

# Association between Interleukin-23 Receptor Polymorphism and Asthma

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## ABSTRACT

"Asthma": A public health problem mainly effects lung ,the main character is inflammation the lead to narrowing of the bronchi creating difficulty breathing,its multifactorial disorder and genetic predisposing one of the etiological and risk factor .one of the genetic study is focusing on signaling through" IL23 receptor (IL23R)". IL-23R is important for signaling of IL-23. Which considered an important role in immune-regulatory pathway. The present study aims to evaluate IL-23R gene polymorphism and its role to increases or decreases tendency to asthma. In this case-control study fifty patients with asthma compared with control group, fifty seemingly healthy the diagnosis made in by specialist consultation at Marjan Medical Hospital in Hilla, Iraq. Analysis of data revealed there are substantial difference in frequency of IL-23 R (rs11209026) G>A polymorphism between asthmatic patients and controls ,opposite to this result ,the another polymorphism (rs1884444) in the same receptor proofed no substantial difference (between) asthmatic patients and (controls) .The results of the work concluded, that patients with GG allele for IL-23 R (rs11209026) have increased susceptibility to asthma compared with GA allel. Also, the present study suggests that IL-23R (rs1884444) is not considered risk factor for asthma.

**Keywords:** asthma, interleukin-23receptor, polymorphism, allele.

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## INTRODUCTION

"Asthma" is an allergic disorder that causes obstruction of airway that impacts people to attack of shortness of breath which is not continues but has manner of reversibility (1) . The condition turns in relatives; therefore, children of asthmatic family are at an expanded hazard of developing asthma (2,3). Episodes of asthma mainly has features of wheezing, coughing, narrowness of the chest, and shortness of breath (4,5). Bronchial asthma is a complicated disorder, its multifactorial etiology has both genetic and environmental factors that influence on the clinical manifestation of asthma. Genetic elements have role and give information whether the person is susceptible to the disease (6). The degree of genetic kinship to an affected family member effects the risk of asthma in a person. If a person in the family is severely affected or affected at an early age, the risk will also be higher in the person's relations (2).

Asthma exhibit character "inflammatory" disease so its connect with T helper 2 (TH2) cell and TH2-type cytokines, such as interleukin-4 (IL-4), IL-5 and IL-13, and this connection are diagnostic criteria of pathology of Asthma. (1). The connection between asthma and T helper cell 2 (Th2) causes inflammation process and leading to airway hyper responsiveness and tissue transformation. The series of event result from activation of Th2 that causes inflammation is expected due to abnormal response to "harmless airborne particles". (6). Inflammatory process of asthma through sensitization of Th2 established mainly through signaling of IL23(7). "Interleukin-23 (IL-23)" is belong to a member of the IL-12 family of "heterodimer cytokines". It is constitute of an unique" p19 subunit" and a common" p40" subunit membership with IL-12 (8)the interaction between IL-23 with "heterodimeric receptor" which included mainly of two type "IL-12 receptor  $\beta$ 1 (IL-12R $\beta$ 1)" and "IL-23 receptor (IL-23R)" and this in consequence causes activation of "Jak2", "Tyk2", and initiation the process of

signal transducers by IL-12, IL-23 play an important role for developing inflammatory diseases including autoimmune, collagen-induced arthritis, and intestinal inflammation (9). Additionally, "polymorphisms" in the gene encoding the IL-23R has considered as potentiation factors for developing these disorder (9). Current study aims to decide whether or not IL23R gene opportunity increases or decreases exposure to bronchial asthma.

## METHOD

### Ethical Issues

The starting this work need for following approval a-Agreement of logical board in College of Medicine (University of Babylon/Iraq).

b-The study design and method of work were described to all subjects.

This type of methodology applying the steps of case control study included hundred subjects, 50 patients with asthma, who attended Marjan Medical City and 50 apparently healthy controls.

All persons are estimated the status of obesity by the body mass index (BMI), [BMI= weight (kg) / height (m)<sup>2</sup>].

### Inclusion Criteria

All patients with pure Asthma.

### Exclusion Criteria

Patient with diabetes mellitus, Patient with hypertension, Smokers, Patients with rheumatoid arthritis, Pregnancy, Any inflammatory diseases, and autoimmune diseases.

### DNA extraction and genotype analysis

Genomic DNA was separated from the peripheral blood of subjects through application of special technique. The DNA was stored in deep freeze condition at temperature at -20°C until date of analysis and the detection of DNA depending on application of agarose gel electrophoresis with aid of Gel loading dye purple to visualized DNA under UV Gel Documentation System.

**PCR-RFLP**

IL23R gene polymorphisms detection depending on amplification the segment that contain polymorphic region using the polymerase chain reaction and then for purpose of genotyping we depend on "PCR-RFLP" technique by using special "restrictive endonuclease".

Briefly, for polymorphism in the IL23R gene amplified by using the sequences of primers used for polymerase chain reaction (PCR) as following:

**Primers Sequences of IL23R gene polymorphism R381Q rs11209026:**

**F:** 5'-CTTTTCTGGCAGGGTCATTTTG-3'

**R:** 5-CAGTCTTTTCCTGCTCCAGACAT-3

**Primers Sequences of IL23R gene polymorphism R381Q rs1884444:**

**F:** 5-AATAAAATCATACTCTTGCCAATGGCCC-3

**R:** 5'-AAGTTGTTTCCTGGGGTAGTTGTG-3'

Genomic DNA was amplified in a final volume of 25µl.

**Reagents used for preparation of PCR reaction:**

Genomic DNA: 5µl, F-"primer": 2.5 µl, R-"primer": 2.5 µl, Promega master mix: 12.5 µl, DDW: 2.5 µl

**PCR conditions that give best result summarized as:**

Initial Denaturation: (95°C, 5 minutes), Denaturation (95 °C, 30 seconds), Annealing: (58 °C, 30 seconds), Extension : (72 °C, 30 seconds),

30 cycles, Final Extension: (72 °C, 5 minutes), Hold (12 °C, ∞).

The amplicon obtains from run PCR was incubated with Restrictive Endonuclease **Hpy188I** for R381Q rs11209026 and **PscI** for R381Q rs1884444.

The RFLP was done in a full volume of 20 µl.

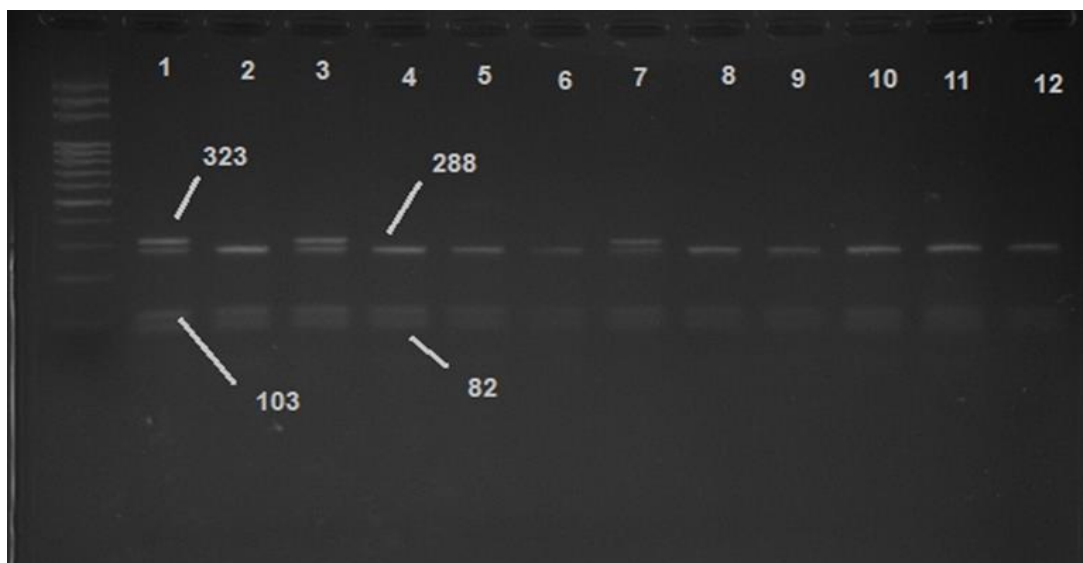
Digestion conditions that give best result were summarized as: 4 µl Water, 5 µl NEB Buffer, 10 µl PCR product, 1 µl enzyme.

The RFLP settings were orientated in 37° C for 90 min .

The restriction-digested fragments were distributed on 2% agarose gels and visualized under UV brightness.

**RESULTS**

There were no significant differences between means of age, body mass index between asthmatic patients and healthy control. The polymorphism of IL-23R (rs11209026) were evaluated in in asthmatic patients and healthy controls ,the analysis of data revealed significant variations have been determined in genotyping frequency of rs11209026 G>A polymorphism between asthmatic patients and control group (OR =2.7 , CI= 1.19-6.1, P-value = <0.05).In all study groups GG and GA were representing in figure no.1



**Figure.1:** Refraction fragment length polymorphism. (1,3,7) heterozygote GA, (2,4,5,6,8,9,10,11,12) homozygote GG

Genotyping and Alleles Frequency of Interleukin-23 Receptor R381Q rs11209026 were calculated in Asthma and control groups, the result was representing in Table no. (1) and (2).

**Table 1:** Comparison of Alleles and Genotype of IL23 R R381Q rs11209026 in asthma patients and healthy control

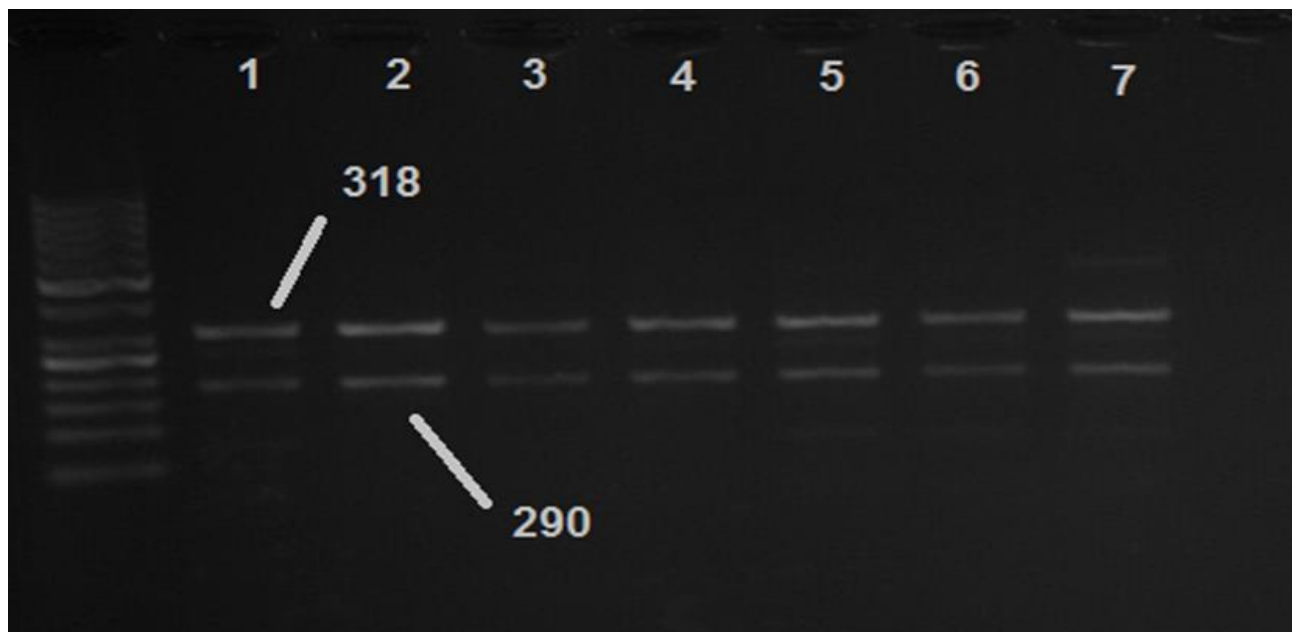
Genotype	Asthma NO.	%	Control NO.	%
GG	34	68%	22	44%
GA	16	32%	28	56%

**Table 2:** Allele distribution of IL-23R (rs11209026) polymorphism gene in patients and controls.

Allele	Asthma	Control
G	84	72
A	16	28

The present study shows that IL23R (rs11209026) gene polymorphism consider as risk factor for Asthma. Odd ratio (2.7), CI (1.19-6.1), P value (<0.05). In the current study the second "polymorphism of IL-23R rs1884444" were evaluated in case asthmatic patients and control

healthy group According to the present study the Case and control group mainly homozygote G allele ,the result was non-significant and this polymorphism is not considered risk factor for asthma.the result of RFLP were representing in Figur 2.

**Fig.2:** Refraction fragment length polymorphism. (1,2,3,4,5,6,7) homozygote GG.

## DISCUSSION

Signaling IL-23 through interaction with IL-23R play an important role in developing the inflammatory process in asthma<sup>(10)</sup>. The IL-23R is a heterodimer and activation of receptor by binding with IL-23 give rise for starting of signaling that association with allergy and inflammatory process<sup>(11)</sup>. The expression IL-23R is done especially upon activation of T cells, T-cell clones and NK cells. Low levels of IL-23R appearance have also been identified on "monocytes, macrophages and dendritic cells", so they considered responsible for IL-23 production<sup>(12)</sup>. IL-23/IL-23R signaling play an important role for differentiation Th17 cell and secretion of IL17 which have a role in the process of inflammatory and autoimmune responses particularly in developing asthma<sup>(13,14)</sup>. The receptor important for signaling process and the present of "polymorphisms" in the IL-23R may cause alteration in amplitude of its responses<sup>(15)</sup>. An allele of the IL-23R" R381Q gene" version instructed that its exhibit defensive effect towards autoimmune sickness changed into pushed through an impairment effector on TH17 cell differentiation and subsequently IL-17A manufacturing.<sup>(16)</sup> The R381Q allele play protection role and effect on outcome of certain inflammatory disorders as in as IBD (including CD and UC)<sup>(17)</sup>, psoriasis<sup>(18)</sup>, ankylosing spondylitis"<sup>(19)</sup>, rheumatoid arthritis, recurrent spontaneous abortion (RSA)<sup>(20)</sup> and asthma<sup>(16)</sup>. Significant variances were seen in distribution of

"rs11209026 G>A" polymorphism between asthmatic patients and controls. In the considered groups, GG and GA genotypes were remarked. The distribution of GA and GG genotypes in patients become 32 % and 68%, respectively, and the dispersion of A and G alleles had been 16% and 84%, respectively, these result indicate that genotype GG in asthma has odd ratio of about 2.7 and considered as risk factor for developing asthma this result agree with study of Mosayebian A, *et al*<sup>(16)</sup>. Another study confirmed that polymorphysim in IL-23 receptor (IL-23R) has role on developing human inflammatory response<sup>(21)</sup>. Numerous research have analyzed the A factor and diverse inflammatory sicknesses such as inflammatory bowel disease (IBD), cron's disease (CD), ulcerative inflammation (UC)<sup>(22)</sup>, psoriasis<sup>(23)</sup>, multiplex induration (MS) and ankylosing spondylitis (AS) in dissimilar populations<sup>(24)</sup>. Another study showed that Th17 cells generated from A allele of IL23R R381Q had significantly reduced IL-23-induced IL-17A production compared to G allele<sup>(25)</sup>. present study shows that rs1884444 gene polymorphism at IL23 R is not considered risk factor for asthma, so at the same receptor there are more than one polymorphism but not all of them has effect on effect of receptor.

**CONCLUSION**

IL-23R gene polymorphism (rs 11209026) increase susceptibility to asthma, while IL-23R gene polymorphism (rs 1884444) was not consider as risk factor for asthma.

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**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**CONFLICT OF INTEREST**

None

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