

Association of Interleukin-10, Interferon-gamma, Transforming Growth Factor-beta, and Tumor Necrosis Factor-alpha Gene Polymorphisms With Long-Term Kidney Allograft Survival

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Introduction. Single nucleotide polymorphisms within promoter or other regulatory sequences of cytokine genes mainly influence the level of production and secretion of proteins. A large amount of evidence has shown that cytokine gene variations alter graft survival length after kidney transplantation. We studied the association of gene polymorphisms in the interleukin-10 gene (*IL10*; -1082 G/A), interferon- γ gene (*IFNG*; +874 T/A), transforming growth factor- β gene (*TGFB*; +869 T/C), and tumor necrosis factor- α gene (*TNFA*; -308 A/G) with kidney allograft survival.

Materials and Methods. The *IL10* (-1082 G/A), *IFNG* (+874 T/A), *TGFB* (+869 T/C), and *TNFA* (-308 A/G) genotypes were determined in 32 kidney allograft recipients with graft rejection during the 1st posttransplant year and 52 without rejection in 5 posttransplant years, using allele-specific oligonucleotides-polymerase chain reaction method.

Results. The *IFNG* +874 A/T genotype showed a significantly higher frequency among kidney recipients of the rejection group than the control group (odds ratio, 2.64, 95% confidence interval, 1.03 to 6.74; $P = .04$). Comparisons between the rejection and control groups in *IL10* (-1082 G/A), *IFNG* (+874 T/A), *TGFB* (+869 T/C), and *TNFA* (-308 A/G) single nucleotide polymorphisms showed no significant difference.

Conclusions. Based on the finding of this study, it seems polymorphisms in the genes that regulate IL-10, IFN- γ , TGF- β , and TNF- α cytokines do not play a major role in kidney allograft survival, and other potential factors in this regard should be considered.

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INTRODUCTION

Kidney transplant rejection is one of the major causes of graft dysfunction. Recent investigations based on epidemiological and genetic studies have defined several susceptible genes in kidney transplant rejection process.¹ In addition, several

single nucleotide polymorphisms (SNPs) in the promoter or other regulatory sequences of cytokines, chemokines, and their receptors have been associated with allograft survival.^{2,3} Cytokine polymorphisms are related to the level of cytokine production. Deregulated production of pro- or anti-inflammatory

cytokines plays an important role in the disease susceptibility and progression.⁴ Interleukin-10 (IL-10) is a pleiotropic anti-inflammatory cytokine, and as an immunosuppressor cytokine, is the main inhibitor of tumor necrosis factor- α (TNF- α). Interleukin-10 could expand T helper 2 cell subsets and tolerance process.⁵ The *IL10* polymorphism at position -1082 is located within the binding site of transcriptional factor and may alter level of IL-10 cytokine production and secretion.⁶ The A allele at position -1082 of *IL10* has been associated with lower production of this cytokine, while the G allele has been associated with higher level of this cytokine. In this regard, the low-producer allele of *IL10* -1082 (A allele) has been associated with a high incidence of kidney and heart transplant rejection.⁷⁻¹⁰ The interferon- γ (*IFNG*) gene is mapped on chromosome 12p24. It has been suggested that there are polymorphisms within the first intron of *IFNG* gene.¹¹ This region has 2 alleles, namely 2 and 3. Allele 2 correlates with high production of this cytokine.¹² The *IFNG* first intron microsatellite has been associated with human immunological diseases, such as kidney transplant rejection.¹³ Transforming growth factor- β (*TGFB*) gene is sited on chromosome 19q13.¹⁴ As a multifactorial cytokine, TGF- β inhibits the inflammatory responses of T helper 1 subsets. Zeller and coworkers in 1999 reported that IL-10 and TGF- β have an additive influence on tolerance induction.¹⁵ Two SNPs in the *TGFB* gene have been studied. They are situated at codon 10 (position +869 T/C) and codon 25 (position +915 G/C) of the *TGFB* gene. The high producer alleles are T at codon 10 and G at codon 25.¹⁶ The pro-inflammatory cytokine *TNFA* gene is localized within the major histocompatibility locus on chromosome 6.¹⁷ A bi-allelic SNP in the promoter sequences of *TNFA* gene is at position -308 (G/A). This polymorphism is the binding site of transcription factor activating protein-2.¹⁸ Two alleles of this polymorphism are *TNFA* -308 G and *TNFA* -308 A. It has been suggested that high levels of TNF- α production may be in relation with *TNFA* -308 A.¹⁹ It has been demonstrated that high-producer phenotype of *TNFA* -308 (A allele) is a risk factor for rejection of kidney and cardiac transplants.^{7,10,20} The *IL10* and *TNFA*, regarding low or high producer phenotypes of the donor as well as the recipient, could impact rejection of organ transplants such as the heart.⁷ Since the level of cytokine secretion is under the

influence of genetics and environmental factors, in this study, we aimed to check the possible role and association of *IL10* (-1082 G/A), *IFNG* (+874 T/A), *TGFB* (+869 T/C), and *TNFA* (-308 A/G) SNPs with kidney allograft survival.

MATERIALS AND METHODS

A total of 84 patients with the history of kidney transplantation were selected from nephrology and kidney transplantation unit of Urmia University of Medical Sciences during a period of 2 years (2005 to 2007). Demographic information for all participants was collected. The rejection group was defined as kidney allograft recipients with either histologically proven acute rejection or an acute rise in serum creatinine of more than 20% responding to antirejection therapy in those patients in whom biopsy was contraindicated. Only rejections in the 1st posttransplant year were considered for this group. The criterion for selecting the control group in this study was the length of allograft survival. Minimum allograft survival of 5 years was set as a threshold for the selection of this group.

We isolated DNA from a 5- to 10-mL whole blood sample of all the participants using the standard method of salting out.²¹ We determined *IL10* (-1082 G/A), *IFNG* (+874 T/A), *TGFB* (+869 T/C), and *TNFA* (-308 A/G) genotypes using allele-specific oligonucleotides-polymerase chain reaction (ASO-PCR) method. Primer sets, PCR conditions, and PCR product size are summarized in the Table 1.²²⁻²⁵ Each PCR reaction was performed in a 20- μ L volume containing 50 ng to 100 ng of genomic DNA, 1x reaction buffer 10 pmol of each primer, 200 μ mol of each deoxyribonucleoside triphosphate, 1 unit of Taq DNA polymerase, and 1.5 mmol of MgCl₂. For visualization of the PCR products, after electrophoresis on 2% agarose gel-contained ethidium bromide stain, ultraviolet transilluminator was used. A polaroid picture was taken and documented. Allele and genotype frequencies were determined by direct counting. Fit to Hardy-Weinberg equilibrium, genotype frequencies of *IFNG* +874, *IL10* -1082, *TNFA* -308, and *TGFB* codon 10 were compared using the chi-square test. The level of statistical significance was defined as *P* values less than .05. The odds ratio and 95% confidence interval were calculated by the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA).

Table 1. Cytokine Primers, Polymerase Chain Reaction (PCR) Conditions, and Product Size

Gene Polymorphism	Sequence of Primers and PCR Conditions	PCR Products	Reference
<i>IFNG</i> +874	Common primer: 5'-TCAACAAAGCTGATACTCCA-3', T allele primer: 5'-TTCTTACAACACAAAATCAAATCT-3', A allele primer: 5'-TTCTTACAACACAAAATCAAATCA-3', 10 cycles: 94°C 30 sec, 62°C 50 sec, 72°C 40 sec, and 20 cycles: 94°C 20 sec, 56°C 50 sec, 72°C 40 sec	261 bp	Pravica et al ²²
<i>IL10</i> -1082	Common primer: 5'-CAGCCCTTCCATTTTACTTTTC-3', G allele primer: 5'-TACTAAGGCTTCTTTGGGAG-3', A allele primer: 5'-CTACTAAGGCTTCTTTGGGAA-3', 30 cycles: 94°C 30 sec, 56°C 30 sec, 72°C 30 sec	550 bp	Huang et al ²³
<i>TNFA</i> -308	Common primer: 5'-TCTCGTTTCTTCTCCATCG-3', G allele primer: 5'-ATAGGTTTTGAGGGGCATGG-3', A allele primer: 5'-ATAGGTTTTGAGGGGCATGA-3', 30 cycles: 94°C 30 sec, 61°C 2.5 min, 72°C 1 min	184 bp	Verjans et al ²⁴
<i>TGFB</i> codon10	Common primer: 5'-TCCGTGGGATACTGAGACAC-3', C allele primer: 5'-GCAGCGGTAGCAGCAGCG-3', T allele primer: 5'-AGCAGCGGTAGCAGCAGCA-3', 10 cycles: 95°C 15 sec, 65°C 50 sec, 72°C 40 sec and 25 cycles: 95°C 20 sec, 59°C 50 sec, 72°C 50 sec	241 bp	Li et al ²⁵

RESULTS

Of the 84 transplant recipients, 52 (61.9%) had at least 5 years of graft survival without rejection (control group). In this group, 32 recipients (61.5%) were men and 20 (38.5%) were women. The mean age was 44.7 years. Thirty-two recipients (38.1%) had a history of acute rejection (rejection group), 10 of whom (31.3%) responded to medical therapy,

but 22 (68.7%) required dialysis. The mean age of the kidney allograft recipients in the rejection group was 35.4 years. This group consisted of 22 men (68.7%) and 10 women (31.3%).

The frequencies of *IL10* (-1082 G/A), *IFNG* (+874 T/A), *TGFB* (+869 T/C), and *TNFA* (-308 A/G) genotypes in the rejection group compared to the control group are summarized in Table 2.

Table 2. Frequencies of Studied Cytokine Gene Polymorphisms in Kidney Transplant Recipients With and Without Allograft Rejection

Genotype	Rejection	No rejection	Odds Ratio (95% Confidence Interval)	P
<i>IFNG</i>				
A	31 (48.4)	47 (45.2)	1.13 (0.61 to 2.12)	.68
T	33 (51.6)	57 (54.8)	0.87 (0.47 to 1.63)	.68
A/A	8 (25.0)	17 (32.7)	0.68 (0.25 to 1.84)	.45
A/T	15 (46.9)	13 (25.0)	2.64 (1.03 to 6.74)	.04
T/T	9 (28.1)	22 (42.3)	0.53 (0.20 to 1.37)	.19
<i>TNFA</i>				
A	32 (50.0)	56 (53.8)	0.85 (0.45 to 1.59)	.62
G	32 (50.0)	48 (46.1)	1.16 (0.62 to 2.17)	.62
A/A	3 (9.4)	7 (13.5)	0.66 (0.15 to 2.78)	.57
A/G	26 (81.3)	42 (80.8)	1.03 (0.33 to 3.17)	.95
G/G	3 (9.4)	3 (5.8)	1.68 (0.31 to 8.93)	.53
<i>IL10</i>				
A	35 (54.7)	53 (51.0)	1.16 (0.62 to 2.16)	.63
G	29 (45.3)	51 (49.0)	0.86 (0.46 to 1.60)	.63
A/A	8 (25.0)	8 (15.4)	1.83 (0.61 to 5.50)	.27
A/G	19 (59.4)	37 (71.1)	0.59 (0.23 to 1.49)	.26
G/G	5 (15.6)	7 (13.5)	1.19 (0.34 to 4.12)	.78
<i>TGFB</i>				
T	34 (53.1)	55 (52.9)	1.01 (0.54 to 1.88)	.97
C	30 (46.9)	49 (47.1)	0.99 (0.53 to 1.84)	.97
T/T	9 (28.1)	14 (26.9)	1.06 (0.391 to 2.84)	.90
T/C	16 (50.0)	27 (51.9)	0.92 (0.38 to 2.23)	.86
C/C	7 (21.9)	11 (21.2)	1.04 (0.35 to 3.04)	.93

As shown in this table, *IFNG* +874 A/T genotype showed a significantly higher frequency among kidney recipients of the rejection group than the control group (odds ratio, 2.64, 95% confidence interval, 1.03 to 6.74; $P = .04$).

DISCUSSION

Correlations between cytokine gene polymorphisms and acute graft rejection after kidney transplantation have been reported.^{26, 27} Interleukin-10, as a pleiothropic anti-inflammatory cytokine, is the main inhibitor of TNF- α cytokine.⁵ The A allele of this cytokine's gene (*IL10* -1082) is related to low levels of production and the G allele is related to high production of this cytokine.⁷ Since the presence of the G allele correlated with a higher rate of this cytokine production, it is expected in our kidney transplant recipients without rejection that we see a higher frequency of this polymorphism. This difference was trivial we compared our rejection and control groups (45.3% versus 49.0%; $P = .63$). Alakulppi and colleagues found in 2004 that the low-producer polymorphism of *IL10* -1082 (A allele) could increase the risk of acute rejection.²⁸ However, opposed to their finding, Sankaran and colleagues reported that the presence of the G/G genotype of *IL10* at position -1082 is a risk factor for acute rejection of kidney transplantation.²⁶ Interestingly, there are many studies that have reported that *IL10* -1082 (A/G) polymorphism is not a risk factor for kidney transplant survival.^{8,10,13,26,29-37,42} These controversial findings show that it is really difficult to assume a definite role for this cytokine in preserving or rejection of the allograft, and we should consider other influential factors, such as gene-gene interaction or gene-environmental action, as well.

Regarding *IFNG* polymorphism at position +874, a significant difference was found at genotypes A/T frequency between our rejection and control groups. Interferon- γ is not mainly associated with acute rejection.³⁸ Asderakis and coworkers reported that high-producer genotypes of *IFNG* in combination with *IL10* are associated with acute rejection.¹³ It has been suggested that IL-10 could enhance humoral responses.³⁹ In this regard, the pattern of cytokine gene polymorphisms leads to screening the responder status of patients that are susceptible to rejection. Based on immunosuppression profile

of patients, they could be treated with greater or minimum dose of immunosuppression drugs. In our study, the frequency of high-producer allele of *IFNG* (T allele) was not significantly different the two groups (51.6% versus 54.8%). However, we observed a significantly higher rate of A/T genotype in our rejection group compared to the control group (46.9% versus 25.0%; $P = .04$). Kidney transplant patients have been shown to be susceptible to graft rejection if they have high-producer phenotype of TNF- α only in the context of human leukocyte antigen DR mismatch.^{10,26} In this study, the frequencies of *TNFA* -308 polymorphisms were not significantly different between the two groups. Also, several studies have reported that *TNFA* -308 polymorphism is not a risk factor for kidney transplant rejection.^{8,10,26,29-35,40} However, there were controversial findings that high-producer polymorphisms of *TNFA* -308 (A allele) could increase the risk of acute rejection.^{28,37,41-43}

Concerning the *TGFB* codon 10 polymorphism, we did not find any significant differences in its frequency between the rejection and control groups. Similar results were observed by Tajik and colleagues.³¹ Further studies on this polymorphism is warranted to confirm our finding.

CONCLUSIONS

Based on the finding of this study, it seems IL-10, IFN- γ , TGF- β , and TNF- α cytokines' gene polymorphisms did not play a major role in kidney allograft survival, and other potential genetic factors in kidney transplant survival should be considered.

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CONFLICT OF INTEREST

As corresponding author and on behalf of all co-authors I have no conflict of interest with any commercial or other associations in connection with the submitted article.

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