GenoMed Connect
ISSN: 3006-9033
2024, Article ID. 2024.0002, Cite as: www.doi.org/10.69709/GenomC.2024.193514

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Research Article

OPEN ACCESS

Diversity of HLA Class I and II Genes in the North Indian Population

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Article History

Submitted: June 24, 2024 Accepted: August 25, 2024 Published: September 12, 2024

Abstract

Introduction: Numerous studies have concentrated on specific populations to explore the extensive polymorphism of class I and II HLA genes. This genetic diversity is crucial for various applications, such as advancing transplantation immunology, understanding genetic population patterns, and uncovering the pathways of different diseases. **Objective:** The objective of the present study was to determine and analyse the frequencies of class I (HLA-A, HLA-B and, HLA-C), and class II (HLA-DRB1, and HLA-DQB1) genes in the North Indian population. **Material and Methods:** To achieve the objective of the study, buccal swab samples from 3648 individuals were collected. All these samples were subsequently tested for the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 genes using the polymerase chain reaction (PCR) sequence-specific oligonucleotide probe (SSOP) typing method and typing result were analyzed to estimate class I and II allele frequencies. **Results:** In the present study, we have identified 16 different variants of HLA-A genes, 28 variants of HLA-B genes, 13 variations of HLA-C genes, 14 variants of HLA-DRB1 genes, and 6 variants of HLA-DQB1 genes. Furthermore, HLA-A*11, HLA-B*35, HLA-C*07, HLA-DRB1*15, and DQB1*06 were observed to be the most frequent alleles within the studied population. **Discussion:** The findings of the studied population highlights the variability exhibited by the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 genes in the North Indian population. Additionally, this also highlights the importance of testing and understanding the prevalence of these specific HLA genes in these populations, which has a greater relevance in both hematopoietic stem cell and solid organ transplantation as well as disease association studies.

Keywords:
HLA; HLA-A; HLA-B; HLA-C; HLA-DRB1; HLA-DQB1

1. Introduction

The human leukocyte antigen (HLA) gene, found on chromosome 6 (6p21. 3), is known for its polymorphism in the human genome. The HLA gene is primarily classified into two classes: class I (HLA-A, -B, and -C) and class II (HLA-DR, -DQ, and DP) [1]. Both the HLA class I and class II genes play an important role in the outcomes of hematopoietic stem cell and solid organ transplantation, as they encode proteins that are vital for antigen presentation [2]. Over 36,000 HLA alleles are currently listed in the IMGT/HLA database (http://hla.alleles.org/nomenclature/stats.html accessed on 12 April 2024) [3]. The primary factors contributing to the polymorphism and molecular sequence variation observed among HLA genes include mutations, selection pressures driven by environmental factors, and the integration of diverse ethnic groups [4–6].

India is well known for its enormous diversity because of the wide range of linguistic and cultural differences that exist among its several regions (North, South, West, Central, and North East). The northern Indian population is distinguished among these areas for having extremely diverse HLA gene polymorphisms [7,8]. Furthermore, in India, various tribal groups, castes and religions exist within various societies. These communities often exhibit unique or limited diversity due to social and cultural barriers that enforce strict endogamy practices. As a result, varied HLA gene complexes have evolved within different Indian communities [7,8]. Despite a growing number of studies focusing on HLA polymorphism in Northern regions, comprehensive data on HLA gene di-

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versity in this population remains scant, especially when larger sample sizes are considered [9–13].

Furthermore, understanding HLA gene diversity is crucial for advancing disease association studies, developing population-specific vaccines, and enhancing the effectiveness of matched unrelated hematopoietic stem cell transplantation (HSCT) programs within stem cell registries worldwide [14,15]. In addition, the HLA gene has also been extensively used to explore the genetic relationships among diverse populations worldwide to understand the demographic events that have shaped these groups. Thus, having access to population-specific HLA gene diversity data is crucial for understanding the history of human settlement and the overall evolution of the human immune system [16]. Therefore, the primary objective of the present study is to determine and analyze the prevalence of HLA-A, B, C, DRB1, and DQB1 gene polymorphisms within the North Indian population, contributing to a deeper understanding of this population's genetic makeup.

2. Materials and Methods

2.1. Study Population

This study is a retrospective analysis conducted on a cohort of 3648 healthy individuals aged between 18 and 55 years from May 2012 to December 2022. The samples for this study were collected from the regions of North Indian States (Delhi, Punjab, Haryana, Jammu, and Uttarakhand). The participants' data were not differentiated into different tribal groups. Any healthy individual between 18 and 55 years old was included in the study. Individuals with a history of blood pressure, diabetes, blood disorders, or major surgery were excluded. The present study was approved by the independent ethical review board of the institute. The study was carried out following the Declaration of Helsinki. Written consent was obtained from each participant, both for participation in the study and for publication purposes. All participants were made aware that their names and initials would not be published, and all standard protocols would be followed to conceal their identity.

2.2. Sample Type

Buccal swab samples were collected from each individual as per the established standard operating procedure (SOP). Two buccal swab sticks were obtained from each person by gently rubbing the inside of each cheek with the swab stick one at a time.

2.3. DNA Extraction and HLA Testing

The DNA extraction from the entire collected buccal swab samples was done by an automated system known as Chemagen MSM I (PerkinElmer, Germany), which is based on electromagnetic iron beads as per the instructions provided in the kit insert (CMG-748 DNA buccal swab kit, PerkinElmer, Baesweiler, Germany). Subsequently, DNA extraction was followed by quantification using a Qubit® 2.0 spectrophotometer (Invitrogen, USA). The average concentration of the extracted DNA ranged between 40–120 ng/μL.

Once the DNA gets extracted and quantified HLA testing was performed by the polymerase chain reaction sequence-specific oligonucleotide probe (PCR-SSOP) low-resolution method using LIFECODES HLA SSO typing (Immucor, USA). The HLA SSOP typing method is based on the hybridization of labeled single stranded polymerase chain reaction (PCR) product to SSO probes. These probes are designed so that each probe preferentially hybridizes to a complementary region that may or may not be present in the PCR amplified DNA. Probes whose measured reaction to a sample was more than 60% of absolute percent deviation known as positive probes were negative in case of less than 60%. In addition, the amplified DNA is also hybridized to one or more consensus probes homologous to sequences present in all the alleles of a locus. The PCR mixture was prepared with 6 μL of Life codes master mix, (provided with kit), 80 ng of genomic DNA, and 0.2 μL 1 U Taq polymerase in a final volume of 20 μL, and then subjected to the following conditions: denaturation at 95 °C for 3 min, 40 cycles of amplification (12 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s, and 28 cycles of 95 °C for 10 s, 63 °C for 30 s, and 72 °C for 30 s) and extension at 72 °C for 2 min. Hybridization was performed in the following conditions: 15 μL probe mix and 5 μL of the PCR product at 97 °C for 2 min, 47 °C for 10 min, and 56 °C for 8 min. The samples were diluted with 170 μL of 1:200 prediluted streptavidin-phycoerythrin solution and analyzed within 30 min using a Luminex® 200 system. Once the run gets completed, the data obtained was export ed. as a comma separated value (csv) file. These files were named "OUTPUT.CSV" and saved in a folder with the Batch Name. Further, these saved data were available for making HLA typing assignments by using MatchIT DNA software.

3. Statistical Analysis of HLA-A, B, C, DRB1and DQB1 Allele

The alleles for HLA-A, -B, -C, -DRB1, and -DQB1 loci were determined using the direct counting method (n/2N

2024, Vol. 1, Article ID. 2024.0004
www.doi.org/10.69709/GenomC.2024.193514

× 100) in Microsoft Excel 2010, where 'n' represents the count of a specific allele and 'N' signifies the total number of samples under study.

4. Results

4.1. HLA-A, B and C Allele Frequency

Following a statistical analysis, we have identified a diverse range of class I alleles: 16 alleles of HLA-A, 28 alleles of HLA-B, and 13 alleles of HLA-C. Out of these identified HLA-A alleles, HLA-A*11 (16.45 %,) was more frequent as compared to others. Subsequently, the most frequent HLA-A alleles were HLA-A*02 (15.28%) and HLA-A*24 (14.41%). Similarly, in HLA-B alleles, HLA-B*35 (14.21%) was found to be the most prevalent, followed by HLA-B*40 (11.47%) and HLA-B*51 (11.31%). Among the HLA-C alleles, HLA-C*07 (26.08%) was identified as more frequent than other variants followed by HLA-C*04 (14.21%) and HLA-C*06 (11.16%). All these findings are shown in Table 1.

Furthermore, in the HLA-A locus, 87.83% of the samples showed heterozygosity and homozygosity was seen only in 12.17%. For the HLA-B locus, 91.25% of the samples were heterozygous, whereas homozygosity was observed in only 8.75% of the cases. Similarly, in the HLA-C locus, 84.64% of the samples were found to be heterozygous, while 15.36% exhibited homozygosity.

4.2. HLA-DRB1 and DQB1 Allele Frequency

In the studied population, a total of 14 alleles of HLA-DRB1 and 6 different alleles of HLA-DQB1 were identified. Among the alleles of HLA-DRB1, the HLA-DRB1*15 (19.13%) was observed to have the highest frequency. This was subsequently followed by the HLA-DRB1*03 (15.49%) and the HLA-DRB1*13 (12.84%). Similarly, in the HLA-DQB1 alleles, the HLA-DQB1*06 (27.86%) allele was found to be the most prevalent. This was followed by HLA-DQB1*02 (24.97%) and HLA-DQB1*03 (24.10%) as shown in Table 2. Furthermore, in the HLA-DRB1 locus, 87.88% of the samples showed heterozygosity and homozygosity was seen only in 12.12%. In contrast, at the HLA-DQB1 locus, 74.92% of the samples were found to be heterozygous, while 25.08% exhibited homozygosity.

5. Discussion

In this study, the aim was to analyze the diversity of allele frequencies for the class I (HLA-A, -B, and -C) and class II (HLA-DRB1, and -DQB1) genes. Limited data exist regarding HLA gene polymorphism at the DNA level within this population on a larger scale, particularly for the HLA-DRB1 and -DQB1 loci.

For the HLA-A locus, most of our results align with earlier studies conducted on the North Indian population [7,13,17]. It has been observed that HLA-A*24 (14.41%) is among the more frequently seen alleles in our study population. Although Mehra et al., did not specifically report A*24 due to their use of serology, which did not distinguish between A*23 and A*24, they reported the frequency of A9 (which includes A24) as 14.5%, closely matching our findings when considering A23 at 1.25% [10]. Similarly, HLA-A*34 was found at a low frequency in the studied population, consistent with Mittal et al., and Rani et al., who reported it at low frequencies in North India. Mehra et al., reported A10 (which includes A25, A26, A34, and A66) at 5.4%, aligning with our findings when considering the more precise PCR-SSOP HLA typing technique used in our study [9–11]. Furthermore, when comparing with South Indian populations, we found HLA-A*24,A*02, A*11, and A*33 to be more prevalent, similar to the North Indian population [18].

HLA-B exhibited significant diversity, with 28 alleles identified in our study, consistent with previous studies conducted on the North Indian population [7,13,17]. In contrast, for HLA-C, our study revealed HLA-C*07 as the most frequent allele, while Rani et al., found it to be less frequent in the North Indian population [13]. In our study, the prevalence of HLA-A*29, A*66, A*69, and A*74 was less than 1%. Similarly, for HLA-B, the HLA-B*14, B*45, B*46, B*47, B*48, and B*49 exhibited frequencies lower than 1%. Whereas HLA-C*17 is the only variant with a prevalence below 1% among identified HLA-C alleles.

Divergent findings emerged in the examination of HLA-DRB1. Our study identified HLA-DRB1*15, DRB1*11, and DRB1*13 alleles with high frequency, consistent with other published literature on the North Indian population [17,19]. In contrast, Mehra et al. did not report these alleles, likely due to their use of serological typing reported frequencies for DR2 (15+16), DR5 (11+12), and DR6 (13+14) making direct comparison difficult [10]. For the HLA-DQB1 locus, DQB1*06, DQB1*02, and DQB1*03 were among the most frequent alleles identified, consistent with a previous study by Agarwal et al. Besides this, no major differences were observed for HLA-DQB1 compared to other North Indian studies [13,19]. The prevalence of HLA-DRB1*02 and DRB1*16 was less than 1%, while for HLA-DQB1, only HLA-DQB1*01 was observed at a frequency of less than 1%.

Analyzing and understanding the frequency of HLA class I and II alleles within a specific population is im-

Table 1: HLA-A, B, and C allele frequency (%) of the studied samples (N = 3648).

HLA-A*	Total Numbers	Frequency (%)	HLA-B*	Total Numbers	Frequency (%)	HLA-C*	Total Numbers	Frequency (%)
A*01	899	12.32	**B*07**	382	5.24	**C*01**	249	3.41
A*02	1115	15.28	**B*08**	690	9.46	**C*02**	85	1.17
A*03	598	8.20	**B*13**	159	2.18	**C*03**	499	6.84
A*11	1200	16.45	**B*14**	4	0.05	**C*04**	1037	14.21
A*23	91	1.25	**B*15**	556	7.62	**C*05**	93	1.27
A*24	1051	14.41	**B*18**	186	2.55	**C*06**	814	11.16
A*26	595	8.16	**B*27**	155	2.12	**C*07**	1903	26.08
A*29	66	0.90	**B*35**	1037	14.21	**C*08**	133	1.82
A*30	98	1.34	**B*37**	216	2.96	**C*12**	1085	14.87
A*31	232	3.18	**B*38**	60	0.82	**C*14**	214	2.93
A*32	321	4.40	**B*39**	35	0.48	**C*15**	899	12.32
A*33	483	6.62	**B*40**	837	11.47	**C*16**	217	2.97
A*66	11	0.15	**B*41**	72	0.99	**C*17**	68	0.93
A*68	523	7.17	**B*44**	521	7.14			
A*69	5	0.07	**B*45**	7	0.10			
A*74	8	0.11	**B*46**	1	0.01			
			B*47	12	0.16			
			B*48	34	0.47			
			B*49	43	0.59			
			B*50	222	3.04			
			B*51	825	11.31			
			B*52	524	7.18			
			B*53	6	0.08			
			B*54	1	0.01			
			B*55	152	2.08			
			B*56	29	0.40			
			B*57	256	3.51			
			B*58	274	3.76			

portant for gaining insights into the susceptibility or resistance to a particular disease [19]. This information underlines its importance in further study for understanding susceptibility to these associated diseases in this region.

Furthermore, a prospective bone marrow transplant (BMT) patient with a common HLA allelic combination has a higher chance of finding a matched unrelated donor (MUD). To the best of our knowledge this is one of comprehensive datasets in terms of sample size, focusing on HLA diversity in the North Indian population. However, it's important to acknowledge the limitations of low-resolution (single digit) HLA typing. Exploring HLA allelic subtypes by using high-resolution HLA typing could offer valuable insights into the genetic diversity of HLA class I and II Genes in the North Indian Population, adding depth to our understanding of such diversity.

6. Conclusions

To conclude, the present study brings attention to the genetic diversity, present within the HLA-class I (HLA-A, B, and C) and class II (HLA-DRB1, and DQB1) genes among the North Indian population. The identified HLA class I and II allele diversity characterizes the North Indian population. Given the heterogeneous nature of the North Indian population and the critical role of HLA genes in both hematopoietic stem cell and solid organ transplantation, the study emphasizes the significance of testing these HLA genes. This, in turn, contributes to improved donor selection and ultimately enhances transplant outcomes. Furthermore, this information also holds potential for further exploration in HLA disease association studies, which could be considered a significant focus in current research endeavors.

Author Contributions

Conceptualization: V.C.M. and V.R.; Methodology: D.C., A.R. and R.S.; Formal Analysis: V.C.M., D.C. and R.S.; Data curation: V.C.M. and D.C.; Writing---Review & Editing: V.C.M., A.R. and R.S.; Visualization: V.R.; Supervision: V.R.; Project administration: V.C.M. and V.R. All authors have read and approved the content of the manuscript as submitted to the journal.

2024, Vol. 1, Article ID. 2024.0004
www.doi.org/10.69709/GenomC.2024.193514

Table 2: HLA-DRB1 and DQB1 allele frequency (%) of the studied samples (N = 3648).

HLA-DRB1*	Total Numbers	Frequency (%)	HLA-DQB1*	Total Numbers	Frequency (%)
DRB1*01	171	2.34	**DQB1*01**	2	0.03
DRB1*02	2	0.03	**DQB1*02**	1822	24.97
DRB1*03	1130	15.49	**DQB1*03**	1759	24.11
DRB1*04	511	7.00	**DQB1*04**	125	1.71
DRB1*07	933	12.79	**DQB1*05**	1555	21.31
DRB1*08	118	1.62	**DQB1*06**	2033	27.86
DRB1*09	53	0.73			
DRB1*10	443	6.07			
DRB1*11	803	11.01			
DRB1*12	126	1.73			
DRB1*13	937	12.84			
DRB1*14	616	8.44			
DRB1*15	1396	19.13			
DRB1*16	57	0.78			

Availability of Data and Materials

All datasets generated for this study are included in the manuscript.

Consent for Publication

Written consent was obtained from each participant, both for participation in the study and for publication purposes. All participants were made aware that their names and initials would not be published, and all standard protocols would be followed to conceal their identity.

Conflict of Interest

The authors report that they have no conflict of inter-est.

Funding

Authors didn't receive any external funding.

Acknowledgments

We are grateful to all the members of the laboratory for their kind support.

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