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Research Article

# Analysis of IFN-γ (+874 A/T) and IL-10 (-1082 G/A) genes polymorphisms with risk of schizophrenia.

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## Abstract

Schizophrenia is a sophisticated mental disability which has affected nearly1.1% of people all over the world. According to recent researches, the key proteins triggered in the immune system are cytokines which might also be taking part in the pathogenesis of schizophrenia. The aim of this study was to evaluate the relationship between the -1082G/A and +874T/A polymorphisms of IL-10 and IFN-γ genes, respectively, in patients with schizophrenia. Total of 94 schizophrenic patients and 97 individuals as control samples were enrolled in this study. All samples were genotyped by amplification mutation refractory system-polymerase chain reaction (ARMS PCR) for candidate SNPs in IFN-γ and IL-10 genes. No significant association was found among various genotypes of IFN-γ and IL-10 in selected SNPs with risk of schizophrenia. As well as there was no significant variation in allelic frequency of IFN-γ and IL-10 genes with the risk of disease**.** These data suggest that the -1082G/A of IL-10 and +874T/A IFNγ genes are not involved in the development of schizophrenia risk. To validate this data, more studies in diverse populations with larger sample size are required.

***Keywords*:** Schizophrenia**,** IFN-γ, IL-10, gene, polymorphism

## Introduction

Schizophrenia is a heterogeneous disorder disclosed by a interruption in cognition and emotion along with negative (abolition, alogia, apathy, poor social functioning) and positive (hallucinations, delusions) symptoms with a worldwide incidence of 1.1% (Rubinov and Bullmore, 2013). Regarding to the macrophage-T cell theory of psychiatric disorders, immune cells (macrophages and T cells) are activated in bipolar disorders and schizophrenia. This hypothesis believed that chronically activated macrophages, microglia and T cells synthesize inflammatory compounds like cytokines that destabilize the brain and lead to schizophrenia (Smith and Maes, 1995) . This possibility that polymorphism of a specific cytokine exhibits susceptible genes for schizophrenia development after infection or ischemia-related insults during the neurodevelopmental process has been proved in vast studies (Paul-Samojedny et al., 2010),(Paul-Samojedny et al., 2011). One of these cytokines that shows alternation in schizophrenia is IL-10 that is located on chromosome 1q31-32, a region previously reported to be linked to schizophrenia in genetic studies ,and is expressed by a variety of cells, such as monocytes, macrophages, microglia, astrocytes, T, B and mast cells (Ekelund et al., 2001),(Peng et al., 2008). IL-10 have various effecs on B and T cells such as initiating B cell differentiation and growth, inhibition the Th1 immune cytokines such as IFN-γ, IL-2 respectively. Also it induces alternative activation of myeloid cells and suppresses lymphocyte effector functions. Morever it was shown recently, that IL-10 prevents glutamate and N-methyl-D-aspartate (NMDA)-mediated cell death in vitro and exerts inhibition of IL-6-mediated excitotoxicity. (Itoh and Hirohata, 1995),(Fiorentino et al., 1991),(Blazevski et al., 2013),(Jun et al., 2003) . The promoter region of this gene has three major putative SNPs; 1082G/A, 819 T/C and -592 C/A. The -1082G/A SNP has known as a higher IL-10-producing allele than others, as well as -G in comparison to A allele has more effect on expression of this anti-inflammatory cytokine (Paul-Samojedny et al., 2010). There is evidence of increased concentration of this Th2 cytokine in the serum of patients with schizophrenia compared to the group of healthy

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(Yu L, 2004 Nov 1),(Ozbey et al., 2009).

Another cytokine express changes in schizophrenia is IFN-γ which is secreted by CD4+ T helper cell, CD8+ cytotoxic lymphocytes, NK cells, B cells, professional antigen-presenting cells (APCs) such as microglia. This pro-inflammatory cytokine that is located on choromosome 12q14 is provital in stimulation of Th1 cytokines and inhibition of Th2 clonal expansion.Furthermore this cytokine prevents of synapse formation, induces class I major histocompatibility complex (MHC) antigen expression on both neuronal and glial cells and also induces MHC class II expression on microglia, some population of astrocytes, and endothelial cells, enhances the function of microglia by increasing the production of some cytokines, nitric oxide, as well as free radicals (Paul-Samojedny et al., 2011),(Kim et al., 2012) .Vast investigations assigned that expression of this Th1 cytokine was significantly reduced due to the functional IFN-γ (+874 A/T) SNP in patients with schizophrenia compared with normal controls (Freudenreich O, 2010 Apr 30). The presence of genotype A/A is linked with low cytokine production, whereas genotype A/T is linked to medium cytokine production, and genotype T/T to high cytokine production, respectively (Pravica et al., 2000). A clear Th2 shift in schizophrenia, may be indicating a possible deregulation of the balance between Th1/Th2 cytokines (Chiang SS, 2013 May). Our present study examines the IFN-γ (+874 A/T) and IL-10 (-1082 G/A) genes polymorphisms with risk of schizophrenia.

## Materials and Methods

Patients were randomly assigned to control and experimental groups.This case-control study was conducted on 94 newly diagnosed and untreated patients with schizophrenia (mean age: 47.53 ± 10.801) and (average of onset age: 20.79 ± 8.729) who were admitted to Azadi and EmamHossein Hospitals during 2010 and 2011 in Tehran, Iran. The control group consisted of 98 subjects with a median age of 46.70+11.716 years who was detached from any signs of neuropsychiatric disorders. All steps of this study were approved by the ethical committee of the university and informed consents were obtained from all patients and healthy in individuals. The demographic characteristics of the participants have been cited in table 1.

### DNA isolation and polymerase chain reaction (PCR)

DNA was extracted from EDTA-collected peripheral whole blood with a method which previously described by Kordi-Tamandani et al (2012) (Kordi-Tamandani et al., 2012).

**Table 1:** The socio-demographic characteristics of the case and control groups

| Variables | Cases | Controls | *P value |
|---|---|---|---|
| Age | 47.53± 10.801 | 46.70± 11.716 | p>0.05 |
| Age of onset | 20.79± 8.729 | - | |
| Sex | | | |
| Females | 26 | 29 | p>0.05 |
| Males | 67 | 70 | |
| Smoking status | | | |
| Non-smokers | - | 54 | P<0.001 |
| Smokers | 93 | 45 | |
| Educational level | | | |
| Illiteracy | 3 | - | |
| Primary school | 8 | 1 | |
| Guidance | 16 | 5 | |
| High school | 53 | 28 | |
| AD | 2 | 26 | P<0.001 |
| BA | 8 | 27 | |
| MA | 1 | 10 | |
| Missing data | 3 | 2 | |
| Marital status | | | |
| Single | 60 | 29 | |
| Married | 17 | 63 | P<0.001 |
| Divorced | 17 | 4 | |

Polymorphisms were analyzed by using amplification mutation refractory system-polymerase chain reaction (ARMS PCR), at positions -597 (rs1800872) in the promoter of the IL10 and +874 IFN-γ genes, the primers have been listed in Table 2. The PCR reaction for these SNPs was carried out in a final volume of 25 µL containing 100 ng genomic DNA, 0.3 mm/L of each primer, 1.5 U Taq DNA polymerase, 2 mm/L $MgCl_2$, 0.25 mm/L dNTPs and 1X PCR buffer. The amplification conditions for IL10 gene were as follows: 94 °C for 5 min, then 35 cycles of 94 °C for 30 Sec, 64 °C for 45 Sec, and 72 °C for 90 Sec, followed by a single cycle of final extension at 72 °C for 10 min. For IFN-γ +874A/T, the reaction was denatured initially at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 48 °C for 30 sec and extension at 72 °C for 30 sec. This response was followed by a final extension step at 72 °C for10 minutes. Finally, PCR products were visualized by 2% agarose gel electrophoresis stained by ethidium bromide. Primer design program was Primer 3.

### Statistical data analyses

The association between polymorphism in IFN-γ and IL-10 genes with the risk of schizophrenia

estimated by computing Odds ratio (OR) and 95% confidence intervals (95% CI) using Epi-Info software (Epi-Info, version3, Center for Disease Control and prevention, Atlanta, GA, USA) and SPSS statistical software version 16 (SPSS, Chicago, IL).

**Table 2.** The list of primers sequence

| Gene | | primer | |
|---|---|---|---|
| IFN-γ +874 A/T | Generic primer | 5'-TCAACAAAGCTGATACTCCA-3' | 262bp |
| | Primer T (sense) | 5'-TTCTTACAACACAAAATCAAATCT-3' | |
| | Primer A (sense) | 5'-TTCTTACAACACAAAATCAAATCA-3' | |
| | Sense: | 5'-GCCTTCCCAACCATTCCCTTA-3' | 429bp |
| | Antisense: | 5'-TCACGGATTTCTGTTGTGTTTC-3' | |
| IL10-1082 G / A | Generic primer (antisense) | 5'-CAGCCCTTCCATTTTACTTC-3' | 550bp |
| | Primer G (sense) | 5'-TACTAAGGCTTCTTTGGGAG-3' | |
| | Primer A (sense) | 5'-CATCTAAGGCTTCTTTGGGAA-3' | 550bp |

## Results

As given in Tables (3&4), TT genotype of IFN-γ was not significant in schizophrenia disorder (OR=4, 95% CI, 0.17-261.5). Also, for AT and combined AT+TT genotypes of IFN-γ have been detected great risk of disease (OR=1.25. 95% CI, 0.20-8.75; OR=1.28, 95% CI, 0.21-8.95) respectively. Analysis of the IL-10 1082G/A genotyping have been highlighted that GA and combined GA +AA genotypes increased the risk of schizophrenia (OR= 0.63, 95% CI, 0.05-5.66; OR=0.64, 95% CI, 0.05-5.72) respectively, but not significant. The allele frequency at T+874A of IFN-γ and G-1082A IL-10 has not demonstrated significant difference between patients and healthy subjects. These data suggest that IL-10 and IFN-γ polymorphism may not confer a susceptibility to the development of schizophrenia at least in the iranian population. Immunological studies in schizophrenia indicated that a shift from Th1 to Th2 immune reactivity has been found to be the most common characteristic immune finding in patients with schizophrenia. A possible mechanisms in the pathogenesis of schizophrenia is that IL-10 is a Th2 cytokine and IFN- γ is a Th1 cytokine that are closely associated with the works of the central nervous system through immunologic, neurochemical, neuroendocrine, and stress-related behavioral activities. Present study has not found any association between IL-10 -1082G/A and IFN-γ +874A/T gene polymorphism with risk of schizophrenia in a sample of the Iranian population. Considering that these two genes do not work alone but functions through a network system and reciprocal interaction with other cytokines, we could suggest that the putative role of IL-10 and IFN-γ influencing the development of schizophrenia would have a complex regulatory function rather than a genetic component. The power of study is important if the negative result comes out.

**Table3.** Allele frequency (%) among individual SCZ cases and healthy controls

| Genes | Cases (%) | Controls (%) | P value |
|---|---|---|---|
| IFN-γ +874 A/T | | | 0.84 |
| T | 93(50%) | 95(48/47%) | |
| A | 93(50%) | 101(51/53%) | |
| IL10-1082 G / A | | | 0.92 |
| G | 92(48/94%) | 95(48/97%) | |
| A | 96(51/06%) | 99(51/03%) | |

**Table 4.** Genotype frequency of IL10-1082 G /A and IFN-γ +874 A/T in SCZ cases and healthy controls.

| SNP | Case | Control | OR | 95%cl | P value |
|---|---|---|---|---|---|
| **IFN-γ** | | | | | |
| AA | 3( 3.22%) | 4(4/08%) | Ref | Ref | Ref |
| AT | 87(93.55%) | 93(94/90%) | 1.25 | 0.20-8.75 | 0.92 |
| TT | 3(3.22%) | 1(1/02%) | 4 | 0.17-261.5 | 0.68 |
| AT+TT | 90(96.77%) | 94(95/92%) | 1.28 | 0.21-8.95 | 0.94 |
| **IL10-1082 G /** | | | | | |
| AA | 3(3/19%) | 2(2/13%) | Ref | Ref | Ref |
| GA | 90(95/74%) | 95(97/94%) | 0.63 | 0.05-5.66 | 0.96 |
| GG | 1(1/1%) | 0 | - | - | 0 |
| GA+AA | 93(98/94%) | 97(1 00%) | 0.64 | 0.05-5.72 | 0.97 |

## Discussion

Several studies have tried to replicate this association in various populations with conflicting results. Samojedny et al (2010) has highlighted that the presence of one or two allele G at position -1082 of IL-10 correlates with increasing risk of paranoid schizophrenia in the Polish population (Paul-Samojedny et al., 2010). In line our data, Ozbey et al (2009) have reported that there is not any association between polymorphism -1082 G/A at the promoter of IL-10 and the risk of schizophrenia (SCZ) in Turkish population (Ozbey et al., 2009). As well as, Yu et al (2004) results have not shown any significant association between IL-10 1082G/A gene polymorphism and progress of SCZ in Chinese population.(Yu L, 2004 Nov 1) A certain study in Spanish population has not uncovered a significant link between the -1082 G/A polymorphic site of the IL-10 gene and risk of schizophrenia (Almoguera B, 2011 Jun 9). These suggest that the contribution of the IL-10 gene promoter polymorphism to the development of schizophrenia may vary among different ethnic groups. Data are sparse regarding the effect of IFN-γ (+874T/A) polymorphism on the development of SCZ. An investigation of the Korean population has been detected a significant difference in genotype distributions and allele frequencies of the IFN-γ +874A/T gene between patients with bipolar disorders and healthy controls (Yoon HK, 2012 Feb). Paul-Samojedny et al (2011) has reported significant difference of IFN-γ (+874T/A) polymorphism between males and female groups in a Polish population (Paul-Samojedny et al., 2011). Frydecka et al (2013) indicated that IL-2, IL-6, IFN-gamma and TGF-beta gene polymorphisms are not linked to the risk of schizophrenia in a sample of Polish population.(Frydecka. et al., 2013) Although, Kim HJ et al (2012) has reported that IFN-γ polymorphisms may be associated with schizophrenia risk in Korean population In conclusion, It seems that the IFN-Gamma (+874 A/T) and IL-10 (-1082 G/A) genotypes studied here do not fully account for the development of SCZ.(Kim et al., 2012) More analyses, with larger sample sizes in various populations are required for more exposing the role of IFN-γ (+874 A/T) and IL-10 (-1082 G /A) in the risk for SCZ.

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