HLA Alleles, Genotype and Haplotype Analyzes from Central Anatolia Region of Turkey

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Background: Although human leukocyte antigen (HLA) data for the Turkish population has been reported, there are no statistics on the HLA-DPB1 locus, which has recently received significant attention, particularly in hematopoietic stem cell transplantation. In addition, there is no study that has reported the 2-6 loci HLA haplotype distribution, 8-digit HLA allele frequency, and genotype frequency in the Turkish population.

Aims: To evaluate the low and high resolution (2-4-8 digits) HLA-A, -B, -C, -DRB1, -DQB1, -DPB1 allele data using the data of 6100 healthy individuals from the Central Anatolian region of Turkey.

Study Design: Retrospective cross-sectional study.

Methods: All tests were performed using molecular HLA techniques: low-resolution DNA-based sequence-specific oligonucleotides, low/ high-resolution DNA-based sequence-specific primer, and highresolution next generation sequencing. A total of 6100 healthy donors with a minimum of 3 loci (HLA-A, -B, -DRB1) were analyzed for their HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 data. Pypop and HLA-net GENE[RATE] were used to analyze the data.

Results: Among the HLA class I alleles, the following were the

most frequently observed alleles: for HLA-A, A*02, A*24, A*03, and A*01; for HLA-B, B*35, B*51, and B*44; and for HLA-C, C*07, C*04, and C*12. Among the HLA class II alleles, the following alelles were the most frequently observed: for HLA-DRB1, DRB1*11, DRB1*04, and DRB1*13; for HLA-DQB1, DQB1*03, DQB1*05, and DQB1*06; and for HLA-DPB1, DPB1*04, DPB1*02, and DPB1*03. The most common alleles among HLA-DPB1 in the 4-digit evaluation were DPB1*04:01, DPB1*02:01, and DPB1*04:02. Among the HLA classes I and II, the following were the most frequently observed 8-digit alleles in HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 were A*02:01:01:01, B*49:01:01:01, C*04:01:01:06, DRB1*07:01:01:01, DQB1*03:01:01:02, and DPB1*02:05, respectively. The most common 6 loci haplotype was A*02~B*35~C*04~DRB1*11~DQB1*03~DPB1*04 (2.71%).

Conclusion: In this study, low and high resolution HLA-DPB1 allele frequency, 6 locus haplotype frequency and genotype frequency were reported for the first time in Turkish population. These new data can be used to map HLA in our country and may provide ideas for potential future studies.

INTRODUCTION

The human leukocyte antigen (HLA) system has the most polymorphic structure. Currently, a total of 35,821 HLA alleles (Class I, n = 25,228; Class II, n = 10,592) are listed in the IMGT/HLA database (version 3.50 version; http://hla.alleles.org/nomenclature/ stats.html). Historically, HLA-A, -B, -C, -DRB1 and -DQB1 loci have been studied before transplantation. Recently, studies have established the importance of HLA-DPB1 in hematopoietic stem cell transplantation (HSCT), especially in the selection of unrelated donors.¹

Previously, typing of HLA genes was determined using 2-digit low-resolution (LR) molecular techniques. Nowadays, with the diversification of molecular methods and widespread use of next generation sequence (NGS) systems, high resolution (HR) 8-digit HLA data can be evaluated, especially before HSCT. With these advancements, it has become important to examine the HLA polymorphism in more detail. The number of studies reporting the *8-digit HLA* gene distribution of populations is gradually increasing.

A literature review revealed that studies from our country²⁻¹⁰ or involving the Turkish population¹¹ have described the distribution



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Yantır E, Gündüz E, Çolak E. HLA Alleles, Genotype and Haplotype Analyzes from Central Anatolia Region of Turkey. *Balkan Med J.*; 2023; 40(5):358-66. *Copyright@Author(s) - Available online at http://balkanmedicaljournal.org/* of HLA -A, -B, -C, -DRB1 and -DQB1 alleles and haplotypes. Our study is the first detailed HLA data analysis report from Turkey regarding LR and HR HLA-DPB1 allele frequency, genotype frequency, HR (4- or 8-digit) 6 loci alleles frequency, and 2-6 loci A:B:C:DRB1:DQB1:DPB1 haplotype frequencies in healthy individuals.

MATERIALS AND METHODS

Population

The data of 6100 healthy bone marrow or solid organ donors who underwent HLA typing at Eskişehir Osmangazi University Tissue Typing Laboratory between 2001 and 2021 were evaluated retrospectively. A total of 6 loci data, including HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1, were assessed in all donors with at least 3 loci (HLA-A, -B, -DRB1) data. In the first step, 2-digit molecular laboratory data from HLA-A, -B, -DRB1 6100, HLA -C 1841, HLA-DQB1 615 and HLA-DPB1 546 donors were evaluated. In the second step, 75 donors within the same group with HR 8-digit HLA data were evaluated. Our study population included 46.7% female and 53.3% male participants; there was no significant sex difference (p > 0.05). The mean age of the participants was 40.53 \pm 16.81 years.

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee (no: 2021-18).

HLA Class I and Class II typing

The LR 2-digit HLA typing was studied using DNA-based sequence-specific oligonucleotides (PCR-SSO) and DNA-based sequence-specific primers (PCR-SSP) as previously described.¹² The 4-digit alleles of each individual were typed using PCR-SSP and/or NGS with an HLA typing kit (MIA FORA NGS FLEX; Bio Array Solution Ltd., New Jersey, USA), and the 8-digit alleles were typed using NGS. The sequenced data were analyzed using MIA FORA NGS FLEX HLA Genotyping Software (version 3.0;). The IMGT/HLA database (version 3.43.0) was used as a reference. All HLA data before 2010 were evaluated after renaming them according to the current nomenclature (https://www.ebi.ac.uk).

Statistical analysis

All HLA data were validated before statistical analysis and analyzed using both GENE[RATE]¹³ (https://hla-net.eu/tools/) and PyPop (version 0.7.0;).¹⁴ Linkage disequilibrium (LD) was calculated in PyPop for each loci pair. One thousand permutations were used to test for significance, and the LD was measured as Wn, D, D' and p values, a multiallelic extension of the correlation measure. 6 loci were tested for Slatkin's implementation of the Ewens-Watterson homozygosity test of neutrality, Hardy-Weinberg Equilibrium (HWE) proportions, and common heterozygotes by allele. The common genotypes were assessed using chi-square test implemented in Pypop and GENE[RATE]. SPSS (version 25) was used to analyze the demographic data.

RESULTS

HLA allele frequencies

The HLA results of 6100 donors were first evaluated using the 2-digit LR data. A total of 21 HLA-A, 41 HLA-B, 16 HLA-C, 14 HLA-DRB1, 5 HLA-DQB1, and 34 HLA-DPB1 alleles were identified. Table 1 shows the allele frequency for the 6 loci.

Among the Class I HLA the most frequently encountered alleles in HLA-A were A*02, A*24, A*03, and A*01; in HLA-B were B*35, B*51, and B*44; and in HLA-C were C*07, C*04, and C*12. Among the Class II HLA the most frequently encountered alleles in HLA-DRB1 were DRB1*11, DRB1*04, and DRB1*13; in HLA-DQB1 were DQB1*03, DQB1*05, and DQB1*06; and in HLA-DPB1 were DPB1*04, DPB1*02, and DPB1*03. A total of 36 HLA-DPB1 alleles were identified in the evaluation of 396 donors with HR 4-digit HLA-DPB1 data. The most common alleles encountered were DPB1*04:01, DPB1*02:01 and HLA-DPB1*04:02 (Supplementary File 1).

In the second phase of our study, evaluation of the 8-digit HLA data of 75 donors in the group revealed 31 HLA-A, 50 HLA-B, 35 HLA-C, 38 HLA-DRB1, 30 HLA-DQB1 and 18 HLA-DPB1 alleles. The most frequently encountered 8-digit alleles in HLA-A were A*02:01:01:01, A*24:02:01:01, and A*01:01:01:01; HLA-B were B*49:01:01:01, B*35:01:01:02, in and B*51:01:01:01; in HLA-C were C*04:01:01:06, C*06:02:01:01, and C*07:01:01:01; in HLA-DRB1 were DRB1*07:01:01:01, DRB1*11:04:01:01, and DRB1*03:01:01:01; in HLA-DQB1 were DOB1*03:01:01:02, DOB1*02:02:01:01, and DOB1*02:01:01:01; and in HLA-DPB1 were DPB1*02:01:02:05, DPB1*04:01:01:06, and DPB1*04:02:01:02. The subgroups of the most frequently observed HLA alleles are summarized in Table 2; the full list is provided in Supplementary File 2.

Haplotype frequencies

Haplotype frequencies were evaluated at 2-6 loci using both Pypop and GENE[RATE]. We identified 2318 A*~B*~C*, 480 DRB1*~DQB1*~DPB1*, A*~B*~C*~DRB1*, 9180 A*~B*~C*~DRB1*~DQB1*, 6,687 and 10987 A*~B*~C*~DRB1*~DQB1*~DPB1* haplotypes. When calculating the 2-digit HLA haplotype data, due to the limitation of not being able to calculate > 5,000 haplotypes using PyPop, A*~B*, A*~DRB1*, B*~DRB1* and A*~B*~DRB1* haplotypes were calculated using GENE[RATE] alone; the other haplotypes were calculated using both programs. The most commonly observed haplotypes are summarized in Tables 3-5, and the full list is provided in Supplementary File 3.

Genotype analysis

The genotypes were the most frequently encountered in HLA-A were A*02:02 and A*02:24; in HLA-B were B *35:51 and B*35:35; in HLA-C were C*04:07 and C*07:07; in HLA-DRB1 were DRB1*04:11 and DRB1*11:11; in HLA-DQB1 were DQB1* 03:03 and DQB1*03:05; and in HLA-DPB1 were DPB1*04:04 and DPB1*02:04 (Table 6). A complete list of the observed genotypes is provided in Supplementary File 4.

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00080 2 8.37 0.4376 7.392 C/5 0.0410 151 D/16 0.0023 00010 2 8.37 0.1451 54 C/0 0.0999 12.00 D/17 0.0036 000011 2 8.37 0.1451 543 C/0 0.0152 56 Total D/17 0.0036 000015 2 8.36 0.1451 54 0.0162 17 0.0152 90 0.0035 00008 8.3 8.44 40*74 0.0035 0.0003 1 D/25 0.00035 000015 1 4.07 56 7 0.015 1 D/25 0.0035 000015 1 4.07 56 7 0.015 1 D/25 0.0035 000015 1 4.07 56 7 0.015 D/26 0.0035 1.00011 1.1 0.00115 1 1 368 0.0013 D/27 0.0015	3 1.117*1 2.22 C/5 0.0401 151 D.R/6 0.0000 7 0.0142 174 0.0142 174 0.0133 0.01179 66 7aut 0.9999 12.00 D.P/5 0.0033 7 0.01431 156 7.0041 1 3632 7 0.0133 0.0133 0.0133 0.0013 D.P/5 0.00135 1 0.0153 1 3632 7.001 3632 0.0013 D.P/5 0.00135 1 0.0153 1 3632 2.2 0.0013 D.P/5 0.0013 1 0.0153 14 1 3632 2.2 D.P/6 0.0033 6 0.0015 14 1 3632 D.P/6 0.0013 1 0.0015 14 2 2 D.P/6 0.0013 1 0.0015 14 2 2 D.P/6 0.0013 1 0.0015 1 2 2	A32	0.04738	578	B34	0.00008	1	CI5	0.06926	255	DR15	0.09484	1157				DP15	0.0119	13
000016 2 837 0.0126 174 0.0139 66 7aul 0.9999 12010 <i>DP17</i> 00138 0.0008 1 338 0.01467 54 7aul 1 368 27 0.0158 0.0008 1 338 74 7aul 1 368 27 0.0053 0.0018 1 338 74 368 0.01667 96 0.0053 0.0158 0.0053	7 0.01426 174 C/7 0.01936 6 Tatul 0.09999 12.00 DP/7 0.0033 8 0.01467 36 7.0012 36 7.0013 0.0013 0.0143 0.0143 0.0013 9 0.01835 41 1 3682 7.0 0.0135 0.0013 0.0143 0.0013 0.0143 0.0013 0.0143 0.0013 0.0143 0.0013 0.013 0.013 0.013 0.0013	A33	0.02869	350	B35	0.18787	2292	C16	0.04101	151	DR16	0.04844	591				DP16	0.00092	-
000041 5 B38 0.0441 14 A62 0.01637 945 Turdi 1 363 0.01637 945 0.00358 74 74 363 0.01637 946 0.00358 74 74 363 0.01637 946 0.00358 74 74 363 0.01637 940 0.00358 74 74 363 0.0035 0.00357 0.00035 1 0.00135 1 0.00135 1 0.00135 1 0.00135 0.00135 1	8 0.04451 543 C/8 0.00453 17 DP/8 0.0035 0 0.01607 156 Tatal 1 362 0.0045 0.0035 0 0.01607 106 Tatal 1 362 0.0035 0 0.0163 14 2 362 0.0035 0 0.0015 14 2 260 0.0035 0 0.0015 14 2 200 0.0035 0 0.0015 14 2 200 0.0035 0 0.0015 14 2 2 200 0 0.0035 36 2 2 2 2 0 0.0135 14 2 2 2 2 2 0 0.0336 410 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	A34	0.00016	2	B37	0.01426	174	C17	0.01793	99	Total	66666.0	12200				DP17	0.02381	26
000008 1 383 0106/1 96 Total 3682 00005 0003 61 349 00383 74 20002 00038 85 342 00008 1 272 00002 00085 85 74 00742 903 1 272 00002 00081 85 0015 1 46 00015 1 272 00002 1 00015 14 207402 903 10 274 00013 10 275 00002 10 272 00002 10 10003 10 10003 10 10003 10 10003 10 10003 10 10003 10 10003 10 10013 14 10013 14 10013 10 10013 10 10013 10 10013 10 10013 11 10013 11 10013 11 10013 11 10013 11 10013 <	0 0.01607 16 Total 1 3682 DP19 0.0056 0 0.0388 744 1 3682 0.0048 DP73 0.0008 2 0.0008 1 4.07402 903 DP74 0.0018 4 4.07402 903 14 DP74 0.0018 6 0.0015 14 DP74 0.0018 DP74 0.0018 6 0.0015 14 DP74 0.0018 DP74 0.0018 6 0.0015 14 DP74 0.0018 DP74 0.0018 6 0.0015 14 DP74 DP74 0.0018 DP74 0.0018 7 0.015 14 DP74 DP74 D0018 DP74 D0018 DP74 D0018 6 0.0035 15 DP74 DP74 D0018 DP74 D0018 DP748 D0018 DP748 D0018 DP748 D0018 DP748 D0018 <t< td=""><td>A36</td><td>0.00041</td><td>5</td><td>B38</td><td>0.04451</td><td>543</td><td>CI8</td><td>0.00462</td><td>17</td><td></td><td></td><td></td><th></th><td></td><td></td><td>DP18</td><td>0.00183</td><td>7</td></t<>	A36	0.00041	5	B38	0.04451	543	CI8	0.00462	17							DP18	0.00183	7
0005 61 B40 0.0385 7.4 DP2.2 0.003 00082 3 B41 0.0743 300 DP2.3 0.0453 00082 3 B44 0.0742 93 0.003 00082 3 B44 0.0742 93 0.003 00062 3 B44 0.0742 93 0.003 1 1.0001 12200 B45 0.0015 14 DP45 0.003 1 1.0001 12200 B45 0.0015 14 DP46 0.0013 1 1.0001 12200 B45 0.0013 14 DP46 0.0013 1 1.0001 12200 B46 0.0013 14 DP47 0.0013 1 1.0001 1230 B44 0.0013 14 DP46 0.0013 1 1.0101 14 DP44 10 DP44 0.0013 1 1.2100 B53 0.0013	0 0.0388 714 DP22 0.0003 1 0.0018 1 DP23 0.0018 4 0.0740 933 00 DP23 0.0018 6 0.0015 14 0.0740 DP23 0.0018 6 0.0015 14 0.0025 32 DP24 0.0018 7 0.0015 14 0.0025 32 DP24 0.0018 7 0.0015 14 DP24 0.0018 DP47 0.0018 6 0.0015 14 DP24 D018 DP47 0.0018 7 0.0015 14 DP24 D018 DP47 D018 7 0.0035 0.013 15 DP41 D018 DP44 D008 6 0.0013 15 DP41 D018 DP41 D018 7 0.0134 15 DP41 D018 DP44 D0012 7 0.0134 15 DP44	A38	0.00008	1	B39	0.01607	196	Total	1	3682							DP19	0.00366	4
003828 467 B41 004449 300 00008 1 D23 00443 00008 83 B42 00008 1 D725 00075 00008 84 00115 14 D747 00035 14 D747 00035 10001 123 043 00115 14 D747 00035 84 00015 14 D744 00035 14 D747 00035 84 00115 14 D744 00035 14 D747 00035 84 00135 14 D744 00135 14 D747 00135 84 00135 14 D744 D744 D744 D747 D746 00135 85 00424 25 D0412 16 D744	1 0.02459 300 DP23 0.0043 2 0.00008 1 DP24 0.0025 4 0.00015 14 DP34 0.00023 6 0.00115 14 DP34 0.0013 7 0.00135 14 DP34 0.0013 8 0.00135 14 DP34 0.0013 9 0.00135 14 DP34 0.0033 6 0.00135 14 DP34 0.0033 9 0.00336 40 D0025 DP47 0.0033 9 0.00336 410 DP34 0.0033 DP41 0.0033 9 0.0336 372 DP34 D934 272 D743 0.00133 9 0.0336 372 D034 372 DP44 0.0033 9 0.0336 25 D743 D743 D0032 D744 0.00133 14 0.0334 372 D0343 D743	A66	0.005	61	B40	0.03885	474										DP22	0.00092	1
0.00058 8.3 B.42 0.00008 1 D226 0.0023 0.00025 3 8.44 0.7470 903 D736 0.0003 1.0001 12200 846 0.00115 14 D747 0.0013 846 0.00115 14 0.07403 063 0.0013 14 0.0013 847 0.00115 14 0.0013 14 0.0013 14 0.0013 846 0.00336 401 0.0013 14 0.0013 14 0.0013 847 0.01356 410 0.00136 410 0.0013 14 0.0013 859 0.00346 312 53 0.00336 1410 0.0033 853 0.00377 46 20 20 20 20 20 20 20 853 0.01371 46 20 20 20 20 20 20 20 20 20 20 20 20	2 0.0008 1 DP36 0.0025 4 0.0742 93 DP36 0.0025 6 0.0015 14 DP36 0.0025 7 0.00115 14 DP37 0.0015 8 0.00155 14 DP47 0.0015 9 0.00155 14 DP47 0.0015 9 0.00155 41 DP47 0.0015 9 0.00425 401 D002 DP47 0.0025 9 0.0043 55 DP44 0.002 DP46 0.0025 1 0.12574 1534 DP44 0.002 DP46 0.002 1 0.1254 15 DP44 0.002 DP44 0.002 2 0.0434 37 DP44 DP46 0.002 DP44 0.002 3 0.0124 45 DP44 DP44 DP44 DP45 DP44 DP44 1 0.0124 1	A68	0.03828	467	B4I	0.02459	300										DP23	0.00458	5
0.00025 3 8.44 0.7402 9.03 1.740 9.13 1.743 0.0002 1.00011 1.2200 B-45 0.0015 1.4 2.743 0.0018 B-47 0.0015 1.4 2.743 0.0018 1.4 2.747 0.0018 B-47 0.0015 1.4 2.0015 1.4 2.741 0.0018 2.747 0.0018 B-47 0.0013 6.4 0.0013 6.4 0.0018 1.4 2.747 0.0018 B-48 0.0013 6.4 0.0013 6.4 0.0018 2.74 0.0018 2.74 0.0018 2.74 0.0018 2.74 0.0018 2.74 0.0018 2.74 0.0018 2.74 0.0018 2.74 0.0012 2.74 0.0012 2.74 0.0012 2.74 0.0012 2.74 0.0012 2.74 0.0012 2.741 0.0012 2.743 0.0012 2.743 0.0012 2.741 0.0002 2.741/3 0.0012 <t< td=""><td>4 0.7402 0.33 DP35 0.0002 6 0.00115 14 DP47 0.0013 7 0.00115 14 DP47 0.0013 6 0.00115 14 DP47 0.0013 7 0.00115 14 DP47 0.0013 9 0.00133 65 DP41 0.0013 9 0.00133 65 DP41 0.0023 9 0.00336 410 DP66 0.0032 9 0.0336 410 DP74 0.0023 8 0.00349 372 DP74 0.0032 9 0.0133 15 DP74 0.0032 4 0.0133 15 DP74 0.0032 4 0.0133 15 DP74 0.0032 6 0.0173 15 DP74 0.0032 7 0.0173 15 DP74 0.0032 10 0.01734 24 0.0036 D</td><td>A69</td><td>0.0068</td><td>83</td><td>B42</td><td>0.00008</td><td>1</td><td></td><td></td><td></td><td></td><td></td><td></td><th></th><td></td><td></td><td>DP26</td><td>0.00275</td><td>Э</td></t<>	4 0.7402 0.33 DP35 0.0002 6 0.00115 14 DP47 0.0013 7 0.00115 14 DP47 0.0013 6 0.00115 14 DP47 0.0013 7 0.00115 14 DP47 0.0013 9 0.00133 65 DP41 0.0013 9 0.00133 65 DP41 0.0023 9 0.00336 410 DP66 0.0032 9 0.0336 410 DP74 0.0023 8 0.00349 372 DP74 0.0032 9 0.0133 15 DP74 0.0032 4 0.0133 15 DP74 0.0032 4 0.0133 15 DP74 0.0032 6 0.0173 15 DP74 0.0032 7 0.0173 15 DP74 0.0032 10 0.01734 24 0.0036 D	A69	0.0068	83	B42	0.00008	1										DP26	0.00275	Э
1.0001 1.200 8.45 0.0026 3.2 DP45 0.0018 8.47 0.0015 1.4 DP47 0.0018 8.47 0.0012 1.4 D0002 DP47 0.0018 8.40 0.00361 410 DP44 0.0002 DP47 0.0018 8.50 0.03402 410 DP44 DP44 D0002 DP47 0.0018 8.51 0.03402 372 DP44 DP44 D002 DP44 D002 8.51 0.03402 372 DP44 DP44 DP44 D002	6 0.0026 32 DP45 0.0083 6 0.00115 14 DP51 0.0083 7 0.00115 14 DP51 0.0083 8 0.0033 65 DP61 0.0083 9 0.013 15 DP74 0.0093 14 0.0133 15 DP74 0.0093 14 0.0133 15 DP74 0.0093 15 0.0034 372 DP74 0.0093 16 0.0031 14 DP74 0.0092 17 0.0154 214 DP74 0.0092 18 0.0164 203 DP74 0.0092 19 0.0006 1 0.0006 DP74 </td <td>A74</td> <td>0.00025</td> <td>Э</td> <td>B44</td> <td>0.07402</td> <td>903</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <th></th> <td></td> <td></td> <td>DP36</td> <td>0.00092</td> <td>1</td>	A74	0.00025	Э	B44	0.07402	903										DP36	0.00092	1
0.00115 14 DP47 0.00135 0.00115 14 0.00135 0.00135 0.00115 14 DP47 0.00135 0.00115 14 0.00135 0.00135 0.00233 65 DP46 0.00135 0.00336 110 DP46 0.00025 0.03304 372 DP44 0.00025 0.03304 372 DP44 0.00025 0.03304 372 DP44 0.00025 0.00434 53 DP74 0.00128 0.00123 15 DP74 0.00128 0.00124 16 DP74 DP749 DP749 0.00125 15 DP744 DP749 DP749 0.01754 214 DD744 DP749 DP749 0.01649 6 D0002 DP744 DP749 D0002 0.0008 1 D0008 1 D00002 DP744 D0002 0.00008 1 D0008 <	6 0.00115 14 0.00135 7 0.00115 14 0.00135 8 0.00533 65 $D^{2}/1$ 0.00135 9 0.00533 65 $D^{2}/1$ 0.00135 9 0.00535 65 $D^{2}/1$ 0.00135 9 0.00355 491 $D^{2}/1$ $D^{2}/1$ 0.00135 9 0.00354 410 $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ 1 0.01354 53 $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ 1 0.00131 15 $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ 1 0.00131 15 $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ 1 0.0154 214 $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ 1 0.0013 1 $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ $D^{2}/1$ 1 0.0003 1 $D^{2}/1$ D^{2	Total	1.00001	12200	B45	0.00262	32										DP45	0.00092	1
0.00115 14 DP51 0.013 0.00533 65 DP61 0.0033 0.00533 65 DP66 0.0033 0.00535 410 DP66 0.0033 0.03356 410 DP66 0.0032 0.03351 100 DP76 0.0032 0.03351 100 DP76 0.0032 0.03343 272 DP74 0.0032 0.03434 53 D0042 16 0.0032 0.03424 46 D0124 16 D0163 0.0043 0.03424 46 D0144 20 D0163 0.0043 0.03424 46 D0164 16 D0163 0.0043 0.00436 6 D0164 20 D0163 0.0003 D164 0.0003 0.00043 6 D00008 1 0.0003 0.0003 0.0003 1 0.0003 1 0.0003 1 0.0003 1 1 0.0003 <	7 0.00115 1.4 DP51 0.0013 8 0.00533 65 DP61 0.0033 9 0.0023 491 DP76 0.0033 10 0.0336 410 DP76 0.0033 11 11.2574 1534 0.0003 12 0.03361 410 DP76 0.0003 13 0.00133 153 DP70 0.0003 14 0.00133 15 DP716 0.0003 14 0.00137 46 D0716 0.0003 15 0.00137 46 D0716 0.0003 16 0.00137 46 D0716 0.0003 16 0.00137 46 D713 0.0013 16 0.00137 46 D713 0.0013 16 0.00137 46 D713 0.0013 16 0.00038 1 D713 0.0003 16 0.00003 1 D713 0.00				B46	0.00115	14										DP47	0.00183	7
0.00533 65 0.00053 0.04025 491 0.0002 0.04025 491 0.0002 0.03361 410 0.0033 0.03361 410 0.002 0.03361 534 0.002 0.03361 534 0.002 0.03049 537 0.0023 0.00123 15 0.0023 0.00123 15 0.0023 0.00123 15 0.0023 0.00123 15 0.0023 0.00123 15 0.0023 0.00123 15 0.0023 0.00377 46 0.0133 0.01754 214 0.0033 0.01754 214 0.0033 0.01754 214 0.0033 0.00038 1 0.0003 0.00008 1 0.0003 0.00008 1 0.0003 0.00008 1 1.0003 0.00016 2 1.0003	8 0.00533 6.5 DP6/1 0.0003 9 0.04025 491 0.0003 DP6/6 0.0003 10 0.03361 410 DP6/6 0.0003 DP7/6 0.0003 11 0.12574 1534 DP7/6 0.0003 DP7/6 0.0003 12 0.00434 53 DP7/6 0.0003 DP7/6 0.0003 14 0.00123 15 DP7/6 0.0013 DP7/6 0.0003 15 0.00123 15 DP7/6 0.0013 DP7/6 0.0003 16 0.00123 15 DP7/6 0.0013 DP7/6 0.0013 17 0.0123 15 DP7/6 DP7/6 0.0003 10 0.00123 16 0.0003 DP7/6 0.0013 12 0.00123 11 0.0013 DP7/6 0.0013 13 0.00064 203 DP7/6 D0002 DP7/6 0.00032 13 0.00				B47	0.00115	14										DP51	0.00183	7
0.04025 491 DP66 00183 0.03361 410 DP70 00022 0.03361 534 0.00022 DP84 0.00022 0.033049 372 DP91 0.00022 DP70 0.00022 0.00123 15 D00123 15 DP70 0.00022 0.00123 15 D00123 16 0.00022 DP70 0.00022 0.00123 15 D00123 16 0.00123 17002 DP703 0.00123 0.00123 15 D00123 12 DP703 0.00022 DP703	0 0.04025 491 DP66 0.0183 0 0.03361 410 DP70 0.0092 1 0.12574 534 0.0092 2 0.03361 410 DP70 0.0092 3 0.0434 53 DP70 0.0092 3 0.0434 53 DP70 0.0092 4 0.0123 15 DP70 0.0092 5 0.03402 415 DP70 0.0092 6 0.00123 15 DP70 0.0092 7 0.0123 15 DP70 0.0092 6 0.0013 16 203 DP70 0.0092 7 0.01754 214 D01 DP70 DP73 0.0092 7 0.0166 203 6 0.0003 6 D0003 DP70 D0002 8 0.00008 1 1 D0003 1 D0003 1 D0003 1 D0003				B48	0.00533	65										DP6I	0.00092	1
0.03361 410 0.0002 0.12574 1534 0.0002 0.12574 1534 0.0002 0.12574 1534 0.0002 0.03049 372 DP91 0.0002 0.00123 15 DP104 0.0002 0.001754 214 D0012 DP131 0.0002 0.100164 203 D0002 DP131 0.0002 DP131 0.0002 0.00018 1 D0002 1 D0002 DP131 D0002 1.0003 0.00016 2 0.00016 2 D0002 1.0003 1.0003 1.0003 0.00016 2 0.00016	0 0.03361 10 DP70 0.0002 1 0.12574 1534 0.0002 DP84 0.0002 2 0.00349 372 DP10 0.0002 DP70 0.0002 3 0.00133 15 DP104 0.0013 DP104 0.0013 4 0.00123 15 DP104 0.0013 DP105 0.0002 6 0.00123 15 DP104 0.0013 DP105 0.0002 7 0.01754 214 D0105 0.0013 DP105 0.0002 7 0.01754 214 D0105 DP103 0.0002 DP103 0.0002 8 0.01664 203 DP103 DP103 DP103 0.0002 9 0.00008 1 D0008 1 D0003 DP103 DP103 DP103 9 0.00008 1 D0008 1 D0003 I D0003 I I D0003 9				B49	0.04025	491										DP66	0.00183	7
0.12574 1534 0.0002 0.03049 372 DP91 0.0002 0.03043 53 DP104 0.0012 0.00123 15 DP104 0.0013 0.031402 415 DP104 0.0012 0.03170 46 DP131 0.0002 0.01541 214 DP131 0.0002 0.01542 214 D0002 DP131 0.0002 0.01543 214 D0002 DP131 0.0002 0.01544 214 D0002 DP131 0.0002 0.01543 214 D0002 DP131 0.0002 0.00037 46 DP131 0.0002 DP131 0.0002 0.00038 1 0.0003 1 D0002 DP363 1.0005 0.00038 1 0.0003 1 0.0003 1 1.0005 1 0.00038 1 0.0003 1 0.0003 1 1.0005 1 0.00016 2 0.00016 2 0.00003 1 1.0005 1	1 0.12574 1534 0.0002 2 0.03049 372 DP91 0.0002 3 0.00133 15 DP104 0.0013 4 0.00123 15 DP104 0.0013 6 0.003402 415 DP103 0.0043 7 0.01754 214 0.0002 8 0.01664 203 0.0002 0 0.00068 1 0.0002 0 0.0008 1 0.0002 0 0.0008 1 1.0005 1 0.0008 1 1.0005 1 0.0008 1 1.0005 1 0.0008 1 1.0005 1 0.0008 1 1.0005 1 0.0008 1 1.0005 1 0.00016 2 1.00016 1 0.00016 2 1.00016 1 0.00016 2 1.00016 1 0.00016 2 1.00016 1 0.00016 2 1.				B50	0.03361	410										DP70	0.00092	1
0.03049 372 DP91 0.0003 0.00123 15 DP104 0.0013 0.00123 15 DP107 0.00438 0.00123 15 DP109 0.00138 0.03402 415 DP109 0.00032 0.03402 415 DP129 0.00032 0.01754 214 0.00032 DP131 0.00032 0.01664 203 0.00032 DP203 0.00032 0.00008 1 0.00038 1 0.0003 0.00008 1 0.0008 1 0.0003 0.00008 1 0.0003 1 0.0003 1 0.00008 1 0.00038 1 0.0003 1 0.00016 2 0.00016 2 1 0.0003 1 0.00016 2 0.00016 2 1 1 1 1 0.00016 2 0.00016 2 1 1 1 1 1 0.00016 2 1 1 1 1 1 <td>2 0.03049 372 DP91 0.0003 3 0.00434 53 DP104 0.0013 4 0.00123 15 DP104 0.0013 5 0.03402 415 DP104 0.0013 6 0.00377 46 DP129 0.0002 7 0.01754 214 DP131 0.0002 8 0.01664 203 0.0002 DP131 0.0002 9 0.00049 6 DP134 0.0002 DP131 0.0002 9 0.00049 6 DP134 0.0002 DP134 0.0002 8 0.00049 6 DP134 0.0002 DP134 0.0002 9 0.0008 1 D0002 DP134 0.0002 DP134 0.0002 3 0.0008 1 D0008 1 D0008 1 3 0.0008 1 D0008 1 1 D0005 1 1</td> <td></td> <td></td> <td></td> <td>B51</td> <td>0.12574</td> <td>1534</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <th></th> <td></td> <td></td> <td>DP84</td> <td>0.00092</td> <td>1</td>	2 0.03049 372 DP91 0.0003 3 0.00434 53 DP104 0.0013 4 0.00123 15 DP104 0.0013 5 0.03402 415 DP104 0.0013 6 0.00377 46 DP129 0.0002 7 0.01754 214 DP131 0.0002 8 0.01664 203 0.0002 DP131 0.0002 9 0.00049 6 DP134 0.0002 DP131 0.0002 9 0.00049 6 DP134 0.0002 DP134 0.0002 8 0.00049 6 DP134 0.0002 DP134 0.0002 9 0.0008 1 D0002 DP134 0.0002 DP134 0.0002 3 0.0008 1 D0008 1 D0008 1 3 0.0008 1 D0008 1 1 D0005 1 1				B51	0.12574	1534										DP84	0.00092	1
0.00434 53 DP104 0.00183 0.00123 15 DP105 0.00458 0.03402 415 DP103 0.00022 0.03402 415 DP131 0.00022 0.00174 214 D0002 0.00022 0.01554 214 0.00022 DP731 0.00022 0.01564 203 0.00022 DP333 0.00022 0.0008 1 0.00032 1 0.00032 0.00082 1 0.0008 1 0.00032 0.00082 1 0.0008 1 0.00032 0.00082 1 0.0008 1 0.00032 0.00082 1 0.00032 1 1 0.00038 1 0.00032 1 1 0.00038 1 0.00032 1 1 0.00038 1 0.00032 1 1 0.00038 1 0.00033 1 1 0.00033 1 <td>3 0.00434 53 DP104 0.0183 4 0.00123 15 DP105 0.00458 5 0.003402 415 DP129 0.00022 6 0.00377 46 DP131 0.00022 7 0.01754 214 0.0002 8 0.01664 203 0.00002 9 0.00049 6 DP340 1.00052 0 0.0008 1 D0008 1.00052 0 0.0008 1 D00062 1.00052 0 0.0008 1 D00062 1.00062 0 0.00063 1 D00062 1.00062 <tr< td=""><td></td><td></td><td></td><td>B52</td><td>0.03049</td><td>372</td><td></td><td></td><td></td><td></td><td></td><td></td><th></th><td></td><td></td><td>DP91</td><td>0.00092</td><td>1</td></tr<></td>	3 0.00434 53 DP104 0.0183 4 0.00123 15 DP105 0.00458 5 0.003402 415 DP129 0.00022 6 0.00377 46 DP131 0.00022 7 0.01754 214 0.0002 8 0.01664 203 0.00002 9 0.00049 6 DP340 1.00052 0 0.0008 1 D0008 1.00052 0 0.0008 1 D00062 1.00052 0 0.0008 1 D00062 1.00062 0 0.00063 1 D00062 1.00062 <tr< td=""><td></td><td></td><td></td><td>B52</td><td>0.03049</td><td>372</td><td></td><td></td><td></td><td></td><td></td><td></td><th></th><td></td><td></td><td>DP91</td><td>0.00092</td><td>1</td></tr<>				B52	0.03049	372										DP91	0.00092	1
0.00123 15 DP105 0.00458 0.03402 415 DP129 0.00022 0.00377 46 DP131 0.00022 0.01544 214 0.00022 DP131 0.00022 0.01664 203 0.00022 DP131 0.00022 0.00008 1 0.00032 10 0.00032 1.00003 0.00008 1 0.00032 10 0.00032 1.00003 0.00016 2 0.00038 1 1.00003 1.00003 0.00016 2 0.00016 2 1.00003 1.00003	i 0.00123 15 $DP105$ 0.00458 $i5$ 0.03402 415 $DP129$ 0.0002 $i6$ 0.00377 46 $DP124$ 0.0002 $i7$ 0.01754 214 $DP129$ 0.0002 $i8$ 0.01664 203 0.0002 $DP131$ 0.0002 $i9$ 0.0008 1 $D0008$ 1 $D0008$ 1 $i0$ 0.0008 1 0.0008 1 0.0008 1 $i0$ 0.0008 1 0.0008 1 1 $i0$ 0.0008 1 0.0008 1 1 $i0$ 0.0008 1 1 1 1 $i0$				B53	0.00434	53										DPI04	0.00183	7
0.03402 415 DP129 0.00092 0.00377 46 DP131 0.00092 0.01754 214 DP731 0.00092 0.01664 203 0.00092 DP760 0.00092 0.00008 1 0.00008 1 0.0006 0.00008 1 0.00008 1 0.0006 0.00008 1 0.00008 1 0.0006 0.00008 1 0.0006 1 0.0006 0.00008 1 0.0006 1 1.0006 0.00008 1 0.0006 1 1.0006 0.00008 1 0.0006 1 1.0006 0.00008 1 0.0006 1 1.0006 0.00016 2 0.0006 1 1.0006 0.00016 2 0.0006 1.0006 1.0006	5 0.03402 415 $DP129$ 0.0002 6 0.00377 46 $DP131$ 0.0002 7 0.01754 214 $DP131$ 0.0002 8 0.01664 203 $DP131$ 0.0002 9 0.00049 6 $D0008$ $Total$ 1.0005 6 0.0008 1 $D0008$ 1 $D0002$ 0.0008 3 0.0008 1 $D0008$ 1 0.0008 1 3 0.0008 1 0.0008 1 0.0008 1 3 0.0008 1 0.0008 1 0.0008 1 3 0.0008 1 0.0008 1 0.0008 1 3 0.0008 1 0.0008 1 0.0008 1 4 0.0008 1 0.0008 1 0.0008 1 3 0.0008 1 0.0008 1 0.0008 1 4 0.0008 </td <td></td> <td></td> <td></td> <td>B54</td> <td>0.00123</td> <td>15</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <th></th> <td></td> <td></td> <td>DPI05</td> <td>0.00458</td> <td>5</td>				B54	0.00123	15										DPI05	0.00458	5
0.00377 46 DP131 0.0002 0.01754 214 DP503 0.0002 0.01664 203 DP703 0.0002 0.00169 6 D760 1 0.0005 0.0008 1 0.0008 1 0.0005 0.0008 1 0.0008 1 0.0005 0.00082 10 0.0008 1 1.0005 0.00083 1 0.0008 1 1.0005 0.00084 1 0.0008 1 1.0005 0.00085 1 0.0008 1 1.0005 0.0008 1 0.0008 1 1.0005 0.00086 1 1.0005 1.0005 1.0005 0.00016 2 0.0005 1.0005 1.0005	6 0.00377 46 $DP131$ 0.0002 7 0.01754 214 $DP303$ 0.0002 8 0.01664 203 $DP303$ 0.0002 8 0.01664 203 $DP303$ 0.0002 6 0.00008 1 $Tatal$ 1.0005 6 0.0008 1 0.0008 1 3 0.0008 1 1.0005 1.0005 2 0.0008 1 1.0005 1.0005 3 0.0008 1 1.0005 1.0005 2 0.0008 1 1.0005 1.0005 3 0.0006 1.0006 1.0006 1.0006 6 0.0008 1.0006 1.0006 1.0006 1.0006 6 0.00008 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1.0006 1				B55	0.03402	415										DP129	0.00092	-
0.01754 214 DP503 0.0002 0.01664 203 70:00 1.0005 0.00008 1 1.00005 1.00005 0.00008 1 0.00082 10 0.00008 1 0.00008 1 0.00008 1 0.00016 1 0.00008 1 0.00016 1 0.00016 2 0.00016 1 0.00016 2 1.2000 1	7 0.01754 214 $DP503$ 0.0002 8 0.01664 203 7001 10005 9 0.00008 1 7001 10005 6 0.00008 1 10005 10005 3 0.0008 1 10005 10005 2 0.0008 1 10005 10005 3 0.0008 1 10005 10005 4 0.0008 1 10005 10005 3 0.00016 2 10005 10005 4 0.00016 2 10005 10005 6 0.00016 2 10005 10005				B56	0.00377	46										DP131	0.00092	-
0.01664 203 Total 1.0005 0.00049 6 0.0008 1 0.00008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1 0.0008 1	8 0.01664 203 Total 1.0005 9 0.00049 6 1.0005 1.0005 6 0.0008 1 1.0005 1.0005 7 0.0008 1 1.0005 1.0005 7 0.0008 1 1.0005 1.0005 7 0.0008 1 1.0005 1.0005 8 0.00016 2 1.0005 1.0005 9 0.00016 2 1.0005 1.0005				B57	0.01754	214										DP503	0.00092	1
0.00049 0.00008 0.000082 0.00008 0.00008 0.00016 0.99998	 (9 0.00049 60 0.00008 66 0.00008 73 0.00082 83 0.00016 7al 0.99998 				B58	0.01664	203										Total	1.00005	1092
0.00008 0.00008 0.00008 0.00008 0.00016 0.99998	60 0.0008 66 0.0008 73 0.0008 73 0.0008 73 0.00016 7al 0.99998				B59	0.00049	9												
0.0008 0.00082 0.00008 0.00016 0.99998	66 0.00008 73 0.00082 82 0.00008 83 0.00016 1al 0.99998				B60	0.00008	1												
0.00082 0.00008 0.00016 0.99998	73 0.00082 82 0.00008 83 0.00016 4al 0.99998				B66	0.00008	1												
0.00008 0.00016 0.99998	22 0.0008 23 0.00016 tal 0.99998				B73	0.00082	10												
0.00016 0.99998	33 0.00016 tal 0.99998				B82	0.00008	1												
0.99998	tal 0.99998				B83	0.00016	2												
	AF, allele frequency; 2n, allele count				Total	0.99998	12200												

TABLE 2. Frequency for the Most Commonly Encountered HLA Alleles and its Subgroups with the NGS Data (2-8 digit) (N = 75).

Two-digit HLA	AF	Four-digit HLA	AF	Eight-digit HLA	AF
4*02	0.32	A*02:01	0.22667	A*02:01:01:01	0.2
				A*02:01:01:05	0.00667
				A*02:01:01:08	0.00667
				A*02:01:01:16	0.01333
		A*02:02	0.00667	A*02:02:01:01	0.00667
		A*02:05	0.04667	A*02:05:01:01	0.04667
		A*02:06	0.02	A*02:06:01:01	0.02
		A*02:09	0.00667	A*02:09:01:01	0.00667
		A*02:17	0.01333	A*02:17:02:01	0.01333
B*35	0.18667	B*35:01	0.08667	B*35:01:01:02	0.06667
				B*35:01:01:05	0.02
		B*35:02	0.04	B*35:02:01:01	0.02667
				B*35:02:01:02	0.01333
		B*35:03	0.04667	B*35:03:01:01	0.02
				B*35:03:01:03	0.02
				B*35:03:01:05	0.00667
		B*35:08	0.01333	B*35:08:01:01	0.01333
C*07	0.22	C*07:01	0.16667	C*07:01:01:01	0.07333
				C*07:01:01:12	0.00667
				C*07:01:01:16	0.05333
				C*07:01:01:68	0.02667
				C*07:01:02:01	0.00667
		C*07:02	0.04667	C*07:02:01:01	0.01333
				C*07:02:01:03	0.03333
		C*07:18	0.00667	C*07:18:01:01	0.00667
DRB1*11	0.24	DRB1*11:01	0.07333	DRB1*11:01:01:01	0.04667
				DRB1*11:01:01:03	0.02667
		DRB1*11:03	0.04	DRB1*11:03:01	0.04
		DRB1*11:04	0.12667	DRB1*11:04:01	0.05333
				DRB1*11:04:01:01	0.07333
DQB1*03	0.36	DQB1*03:01	0.24667	DQB1*03:01:01:01	0.02
				DQB1*03:01:01:02	0.13333
				DQB1*03:01:01:03	0.07333
				DQB1*03:01:01:07	0.00667
				DQB1*03:01:01:09	0.01333
		DQB1*03:02	0.07333	DQB1*03:02:01:01	0.06667
				DQB1*03:02:01:02	0.00667
		DQB1*03:03	0.02	DQB1*03:03:02:01	0.00667
				DQB1*03:03:02:02	0.01333
		DQB1*03:04	0.01333	DQB1*03:04:01:01	0.01333
		DQB1*03:05	0.00667	DQB1*03:05:01	0.00667
DPB1*04	0.47826	DPB1*04:01	0.34783	DPB1*04:01:01:01	0.06522
				DPB1*04:01:01:05	0.13043
				DPB1*04:01:01:06	0.15217
		DPB1*04:02	0.13043	DPB1*04:02:01:02	0.13043

Test for neutrality and HWE analysis

The heterozygosity of the most frequently observed alleles is shown in Table 7. The complete list of the heterozygosity assessment is provided in Supplementary File 5.

The result for the Slatskin's implementation of Ewens-Watterson homozygosity test of neutrality are given in Suplementary File 6 Table S1. The result showed negative and significant Fnd values for all loci 2-digit HLA data expected DPB1 locus. This indicates homozygosity, which is suggestive of a balanced selection at these loci.

There was no detectable deviation of the HWE proportion for heterozygosity in all loci, except at HLA-A and HLA-C (Supplementary File 6 Table S2).

The haplotype/LD statistics used in the multilocus analysis are listed in Supplementary File 6 Table S3-S4. Pairwise LD measured by Log-likelihood under linkage equilibrium $[ln(L_0)]$, Log-likelihood obtained via the EM algorithm $[ln(L_1)]$, Hedrick's D' and Cramer's V Statistics (Wn) were significant (p < 0.05) for all loci, except for the A:DQB1 loci pairs.

DISCUSSION

Our study is the first report from Turkey that has evaluated the haplotype frequency of 6 loci A*~B*~C*~DRB1*~DQB1*~DPB1*, genotype frequency, and frequency distribution of the HR 8-digit HLA-A, -B, -C, -DRB1,-DQB1 and -DPB1 alleles. Our study has included the highest number of healthy cases in Turkey till date. Since our results were obtained healthy donors from Central Anatolia and surrounding provinces, it was understood that our study results would not reflect that of the entire population of Turkey. The allele and some haplotype frequencies of our study were similar to those of most previous studies conducted in our country.³⁻¹⁰ A comparison of the 8-digit HLA data frequency could not be conducted due to the lack of previous studies reporting the same in our nation.

In our study, the frequency of six alleles was > 5% at the A locus. The most common 2-digit alleles were A*02, A*24, and A*03. The most common 4-digit alleles were A*02:01, A*24:02, and A*01:01, and the most common 8-digit alleles were A*02:01:01:01,

TABLE 3. Most Common Two-loci Haplotype Frequency.

		Haplotype				Haplotype				Haplotype	
Loci	HN	name	HF	Loci		name	HF	Loci	HN	name	HF
A*∼B*	25	02~51	0.0503	B*~DRB1*	22	35~11	0.0544	C*~DQB1*	28	04~03	0.1066
A*~B*	25	24~35	0.0390	B*~DRB1*	22	51~11	0.0342	C*~DQB1*	28	07~03	0.0666
A*~B*	25	03~35	0.0302	B*~DRB1*	22	08~03	0.0309	C*~DQB1*	28	06~02	0.0586
A*~DRB1*	31	24~11	0.0448	B*~DQB1*	32	35~03	0.1030	C*~DPB1*	21	07~04	0.1370
A*~DRB1*	31	02~11	0.0430	B*~DQB1*	32	51~03	0.0563	C*~DPB1*	21	04~04	0.1088
A*~DRB1*	31	02~04	0.0365	B*~DQB1*	32	35~05	0.0525	C*~DPB1*	21	12~04	0.0760
A*~C*	32	02~07	0.0581	B*~DPB1*	22	35~04	0.1183	DQB1*~DPB1*	14	03~04	0.2278
A*~C*	32	24~04	0.0339	B*~DPB1*	22	51~04	0.0729	DQB1*~DPB1*	14	05~04	0.1255
A*~C*	32	02~04	0.0333	B*~DPB1*	22	44~04	0.0477	DQB1*~DPB1*	14	06~04	0.1076
A*~DQB1*	31	02~03	0.1112	C*~B*	28	04~35	0.1634	DRB1*~DPB1*	22	11~04	0.1413
A*~DQB1*	31	24~03	0.0766	C*~B*	28	07~49	0.0475	DRB1*~DPB1*	22	13~04	0.0655
A*~DQB1*	31	02~05	0.0558	C*~B*	28	15~51	0.0455	DRB1*~DPB1*	22	04~04	0.0605
A*~DPB1*	20	02~04	0.1472	C*~DRB1*	27	04~11	0.0525	DRB1*~DQB1*	16	11~03	0.2115
A*~DPB1*	20	24~04	0.0890	C*~DRB1*	27	07~11	0.0515	DRB1*~DQB1*	16	04~03	0.118
A*~DPB1*	20	02~02	0.0682	C*~DRB1*	27	06~07	0.0466	DRB1*~DQB1*	16	15~06	0.0894

TABLE 4. The Five Most Common Three-loci haplotype Frequencies Identified Using Pypop and GENE[RATE].

		HF				HF			HF	
A*~B*~C*	HN	GENE [RATE]	PyPOP		HN	GENE [RATE]	DRB1*~DQB1*~DPB1	HN	GENE [RATE]	PyPOP
24~35~04	23	0.0334	0.03337	24~35~11	4	0.0163	11~03~04	20	0.1387	0.13827
02~35~04	23	0.0321	0.03209	02~51~11	4	0.0135	15~06~04	20	0.0551	0.05513
03~35~04	23	0.0265	0.02657	02~51~04	4	0.0116	03~02~04	20	0.0523	0.05245
23~49~07	23	0.0213	0.02133	01~08~03	4	0.0111	13~06~04	20	0.0511	0.05107
01~35~04	23	0.0206	0.02057	01~35~11	4	0.0097	04~03~04	20	0.0484	0.04836

HF. haplotype frequency; HN. number of haplotypes with HF > 0.0100. *When calculating the HLA haplotype data. due to the limitation of not being able to calculate > 5.000 haplotypes with PyPop. these haplotypes were calculated using GENE[RATE]. The full list is provided in Supplementary File 3.

A*24:02:01:01 and A*01:01:01:01. The A*02 was the most frequently identified allele, which is consistent with the findings of previous studies.³⁻¹⁰ The A*24 allele was the second most frequently observed allele. This was finding was consistent with those of most previous studies, except those of the study conducted by Pala et al.⁷ in the Thracian population. Six A*02 allele subtypes

were observed for 4-digit alleles; A*02:01 was the most common subtype. Nine A*02 allele subtypes were observed in the 8-digit alleles; A*02:01:01:01 was the most common subtype. Our study findings are similar to those of studies by Oguz et al.¹⁵ and Uyar et al.⁹ for the HLA-A alleles. Furthermore, Pingel et al.¹¹ determined that A*02:01, A*24:02, and A*01:01g were often observed among

TABLE 5. Most Common Four-. Five-. and Six-loci Haplotype Frequencies Calculated Using GENE[RATE] and Pypop (HF > 0.0100).

Four-loci haplotype	HF		Five-loci haplotype	HF		Six-loci haplotype	HF	
A*~B*~C*~DRB1*	GENE [RATE]	РуРОР	A*~B*~C*~DRB1*~DQB1*	GENE [RATE]	РуРОР	A*~B*~C*~DRB1*~DQB1*~DPB1*	GENE [RATE]	РуРОР
02~35~04~11	0.0133	0.01334	02~35~04~11~03	0.0246	0.02464	02~35~04~11~03~04	0.0273	0.02711
24~35~04~11	0.0133	0.01327	24~35~04~11~03	0.0198	0.01978	23~49~07~11~03~04	0.0139	0.01389
23~49~07~11	0.0115	0.01152	02~35~04~04~03	0.0170	0.01696	24~35~04~11~03~04	0.0126	0.01389
01~08~07~03	0.0111	0.01113	02~50~06~07~02	0.0139	0.01389	11~52~12~15~06~04	0.0114	0.01263
02~50~06~07	0.0101	0.01011	23~49~07~11~03	0.0128	0.01282	01~35~04~13~06~04	0.0101	0.01136
			01~35~04~13~06	0.0127	0.01271	02~51~15~11~03~04	0.0101	0.0101
			01~08~07~03~02	0.0118	0.01175	26~08~07~03~02~04	0.0101	0.0101
			03~35~04~01~05	0.0118	0.01175	02~13~06~07~02~02	0.0101	0.0101
			02~13~06~07~02	0.0117	0.01170	02~07~07~15~06~04	0.0100	0.0101
			11~52~12~15~06	0.0114	0.01144	02~35~04~04~03~02	0.0088	0.00884
			02~07~07~15~06	0.0107	0.01068			

HF, haplotype frequency; Pypop, from PyPop software; GENE[RATE: from GENE[RATE] tools. The full list is provided in Supplementary File 3.

TABLE 6. Most Frequently Observed HLA Genotypes.

	1						
Locus	Observed	Expected	p-value	Locus	Observed	Expected	p-value
A*				DRB1*			
02:02	431	382.00	0.0122*	04:11	401	377.62	0.2290
02:24	436	455.20	0.3682	11:11	273	270.27	0.8662
B*				DQB1*			
35:51	283	288.19	0.7598	03:03	107	100.41	0.5108
35:35	240	215.30	0.0923	03:05	102	107.08	0.6237
C*				DPB1*			
04:07	138	150.99	0.2906	04:04	158	165.39	0.5658
07:07	113	88.25	0.0011***	02:04	120	111.72	0.4337

*, significant at the 5% level; **, significant at the 1% level; ***, significant at the 0.1% level. The full list is provided in Supplementary File 4. HLA, human leukocyte antigen.

TABLE 7. Heter	rozygotes of the M	ost Common HLA Allele.
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Allele	Observed	Expected	p-value	Allele	Observed	Expected	p-value
A*02	2191	2289.25	0.0405*	DRB1*11	2022	2027.46	0.9035
A*24	1497	1547.79	0.1967	DRB1*04	1470	1540.19	0.1239
B*35	1812	1861.40	0.2522	DQB1*03	283	296.18	0.4438
B*51	1302	1341.12	0.2854	DQB1*05	197	207.91	0.4494
C*07	557	616.49	0.0166*	DPB1*04	285	270.23	0.3689
C*04	522	573.09	0.0328*	DPB1*02	165	165.26	0.9937

*, significant at the 5% level. The full list is given in Supplementary File 5.

HLA, human leukocyte antigen.

Turkish donors at a German bone marrow bank; this finding was similar to that of our study finding.

The B locus was found to be the most polymorphic locus; only four allele frequencies were > 5%. In our study, the 2-digit B*35, B*51, and B*44 alleles, 4-digit B*51:01, B*35:01, and B*49:01 alleles, and 8-digit B*49:01:01:01, B*35:01:01:02, and B*51:01:01:01 were the most frequently observed. Four B*35 allele subtypess were observed among the 4-digit alleles; B*35:01 was the most common. Eight B*35 allele subtypes were observed among the 8-digit alleles; B*35:01:01:02 was the most common. B*35 has been previously reported as the most frequently observed allele.³⁻¹⁰ In the study by Pingel et al.,¹¹ the most frequently observed 2-digit alleles were B*35, B*51, and B*44 and 4-digit alleles were B*51:01, B*35:01, and B*18:01; these results are similar to those of our study.

In the C locus, the frequency of five alleles was > 5%. In our study, the 2-digit C*07, C*04, and C*12 alleles, 4-digit C*07:01, C*04:01, C*06:02, and C*12:03 alleles, and 8-digit C*04:01:01:06, C*06:02:01:01, and C*07:01:01:01 alleles were the most common alleles observed. Three C*07 allele subtypes were observed among the 4-digit alleles; C*07:01 was the most common subtype. Eight C*07 allele subtypes were observed among the 8-digit alleles; C*07:01:01:01 was the most common allele; in two studies conducted in our country,^{3,5} C*04 was the most common. Oguz et al.¹⁵ determined that the top three alleles were C*04:01, C*07:01, and C*12:03.⁹ The most frequently observed alleles in the study by Pingel et al.¹¹ were the 2-digit C*07, C*04, and C*12 alleles and 4-digit C*04:01, C*07:01, and C*12:03 alleles.

At the DRB1 locus, eight allele frequencies were > 5%. The DRB1*11 allele was the most frequently observed allele, which is consistent with the findings of previous studies.³⁻¹⁰ In our study, the 2-digit DRB1*11, DRB1*04, and DRB1*13 alleles, 4-digit DRB1*11:04, DRB1*07:01g, DRB1*03:01, and DRB1*15:01 alleles, and 8-digit DRB1*07:01:01:01, DRB1*11:04:01:01, and DRB1*03:01:01:01 alleles were the most common alleles detected. Three DRB1*11 allele subtypes were observed among the 4-digit allele; DRB1*11:04 was the most common. Five DRB1*11 allele subtype were observed among the 8-digit alleles; DRB1*11:04:01:01 was the most common. DRB1*11:01/12 was the most frequently observed allele in one study² from our country; in another study it was DRB1*07:01.15 Similar to our study findings, Pingel et al.¹¹ determined that DRB1*11, DRB1*04, and DRB1*13 were the most frequently observed 2-digit alleles, while DRB1*11:04, DRB1*07:01, and DRB1*11:01g were the most frequently observed 4-digit alleles.

The DQB1 locus had the least polymorphism. There were five different alleles, of which four had frequencies > 5%. The DQB1*03 allele was the most common allele identified, which is similar to the findings of previous studies.²⁻⁵ In our study, the 2-digit DQB1*03, DRB1*05, and DQB1*06 alleles, 4-digit DQB1*03:01, DQB1*05:01, and DQB1*02:02 alleles, and 8-digit DQB1*03:01:01:02, DQB1*02:02:01:01, and DQB1*02:01:01:01 were the most frequently encountered alleles. There were five

DQB1*03 subtypes among the 4-digit alleles; DQB1*03:01 was the most prevalent. There were 11 DQB1*03 subtypes among the 8-digit alleles; DQB1*03:01:01:02 was the most common. Saruhan-Direskeneli et al.² and Oguz et al.¹⁵ also reported that DQB1*03:01 as the most frequently observed allele in our country. Similar to our study findings, Pingel et al.¹¹ also determined the 2-digit DQB1*03, DQB1*05, and DQB1*06 alleles and 4-digit DQB1*03:01, DQB1*02:01, and DQB1*03:02 alleles were the most frequently observed alleles.

Our study is the first to report HLA-DPB1 locus information in the Turkish population.

After HLA-B, HLA-DPB1 had the most polymorphic structure. with 34 different alleles detected in our LR group study. Moreover, only three alleles had frequencies > 5%. DPB1*04 (AF = 0.55037) was the most prevalent, followed by DPB1*02 (AF = 0.1859) and DPB1*03 (AF = 0.087). Among the 4-digit DPB1*04 alleles, DPB1*04:01, DPB1*02:01, and DPB1*04:02 were the most frequently observed alleles. Among the 8-digit DPB1*04 alleles, DPB1*04:01:01:06 and DPB1*02:01:02:05, followed by DPB1*04:01:01:05 and DPB1*04:02:01:02 were the most frequently observed alleles. In a study conducted in patients with granulomatosis in Turkey,¹⁶ the DPB1*04:01 ratio was 36% higher in the healthy control group than in the disease group, when compared to other alleles. According to the Allele Frequency Net Database (AFND; http://www.allelefrequencies. net/) literature, the most frequently reported DPB1 allele from other countries are as follows: DPB1*02:01:02 (AF = 0.1176-0.6000) and DPB1*04:01 (AF = 0.1078-0.4200) in some regions of Russia; DPB1*04 (AF = 0.560) and DPB1*04:01:01G (AF = 0.445) in Norway;¹⁷ DPB1*04:01 (30%-50%) in Europe and beyond;¹⁸ DPB1*04:01 (AF = 0.4342) in the UK;¹⁹ DPB1*04:01:01 (AF = 0.351851) in Saudi Arabia;²⁰ DPB1*01:01:01 (AF = 0.260) in Guadeloupe;²¹ DPB1*05:01 (AF = 0.360)²² and 41.87%²³ in China; DPB1*05:01:01 (34.1%) in South Korea;²⁴ DPB1*05:01 (AF = 0.4530) in Australia; and DPB1*13:01 in Thailand.25

Our study group data were compatible with those of populations in Europe and its surrounding region, as well as some populations in neighborhoods of Saudi Arabia and Russia.

To clearly analyze the haplotypes, statistical analyses need to be conducted after the haplotypes inherited from both the mother and father have been determined precisely by an HR family study. However, because these analyses are limited by cost and accessibility, haplotype analysis is performed in population genetics analyses using certain programs, and it is commonly used to compare populations. Both of these population genetics programs were used in our study, and the calculations were performed separately using each program. The data from both programs were similar.

Several differences were identified in the top three alleles detected in our study and in previous studies. Among the $A^* \sim B^*$ haplotypes, the 02~51 ranked first in our study; in other studies from different regions it was ranked second,¹⁰ third,⁹ or fourth.⁸ Among the B*~C* haplotypes, the 35~04 ranked first, which is similar to the finding of another study.⁹ The most common A*~DRB1* and B*~DRB1* haplotypes in our study were 24~11 and 35~11, respectively; this differed from the findings of a study conducted in a population located near the Black Sea.¹⁰ In our study, the 11~03 was the most common DRB1*~DQB1* haplotype, which was similar to the finding in another study.² Our study is the first to report the haplotype frequencies for A*~C*, A*~DQB1*, A*~DPB1, B*~DQB1, B*~DPB1, C*~DRB1*, C*~DQB1*, C*~DPB1*, and DQB1*~DPB1*.

The 24~35~04 was the most frequently identified A*~B*~C* haplotype, which was similar to the finding of another study carried out in our country.⁹ The 24~35~11 was most the frequently identified A*~B*~DRB1* haplotype, which has been previously reported.^{4,8} However, this finding differed from the finding conducted in the Black Sea region.¹⁰ The most common DRB1*~DQB1*~DPB1* haplotype in our study was 11~03~04, which has never been reported in our country or the Turkish population.

The most common 4 loci $A^* \sim B^* \sim C^* \sim DRB1^*$ and 5 loci $A^* \sim B^* \sim C^* \sim DRB1^* \sim DQB1^*$ haplotypes in our study were $02 \sim 35 \sim 04 \sim 11$ and $02 \sim 35 \sim 04 \sim 11 \sim 03$, respectively; these findings are similar to those of previous studies.^{4,11}

The most common 6 loci A*~B*~C*~DRB1*~DQB1*~DPB1* haplotypes were $02\sim35\sim04\sim11\sim03\sim04$ (HF = 0.02711), followed by $23\sim49\sim07\sim11\sim03\sim04$ (HF = 0.01389). $24\sim35\sim04\sim11\sim03\sim04$ (HF = 0.01389) had twice the frequency of haplotypes. Our study is significant because it is the first to document the frequencies of 6 loci HLA haplotypes in Turkey. Analysis of AFND data of 6 loci A*~B*~C*~DRB1*~DQB1*~DPB1* haplotypes revealed that $02\sim35\sim04\sim11\sim03\sim04$ (2.711%) was detected at different frequencies in Russia (0.113%), Germany (0.0462%), Norway (0.0100%), and Brazil (3.3891%). Furthermore, the haplotype $23\sim49\sim07\sim11\sim03\sim04$ (1,389%) was determined to be the second most prevalent type in our study, and less so in Russia (0.1724%), Germany (0.0979%), and Norway (0.0400%).

A limitation of our study was that there were not enough participants with 8-digit HLA data to calculate the haplotype frequencies. Thus, the results might not adequately reflect the real haplotype frequency in the Turkish population. Future studies with more participants are required to analyze the 8-digit haplotype frequency. The 8-digit haplotypes identified in this study could offer guidance for future studies. According to 4-digit HLA data, the most common haplotypes for the 5 loci A*~B*~C*~DRB1*~DOB1* haplotype were 01:01~08:01~07:01~03:01~02:01 and 24:02~49:01~07:01~11:01~03:01 and that for the 6 loci A*~B*~C*~DRB1*~DQB1*~DPB1* haplotype was 01:01~35 :02~06:02~11:04~03:01~04:01. Pingel et al.11 also reported that 01:01~08:01~07:01~03:01~02:01 was the most common 5 loci haplotype in a Turkish population.

The most frequently observed 5 loci A*~B*~C*~DRB1*~DQB1* haplotype and 6 loci A*~B*~C*~DRB1*~DQB1*~DPB1* haplotype were 01:01:01:01-08:01:01:01-07:01:01:01-03:01:01: 01~02:01:01:01 and 01:01:01:01-35:02:01:01-03:04:01:02~11:0 4:01:01-03:03:02:02-04:01:01:06, respectively, according to the 8-digit HLA data analysis in our study.

Previous studies from our country have not reported the frequencies of HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci in 2-8-digit alleles, 2-6 loci haplotypes, and genotypes in our region's healthy population using the data we evaluated. Polymorphism studies should be conducted with considerably larger populations and data from a broader geographical region. Although our study does not include the complete Turkish population, it can help map HLAs in our country and provided a basis for future studies.

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee (no: 2021-18).

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship Contributions: Concept- E.Y., E.G., E.Ç.; Design- E.Y., E.G., E.Ç.; Analysis or Interpretation- E.Y., E.Ç.; Critical Review- E.Ç.

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Supplementary File 1.

http://balkanmedicaljournal.org/uploads/pdf/supp-1.pdf

Supplementary File 2.

http://balkanmedicaljournal.org/uploads/pdf/sup-2.pdf

Supplementary File 3.

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Supplementary File 4.

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Supplementary File 5.

http://balkanmedicaljournal.org/uploads/pdf/sup-5.pdf

Supplementary File 6.

http://balkanmedicaljournal.org/uploads/pdf/sup-6.pdf

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