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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 high-resolution 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*02:01:02:05*, 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 country[2-10] or involving the Turkish population[11] have described the distribution

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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 ± 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; in HLA-B were B*49:01:01:01, B*35:01:01:02, 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 DQB1*03:01:01:02, DQB1*02:02:01:01, and DQB1*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*, 9180 A*~B*~C*~DRB1*, 6,687 A*~B*~C*~DRB1*~DQB1*, 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.

TABLE 1. Allele Frequency for HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 at 1st Field Resolution (Two-digit).

	AF	2n		AF	2n		AF	2n		AF	2n		AF	2n		AF	2n
A01	***0.11205***	***1367***	*B03*	0.00016	2	*C01*	0.03992	147	*DR01*	0.07041	859	*DQ02*	0.16667	205	*DP01*	0.01374	15
A02	***0.25025***	***3053***	*B04*	0.00008	1	*C02*	0.03449	127	*DR02*	0.00008	1	***DQ03***	***0.40407***	***497***	***DP02***	**0.1859**	**203**
A03	***0.11443***	***1396***	*B07*	0.05213	636	*C03*	0.05323	196	*DR03*	0.08082	986	*DQ04*	0.02846	35	***DP03***	**0.087**	**95**
A11	0.07607	928	*B08*	0.03951	482	***C04***	***0.19283***	***710***	***DR04***	***0.14705***	***1794***	***DQ05***	***0.21545***	***265***	***DP04***	**0.55037**	**601**
A22	0.00008	1	*B13*	0.03615	441	*C05*	0.02526	93	*DR07*	0.09344	1140	***DQ06***	***0.18537***	***228***	*DP05*	0.01923	21
A23	0.03344	408	*B14*	0.02336	285	*C06*	0.10456	385	*DR08*	0.02434	297	***Total***	**1.00002**	**1230**	*DP06*	0.00183	2
A24	***0.1491***	***1819***	*B15*	0.03549	433	***C07***	***0.21266***	***783***	*DR09*	0.01262	154				*DP08*	0.00092	1
A25	0.01066	130	*B18*	0.06016	734	*C08*	0.03748	138	*DR10*	0.02336	285				*DP09*	0.01465	16
A26	0.05787	706	*B19*	0.00008	1	*C10*	0.00027	1	***DR11***	***0.21049***	***2568***				*DP10*	0.01648	18
A29	0.02033	248	*B24*	0.00008	1	***C12***	***0.12466***	***459***	*DR12*	0.01541	188				*DP11*	0.00366	4
A30	0.03311	404	*B27*	0.0327	399	*C13*	0.00027	1	***DR13***	***0.11467***	***1399***				*DP13*	0.02198	24
A31	0.01557	190	*B32*	0.00016	2	*C14*	0.04155	153	*DR14*	0.06402	781				*DP14*	0.01374	15
A32	0.04738	578	*B34*	0.00008	1	*C15*	0.06926	255	*DR15*	0.09484	1157				*DP15*	0.0119	13
A33	0.02869	350	***B35***	***0.18787***	***2292***	*C16*	0.04101	151	*DR16*	0.04844	591				*DP16*	0.00092	1
A34	0.00016	2	*B37*	0.01426	174	*C17*	0.01793	66	***Total***	**0.99999**	**12200**				*DP17*	0.02381	26
A36	0.00041	5	*B38*	0.04451	543	*C18*	0.00462	17							*DP18*	0.00183	2
A38	0.00008	1	*B39*	0.01607	196	**Total**	**1**	**3682**							*DP19*	0.00366	4
A66	0.005	61	*B40*	0.03885	474										*DP22*	0.00092	1
A68	0.03828	467	*B41*	0.02459	300										*DP23*	0.00458	5
A69	0.0068	83	*B42*	0.00008	1										*DP26*	0.00275	3
A74	0.00025	3	***B44***	***0.07402***	***903***										*DP36*	0.00092	1
Total	**1.00001**	**12200**	*B45*	0.00262	32										*DP45*	0.00092	1
			B46	0.00115	14										*DP47*	0.00183	2
			B47	0.00115	14										*DP51*	0.00183	2
			B48	0.00533	65										*DP61*	0.00092	1
			B49	0.04025	491										*DP66*	0.00183	2
			B50	0.03361	410										*DP70*	0.00092	1
			B51	***0.12574***	***1534***										*DP84*	0.00092	1
			B52	0.03049	372										*DP91*	0.00092	1
			B53	0.00434	53										*DP104*	0.00183	2
			B54	0.00123	15										*DP105*	0.00458	5
			B55	0.03402	415										*DP129*	0.00092	1
			B56	0.00377	46										*DP131*	0.00092	1
			B57	0.01754	214										*DP503*	0.00092	1
			B58	0.01664	203										***Total***	**1.00005**	**1092**
			B59	0.00049	6												
			B60	0.00008	1												
			B66	0.00008	1												
			B73	0.00082	10												
			B82	0.00008	1												
			B83	0.00016	2												
			Total	**0.99998**	**12200**												

AF, allele frequency; 2n, allele count

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
___A*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

AF. allele frequency; 2n. allele count. **Bold** text indicates the alleles with the highest frequency. The full list is provided in Supplementary File 2.

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.

Loci	HN	Haplotype name	HF	Loci		Haplotype name	HF	Loci	HN	Haplotype 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	0.1181
A*~DPB1*	20	02~02	0.0682	**C*~DRB1***	27	06~07	0.0466	**DRB1*~DQB1***	16	15~06	0.0894

HF, haplotype frequency; HN, number of haplotypes with HF > 0.0100 (obtained using GENE[RATE]). The full list is provided in Supplementary File 3.

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	A*~B*~DRB1*	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 caluclated 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]	PyPOP	A*~B*~C*~DRB1*~DQB1*	GENE [RATE]	PyPOP	A*~B*~C*~DRB1*~DQB1*~DPB1*	GENE [RATE]	PyPOP
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.

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. Heterozygotes of the Most Common HLA Allele.

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 subtype. In two studies conducted in our country,[3,5] C*04 was the most common allele; in two other studies.[9,4] C*07 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 study[2] 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*~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 popluation 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*~B*~C*~DRB1* and 5 loci A*~B*~C*~DRB1*~DQB1* haplotypes in our study were 02~35~04~11 and 02~35~04~11~03, 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 02~35~04~11~03~04 (HF = 0.02711), followed by 23~49~07~11~03~04 (HF = 0.01389). 24~35~04~11~03~04 (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 02~35~04~11~03~04 (2.711%) was detected at different frequencies in Russia (0.113%), Germany (0.0462%), Norway (0.0100%), and Brazil (3.3891%). Furthermore, the haplotype 23~49~07~11~03~04 (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*~DQB1* 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 01:01:01:01~35:02:01:01~03:04:01:02~11:04:01:01~03:03:02:02~04:01:01:06, 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.

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

Supplementary File 2.
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Supplementary File 5.
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Supplementary File 6.
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