Research Article

Suleyman Rustu Oguz*, Hayriye Senturk Ciftci, Muge Gokce, Yeliz Ogret, Demet Kivanc, Kursat Oezdilli, Avni Atay, Fatma Savran Oguz and Filiz Aydin

HLA DRB1 alleles, IFN-γ and TGF-β Gene Variants in childhood ALL patients

Çocukluk Çağında ALL Hastalarında HLA DRB1 Alelleri, IFN-γ ve TGF-β Gen Varyantları

URL: https://doi.org/10.1515/tjb-2021-0202
Received September 10, 2021; accepted April 11, 2022; published online May 24, 2022

Abstract

Objectives: Graft-versus-host disease (GvHD) is a complex clinical syndrome with organ dysfunction as a consequence of a severe immunological reaction mediated by mainly T cells after hematopoietic stem cell transplantation. Our aim is to evaluate the association of HLA-DRB1 alleles, IFN-γ and TGF-β gene variations, with childhood ALL (c-ALL) patients and with GvHD after transplantation.

Methods: This study included 30 high-risk c-ALL patients and 100 controls. HLA-DRB1 alleles were studied by the NGS method, and TGF-β and IFN-γ variations were studied by the PCR-RFLP method.

Results: The rates of HLA-DRB1*15 alleles and IFN-gamma CC genotype were significantly higher in c-ALL patients (p=0.004, p=0.036 respectively). Association of the HLA-DRB1*15 alleles with the TGF-β TC genotype was found with a higher rate in the patient group (p=0.031). Association of the DRB1*04 allele with the IFN-γ CC genotype was found with a higher rate in the patient group (p=0.028). Acute GvHD developed in eight of 19 patients who underwent transplantation. IFN-γ CT was found to have a protective role in occurrence of aGvHD (p=0.044). Association of the DRB1*15 allele with IFN-γ TT was found with a higher rate in a GvHD (p=0.050).

Conclusions: It is thought that polymorphism of HLA-DR15 and IFN-γ CC may contribute to the development of c-ALL, while IFN-γ CT might be protective for aGvHD.

Keywords: acute lymphoblastic leukemia; aGvHD; cytokine; genetic polymorphism; HLA.

Özet

Amaç: Graft-versus-host hastalığı (GvHD), hematopoietik kök hücre transplantasyonundan sonra esas olarak T hücrelerinin aracılığı ile organ disfonksiyonuna neden olan kompleks bir klinik sendromdur. Amacı c-ALL hastaların ve GvHD’den etkilenen hastalarda HLA-DRB1 allelleri, IFN-γ ve TGF-β gen varyasyonlarının ilişkisi araştırılmasıdır.

Gereç ve Yöntemler: Çalışıma 30 yüksek riskli c-ALL hastası ve 100 kontrol dahil edildi. HLA-DRB1 allelleri NGS, TGF-β ve IFN-γ varyasyonları PCR-RFLP yöntemleriyle tespit edildi.

Bulgular: HLA-DRB1*15 allelleri ve IFN-γ CC genotipi, c-ALL hastasında anlamlı olarak daha yüksek sıklıkta (srasıyla p=0,004, p=0,036). HLA-DRB1*15 allelleri ile TGF-β TC genotipi ile birlikteliği hastada daha yüksekti bulundu...
(p=0.031). DRB1*04 allelinin IFN-γ CC genotipi ile birlikte daha yüksek bulundu (p=0.028). Akut GvHD, nakledilen 19 hastanın 8’inde gelişti. IFN-γ CT genotipinin GvHD’nin ortaya çıkmamasında korumucu bir rolü olduğu bulundu (p=0.044). DRB1*15 allelinin IFN-γ TT ile birlikteği akut GvHD’de daha yüksek (p=0.050).

**Sonuç:** HLA-DR15 ve IFN-γ CC polymorfoliminin c-ALL gelişimine katkı sağlayabileceği, IFN-γ CT’nin ise akut GvHD’den korumucu olabileceği düştülmektedir.

**Anahtar Kelimeler:** Akut lenfoblastik lösemi; sitokin; genetik polymorfizm; akut GvHD; HLA.

**Introduction**

Graft-versus-host disease (GvHD) is a clinical condition that occurs as a consequence of the immunologic mismatch for minor antigens, and for other unknown antigens in patients who are matched for Human Leukocyte Antigen (HLA) antigens to the immunocompetent lymphohematopoietic cells received from a healthy donor. GvHD occurs during the response of the donor’s T cells to the genetically described proteins on the host cells [1]. HLA genes in the Major Histo-compatibility Complex (MHC) gene complexes have a significant effect on the cause of GvHD after stem cell transplant. In addition to the HLA genes, other causative factors for GvHD include age, sex difference, and pregnancy. The prevalence of acute GvHD has been reported to range between 30 and 50% in fully HLA matched allogeneic hematopoietic stem cell transplant (AHSCT) series [2–5].

The prevalence of acute GvHD is directly associated with HLA mismatch. Therefore, HLA A, B, C, and DR must be matched in patients and in related donors. In addition, some γ pathways other than direct T cell-mediated cytotoxicity have been shown to be important in the pathogenesis of GvHD. Higher tumor necrosis factor (TNF-α), and lower interleukin-10 (IL-10) levels in pretransplant patients have been shown to cause GvHD [1, 6].

Acute GvHD is characterized by extreme inflammatory response during the production of various proinflammatory cytokines. Production of various inflammatory cytokines contributes to cytokine storm exacerbating GvHD and also causing a higher level of proinflammatory cytokine production by T lymphocytes, maintaining the disease period, and enhancing the ability to attack the host tissue [7]. HLA class II proteins (HLA-DR, HLA-DP, and HLA-DQ) play a key role in the regulation of immune reactions. HLA class II molecules are expressed by B cells, macrophages, and dendritic cells, all of which have the antigen-presenting ability. The expression level of human leukocyte antigen (HLA) class II antigens is regulated by interferon-gamma (IFN-gamma) depending on the status of class II trans-activator protein (CIITA) which is a coactivator of MCH class II. Interferon-gamma (IFN-γ) is an innate proinflammatory pleiotropic cytokine that has a key role in encouraging adaptive host defense mechanisms [1, 2]. Interferon-gamma (IFN-γ) released by T cells and natural killer (NK) cells is important in the conduction of T helper cell type 1 (Th1) responses [8].

Transforming growth factor-β (TGF-β) is a pleiotropic cytokine that regulates cell growth, differentiation, apoptosis, migration, cell adhesion, and immune response. TGF-β activates Smad signaling via its two cell surface receptors, TbetaRII and ALK5/TbetaRI, in the classical TGF-beta signaling pathway. Disruption of the TGF-β pathway has been implicated in many human diseases, including solid and hematopoietic tumors. In children with T-cell acute lymphoblastic leukemia (ALL), SMAD3 protein is absent or significantly reduced, but SMAD3 mRNA is detected at similar levels in T-cell ALL and normal T cells. The SMAD3 level is decisive in terms of T-cell response to TGF-β. It was proven that the loss of Smad3 and p27KIP1, which is also frequently altered in human T-cell ALL, promoted T-cell leukemogenesis in mice [9, 10]. It was demonstrated that TGF-β down-regulated IFN-gamma-induced CIITA and HLA-DR expression in cultured human glomerular endothelial cells (HGECs) and it is thought that there is an antagonism between IFN-γ and TGF-β1 [11].

Although the role of Th1 and Th2 in the pathogenesis of GvHD is yet controversial, classically, Th1 cells are suggested to mediate the acute form of GvHD. Allthough this paradigm is controversial, acute GvHD may be stimulated by Th2 cells, and by T cells with a deficiency of Signal transducer and activator of transcription-4 (STAT-4) or (IFN-γ). These data suggested that not only was IFN-γ not critical for the generation of acute GvHD, but that its absence also exacerbated GvHD lethality [12].

We aimed to investigate the contribution of HLA-DRB1 alleles, IFN-γ and TGF-β gene variations in patients with childhood ALL (c-ALL), and the development of GvHD after AHSCT in our study.

**Materials and methods**

Thirty (19 Males/11 Females) high-risk c-ALL patients, and 100 Controls (36 Males/64 Females) who were consecutively admitted to the Pediatric Hematology Unit of Yeniyüzü University, Medical Faculty Gaziosmanpaşa Hospital were included in the study. The mean age of the patients was 9.77 ± 5.20 years and the interval was 2–17 years. The mean age of the control group was 35.29 ± 8.4 years, and the interval
was 20–35 years. The control group was from the same geographic region. Demographic information of the patients and controls is given in Supplementary Table 1. The HLA-DRB1 alleles for the patients and controls were studied by using the Next Generation Sequencing (NGS) technology Illumina Miseq Sequencing system.

**Next Generation Sequencing (NGS) technology Illumina Miseq sequencing system**

To generate targeted sequencing data, all samples of total DNA were extracted from white blood cells using the Blood DNA Extraction kit (Qiagen). Contiguous full-length PCR amplifications of six HLA loci were carried out for the patients (30) and controls (100). Samples were prepared using the GenDX Library Prep kit for NGS for up to seven loci (full length HLA-A, B, C and DQB1, and intron 1 to intron four of DRB1, and intron 1–3’ UTR of DPB1) and were sequenced in both an individual and pooled amplicon approach using 2 × 251 bp reads on the Illumina MiSeq. Analysis programs were used to genotype the NGS data, NGSengine by GenDX [13].

IFN-γ rs2069705 (−1616/C/T) and TGF-β1 [T869C (Leu10Pro)] polymorphisms; The products were digested with the restriction enzyme PvuII for IFN-γ. The PCR product was digested overnight at 37 °C in a water bath with a restriction enzyme. IFN-γ (−1616/C/T) homozygous T/T alleles give three bands (193, 110, and 19 bp) on gel. Subjects bearing the IFN-γ homozygous C/C alleles also give three bands (174, 110, and 19 bp). However, the same analysis performed on subjects bearing the IFN-γ heterozygous T/C alleles gives four bands (193, 174, 110, and 19 bp) [14].

The products were digested with the restriction enzyme MspA1 for TGF-β1. MspA1 digestion was performed at 37 °C for overnight incubation for TGF-β1. After enzyme digestion, products were visualized by electrophoresis on 3% agarose gel. The MspA1 restriction site on the T allele yields fragments 161, 67, 40, and 26 bp; the C allele yields fragments 149, 67, 40, 26, and 12 bp [14].

For PCR-RFLP TGF-β1 (T869C (Leu10Pro) C/T codon 10); 5’-TTC AAG ACC ACC CAC CCT CT-3’
5’-TCG CGG GTG CTG TTGATAC A-3’ primers, and MspA1 enzyme were used.

For IFN-γ rs2069705 (−1616/C/T); 5’-TAGCACTTTATGAGGATTAC-3’
5’-AGGTAAT CCTCATAAAGTGC-3’ primers, and Pvu II enzyme were used.

Following genotyping process, five samples were selected randomly and sequenced for verification.

**Statistical method**

Statistical Package for the Social Sciences (SPSS) 21.0 software was used for the statistical analysis of the data. Descriptive analyses, and descriptive statistical methods were applied for the data. The comparison of categorical variables was identified with frequency, and ratios and the results were represented as percentages. The chi-square test or Fisher’s exact test was used to detect the differences between grouped data. The Bonferroni correlation was used for the posthoc test in pairwise comparisons to determine which groups showed statistically significant differences. The Hardy-Weinberg Equilibrium (HWE) analysis was performed for IFN-gamma and TGF-β genotype distributions in the patients and healthy controls. p values of <0.05 were considered statistically significant.

**Results**

The CC genotype in IFN-γ C/T polymorphism (has high expression) in the patient group (73.3%) was found to be higher compared to the control group (51.0%) (p=0.036). On the other hand, the CT genotype (has moderate expression) was found to be lower in the patient group (16.7%) compared to the control group (42.0%) (p=0.016). No statistical significance was detected in TGF-β variations between the patient and control groups (Supplementary Table 2). The frequencies of HLA-DRB1, DRB3, 4, 5 alleles belonging to the patient and control groups are given in Supplementary Table 3. Among the HLA-DRB1 alleles shown in the table, DRB1*01:01 and DRB1*07:01 alleles were found with a high frequency in the patient group with a high-resolution tissue typing. For the HLA-DRB1*04 allele which was found with the highest frequency in the patient group, HLA-DRB1*04:02 and HLA-DRB1*04:04 alleles were found with a high rate. In the control group, DRB1*03:01 and DRB1*07:01 alleles were found with a high frequency. For the HLA-DRB1*04 allele, which was found with the highest frequency in the control group, HLA-DRB1*04:03 and HLA-DRB1*04:08 alleles were found with a high frequency.
	aGvHD developed in eight out of 19 patients (42.1%) who underwent a transplant. No association was detected between the TGF-β variations and aGvHD in the patient group. However, the (CC) genotype of IFN-γ in the patients who developed aGvHD (87.5%) was detected with a higher level compared to the patients who did not develop aGvHD (54.5%), though the difference was not statistically significant (p=0.177). The frequency of CT genotype of IFN-γ was found to be lower in the patients who developed aGvHD (25.0%) compared to the patients who did not develop aGvHD (36.3%). It was found that the IFN-γ (CT) genotype had a protective role in the emergence of aGvHD (p=0.044) (Supplementary Table 4).

The association between DRB5 (DR51) and the IFN-γ (CT) genotype which are inherited from DRB1*15 and *16 alleles was found with a higher rate in the control group (54.1%) compared to the patient group (28.2%), and this difference was found to be statistically significant (p=0.023) (Table 1).

No association between the HLA-DRB1*15 alleles, and IFN-γ variations was shown in the patient and control groups. However, the association of HLA-DRB1*15 alleles with the TGF-β (CT) genotype was found with a higher rate
Table 1: HLA-DR supertype antigens of the patients, and the association with TGF-β, IFN-γ variations.

| DR51+ | Patient (n:30) | Control (n:100) | Fisher’s p |
|-------|----------------|-----------------|------------|
| DR51+ |                 |                 |            |
| DR51 + IFN-γ/C | 62.6% | 35.9% | 0.245 |
| DR51 + IFN-γ/T | 28.2% | 54.1% | 0.023 |
| DR51 + IFN-γ/T | 9.2% | 10.0% | 0.745 |
| DR51+ | Patient (n:30) | Control (n:100) | p |
| DR51 + TGF-β/C | 55.2% | 44.2% | 0.678 |
| DR51 + TGF-β/T | 34.7% | 48.1% | 0.324 |
| DR51 + TGF-β/T | 10.1% | 7.7% | 0.145 |
| DR52+ | Patient (n:30) | Control (n:100) | p |
| DR52 + IFN-γ/C | 59.1% | 52.2% | 0.876 |
| DR52 + IFN-γ/T | 27.8% | 32.1% | 0.528 |
| DR52 + IFN-γ/T | 13.1% | 15.7% | 0.941 |
| DR52+ | Patient (n:30) | Control (n:100) | p |
| DR52 + TGF-β/C | 61.0% | 58.7% | 0.423 |
| DR52 + TGF-β/T | 30.4% | 33.1% | 0.634 |
| DR52 + TGF-β/T | 8.6% | 8.2% | 0.937 |
| DR53+ | Patient (n:30) | Control (n:100) | p |
| DR53 + IFN-γ/C | 61.7% | 65.6% | 0.687 |
| DR53 + IFN-γ/T | 36.7% | 34.6% | 0.746 |
| DR53 + IFN-γ/T | 11.6% | 7.8% | 0.068 |
| DR53+ | Patient (n:30) | Control (n:100) | p |
| DR53 + TGF-β/C | 59.6% | 61.4% | 0.832 |
| DR53 + TGF-β/T | 24.5% | 24.4% | 0.999 |
| DR53 + TGF-β/T | 15.9% | 14.2% | 0.831 |

Table 2: Comparison of the HLA-DRB1*15, 16, 04, 07, 09 alleles and the association with the TGF-β, IFN-γ variations in the patients and control.

| DRB1*15+ | Patient (n:30) | Control (n:100) | Fisher’s p |
|----------|----------------|-----------------|------------|
| DRB1*15+ |                 |                 |            |
| DRB1*15 + IFN-γ/C | 5.0% | 3.0% | 0.614 |
| DRB1*15 + IFN-γ/T | 3.3% | 1.5% | 0.838 |
| DRB1*15 + IFN-γ/T | 3.3% | 0.5% | 0.074 |
| DRB1*15+ | Patient (n:30) | Control (n:100) | p |
| DRB1*15 + TGF-β/C | 1.7% | 0.5% | 0.567 |
| DRB1*15 + TGF-β/T | 8.3% | 1.5% | 0.031 |
| DRB1*15 + TGF-β/T | 1.7% | 2.5% | 0.090 |
| DRB1*16+ | Patient (n:30) | Control (n:100) | p |
| DRB1*16 + IFN-γ/C | 8.3% | 2.5% | 0.047 |
| DRB1*16 + IFN-γ/T | 0.0% | 2.0% | - |
| DRB1*16 + IFN-γ/T | - | - | - |
| DRB1*16+ | Patient (n:30) | Control (n:100) | p |
| DRB1*16 + TGF-β/C | 0.0% | 0.5% | 0.600 |
| DRB1*16 + TGF-β/T | 6.7% | 2.0% | 0.097 |
| DRB1*16 + TGF-β/T | 1.7% | 2.0% | 0.360 |
| DRB1*16+ | Patient (n:30) | Control (n:100) | p |
| DRB1*16 + IFN-γ/C | 16.7% | 8.5% | 0.028 |
| DRB1*16 + IFN-γ/T | 3.3% | 7.5% | 0.116 |
| DRB1*16 + IFN-γ/T | 0.0% | 2.0% | 0.559 |
| DRB1*16+ | Patient (n:30) | Control (n:100) | p |
| DRB1*16 + TGF-β/C | 0.0% | 2.0% | 0.645 |
| DRB1*16 + TGF-β/T | 6.7% | 5.5% | 0.857 |
| DRB1*16 + TGF-β/T | 13.3% | 10.5% | 0.609 |
| DRB1*17+ | Patient (n:30) | Control (n:100) | p |
| DRB1*17 + IFN-γ/C | 5.0% | 5.5% | 0.932 |
| DRB1*17 + IFN-γ/T | 1.7% | 3.5% | 0.477 |
| DRB1*17 + IFN-γ/T | 1.7% | 0.5% | 0.288 |
| DRB1*17+ | Patient (n:30) | Control (n:100) | p |
| DRB1*17 + TGF-β/C | 0.0% | 2.0% | 0.663 |
| DRB1*17 + TGF-β/T | 1.7% | 2.5% | 0.771 |
| DRB1*17 + TGF-β/T | 6.7% | 5.0% | 0.269 |
| DRB1*19+ | Patient (n:30) | Control (n:100) | p |
| DRB1*19 + IFN-γ/C | 1.7% | 0.5% | 0.899 |
| DRB1*19 + IFN-γ/T | - | - | - |
| DRB1*19 + IFN-γ/T | - | - | - |
| DRB1*19+ | Patient (n:30) | Control (n:100) | p |
| DRB1*19 + TGF-β/C | 1.7% | 0.5% | 0.899 |
| DRB1*19 + TGF-β/T | - | - | - |
| DRB1*19 + TGF-β/T | - | - | - |

HLA, Human Leukocyte Antigen; TGF-β, Transforming growth factor-β; IFN-γ, Interferon-gamma. Bold values indicate significant values (p<0.05).

in the patient group (8.3%) compared to the control group (1.5%) (p=0.031). The association of HLA-DRB1*16 alleles with the IFN-γ (CT) genotype was found with a higher rate in the control group (2.0%) compared to the patient group (0.0%) (p=0.047). The association of DRB1*04 plus + IFN-γ C/C was found with a higher rate in the patient group (16.7%) compared to the control group (8.5%) (p=0.028) (Table 2).

The association of the DRB1*15 allele with the IFN-γ (CC) genotype was found with a higher rate in the group who did not develop aGVHD (p=0.024). The association of the DRB1*15 allele with the IFN-γ (TT) genotype was found with a higher rate in the aGVHD group and this difference was statistically significant (p=0.050) (Table 3).

Discussion

GvHD is a significant complication that should be managed in the best way, because it is one of the most important causes of morbidity and mortality after hematopoietic stem cell transplantation.

Therefore, clarification of the GvHD pathogenesis is important for better management and prevention, and for developing treatment strategies [2, 15]. Cytokines are the significant effector and regulatory molecules playing an important role in the pathogenesis of GvHD. The proinflammatory cytokines, which are released by activated immune cells including T cells, resulting in the rapid initiation of GvHD, and rapid exacerbation by creating a ‘cytokine storm’ [16].

The donor naive CD4+ T cells in GvHD recognise the allo-antigens presented by the antigen-presenting cells
Table 3: HLA-DRB1*15, 16, 04, 07, 09 alleles of the patients, and the correlation with TGF-β, IFN-γ variations between the subjects with and without aGvHD.

| DRB1*15 | With aGvHD (n:8) | Without aGvHD (n:11) | Fisher's p |
|---------|------------------|----------------------|------------|
| DRB1*15 + IFN-γ/C | 0.0% | 10.0% | 0.024 |
| DRB1*15 + IFN-γ/T | 0.0% | 5.0% | 0.102 |
| DRB1*15 + IFN-γ/T | 6.3% | 0.0% | 0.050 |
| DRB1*15 + TGF-β/C | 5.0% | 0.0% | 0.102 |
| DRB1*15 + TGF-β/T | 6.3% | 10.0% | 0.247 |
| DRB1*15 + TGF-β/T | 0.0% | 5.0% | 0.349 |
| DRB1*16 | With aGvHD (n:8) | Without aGvHD (n:11) | p |
| DRB1*16 + IFN-γ/C | 0.0% | 5.0% | 0.102 |
| DRB1*16 + IFN-γ/T | – | – | – |
| DRB1*16 + IFN-γ/T | – | – | – |
| DRB1*16 + TGF-β/C | – | – | – |
| DRB1*16 + TGF-β/T | 6.3% | 5.0% | 0.514 |
| DRB1*16 + TGF-β/T | 6.3% | 0.0% | 0.051 |
| DRB1*04 | With aGvHD (n:8) | Without aGvHD (n:11) | p |
| DRB1*04 + IFN-γ/C | 0.0% | 5.0% | 0.102 |
| DRB1*04 + IFN-γ/T | 6.3% | 0.0% | 0.051 |
| tDRB1*04 + IFN-γ/T | – | – | – |
| DRB1*04 | With aGvHD (n:8) | Without aGvHD (n:11) | p |
| DRB1*04 + TGF-β/C | – | – | – |
| DRB1*04 + TGF-β/T | – | – | – |
| DRB1*04 + TGF-β/T | 0.0% | 15.0% | 0.037 |
| DRB1*07 | With aGvHD (n:8) | Without aGvHD (n:11) | p |
| DRB1*07 + IFN-γ/C | 0.0% | 5.0% | 0.102 |
| DRB1*07 + IFN-γ/T | 1.7% | 3.5% | 0.477 |
| DRB1*07 + IFN-γ/T | 12.5% | 0.0% | 0.024 |
| DRB1*07 + TGF-β/C | – | – | – |
| DRB1*07 + TGF-β/T | – | – | – |
| DRB1*07 + TGF-β/T | 12.5% | 5.0% | 0.076 |
| DRB1*09 | With aGvHD (n:8) | Without aGvHD (n:11) | p |
| DRB1*09 + IFN-γ/C | – | – | – |
| DRB1*09 + IFN-γ/T | – | – | – |
| DRB1*09 + IFN-γ/T | – | – | – |
| DRB1*09 + TGF-β/C | – | – | – |
| DRB1*09 + TGF-β/T | – | – | – |
| DRB1*09 + TGF-β/T | – | – | – |

HLA: Human Leukocyte Antigen; TGF-β: Transforming growth factor-β; IFN-γ: Interferon-gamma; aGvHD: acute Graft versus Host Disease. Bold values indicate significant values (p<0.05).

DE GRYFTER

Oguz et al.: HLA DRB1 alleles, IFN-γ and TGF-β gene variants in c-ALL patients — 5

The cytokines including IFN-γ are known to stimulate the regulation of the chemokines and receptors which have an important role in the occurrence of GvHD. In addition, IFN-γ is required for the development of T-regs. Production of high levels of IFN-γ by the recipient cells may affect the donor cell engraftment, and development and/or suppression of the Treg cells [14]. Studies reported that cytokine gene polymorphisms (IL-1, TNF, IL-10, TGF-β, IL-8, HSP70, IL-10, IFN-γ), had an important role in the occurrence of aGvHD [16, 17].

Some studies showed the association between the IFN-γ microsatellite alleles and IFN-γ (+874) high productive genotype with aGvHD [17–19]. In this study, the IFN-γ CC (rs: 2069705) genotype was found to be a high factor in acute GvHD. A Meta-Analysis showed the Association of Interferon Gamma +874T/A Polymorphism with Leukemia Risk. Looking at the results based on 420 leukemia cases and 767 matched controls from eight studies, it was revealed that the IFN-γ +874T/A polymorphism was associated with CML and CLL susceptibility [20–23].

In our study, the CC genotype (having high expression) in the patient group was found with a significantly higher rate as compared with the level in the control group, and the CT genotype (having moderate expression) in the patient group was found to a lower rate compared with the level in the control group in IFN-γ rs 2069705 (+1616/C/T) polymorphism.

Kamel et al. studied the TGFβ1-codon 10 and 25 and IFN-γ polymorphism in 106 patients in their study and
found GVHD in 26 patients out of 106 patients. Kamel et al. reported that the patients' IFN-γ +874 polymorphism showed a significant relationship with the development of chronic GVHD. Patients with the high producer IFN-γ +874 genotype showed an 8-fold increased probability of developing chronic GVHD compared to those with the low producer IFN-γ +874 genotype. The patients producing high/intermediate TGFβ1-codon 10 and 25 were reported to have a lower incidence of acute GVHD, although this was not statistically significant [24].

The prevalence of acute GVHD is directly associated with the degree of HLA match. There are many studies proving the importance of the match of the HLA antigens in bone marrow transplants [25]. Several studies have evaluated the hypothesis that DR15 is involved in alloimmune reactions after unrelated donor transplantation. The antigen-free mechanism of HLA DRB1*15 providing protection against acute GVHD is unknown. DR15 may mediate immune dysregulation by linking the imbalance to other related genes. One of the HLA-DRB1 alleles of HLA-DR15 was reported to be associated with low-grade II-IV acute GVHD risk in some studies [25–27]. Stern et al. mentioned the association of DR15 with a lower risk of disease recurrence and better survival after HSCT for patients with leukemia or non-Hodgkin lymphoma [28]. Öğüz et al. reported that HLA-DR15 was associated with autoimmune cytopenia in patients with aplastic anemia, myelodysplastic syndrome, and paroxysmal nocturnal hemoglobinuria. They also stated that the presence of the HLA-DR15 antigen might be associated with the response to immunosuppressive therapy [29]. On the other hand, Morishima et al. analyzed 1790 leukemia patients who underwent HSCT transplant between 1993 and 2000, and reported that HLA-A, HLA-B, and HLA-C were associated with severe acute GVHD. However, no association was detected with HLA-DRB1 mismatch. HLA-DRB1*04 was found with a higher rate in the patient group [30].

In this study, the HLA-DRB1*04 allele was found with a higher rate in the patient group, and also the association with DRB1*04 + plus IFN-γ CC was found with a higher rate in the patient group. Researchers have reported a significant association of the HLA-DRB1 allele with various leukemias. HLA-DRB1*11 was found with a significantly higher rate in the control group. HLA-DRB1*15 was found with a significantly higher rate in the patient group. Oguz et al. found that HLA-DRB1*15 was a risk indicator in patients with aplastic anemia. In addition, the frequency of relapse was shown to be lower compared to HLA-DR15 negative patients [29].

Dorak et al. reported that HLA-DRB4 was found with a higher rate in male patients compared to the female patients among pediatric ALL patients [31]. Several investigators reported that the DRB1*04 and DRB1*07 alleles might be associated with a higher risk in ALL patients [31–34].

Morrison et al. showed a correlation between rs3135388 and DRB1*1501 in HLA typing reference cell lines. They observed a female-specific risk association in pediatric ALL patients, similar to the stronger association of DRB1*15:01 in female patients with Multiple Sclerosis [35]. In our study, HLA-DRB1*11 and *03 were found with a higher rate in the control group, and HLA-DRB1*15 was found with a significantly higher rate in the patient group. In this study, no significant difference was detected in the ratio of DRB5 (DR51) which is inherited with DRB1*15, and in the ratio of DRB1*16 in the patients who developed aGVHD compared to the patients who did not develop GVHD.

In our study, an association of DRB5 (DR51) with the IFN-γ (CT) genotype was found with a higher rate in the control group compared to the patient group, and this difference was statistically significant. The IFN-γ (CT) genotype was found to have a protective role in the occurrence of GVHD. Association of the DRB1*15 allele with the IFN-γ (CC) genotype was found with a higher rate in the patients in this study compared to the non-acute GVHD group. Association of the DRB1*15 allele with the IFN-γ (TT) genotype was found with a higher rate in the acute GVHD group, however, this difference showed a statistically liminal significance.

Conclusion

Cytokines including IFN-γ are known to trigger the regulation of chemokines and receptors which have an important role in the emergence of GVHD. In addition, IFN-γ is required for the development of T-reg cells, and production of high levels of IFN-γ by the recipient cells may affect the donor cell engraftment, and development and/or suppression of Treg cells. An association was shown between the IFN-γ microsatellite alleles and the high producer IFN-γ (+874) genotype, and acute GVHD in some studies. The present study showed that some cytokine gene variations might contribute to the development of ALL, and the IFN-γ (CT) genotype had a protective role in the emergence of GVHD. In addition, we suggest that inheritance of IFN-γ (CT) in association with the HLA-DRB1*15 allele might be protective against GVHD. We suggest that investigations in larger groups will be useful in reveal the genetic indicators which create sensitivity against childhood leukemia, and in better understanding the immunopathogenesis of GVHD.

This study has several limitations. The first limitation of this study is the low number of patients. The second
limitation is that the number of patients with GvHD among transplant patients is small.

Acknowledgment: 1. Medipol Hematology Symposium, 6–7 Ekim 2018. 16. Medical Biology and Genetics Congress with International Participation. 27–30 Ekim 2019.

Research funding: The institution’s facilities were used as a financial resource.

Author contributions: Conception/Design of Study-R.O., H.S.C., F.S.O.; Data Acquisition- F.S.O., R.O., Data Analysis/Interpretation- F.S.O., Y.O., H.S.C., K.O., Drafting Manuscript-K.O., F.S.O., H.S.C., D.K., Critical Revision of Manuscript- F.S.O., K.O., Final Approval and Accountability- F.S.O., K.O., Technical or Material Support- R.O., M.G., Supervision- F.S.O., F.A., A.A., M.G., R.O.

Competing interests: The authors declare no conflict of interest.

Informed Consent: Informed consent was not received due to the retrospective nature of the study.

Ethical approval: Ethics committee approval was not received due to the retrospective nature of the study. This work was supported by the Clinical Research Ethics Committee of Istanbul University (13.11.2020, no:28). This study was approved by the ethical review boards of the Istanbul University, Istanbul, Turkey, and conducted in accordance with the standards of the Declaration of Helsinki.

References

1. Devergie A. Graft versus host disease. In: Apperley J, Carreras E, Gluckman E, Gratwohl A, Masszi T, editors. The EBMT handbook: hematopoietic stem cell transplantation, 5th ed. Cham (CH): Springer; 2008:219–33 pp.

2. Penack O, Marchetti M, Ruutu T, Aljurf M, Bacigalupo A, Bonifazi F, et al. Prophylaxis and management of graft versus host disease after stem-cell transplantation for haematological malignancies: updated consensus recommendations of the European society for blood and marrow transplantation. Lancet Haematol 2020;7:157–67. PMID:32004485.

3. Jagasia M, Arora M, Flowers ME, Chao NJ, McCarthy PL, Cutler CS, et al. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. Blood 2012;119:296–7. PMID:22010102.

4. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol 2012;12: 443–58.

5. Özçelik T. Kık hücre transplantasyonu hazırlama rejimleri ve bunların seçiminde dikkat edilecek hususlar. Türk Hematoloji Dergisi 2011;43–56.

6. Zeiser R, Blazar BR. Pathophysiology of chronic graft versus host disease and therapeutic targets. N Engl J Med 2017;28377: 2565–79. PMID:29281578.

7. Reddy P. Pathophysiology of acute graft versus host disease. Hematol Oncol 2003;21:149–61. PMID:14735553.

8. Rodríguez T, Méndez R, Del Campo A, Aptsiauri N, Martín J, Orozco G, et al. Patterns of constitutive and IFN-gamma inducible expression of HLA class II molecules in human melanoma cell lines. Immunogenetics 2007;59:123–33. PMID:17180681.

9. Wolffram LA, Fernandez TM, Mamura M, Fuller WL, Kumar R, Cole DE, et al. Loss of Smad3 in acute T-cell lymphoblastic leukemia. N Engl J Med 2004;351:552–9.

10. Yan X, Liu Z, Chen Y. Regulation of TGF-beta signaling by Smad7. Acta Biochim Biophys Sin 2009;41:263–72.

11. Yang WS, Han NJ, Kim CS, Ahn H, Lee SK, Lee KU, et al. STAT1-independent down-regulation of interferon-gamma-induced class II transactivator and HLA-DR expression by transforming growth factor beta-1 in human glomerular endothelial cells. Nephron Exp Nephrol 2005;100:e124–31.

12. Coghill JM, Sarantopoulos S, Moran TP, Murphy WI, Blazar BR, Serody JS. Effector CD4+ T cells, the cytokines they generate, and GVHD: something old and something new. Blood 2011;117: 3268–76.

13. Gabriel C, Fürst D, Faé I, Wenda S, Zollokofer C, Mytilineous J, et al. HLA typing by next-generation sequencing - getting closer to reality. Tissue Antigens 2014;83:65–75. PMID:24447174.

14. Wood NA, Thomson SC, Smith RM, Bidwell JL. Identification of human TGF-beta1 signal (leader) sequence polymorphisms by PCR-RFLP. J Immunol Methods 2000;3:117–22.

15. Carpenter PA, MacMillan ML. Management of acute graft-versus-host disease in children. Pediatr Clin 2010;57:273–95. PMID: 20307721.

16. Henden AS, Hill GR. Cytokines in graft-versus-host disease. J Immunol 2015;194:4604–12. PMID:25934923.

17. Kumar S, Mohammadpour H, Cao X. Targeting cytokines in GVHD therapy. J Immunol Res Ther 2017;2:90–9. PMID:28819653.

18. Yi T, Chen Y, Wang L, Du G, Huang D, Zhao D, et al. Reciprocal differentiation and tissue-specific pathogenesis of Th1, Th2, and Th17 cells in graft-versus-host disease. Blood 2009;114:3101–12. PMID:19602708.

19. Lai HY, Chou TY, Tzeng CH, Lee OK. Cytokine profiles in various graft-versus-host disease target organs following hematopoietic stem cell transplantation. Cell Transplant 2012;21:2033–45.

20. Wu Z, Sun Y, Zhu S, Tang S, Liu C, Qin W. Association of interferon gamma +874T/A polymorphism and leukemia risk. Medicine (Baltim) 2016;95:e3129. PMID:27015189.

21. Jiang H, Fu D, Bidgoli A, Paceszny S, T cell subsets in graft versus host disease and graft versus tumor. Front Immunol. 2021;12: 761448.PMID:34675938.

22. Carli C, Giroux M, Delisie JS. Roles of transforming growth factor-β in graft-versus-host and graft-versus-tumor effects. Biol Blood Marrow Transplant 2012;18:1329–40.

23. Zhang Q, Fu L, Liang Y, Guo Z, Wang L, Ma C, et al. Exosomes originating from MSCs stimulated with TGF-β and IFN-γ promote Treg differentiation. J Cell Physiol 2018;233:6832–40.

24. Kamel AM, Gameel A, Ebid GTA, Radwan ER, Mohammed Saleh MF, Abdelfattah R. The impact of cytokine gene polymorphisms on the outcome of HLA matched sibling hematopoietic stem cell transplantation. Cytokine 2018;110: 404–11. PMID:29801972.

25. Morishima S. Implications of HLA in allogeneic stem cell transplantation. Rinsho Ketsueki 2019;60:1324–30.
26. Taumoz R, Rocha V, Cook M. Association of HLA E polymorphism with severe bacterial infection and early transplant related mortality in matched unrelated bone marrow transplantation. Transplantation 2005;80:140–4. PMID:16003246.

27. Battiwalla M, Hahn T, Radovic M, Roy H, Wahab A, Duman E, et al. Human leukocyte antigen (HLA) DR15 is associated with reduced incidence of acute GVHD in HLA-matched allogeneic transplantation but does not impact chronic GVHD incidence. Blood 2006;107:1970–3. PMID:16282347.

28. Stern M, Passweg J, Tiercy JM, Genitsch A, Meyer-Monard S, Heim D, et al. Human leukocyte antigen DR15 is associated with reduced relapse rate and improved survival after human leukocyte antigen-identical sibling hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2006;12:1169–75. PMID:17085310.

29. Oguz FS, Yalman N, Diler AS, Oguz R, Anak S, Dorak MT. HLA-DRB1*15 and pediatric aplastic anemia. Haematologica 2002;87:772–4. PMID:12091130.

30. Morishima Y, Yabe T, Matsuo K, Kashiwase K, Inoko H, Saji H, et al. Japan Marrow Donor Program. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. Biol Blood Marrow Transplant 2007;13:315–28. PMID:17317585.

31. Dorak MT, Oguz FS, Yalman N, Diler AS, Kalayoglu S, Anak S, et al. A male-specific increase in the HLA-DRB4 (DR53) frequency in high-risk and relapsed childhood ALL. Leuk Res 2002;26:651–6. PMID:12008082.

32. Patiroğlu T, Akar HH. The frequency of HLA-A, HLA-B, and HLA-DRB1 alleles in patients with acute lymphoblastic leukemia in the Turkish population: a case-control study. Turk J Haematol 2016;1:339–45. PMID:27095065.

33. Yavuz C, Oguz F, Cinar C, Senturk Ciftci H, Kivanc D, Karakas Z. The association of PRKRAP1 pseudogene with acute lymphoblastic leukemia risk. J Clin Haematol 2021;2:18–23.

34. Ozdilli K, Oguz FS, Anak S, Kekik C, Carin M, Gedikoglu G. The frequency of HLA class I and II alleles in Turkish childhood acute leukaemia patients. J Int Med Res 2010;38:1835–44. PMID:21309500.

35. Morrison BA, Ucisik-Akkaya E, Flores H, Alaez C, Gorodezky C, Dorak MT. Multiple sclerosis risk markers in HLA-DRA, HLA-C, and IFNG genes are associated with sex-specific childhood leukemia risk. Autoimmunity 2010;43:690–7. PMID:21067287.

**Supplementary Material:** The online version of this article offers supplementary material (https://doi.org/10.1515/tjb-2021-0202).