

HLA Allelic and Haplotypic Organization in Common ABO Blood Group Phenotypes: A Single-Center Cross-Sectional Analysis

Yaygın ABO Kan Grubu Fenotiplerinde Alelik ve Haplotipik HLA Organizasyonu: Tek Merkezli Kesitsel Bir Analiz

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Abstract

Objective: This study aimed to investigate the relationship between the human leukocyte antigen (HLA) system and ABO blood groups in healthy donors. A comparative analysis of HLA immunogenetic architecture was performed between A Rh(+) and O Rh(+) individuals to examine potential population-level variations.

Materials and Methods: This retrospective study included 346 unrelated healthy donors registered with the Eskişehir Osmangazi University Tissue Typing Laboratory between 2017 and 2025. Participants were 50.7% female and 49.3% male, with a mean age of 41.2±14.8 years. Detailed HLA immunogenetic analyses were performed for 249 donors, including 142 A Rh(+) and 107 O Rh(+) individuals. The HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci were genotyped using the DNA-based polymerase chain reaction sequence-specific oligonucleotide method. Allele frequencies, haplotype distributions, Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium (LD) patterns were analyzed using PyPop software.

Results: Allele distributions were broadly similar between groups. The HLA-DRB1*16 allele was enriched in A Rh(+) donors (8.8% vs. 4.2%, $p=0.049$), while HLA-B*49, HLA-DPB1*13, and HLA-DPB1*01 alleles were enriched in O Rh(+) donors ($p=0.032$, 0.022, and 0.015, respectively). LD patterns differed between the groups [A Rh(+): DQB1~DPB1, $p=0.038$; O Rh(+): HLA-A~DQB1, $p=0.019$], and all loci were in HWE ($p>0.05$).

Conclusion: The analysis indicated that A Rh(+) and O Rh(+) donors share a largely similar HLA genetic background, with modest but detectable differences in selected class I and class II HLA alleles and LD patterns. These subtle variations likely reflect minor population heterogeneity rather than distinct ancestry and may aid in donor matching efforts and the interpretation of population-based immunogenetic association studies.

Keywords: Human leukocyte antigen, Immunogenetics, ABO blood groups, Haplotype frequencies, Linkage disequilibrium

Öz

Amaç: Bu çalışma, sağlıklı donörlerde insan lökosit antijen (HLA) sistemi ile ABO kan grupları arasındaki ilişkiyi araştırmayı amaçlamıştır. A Rh(+) ve O Rh(+) bireyler arasında, popülasyon düzeyinde olası farklılıkları incelemek amacıyla HLA immünogenetik mimarisinin karşılaştırmalı analizi yapılmıştır.

Gereç ve Yöntemler: Bu retrospektif çalışmaya, 2017-2025 yılları arasında Eskişehir Osmangazi Üniversitesi Doku Tipleme Laboratuvarı'na kayıtlı 346 akraba olmayan sağlıklı donör dahil edilmiştir. Katılımcıların %50,7'si kadın, %49,3'ü erkek olup, yaş ortalaması 41,2±14,8 yıldır. Ayrıntılı HLA immünogenetik analizleri 249 donör üzerinde gerçekleştirilmiştir [142 A Rh(+) ve 107 O Rh(+)]. HLA-A, -B, -C, -DRB1, -DQB1 ve -DPB1 lokusları DNA temelli diziyeye özgü oligonükleotid temelli polimeraz zincir reaksiyonu yöntemi ile genotiplenmiştir. Alel frekansları, haplotip dağılımları, Hardy-Weinberg dengesi (HWE) ve bağlantı dengesizliği (LD) örüntüleri PyPop yazılımı kullanılarak analiz edilmiştir.

Bulgular: Genel olarak alel dağılımları iki grup arasında büyük ölçüde benzer bulunmuştur. HLA-DRB1*16 aleli A Rh(+) donörlerde daha yüksek sıklıkta saptanırken (%8,8'e karşı %4,2; $p=0,049$), HLA-B*49, HLA-DPB1*13 ve HLA-DPB1*01 alelleri O Rh(+) grubunda daha yüksek sıklıkta saptanmıştır (sırasıyla $p=0,032$, 0,022 ve 0,015). LD örüntüleri gruplar arasında farklılık göstermiştir [A Rh(+): DQB1~DPB1, $p=0,038$; O Rh(+): HLA-A~DQB1, $p=0,019$] ve incelenen tüm lokuslar HWE'ye uygun bulunmuştur ($p>0,05$).

Sonuç: Analizler, A Rh(+) ve O Rh(+) donörlerin büyük ölçüde benzer bir HLA genetik yapısına sahip olduğunu, ancak seçilmiş sınıf I ve sınıf II HLA alelleri ile LD örüntülerinde sınırlı fakat istatistiksel olarak saptanabilir farklılıklar bulunduğunu göstermektedir. Bu ince immünogenetik varyasyonların, belirgin atasal ayrışmalardan ziyade popülasyon düzeyinde hafif bir genetik heterojeniteyi yansıttığı düşünülebilir. Bu farklılıkların anlaşılması, donör eşleştirme stratejilerinin optimize edilmesine ve popülasyon temelli immünogenetik ilişkilendirme çalışmalarının daha doğru yorumlanmasına katkı sağlayabilir.

Anahtar Sözcükler: İnsan lökosit antijeni, İmmünogenetik, ABO kan grupları, Haplotip frekansları, Bağlantı dengesizliği



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Introduction

The human leukocyte antigen (HLA) system (chromosome 6) is highly polymorphic and critical for immune response and tissue compatibility in transplantations [1]. Similarly, the ABO and Rh blood group systems (chromosomes 9 and 1) are fundamental to transfusion medicine and clinical outcomes [2]. Managing HLA mismatches and blood group incompatibilities is essential for preventing graft rejection and acute hemolytic reactions [2,3,4].

Studies have suggested potential associations between HLA profiles and ABO phenotypes; however, findings remain inconsistent and the immunogenetic relationship remains unclear in healthy individuals. Understanding subtle genetic differences between common blood groups, such as A Rh(+) and O Rh(+), may refine donor selection and improve the interpretation of population-based disease association studies. Currently, there is a lack of regional data on how these associations manifest, restricting our understanding of the extent to which ABO groups reflect underlying immunogenetic variation [3,4,5].

In this study, we hypothesized that common ABO phenotypes, specifically A Rh(+) and O Rh(+), coincide with subtle but measurable variations in HLA allelic and haplotypic distributions, reflecting a degree of immunogenetic heterogeneity within a healthy regional population. To test this hypothesis, we performed a comparative analysis of the HLA immunogenetic architecture between healthy A Rh(+) and O Rh(+) donors in a Western Central Anatolian Turkish population. We aimed to detect differences in allele frequencies, haplotype distributions, and linkage disequilibrium (LD) patterns to provide insight into regional immunogenetic diversity and offer potential implications for donor matching and immunogenetic research.

Materials and Methods

Study Population

This retrospective cross-sectional study initially evaluated 346 healthy donors registered with the Eskişehir Osmangazi University Tissue Typing Laboratory in 2017-2025. Based on the simultaneous availability of ABO/Rh and HLA genotyping data, 249 donors were included in the final analysis. The cohort comprised 142 A Rh(+) and 107 O Rh(+) individuals, representing the region's most prevalent phenotypes.

The participants were 50.7% female and 49.3% male, with a mean age of 41.2 ± 14.8 years. No statistically significant differences in age or sex distribution were detected between the A Rh(+) and O Rh(+) groups ($p > 0.05$), ensuring demographic homogeneity for immunogenetic comparisons.

The study protocol was approved by the Eskişehir Osmangazi University Clinical Research Ethics Committee (protocol no: 08; date: 06.01.2026) and conducted in accordance with the

principles of the Declaration of Helsinki. Due to the retrospective nature of the study, all data were anonymized to ensure donor confidentiality.

Inclusion/Exclusion Criteria

The study population was selected based on the following inclusion criteria: (i) unrelated healthy blood donors aged 18-65 years, (ii) availability of HLA genotyping results, and (iii) documented ABO and RhD blood group data. The exclusion criteria were as follows: (i) presence of known chronic systemic, inflammatory, or autoimmune diseases and (ii) incomplete or ambiguous immunogenetic records.

Laboratory Methods

ABO and RhD blood grouping tests were performed at the institutional transfusion laboratory using the gel centrifugation hemagglutination method (Bio-Rad ID-System, Bio-Rad Laboratories, Fribourg, Switzerland). In accordance with standard quality protocols, both forward (cell) and reverse (serum) groupings were performed for all samples. To ensure phenotypic accuracy, only donors demonstrating 100% concordant forward and reverse typing results were eligible for inclusion in the study.

For HLA typing, genomic DNA was extracted from ethylenediaminetetraacetic acid-anticoagulated peripheral blood samples using standard procedures. Genotyping was performed for six loci (HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1) using the LIFECODES sequence-specific oligonucleotide (SSO) typing kit (Immucor, Stamford, CT, USA) according to the manufacturer's instructions [6]. Allele assignment and nomenclature were based on the IMGT/HLA database (version 3.43.0) [7].

Statistical Analysis

Population genetic analyses were performed specifically for the A Rh(+) and O Rh(+) donor groups to ensure statistical robustness; other blood groups were excluded from the comparative immunogenetic analysis due to insufficient sample sizes. Allele and haplotype frequencies were estimated using PyPop software (version 1.3.0) [8,9]. Haplotype frequencies for multilocus genotypes were inferred using the expectation-maximization algorithm.

Deviation from Hardy-Weinberg equilibrium (HWE) was evaluated for each locus using both the chi-square test and the Guo-Thompson exact test based on a Markov chain Monte Carlo approach with 1000 iterations. To assess selective neutrality and the distribution of allele frequencies, the Ewens-Watterson homozygosity test and Slatkin's exact test were applied.

LD between all pairs of loci was assessed by calculating D and D' coefficients. The statistical significance of LD was determined using permutation testing to obtain empirical p values. A D' threshold of ≥ 0.5 was considered indicative of strong LD.

Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using Fisher’s exact test for alleles showing intergroup differences. Because alleles within the major histocompatibility complex are correlated through strong LD, the effective number of independent tests is lower than the nominal number of allele comparisons; therefore, we reported unadjusted p values and interpreted results as hypothesis-generating signals, with emphasis on effect sizes, CIs, and biological plausibility. Demographic comparisons were performed using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

Results

General Profile of the Study Population

A total of 346 unrelated healthy donors were included in the initial screening process. The most prevalent blood groups in the total donor pool were A Rh(+) (n=142, 41.0%) and O Rh(+) (n=107, 30.9%), followed by B Rh(+) (11.0%), O Rh(-) (6.1%), and AB Rh(+) (5.2%) (Table 1; Figure 1).

Blood group	Percentage (%)
A Rh(+)	41.0
O Rh(+)	30.9
B Rh(+)	11.0
O Rh(-)	6.1
AB Rh(+)	5.2
A Rh(-)	3.5
B Rh(-)	1.7
AB Rh(-)	0.6
Total	100.0

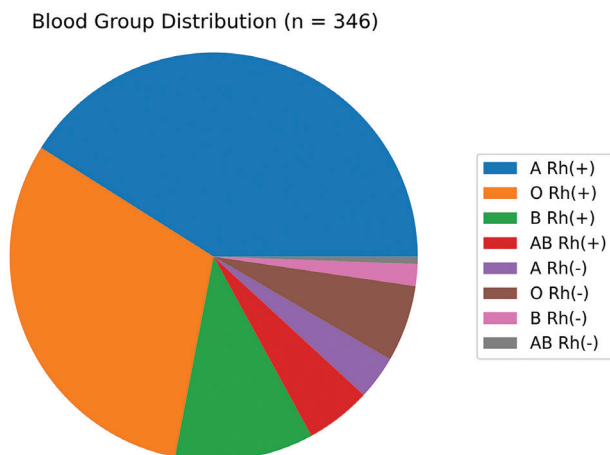


Figure 1. Blood group distribution.

A detailed immunogenetic comparative analysis was conducted for 249 donors representing A Rh(+) and O Rh(+) as the two most frequent phenotypes. Across the analyzed cohort, the most frequent HLA alleles were HLA-A*02, HLA-B*35, HLA-C*07, HLA-DRB1*11, HLA-DQB1*03, and HLA-DPB1*04, providing a representative overview of regional HLA profiles.

Basic Population Structure and Heterozygosity

Both donor groups demonstrated genetically stable population structures in this study. All examined HLA loci conformed to HWE ($p > 0.05$), indicating no significant deviation from the expected genotype distribution. The observed heterozygosity (H_o) values exceeded 85% at most loci, reflecting extensive allelic variation. The O Rh(+) group showed slightly higher heterozygosity at HLA-A and HLA-B, whereas the A Rh(+) group demonstrated marginally higher diversity at HLA-DQB1. The lowest heterozygosity was observed at the HLA-DPB1 locus in both groups (Table 2).

Allele Frequency Differences Between A Rh(+) and O Rh(+) Donors

Although the overall allele distributions were broadly similar between the two donor groups, several statistically significant differences were identified at specific loci.

The HLA-B*49 allele was significantly enriched in O Rh(+) individuals (7.0% vs. 2.8%), corresponding to significantly lower odds in A Rh(+) donors (OR: 0.38, 95% CI: 0.16-0.92, $p = 0.032$). Conversely, HLA-DRB1*16 was more common in A Rh(+) individuals (8.8%) than in O Rh(+) individuals (4.2%), representing a more than twofold increased likelihood in the A Rh(+) group (OR: 2.20, 95% CI: 1.00-4.81, $p = 0.049$). Additionally, DPB1*13 was markedly enriched in the O Rh(+) group (5.8%) compared to the A Rh(+) group (0.6%) (OR: 0.10, 95% CI: 0.01-0.90, $p = 0.022$). Furthermore, DPB1*01 was observed exclusively in the O Rh(+) group (4.7%), representing significantly lower odds in A Rh(+) donors (OR: 0.06, 95% CI: 0.003-1.09, $p = 0.015$). Other frequent alleles, including A*02, B*35, C*07, DRB1*11, DQB1*03, and DPB1*04, did not differ significantly between the groups and represented the shared immunogenetic backbone of the study population (Table 3).

HLA locus	A Rh(+) H_o	O Rh(+) H_o
A	89.4	92.1
B	91.2	93.0
C	86.0	87.9
DRB1	88.1	89.7
DQB1	84.5	82.2
DPB1	58.3	62.4

HLA: Human leukocyte antigen.

Table 3. HLA alleles showing significant frequency differences and odds ratios between donor groups.

HLA allele	A Rh(+) (2n=284), n (%)	O Rh(+) (2n=214), n (%)	OR	95% CI	p
DRB1*16	25 (8.8%)	9 (4.2%)	2.20	1.00-4.81	0.049
B*49	8 (2.8%)	15 (7.0%)	0.38	0.16-0.92	0.032
DPB1*13	2 (0.6%)	12 (5.8%)	0.10	0.01-0.90	0.022
DPB1*01	0 (0.0%)	10 (4.7%)	0.06	0.00-1.09	0.015

OR values were calculated with O Rh(+) as the reference group; p values were calculated using Fisher's exact test with significance at $p < 0.05$.
HLA: Human leukocyte antigen; OR: odds ratio; CI: confidence interval.

Table 4. Comparison of top haplotypes across increasing locus resolution.

Locus level	Group	Rank 1 haplotype	Rank 2 haplotype	Freq. (%)	Freq. (%)
3-Locus (A~B~C)	A Rh(+)	A*02~B*35~C*04	A*24~B*35~C*04	6.98	2.51
	O Rh(+)	A*01~B*35~C*04	A*11~B*35~C*04	4.08	4.08
4-Locus (A~B~C~DRB1)	A Rh(+)	A*02~B*35~C*04~DRB1*11	A*02~B*07~C*07~DRB1*15	3.05	2.44
	O Rh(+)	A*11~B*35~C*04~DRB1*15	A*03~B*07~C*07~DRB1*15	3.06	3.06
5-Locus (A~B~C~DRB1~DQB1)	A Rh(+)	A*02~B*35~C*04~DRB1*11~DQB1*03	A*24~B*35~C*04~DRB1*04~DQB1*03	3.05	2.44
	O Rh(+)	A*11~B*35~C*04~DRB1*15~DQB1*06	A*02~B*44~C*14~DRB1*11~DQB1*03	3.19	3.19
6-Locus	A Rh(+)	A*02~B*35~C*04~DRB1*11~DQB1*03~DPB1*04	A*24~B*35~C*04~DRB1*04~DQB1*03~DPB1*04	3.21	2.56
	O Rh(+)	A*01~B*35~C*03~DRB1*07~DQB1*02~DPB1*04	A*23~B*49~C*07~DRB1*11~DQB1*03~DPB1*04	2.44	2.44

Haplotype Profiles Across Increasing Locus Resolution

Haplotype frequency analysis demonstrated both shared conserved haplotypic blocks and group-specific distribution patterns, indicating modest but detectable population-level differentiation between A Rh(+) and O Rh(+) donors (Table 4).

Three-Locus Level (HLA-A~B~C)

At the class I three-locus level, the A Rh(+) group exhibited a pronounced and dominant haplotype structure. The most frequent combination was HLA-A*02~B*35~C*04 (6.98%), which clearly exceeded all other identified haplotypes. In contrast, the O Rh(+) group showed a more evenly distributed profile, with two co-dominant haplotypes, HLA-A*01~B*35~C*04 and HLA-A*11~B*35~C*04, each occurring at a rate of 4.08%. Notably, haplotypes containing B*49 (for example, HLA-A*23~B*49~C*07) appeared among the top combinations exclusively in the O Rh(+) group, consistent with the allele-level enrichment of HLA-B*49 in this subgroup.

Four-Locus Level (HLA-A~B~C~DRB1)

In the A Rh(+) cohort, the haplotypic structure remained centered around a single dominant block, HLA-A*02~B*35~C*04~DRB1*11 (3.05%). Conversely, the O Rh(+) group demonstrated multiple co-dominant haplotypes at comparable frequencies (~3.06%), including HLA-A*11~B*35~C*04~DRB1*15 and HLA-A*03~B*07~C*07~DRB1*15.

Five-Locus Level (HLA-A~B~C~DRB1~DQB1)

The dominant haplotype in the A Rh(+) group was HLA-A*02~B*35~C*04~DRB1*11~DQB1*03 (3.05%). In contrast, the O Rh(+) group again showed a more distributed profile, with two leading haplotypes, HLA-A*11~B*35~C*04~DRB1*15~DQB1*06 and HLA-A*02~B*44~C*14~DRB1*11~DQB1*03, each occurring at a rate of 3.19%.

Six-Locus Level (HLA-A~B~C~DRB1~DQB1~DPB1)

The A Rh(+) group retained a clearly leading extended haplotype, HLA-A*02~B*35~C*04~DRB1*11~DQB1*03~DPB1*04 (3.21%), indicating a relatively centralized haplotypic architecture. In contrast, the O Rh(+) group did not show a single dominant extended haplotype. Several combinations shared the highest observed frequency (2.44%), including HLA-A*23~B*49~C*07~DRB1*11~DQB1*03~DPB1*04, again highlighting the contribution of B*49-bearing chromosomes to the O Rh(+) immunogenetic structure.

Shared Class II Backbone

Despite the class I-driven differences, both groups shared a highly conserved class II haplotype. HLA-DRB1*11~DQB1*03~DPB1*04 was the most frequent DR~DQ~DP combination in both A Rh(+) (13.72%) and O Rh(+) (13.82%) individuals.

Analysis of LD Profiles

Pairwise LD analysis was performed to evaluate the strength of non-random genetic associations between HLA loci in the A Rh(+) and O Rh(+) donor groups (Table 5; Figure 2).

In both groups, strong and highly significant LD ($p < 0.001$) was consistently observed between neighboring loci, particularly for

HLA-C~HLA-B, HLA-B~HLA-DRB1, and HLA-DRB1~HLA-DQB1. These findings confirm the conserved inheritance of HLA genes as haplotypic blocks in this population.

However, differences emerged when long-range associations between distant loci were examined. A statistically significant LD between HLA-A and HLA-DQB1 was detected in the O Rh(+) group ($p = 0.019$), while this association did not reach statistical significance in the A Rh(+) group. Conversely, the HLA-DQB1~HLA-DPB1 pair showed significant LD in the A Rh(+) group ($p = 0.038$), but not in the O Rh(+) group.

Table 5. Linkage disequilibrium for groups.

Locus pair	A Rh(+) D'	A Rh(+) p	O Rh(+) D'	O Rh(+) p
HLA-A~HLA-C	0.452	0.018*	0.498	0.027*
HLA-A~HLA-B	0.427	0.002*	0.548	0.0003*
HLA-A~HLA-DRB1	0.295	0.198	0.318	0.176
HLA-A~HLA-DQB1	0.284	0.087	0.412	0.019*
HLA-C~HLA-B	0.801	<0.0001*	0.836	<0.0001*
HLA-C~HLA-DRB1	0.446	0.002*	0.489	0.006*
HLA-C~HLA-DQB1	0.351	0.011*	0.372	0.041*
HLA-B~HLA-DRB1	0.552	<0.0001*	0.563	<0.0001*
HLA-B~HLA-DQB1	0.443	<0.0001*	0.497	<0.0001*
HLA-DRB1~HLA-DQB1	0.941	<0.0001*	0.879	<0.0001*
HLA-DRB1~HLA-DPB1	0.392	0.028*	0.541	0.031*
HLA-DQB1~HLA-DPB1	0.268	0.038*	0.473	0.211

*: Statistically significant at $p < 0.05$; D': pairwise linkage disequilibrium.

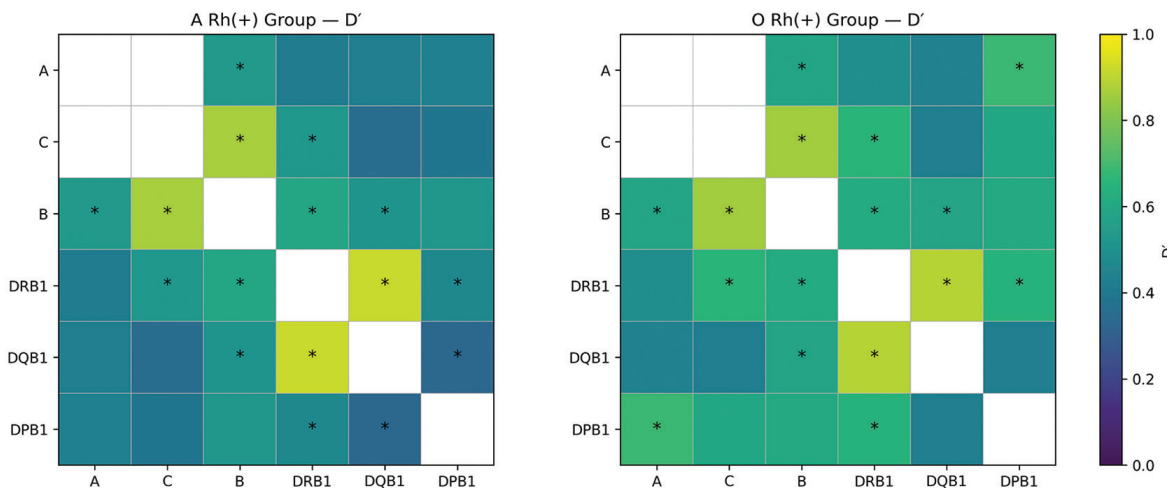


Figure 2. Linkage disequilibrium (LD) heatmaps for human leukocyte antigen (HLA) loci in the A Rh(+) and O Rh(+) groups. Heatmaps illustrate the pairwise linkage disequilibrium (D') values among the HLA loci. The color intensity corresponds to the magnitude of D'. Cells marked with an asterisk (*) indicate statistically significant LD ($p < 0.05$).

Discussion

This study demonstrates that healthy A Rh(+) and O Rh(+) blood donors from a regional Turkish population exhibit subtle but detectable differences in HLA allele frequencies, haplotypic patterns, and LD structure. Although the ABO and HLA systems are located on different chromosomes, the multilayered differences observed across alleles, haplotypes, and LD levels indicate that ABO phenotypes align with subtle immunogenetic heterogeneity within a regional donor population rather than reflecting direct genetic linkage [2,3].

The predominance of A Rh(+) and O Rh(+) phenotypes in our donor pool is consistent with the national ABO distribution data in Türkiye [4,10,11]. However, the principal contribution of this study lies in demonstrating that these two common blood groups differ not only in individual allele frequencies but also in the structural organization of their immunogenetic backgrounds. This organization includes differences in dominant haplotype blocks, degree of haplotypic centralization versus dispersion, and extent of long-range versus localized LD patterns.

Allelic Differences Reflecting Immunogenetic Heterogeneity

Allele-level variations provide the first layer of evidence for genetic differentiation between the studied groups. The enrichment of B*49 and DPB1*13 in O Rh(+) donors, alongside the higher frequency of DRB1*16 in A Rh(+) individuals, suggests subtly differing immunogenetic patterns within the same geographic population. Notably, the markedly lower odds of DPB1*13 in A Rh(+) donors (OR: 0.10) and the exclusive detection of DPB1*01 in the O Rh(+) group support this variation. However, due to low absolute frequencies, these findings should be regarded as preliminary observations requiring cautious interpretation.

It should also be noted that ABO blood groups are not classical markers of deep ancestry. The differences observed in this study most likely reflect regional sampling variability or subtle shifts in the immunogenetic background of the donor registry rather than ancestral stratification. Given that the HLA and ABO systems are located on separate chromosomes (9 and 6, respectively), these associations are interpreted as population-level co-occurrences within a defined geographic area.

Given Türkiye's history of migration and admixture, these variations likely reflect historical population structure rather than recent selection. Since these patterns were identified in healthy individuals, they represent baseline genetic variation. Overall, these findings are most consistent with subtle immunogenetic heterogeneity (or sampling-level variation) within the regional donor registry rather than distinct ancestry-defined strata [5,10].

Haplotype Architecture: Relative Centralization Versus Dispersion

The A Rh(+) group exhibited a centralized haplotypic pattern dominated by the HLA-A*02~B*35~C*04~DRB1*11~DQB1*03~DPB1*04 extended haplotype. In contrast, the O Rh(+) group showed a flatter dispersed organization with multiple co-dominant haplotypes. These structural variations, evidenced by the cumulative frequency of top haplotypes, likely reflect distinct historical patterns of genetic drift and recombination within the regional population.

Structural Organization Revealed by LD

While both groups shared strong LD between neighboring loci (e.g., HLA-C~B and DRB1~DQB1), long-range associations diverged. The HLA-A~DQB1 linkage was significant only in O Rh(+) donors, whereas the DQB1~DPB1 linkage was significant only in the A Rh(+) group. This suggests subtle differences in chromosomal coupling along chromosome 6, supporting the presence of a mild immunogenetic substructure.

Implications for Immunogenetic Diversity and Clinical Practice

Although the observed differences were modest, they may have practical relevance. In hematopoietic stem cell transplantation (HSCT), donor matching depends heavily on haplotype frequencies in regional registries. The more centralized haplotypic structure in A Rh(+) individuals may increase the probability of identifying matches for common haplotypes, whereas the more dispersed O Rh(+) structure indicates broader diversity that could be advantageous for matching rarer haplotypic combinations. These findings may also contribute to the interpretation of unexpected donor-recipient mismatches in HSCT registries from genetically heterogeneous populations.

Beyond transplantation, these findings may also help contextualize the reported associations between ABO blood groups and infectious or immune-mediated diseases. Associations reported between specific ABO phenotypes and disease outcomes, such as coronavirus disease-2019 severity, may not be attributable solely to ABO antigens but could be partially influenced by the underlying HLA background that co-occurs within the ABO-defined subpopulation [12]. Similarly, a study by Mora-Buch et al. [13] showed that RhD status and HLA genotypes may influence the T-cell response to pathogens such as the BK virus. Therefore, our results provide a population genetic framework for interpreting ABO-disease associations with greater nuance and caution.

Limitations and Future Directions

Several limitations of this study should be acknowledged. First, the moderate sample size and single-center design limited the statistical power and generalizability of our findings,

particularly for less frequent blood groups and rare alleles. Consequently, associations involving low-frequency variants should be interpreted with caution, as small count variations can disproportionately skew OR estimates and significance levels.

Furthermore, no formal correction for multiple comparisons was applied. Given the exploratory aim of identifying potential immunogenetic patterns within a regional population, raw *p* values were presented to minimize the risk of overlooking potentially relevant signals. Additionally, since HLA loci exhibit strong LD, these alleles are not independent variables, making strict correction methods potentially overly conservative in this context. Accordingly, the reported findings should be regarded as preliminary observations that require validation in larger, multicenter cohorts.

The donor pool consisted of voluntary donors rather than a strictly random population sample, which may have introduced some degree of selection bias. Another primary limitation is the use of the polymerase chain reaction (PCR) SSO typing method, which provides low-to-intermediate resolution. While this approach is acceptable for regional registry-level analyses, it inevitably limits the confidence in haplotype inference and the precise characterization of LD patterns compared to contemporary high-resolution sequencing. Specifically, intermediate resolution may mask rare allelic variants and finer-scale diversity, potentially leading to an oversimplification of the haplotypic architecture.

Additionally, the A blood group was not further subdivided into A1 and A2 phenotypes using A1 lectin. Since A1 and A2 subgroups differ in antigen density and potentially in their clinical and immunogenetic associations, future studies incorporating such subtyping may provide more granular insights into specific HLA-ABO interactions.

Future studies conducted with larger and more diverse populations, encompassing all ABO phenotypes, are required to validate these findings and determine whether the observed structural differences in HLA architecture translate into functional immunological consequences or disease susceptibility patterns.

Conclusion

Healthy A Rh(+) and O Rh(+) donors in this regional Turkish cohort shared a largely similar HLA background, while selected alleles (e.g., DRB1*16, B*49, DPB1*13, DPB1*01) and LD patterns showed modest but detectable differences. Because the ABO and HLA loci are on different chromosomes, these observations most likely reflect sampling-level immunogenetic heterogeneity within the donor registry rather than distinct ancestry-defined differences. Recognizing this variation may contribute to improved interpretation of population-based

association studies and provide supportive information for donor matching. Given the exploratory design and intermediate-resolution PCR-SSO typing, these findings should be regarded as hypothesis-generating observations that warrant validation in larger multicenter cohorts using high-resolution genotyping.

Ethics

Ethics Committee Approval: The study protocol was approved by the Eskişehir Osmangazi University Clinical Research Ethics Committee (protocol no: 08; date: 06.01.2026) and conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all participants prior to their enrolment in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.G.; Concept: E.Y.; Design: E.Y., E.G.; Data Collection or Processing: E.Y., E.G.; Analysis or Interpretation: E.Y., E.G.; Literature Search: E.Y.; Writing: E.Y.

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