# Association of IL-12 and TNF-α Polymorphisms with Graft-Versus-Host Disease in Allogeneic Hematopoietic Stem Cell Transplant Recipients

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## ABSTRACT

Background: Cytokines are important factors determining the outcome of transplantation. The host ability in cytokine production may be affected by cytokine genes polymorphisms.

Objective: To investigate the effect of IL-12 and TNF-α gene polymorphisms on outcome of hematopoietic stem cell transplantation.

Methods: 90 bone marrow transplant recipients were included in this study. 30 (33%) of 90 recipients experienced graft-versus-host disease (GVHD). IL-12 and TNF-α gene polymorphisms were evaluated by PCR-RFLP and ARMS-PCR method, respectively.

Results: No significant difference in the distribution of IL-12 (rs3212227 +1188 A/C) and TNF-α (rs 1800629 -308 G/A) genotypes and alleles was observed between those with and without GVHD. There was no significant association between the distribution of genotypes and the recipient sex.

Conclusion: IL-12 (rs3212227 +1188 A/C) and TNF-α (rs 1800629-308 G/A) genotypes and alleles were not risk factors for development of GVHD.

**KEYWORDS:** Interleukin 12; TNF-α; Hematopoietic stem cell transplantation; Graft-versus-host disease; Polymorphism

## INTRODUCTION

The most effective treatment for patients with hematologic malignancies, inherited disorders, and complicated bone marrow failures is allogeneic hematopoietic stem cell transplantation (HSCT) [1, 2]. A major complication after allogeneic HSCT is acute and chronic graft-versus-host disease (GVHD) [3, 4]. The pathophysiology of GVHD includes complex interactions between cellular immune effectors, like donor T cells and host antigen-presenting cells (APC), and soluble effectors, such as inflammatory cytokines, tumor necrosis factor (TNF-α) and interleukin-12 (IL-12) [5]. The proinflammatory cytokine, TNF-α, is produced by monocytes/macrophages and to a lesser extent T cells and B cells. The role of TNF-α is to stimulate macrophage function and increases MHC II antigen expression, which may result in the reactivity of post-transplant alloimmune reactivity [6, 7]. The TNF-α gene is placed on chromosome 6p21.3 [6, 7]. TNF-α expression is firmly controlled at both transcriptional and post-transcriptional levels [6, 7]. Six diallelic polymorphisms in the TNF-α promoter are thought to influence TNF-α production; it has been reported that they occur at positions -1031, -863, -857, -376, -308, and -238 [7, 8]. Some of them are reported to be associated with a difference in their TNF-α production. Kroeger, *et al*, stated that the -308A (adenine) allelic form has a two-fold greater level of transcription than the -308G

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**Table 1:** The primers, types of PCR, restriction enzyme and thermocycling programs for the IL-12 and TNF-α genotyping

| | | | | |
|---|---|---|---|---|
| IL-12 (rs82122227+1188A/C) | Forward primer: 5' CTG ATC CAG GAT GAA AAT TTG G 3' Reverse primer: 5' CCC ATG GCA ACT TGA GAG CTG G 3' | PCR-RFLP (TaqI) | 95 °C 5 min; 35 cycles. 95 °C, 30 s. 60.5 °C, 45 s. 72 °C, 1 min; 72 °C, 5 min | AA: 233 bp CC: 165+68 bp AC: 233, 165, 68 bp |
| TNF-α (rs1800629-308 G/A) | Forward Primer (A allele): 5´-ATAGGTTTTGAGGGGCATGA-3´ Forward Primer (G allele): 5´-ATAGGTTTTGAGGGGCATGG-3´ Common primer (Reverse): 5´-AAGAATCATTCAACCAGCGA-3´ Forward primer (β-acin): 5' GGC GGC ACC ACC ATG TAC CC 3' Reverse primer (β-actin): 5' GGA GGG GCC GGA CTC GTC AT 3' | ARMS-PCR | 95oC, 2 min; 35cycles. 95 oC, 30 sec. 60.3oC, 30 sec. 72 oC, 30 sec ; 72 o C, 5 min | GG: 272 bp AA: 272 bp AG: 272 bp β-actin (internal control): 203bp |

(guanine) form [9]. The high TNF-α production genotype is significantly related to acute rejections of heart [10] and renal transplants [11], and severity of acute GVHD after bone marrow transplant (BMT) [12]. However, cytokines also play an important role in its development. Lately, it has been recognized that some cytokines are produced under genetic control. In the regulatory regions of several cytokine genes, polymorphisms may cause inter-individual differences in cytokine production [13]. As these polymorphism segregate independently, each individual is a mosaic of high-, intermediate-, and low-producing phenotypes. These cytokine polymorphisms are famous for their functional relevance in post-transplant outcome, rejection and GVHD, following solid organ [14-16] and HSCT, respectively [17, 18].

IL-12 is produced primarily by activating inflammatory cells including neutrophils, microglia and dendritic cells (DCs) while these cells are stimulated by various pathogenic or inflammatory agents [19, 20]. It can be produced by keratinocytes and airway epithelial cells as well [21]. IL-12 is a heterodimeric proinflammatory cytokine made up of two disulfide-bounded polypeptide chains, p35 and p40, each encoded by different genes [20, 22]. The gene for the IL-12p40 subunit (IL-12B), is placed on chromosome 5 at 5q31–33 in humans [23, 24]. The p40 subunit is a constituent of both IL-12 p70 and IL-23. The IL-12 heterodimer production requires the coordinated expression of both p40 and p35 chains: when associated with p40, p35 can only be concealed [20]. Several promoter, intron and 3' untranslated region (UTR) polymorphisms are known at IL-12 p40 gene and IL-12B [23, 24]. Lately, single nucleotide polymorphism (SNP) (+1188A/C) was identified at position +1188 in the 3' untranslated region (UTR) of IL-12 p40 subunit gene (IL-12B) [24]. Cytokines gene polymorphisms and their receptors are important candidates for genetic factors in immune-mediated diseases and have been stated to be related to disease susceptibility to autoimmune, inflammatory and infectious diseases [24]. Considering the importance of Th1 and its related cytokines in the HSCT

outcome, and the importance of polymorphism in the function of each gene can lead to change in gene operation, cytokine production and the outcome of HSCT. Therefore, the present study was conducted to investigate the association of SNPs in the genes of TNF-α (rs1800629-308G/A) and IL-12 (rs3212227 +1188 A/C) with GVHD in HSCT recipients.

## MATERIALS AND METHODS

### Patients

A total of 90 HSCT recipients who underwent surgery at Namazi Hospital, Shiraz, southern Iran, was consecutively recruited from 2005 to 2011. All of the patients were Iranian and had transplantation at the Transplant Center of Namazi Hospital affiliated to Shiraz University of Medical Sciences. In this study, the patients were divided into two groups with respect to the presence or absence of GVHD. Acute GVHD was graded according to the classic Glucksbeg-Seattle criteria (GSC) and the International HSCT Registry (IBMTR) [25-27]. Those without GVHD were considered the control group. Signs and symptoms were identified by an expert hematologist team based on the European group for blood and marrow transplantation criteria. This study was approved by Research Ethics Committee of our institute. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Conditioning chemotherapy regimen included busulfan 16 mg/kg or busulfex IV (80% of oral dose) and cyclophosphamide 120–200 mg/kg in leukemia patients (acute myelogenous leukemia [AML], acute lymphogenous leukemia [ALL], and chronic myelogenous leukemia [CML]) and cyclophosphamide 60–120 mg/kg + anti-thymocyte globulin (ATG) 90 kg/mg for severe aplastic anemia and Fanconi's anemia. GVHD prophylaxis consisted of cyclosporine and methotrexate. Prophylactic antibiotic, antifungal, and antiviral drugs were prescribed for all patients. All blood products were irradiated with gamma rays to prevent post-transfusion GVHD. The HLA typing was routine in our center.

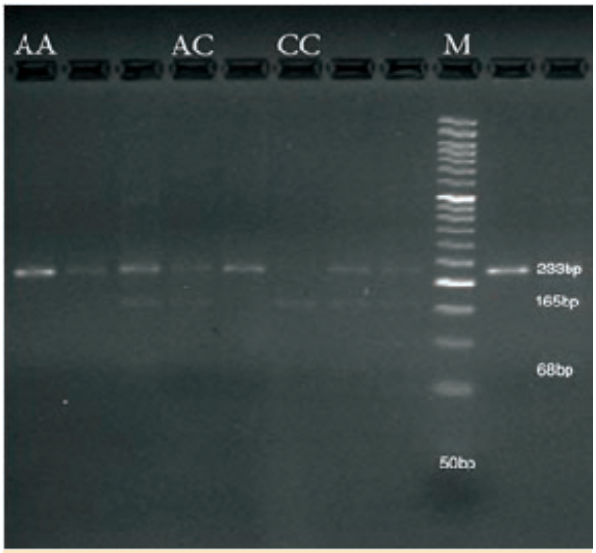


**Figure 1:** Genotyping of the IL-12 (rs3212227+1188C/A) polymorphism using TaqI by RFLP. Lane 1 AA genotype (233 bp), lane 4 indicate AC genotype (233,165, 68 bp), lane 6 CC genotype (165, 68 bp), and lane 9 M DNA size marker (100 bp ladder), respectively.

### DNA Extraction

The buffy coat of the whole blood from bone marrow transplanted patients was available in the sample bank affiliated to Shiraz Transplant Research Center. Genomic DNA was extracted from the buffy coat, using a QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's instructions.

### IL-12, TNF-α Genotyping Protocols

We selected one SNP from the IL-12 (rs3212227 +1188 A/C) gene in chromosome 5q31–33 and one SNP from the TNF-α (rs1800629-308G/A) gene in chromosome 6p21.3. Cytokine gene polymorphisms were evaluated by polymerase chain reaction (PCR) using a thermal cycler (Techne, Genius, UK). PCR conditions, PCR cycles and primers are summarized in Table 1. An in-house ARMS-PCR method was optimized for TNF-α (rs 1800629 -308 G/A) in 25 µL reaction mixture. A β-globin gene primer was used as an internal control. PCR-RFLP was optimized for determining the IL-12 (rs3212227 +1188 A/C) gene polymorphism. The PCR products were digested by TaqI restriction enzyme (Fermentas, Lithunia). The amplified products were monitored by agarose gel electrophoresis and rima staining (Figures 1 and 2).

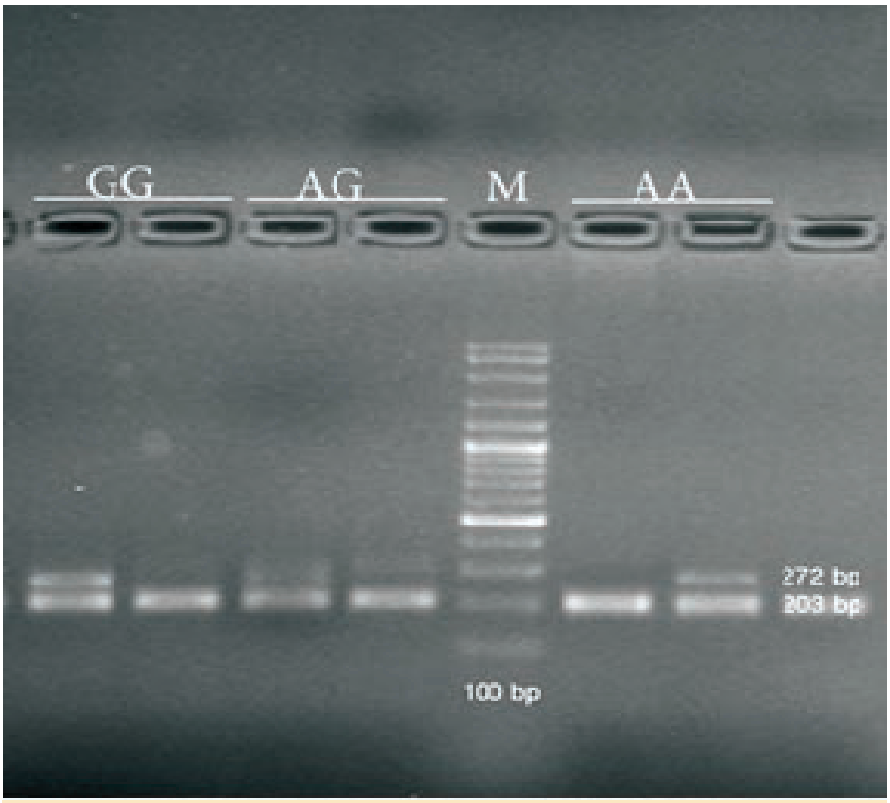


**Figure 2:** Electrophoretic scheme of ARMS-PCR generated bands for determination of TNF-α (rs1800629-308 G/A) gene polymorphism. Left to right: wells #1 and 2: GG homozygous (272 bp); wells #3 and 4: heterozygous AG (272 bp); 5 M: DNA size marker (100 bp); well #6 and 7: homozygous AA (272 bp); and β-actin (internal control) 203 bp.

## Statistical Analysis

Allele and genotype frequencies were calculated in patient and control groups by direct gene counting. Statistical evaluation was carried out using the SPSS® for Windows® *ver* 15. The frequencies of the alleles/genotypes were compared in cases and controls by $\chi^2$ and Fisher's exact tests. A p value <0.05 was considered statistically significant.

**Table 2:** The frequencies of IL-12 and TNF-α genotypes and alleles in HSCT patients with different grades of GVHD

| Locus | Genotype | GVHD (0-I) n (%) | GVHD (II-IV) n (%) | p value | OR (95% CI) |
|---|---|---|---|---|---|
| IL-12 (rs3212227+1188A/C) | AA | 6 (67) | 12 (57) | 0. 62 | 1.50 (0.23–10.45) |
| | CC | 1 (11) | 2 (10) | 0.89 | 1.19 (0–21.39) |
| | AC | 2 (22) | 7 (33) | 0.54 | 0. 57 (0.06–4.49) |
| | A allele | 14 (78) | 31 (74) | 0.74 | 1.24 (0.29–5.63) |
| | C allele | 4 (22) | 11 (26) | | |
| TNF-α (rs 1800629 -308G/A) | GG | 1 (11) | 5 (24) | 0.42 | 0.40 (0.01–4.87) |
| | AA | 1 (11) | 2 (10) | 0.89 | 1.19 (0–21.39) |
| | GA | 7 (78) | 14 (67) | 0.54 | 1.75 (0.22–16.24) |
| | G allele | 9 (50) | 24 (57) | 0.61 | 0.75 (0.21–2.61) |
| | A allele | 9 (50) | 18 (43) | | |

For genotypes, each p value comes from comparing the corresponding row with the sum of other rows.

**Table 3:** The frequencies of IL-12 (+1188 A/C) (rs3212227) and TNF-α (-308G/A) (rs1800629) genotypes and alleles in patients with and without GVHD.

| Locus | Genotype | GVHD n (%) | Non-GVHD n (%) | GVHD Male n (%) | Non-GVHD Male n (%) | GVHD Female n (%) | Non-GVHD Female n (%) | $p_1$ value* | $p_2$ value* | $p_3$ value* |
|---|---|---|---|---|---|---|---|---|---|---|
| IL-12 (rs3212227+1188A/C) | AA | 19 (63) | 42 (70) | 13 (65) | 25 (78) | 6 (60) | 17 (61) | 0.52 | 0.29 | 0.96 |
| | CC | 2 (7) | 5 (8) | 2 (10) | 2 (6) | 0 (0) | 3 (11) | 0.78 | 0.62 | 0.28 |
| | AC | 9 (30) | 13 (22) | 5 (25) | 5 (16) | 4 (40) | 8 (29) | 0.38 | 0.40 | 0.50 |
| | A allele | 47 (78) | 97 (81) | 31 (78) | 55 (86) | 16 (80) | 42 (75) | 0.69 | 0.26 | 0.65 |
| | C allele | 13 (22) | 23 (19) | 9 (23) | 9 (14) | 4 (20) | 14 (25) | | | |
| TNF-α (rs 1800629 -308G/A) | GG | 8 (27) | 14 (23) | 6 (23) | 6 (19) | 1 (10) | 8 (29) | 0.72 | 0.34 | 0.23 |
| | AA | 3 (10) | 3 (5) | 3 (5.6) | 1 (3) | 0 (0) | 1 (4) | 0.37 | 0.11 | 0.54 |
| | GA | 19 (63) | 43 (72) | 11 (71) | 25 (78) | 9 (90) | 19 (68) | 0.42 | 0.07 | 0.17 |
| | G allele | 35 (58) | 71 (59) | 23 (58) | 37 (58) | 11 (55) | 35 (63) | 0.91 | 0.97 | 0.55 |
| | A allele | 25 (42) | 49 (41) | 17 (43) | 27 (42) | 9 (45) | 21 (38) | | | |

*$p_1$ value: GHVD *vs* non-GHVD; $p_2$ value: GHVD *vs* non-GHVD in male patients; $p_3$ value: GHVD *vs* non-GHVD in female patients
For genotypes, each p value comes from comparing the corresponding row with the sum of other rows.

**Table 4:** Relationship between GVHD and the underlying disease

| Underlying disease | GVHD, n (%) | Non-GVHD, n (%) | p Value |
|---|---|---|---|
| AML | 12 (40) | 22 (37) | 0.75 |
| AML ($M_4$) | — | 3 (5) | 0.21 |
| ALL | 7 (23) | 16 (27) | 0.73 |
| CML | 3 (10) | 3 (5) | 0.37 |
| Cooley's anemia | 4 (13) | 5 (8) | 0.45 |
| Aplastic anemia | — | 6 (10) | 0.07 |
| Thalassemia | 2 (7) | 4 (7) | 1 |
| MDS | 2 (7) | — | 0.04 |
| Lymphoma | —— | 1(1.7) | 0.47 |

For genotypes, each p value comes from comparing the corresponding row with the sum of other rows.

## RESULTS

### GVHD Rate

The age of bone marrow transplant recipients ranged between 6 and 55 years. The mean±SD age of the study participants was 24.3±11.4 years. More than half (58%, 52 of 90) of the allogeneic HSCT recipients were male. The male to female ratio (M/F) was 2 in GVHD group and 1.14 in the non-GVHD group. All patients received a graft from related donors. One-third of 90 transplant recipients developed GVHD—nine had grade 0–I, and 21 had grade II–IV GVHD.

### Alleles and Genotypes Frequencies

Alleles and genotypes frequencies for IL-12 (rs3212227 +1188 A/C) and TNF-α (rs 1800629 G/A), were determined in the recipients. No significant difference was observed in the distribution of IL-12 (rs3212227 +1188 A/C) and TNF-α (rs 1800629 G/A) genotypes and alleles between the groups with and without GVHD; nor did it associate with severity of GVHD or the recipient sex (Tables 2 and 3).

### GVHD Rate and the Underlying Diseases

The indications of HSCT are summarized in Table 4. There was a significant (p=0.04) association between MDS, an underlying disease, and GVHD in HSC transplanted patients.

## DISCUSSION

Point mutations or SNPs in the regulatory regions of cytokine genes, namely Th1 and Th2, have been associated with susceptibility to different infectious agents [28-30], autoimmune diseases [31] and allograft outcome [16]. Polymorphisms existing within the 5' or 3' regulatory sequences of genes may change the transcription or translation of the genes. Therefore, they may alter the amounts of cytokine's production [32]. Many studies done in the field of the stem cell transplantation have indicated the relationships between SNPs and HSCT-related complications, including GVHD and early death [33, 34]. Despite the fact that HLA typing is the central means of selecting donors for transplantation, SNPs in cytokine genes determine the immunological state of individuals. Different studies have shown that polymorphisms can be responsible for transplantation outcome in cytokine genes [35]. Other nucleotide variations encoding these molecules in genes may influence the secretion or function of the corresponding proteins [35]. In our study, no difference in the distribution of the IL-12 (rs3212227 +1188 A/C) genotypes and alleles was observed in recipients with and without GVHD; nor did it associate with recipient sex. Gan, *et al*, in their study found that cytokine gene polymorphism can affect the outcome of patients undergoing autologous stem cell transplant (ASCT), especially IL-10 and possibly IL-12 and IL-2 [36]. Reddy, *et al*, showed that after bone marrow transplantation, high levels of IL-12 are related to the improved relapse-free survival without increasing the risk of GVHD [37]. Previous studies showed that individuals with

CC polymorphism of IL-12B +1188 had a significantly higher IL-12 secretion levels compared with those with AC and AA genotypes. They concluded that the existence of the common A allele at +1188 is correlated with lower IL-12 secretion [38]. It has been shown IL-12 has an anti-tumor effect in murine studies and consequently it is possible that a high level of IL-12 may be correlated with a reduction in the risk of relapse [39]. In our study, no significant association was observed in the distribution of the TNF-α (rs 1800629 -308 G/A) genotypes and alleles and the status of GVHD or sex. Several reports have dealt with the measurement of serum TNF-α and its function in predicting GVHD [40]. Some TNF-α direct associations/alleles with acute GVHD were explained for both microsatellite polymorphisms and -308 SNP [12, 41]. In some studies, no correlation between acute and chronic GVHD and patients and donors with TNF-α (rs 1800629 -308 G/A) genotypes could be found [12, 18, 42]. Bogunia-Kubik, *et al*, found TNF polymorphisms correlated with toxic but not with acute GVHD complications [6]. This observation could possibly be related to use of cyclosporine as a therapeutic choice, which has been stated to remove the higher response seen in TNF-α (-308G/A or A/A) subjects [43]. It is possibly related to the interruption of 5' regulation of transcription of TNF-α in T cells and macrophages [44]. Mayer, *et al*, have recently completed a study on 53 CML allograft recipients showing a potential role of the -308 polymorphism in severe GVHD [45]. That study was limited to CML patients and consisted of matched unrelated donor transplants as well as HLA-identical siblings and therefore, may not be compared with the present direct study [45]. In summary, donor- or recipient-derived TNF-α (-308) and donor-derived IL-10·G may contribute to the development of severe acute and chronic GVHD, respectively [12]. The difference in the cytokine gene polymorphism connected with each GVHD type may show differences in the pathogenesis and the clinical features of each type [12]. In conclusion, GVHD is a multifactor phenomenon, and cytokines have major roles in this process. We did not find any association between IL-12 and TNF-α gene polymorphism and GVHD outcome. However, this could be attributed to our low study power. The study should be conducted on larger samples and in different populations.

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## REFERENCES

1. Ferrara JL. Advances in the clinical management of GVHD. *Best Pract Res Clin Haematol* 2008;**21**:677–682.
2. Markey KA, MacDonald KP, Hill GR. Impact of cytokine gene polymorphisms on graft-vs-host disease. *Tissue Antigens* 2008;**72**:507–516.
3. Noori-Daloii MR, Jalilian N, Izadi P, *et al*. Cytokine gene polymorphism and graft-versus-host disease: a survey in Iranian bone marrow transplanted patients. *Mol Biol Rep* 2013;**40**:4861-7.
4. Resende RG, Correia-Silva Jde F, Arão TC, *et al*. Investigation of Functional IL-10 Gene Polymorphism and IL-10 Levels in Acute Graft-Versus-Host Disease. *J ClinImmunol* 2010;**30**:465-73.
5. Hill GR, Crawford JM, Cooke KR, *et al*. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. *Blood* 1997;**90**:3204-13.
6. Bogunia-Kubik K, Polak M, Lange A. TNF polymorphisms are associated with toxic but not with aGVHD complications in the recipients of allogeneic sibling haematopoietic stem cell transplantation. *Bone Marrow Transplant* 2003;**32**:617-22.
7. Ziaei R, Ayatollahi M, Yaghobi R, *et al*. Involvement of TNF-α in differential gene expression pattern of CXCR4 on human marrow-derived mesenchymal stem cells. *Molecular Biology Reports* 2014;**41**:1059-66.
8. Bidwell J, Keen L, Gallagher G, *et al*. Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* 1999,**1**:3-19.
9. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol Immunol* 1997; **34**:391–399.
10. Turner D, Grant SC, Yonan N, *et al*. Cytokine gene polymorphismand heart transplant rejection. *Transplantation* 1997;**64**:776-9.
11. Sankaran D, Asderakis A, Ashraf S, *et al*. Cytokine gene polymorphisms predict acute graft rejection following renal transplantation. *Kidney Int* 1999;

**56**:281-8.

12. Iravani-Saadi MI, Karimi MH, Yaghobi R, *et al*. Polymorphism of costimulatory molecules (CTLA4, ICOS, PD.1 and CD28) and allogeneic hematopoietic stem cell transplantation in Iranian patients. *Immunol Invest* 2014;**43**:391-404.
13. Saadi MI, Yaghobi R, Karimi MH, *et al*. Association of the costimulatory molecule gene polymorphisms and active cytomegalovirus infection in hematopoietic stem cell transplant patients. *Mol Biol Rep* 2013;**40**:5833-42.
14. Karimi MH, Daneshmandi S, Pourfathollah AA, *et al*. The IFN-gamma allele is correlated to moderate-to-severe acute graft-versus-host disease after allogeneic stem cell transplant. *Exp Clin Transplant* 2010;**8**:125-9.
15. Karimi MH, Hejr S, Geramizadeh B, *et al*. Combined analysis of cytokine gene polymorphism and the level of expression with allograft function in kidney transplant recipients. *Transpl Immunol* 2014;**30**:46-51.
16. Fernandes H, Koneru B, Fernandes N, *et al*. Investigation of promoter polymorphisms in the tumor necrosis factor-a and interleukin-10 genes in liver transplant patients. *Transplantation* 2002;**73**:1886-91.
17. Tambur AR, Yaniv I, Stein J, *et al*. Cytokine gene polymorphism in patients with graft-versus-host disease. *Transplant Proc* 2001;**33**: 502-3.
18. Socie´ G, Loiseau P, Tamouza R, *et al*. Both genetic and clinical factors predict the development of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Transplantation* 2001;**72**: 699-706.
19. Trinchieri G, Pflanz S, Kastelein RA, *et al*. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity* 2003;**19**:641-4.
20. Trinchieri, G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003;**3**:133-46.
21. Mikols CL, Yan L, Norris JY, *et al*. IL-12 p80 is an innate epithelial cell effector that mediates chronic allograft dysfunction. *Am J Respir Crit Care Med* 2006;**174**:461-70.
22. Hoelscher C. The power of combinatorial immunology: IL-12 and IL-12-related dimeric cytokines in infectious diseases. *Med MicrobiolImmunol* 2004;**193**:1-17.
23. Hall MA, McGlinn E, Coakley G, *et al*. Genetic polymorphism of IL-12 p40 gene in immunemediated disease. *Genes Immun* 2000;**1**:219-24.
24. Huang D, Cancilla MR, Morahan G. Complete primary structure, chromosomal localization and definition of polymorphisms of the gene encoding the human interleukin-12 p40 subunit. *Genes Immun* 2000;**1**:515-20.
25. Glucksberg H, Storb R, Fefer A, *et al*. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA matched sibling donors. *Transplantation* 1974;**18**:295-304.
26. Thomas ED, Storb R, Clift RA, *et al*. Bone-marrow transplantation (second of two parts). *N Engl J Med* 1975;**292**:895-902.
27. Rowlings PA, Przepiorka D, Klein JP, *et al*. IBMTR severity index for grading graft-versus-host disease: retrospective comparison with Glucksberg grade. *Br J Haematol* 1997;**97**:855-64.
28. Cipriano C, Caruso C, Lio D, *et al*. The -308G/A polymorphism of TNF-alpha influences immunological parameters in old subjects affected by infectious diseases. *Int J Immunogenet* 2005;**32**:8-13.
29. Santos AR, Suffys PN, Vanderborght PR, *et al*. Role of tumor necro- sis factor-a and interleukin-10 promoter gene polymorphisms in leprosy. *J Infect Dis* 2002;**186**:1687-91.
30. Lu LP, Li XW, Liu Y, *et al*. Association of -238G/A polymorphism of tumor necrosis factor-alpha gene promoter region with outcomes of hepatitis B virus infection in Chinese Han population. *World J Gastroenterol* 2004;**10**:1810-14.
31. Roxo VM, Pereira NF, Pavoni DP, *et al*. Polymorphisms within the tumor necrosis factor and lymphotoxin-alpha genes and endemic pemphigus foliaceus – are there any associations? *Tissue Antigens* 2003;**62**:394-400.
32. Karabon L, Wysoczanska B, Bogunia-Kubik K, *et al*. IL-6 and IL-10 promoter gene polymorphisms of patients and donors of allogeneic sibling hematopoietic stem cell transplants associate with the risk of acute graft-versus-host disease. *Hum Immunol* 2005;**66**:700-10.
33. Dickinson AM, Charron D. Non-HLA immunogenetics in hemato- poietic stem cell transplantation. *Curr Opin Immunol* 2005;**17**:517-25.
34. Bettens F, Passweg J, Gratwohl A, *et al*. Association of TNFd and IL-10 polymorphisms with mortality in unrelated hematopoietic stem cell transplantation. *Transplantation* 2006;**81**:1261-7.
35. Fishman D, Faulds G, Jeffery R, *et al* . The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998;**102**:1369-76.
36. Gan G.G, Leong Y.C, Bee P.C, *et al*. Influence of genetic Polymorphisms of cytokine genes in the outcome of HLA-matched allogeneic stem cell transplantation in a South East Asian population. *Cytokine* 2016;**78**:55-61.
37. Reddy V, Winer AG, Eksioglu E, *et al* . Interleukin 12 is associated with reduced relapse without increased incidence of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transpl* 2005;**11**:1014–21.
38. Yilmaz V, Yentur S.P, Saruhan-Direskeneli G. IL-12 and IL-10 polymorphisms and their effects on cytokine production. *Cytokine* 2005;**30**:188-194.

39. Cocco C, Ferretti E, Airoldi I, *et al*. Cytokines as anti-angiogenic agents in hematological malignancies, Curr. *Cancer Drug Targets* 2011;**11**:997–1004.

40. Holler E, Ferrara JML. Antagonists of inflammatory cytokines: prophylactic and therapeutic applications, in Ferrara JLM, Deeg HJ, Burakoff SJ (eds): *Graft versus Host Disease*. New York, NY, Dekker, **1997**; 667.

41. Nordlander A, Uzunel M, Mattsson J, *et al*. The TNFd4 allele is correlated to moderate-to-severe acute graft-versus-host disease after allogeneic stem cell transplantation. *British J Haematol* 2002;**119**: 1133-6.

42. Tambur AR, Yaniv I, Stein J, *et al*. Cytokine gene polymorphism in patients with graft-versus-host disease. *Transplant Proc* 2001;**33**: 502-503.

43. Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. *Immunol Today* 1992;**13**: 136-142.

44. McCaffrey PG, Goldfeld AE, Rao A. The role of NFATp in cyclosporine A-sensitive tumor necrosis factor-alpha gene transcription. *J BiolChem* 1994;**269**: 30445-50.

45. Mayer FR, Messer G, KnabeW, *et al*. High response of TNFa secretion in vivo in patients undergoing BMT may be associated with the 2308 bp TNF-a-gene enhancer-polymorphism. *Bone Marrow Transplant* 1996;**17**:s101.