**RESEARCH ARTICLE**

# Analysis of Some Candidate Genes for Rheumatoid Arthritis of the Iraqi Population

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**ABSTRACT**

This study was designed to determine different genes of the frequencies of polymorphic of several functional groups, cytokine genes (IL-2, IL-17), and protein tyrosine phosphatases (PTPN22) in patients with Rheumatoid Arthritis. The extraction of DNA was done by using a special reagent from Whole Blood Leukocytes with the help of a reagent (DNA Express-blood). This reagent was used to isolate the DNA from natural sources. After extracting the DNA carried out the amplification reaction using (SNP-express) by PCR and specific kits for every gene. Detection of amplification products by horizontal electrophoresis method and then visualization and identification of amplification products to investigate the alleles in various genes its used and finally combination between the genes by MDR method. The results were shown that different polymorphic markers of three genes IL2, IL17, and PTPN22 for two groups, patients with Rheumatoid arthritis and control group (healthy individuals ) of Baghdad people which presence of an association between Rheumatoid arthritis and a number of polymorphic markers of the genes IL2, IL17, and PTPN22. The association of the polymorphic marker A < C of the IL-2 gene, however, the association of the polymorphic marker T > G of the IL-17 gene, while The association of the polymorphic marker T > C of the IL-17 gene.

**Keywords:** DNA, Interleukin-2, Interleukin-17, Multifactorial diseases, Rheumatoid arthritis.

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## INTRODUCTION

There are many autoimmune diseases, one of them, Rheumatoid arthritis, is a common multifactor disorder.[1] In this time, there are many diseases, such as rheumatoid arthritis, cardiovascular, cancer, and diabetes, conceders a hereditary disorder and called multifactorial diseases because this type of disease is caused not just by a mutation in one gene. Still, this disease is caused by a combination of genetic and environmental factors.[2] The studies of genetics screening for those mutations in those diseases are considered as an indicator of the future risk. It cannot predict exactly the cause of the diseases because there are many other factors such as biological factors, environmental and lifestyle.[3]

There are a huge group of proteins called cytokines. It has a subgroup of natural proteins known as interleukins which cell growth regulated, motility, and cell differentiation; there are a very important roles in immune responses stimulated when inflammation happened.[4] Interleukins cannot be stored between the cells but rapidly secreted as immune responses when any infection or inflammation in the body.[5] When production of interleukins. It travels into the target cell and contacts it by a special receptor found on the cell's surface. This interaction between interleukins and target cell will change the behavior of the cell.[6]

Cytokine family has many members like IL-1, IL-2, IL-3, IL-4, IL-5,IL-6, IL-7, IL-8, and others; some family members have a four alpha bundle as helix shape while some consist of three chains alpha, beta, and gamma. Interleukin -2 is made by one of the white blood cells that is T lymphocyte, which increases the activity and growth of B lymphocytes and T-lymphocytes when developing the immune system.[7]

The cytokines family has subgroup recently is interleukin -17. This interleukin with his receptors new understanding how working in infection of autoimmune diseases. There are six ligands for this interleukin from A to F and five receptors. The role of interleukin 17 is linked between the T- cell and neutrophile; therefore, its plays very important role in innate immunity.[8]

There are four classes for protein tyrosine phosphatases some of them has receptor called classical receptor PTPS and others non-receptor PTPS. One of the families of protein tyrosine phosphatases is the protein tyrosine phosphatase N-22 (*PTPN-22*) gene located on chromosome 1p13. This gene encodes PTPN22, and this enzyme is uttered in lymphoid tissue and is implicated with some immune response pathways.[9]

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## SUBJECTS AND METHODS

### Collection of Blood Samples

Thirty-seven patients (23 males and 14 females), their ages from 45 to76 years old, were diagnosed with Rheumatoid Arthritis, and 13 healthy individuals as the control group (7 females and 6 male) were registered in this study. Blood samples were collected from many of Hospitals in Baghdad city from June to July 2018.

### Extraction of DNA

The extraction of DNA was done by using a special reagent from Whole Blood Leukocytes with the help of a reagent (DNA Express-blood). This reagent was used to isolate the DNA from natural materials. After extracting the DNA, carried out the amplification reaction using (SNP-express) by PCR and specific kits for every gene. Detection of amplification products by horizontal electrophoresis method and then visualization and identification of amplification products to identify the alleles in various genes its used.

Using the MDR method Multifactor Dimensionality Reduction, this program estimated the incorporation of polymorphic variants between three candidate genes related to the development of Rheumatoid Arthritis.[10]

## RESULTS AND DISCUSSION

The results were shown that different polymorphic markers of three genes IL2, IL17, and PTPN22 for two groups, patients with Rheumatoid Arthritis and control group (healthy individuals) of Baghdad people which presence of an association between Rheumatoid Arthritis and a number of polymorphic markers of the genes IL2, IL17 and PTPN22.

The association of the polymorphic marker A < C of the IL-2 gene the development of Rheumatoid Arthritis in the inhabitants of the Baghdad region was found. It has been shown that carriers of the C allele of the C/C and A of the A/A genotype have a reduced risk of developing Rheumatoid Arthritis, while carriers of the alleles of the A/C genotype increased risk. However, the association of the polymorphic marker T > G of the IL-17 gene has been shown that carriers of the G allele of the G/G genotype have a reduced risk of developing Rheumatoid Arthritis, while carriers of the alleles are Genotypes T/G increased risk , while The association of the polymorphic marker T and C of the IL-17 gene has been shown that Non-significant and carriers of the C allele of the C/C genotype have a reduced risk of developing Rheumatoid Arthritis, while carriers of the alleles of the C/T genotype increased risk (Tables 1 to 3).

**Table 1:** Distribution of sample study according to allele and genotype of IL-2 (A/C).

| | *Frequency of alleles and genotypes* | | *value χ2* | | *Level of* |
|---|---|---|---|---|---|
| *Alleles and genotypes* | *Cases (NO = 37)* | *Controls (NO = 13)* | *(p)* | *OR[CI 95%]* | *significance* |
| Allele A | 0.500 | 0.731 | 4.16(0.04)* | 0.14–0.98 | 0.05 |
| Allele C | 0.500 | 0.269 | | 1.02–7.23 | |
| Genotype A/A | 0.081 | 0.615 | | 0.01 – 0.28 | |
| Genotype A/C | 0.838 | 0.231 | 6.71(0.01)* | 3.62 – 81.83 | |
| Genotype C/C | 0.081 | 0.154 | | 0.07–3.29 | |

* (p < 0.05), NS: Non-significant.

**Table 2:** Distribution of sample study according to allele and genotype of IL-17(T/G)

| | *Frequency of alleles and genotypes* | | *value χ2* | | *Level of* |
|---|---|---|---|---|---|
| *Alleles and genotypes* | *cases (No = 37)* | *Controls (No = 13)* | *(p)* | *OR[CI 95%]* | *significance* |
| Allele T | 0.527 | 0.731 | 3.28(0.07) NS | 0.15–1.09 | |
| Allele G | 0.473 | 0.269 | | 0.91–6.49 | |
| Genotype T/T | 0.108 | 0.615 | | 0.02–0.35 | 0.05 |
| Genotype T/G | 0.838 | 0.231 | 5.43(0.02)* | 3.62–81.83 | |
| Genotype G/G | 0.054 | 0.154 | | 0.04 – 2.50 | |

* (p < 0.05), ** (p < 0.01), NS: Non-significant.

**Table 3:** Distribution of sample study according to allele and genotype of PTPN-22 (C/T)

| | *Frequency of alleles and genotypes* | | *value χ2* | | *Level of* |
|---|---|---|---|---|---|
| *Alleles and genotypes* | *cases (NO. = 37)* | *Controls (NO = 13)* | *(p)* | *OR[CI 95%]* | *significance* |
| Allele C | 0.541 | 0.731 | 2.88(0.09) NS | 0.16–1.15 | |
| Allele T | 0.459 | 0.269 | | 0.87–6.15 | |
| Genotype C/C | 0.135 | 0.615 | | 0.02–0.42 | 0.05 |
| Genotype C/T | 0.811 | 0.231 | 4.53(0.03)* | 3.09–65.99 | |
| Genotype T/T | 0.054 | 0.154 | | 0.04–2.50 | |

* (p < 0.05), ** (p < 0.01), NS: Non-significant.

By using the MDR method Multifactor Dimensionality Reduction , by this program estimated the incorporation of polymorphic variants between three candidate genes related to the development of Rheumatoid Arthritis. A two-locus model of gene interaction indicates the importance of the combination in the IL2 and PTP in heterozygote's alleles Figure 1. While the three-locus model of gene interaction indicates the significance of the combination of the allelic variants, an increased risk of developing the disease is established if the polymorphism of the IL2 and IL17 and PTP genes in one genome (Figure 2).

The statistical analyses of the results show that the difference is insignificant between polymorphism of gene PTPN -22 and rheumatoid arthritis patients in the Baghdad population. Our result disagrees with those who presented that no association has been seen in many.[11-14] On the other hand, our result agrees with those who reported for the C / T(1858 ) polymorphism in populations.[15,16] According to the result in data on the polymorphic marker of the IL-2 gene show that there is a significant difference between the mutant homozygous A/ A and the mutant heterozygous A/C between the patients of Rheumatoid Arthritis in Baghdad population, this result is identical with reported in many studied.[17-20] This study presented the mutant homozygous T/ T and the mutant heterozygous T / G on the polymorphic marker of the IL17 gene in a sample of patients with RA and showed a considerable increase compared to controls. Identical with which reported by other studied [21] and found significantly in frequencies of the polymorphic marker of the IL17 gene and rheumatoid arthritis patients.[22,23]

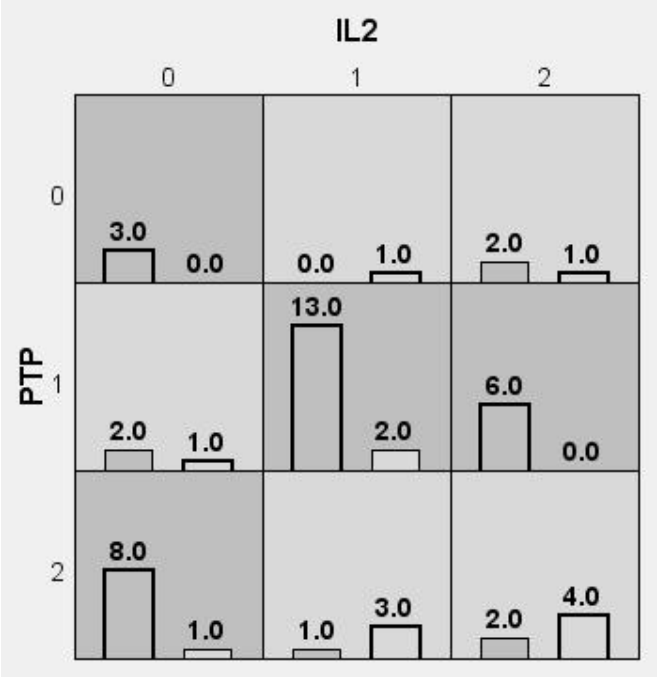


**Figure 1:** Distribution and combination of two-locus genotypes IL-2 and PTPN22 (dark gray mean high-risk genotype, light gray mean low risk)

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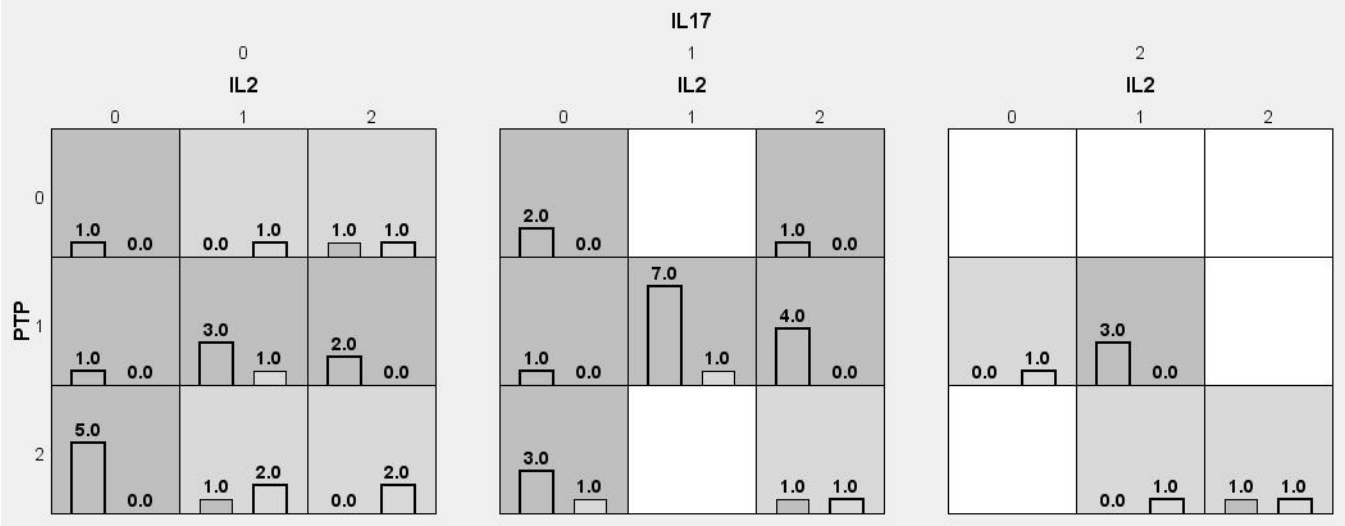


**Figure 2:** Distribution and combination of three locus genotypes IL-2, IL-17, and PTPN22 (dark gray mean high-risk genotype, light gray mean low risk)

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