42%) in control group whereas A allele frequency was (65.3%) in asthmatic group and (58%) in control group.

Association between Genotypes of IL-13 rs 20541 and with serum IL-13 and IgE Levels

In asthmatic patients and controls, the relationship between the IL-13rs 20541 genotype and IgE and IL-13 was studied. The presence of IgE and IL-13 was shown to be significantly linked with IL-13 rs20541 genotype (GG, AG, AA) among patients and controls in this investigation ($p \leq 0.05$) (Table 3).

Table 3. Genotype association of *IL-13(*rs 20541**)** with serum IgE and IL-13 in patients and control groups.

		IgE	IL-13
Group	**IL-13 rs 20541**	**Mean±SD**	**Mean±SD**
Patient	**GG**	395.11±219.811	67.2088±17.10362
	AG	422.62±292.181	54.4905±19.03138
	AA	509.66±303.786	62.8842±22.99617
Control	**GG**	22.56±26.651	6.7386±3.61852
	AG	30.88±24.210	6.0287±3.78327
	AA	32.94±24.211	7.6375±4.78388
P-value		**0.009***	**0.019***

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*** Significance differences (p≤ 0.05).**

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Discussion

Patients with bronchial asthma typically acquire the disease early in life, usually in infancy or childhood. The attack usually occurs upon exposure to allergens, the total serum Immunoglobulin-E (IgE) concentration is frequently elevated but sometimes remain normal (10). Allergen-induced IgE synthesis can trigger eosinophils, basophils and mast cells to release cytokines for the differentiation of T-helper 2 (Th2) cells to secrete Interleukine-13 as well as the release of proinflammatory, vasoactive and fibrogenic factors that are responsible for symptoms of asthma (12). Interleukine-13 (IL-13) is an immunoregulatory cytokine generated primarily by activated Th2 cells. It has been identified as a main cytokine mediating allergic airway inflammation and remodeling in asthma, which is characterized by mucus hypersecretion, airway hyperresponsiveness, and subepithelial fibrosis (12). The relationship between IgE and asthma expression includes multiple cofactors influencing airway and disease persistence and the probabilities of wheezing and reduced lung function, in parallel with serum IgE values, may undoubtedly mediate asthma reactions in allergic individuals (13, 14).

In genetic analysis, the human IL-13 gene has been associated to the development of atopy and asthma. Previous study demonstrated the link between IL-13 and high IgE total serum concentrations, bronchial hyperreaction and asthma (15). SNPs in IL-4 and IL-13 play a role in Allergic Rhinitis in different groups, according to a prior study by Shirkani et al (16).

A genetic study in humans involving *IL-13* gene polymorphism has clearly shown a relationship between susceptibility to asthma which was independent on serum IgE levels (17). The immune system undergoes characteristic changes with aging, and serum IgE levels decrease with age in the general population. In the aged, most T-cell activity is lowered, and the accumulation of CD45RO+memory cells leads in a reduced ability to react to novel antigens but a retained ability to respond to recall antigens as long as the memory cells are present and functioning (5).

The result show protective role of asthma for wide type homozygous and heterozygous genotype. Whereas the AA genotype in case and control mutant homozygous were risk factors of asthma among individuals. It's possible that this has a predisposing impact on the development of asthma.

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The authors have declared no conflict of interest.

Association of IL- 13 rs20541 and rs 1295686

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