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Review Article

HLA Type Variation Across Populations

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Abstract

Background: Human Leukocyte Antigen (HLA) is highly polymorphic and it is commonly used as a marker for core population genital aspects such as human migration, predisposition to diseases and in tissue transplantation compatibility.

Methodology: For our study authors have taken up sample size of 86 for meta- analysis of high-resolution frequency data of the HLA allele. North Indian patients from tertiary centre were analyzed for identification of HLA-A, B, C, DRB1 and DQB1 alleles by sing serological and molecular typing techniques. A plethora of known alleles were identified at different loci e.g., HLA A 18, HLA B 36, HLA C 5, HLA DRB1 28 and HLA DQB1 8. This study focusing on frequencies of HLA alleles and their homozygosity and heterozygosity have been reported, but HLA data in the North Indian population living in Delhi, Haryana, Uttarakhand , Uttar Pradesh, Punjab and Himachal Pradesh, are reported here characterized. Allele frequencies were analyzed by using the Hardy-Weinberg equilibrium.

Results: Allele heterozygosity was observed in 96.51% individuals for locus HLA-A, 97.67% individuals for locus HLA-B and 96.51% for HLA-DRB1,overallheterozygosity for all three loci was 89.5% (77/86 individuals). Among individuals showing homozygosity (9 individuals), two showed homozygosity at two loci.

Conclusion: The increased heterozygosity for class 1 and class 2 is associated with selective advantage against infectious diseases. The phenotypic frequency of B*35 alleles (B*35:01, B*35:03 and B*35:08) in our study is 31.39%. Comparable B*35 allele phenotypic frequency (27.5%) was reported in north Indian patients.

Keywords: Human leukocyte antigen (HLA); Major Histocompatibility Complex (MHC); Sequence specific oligonucleotide (SSO); Sequence specific primer (SSP); Sequence based typing (SBT); PCR; DNA; HSCT; RBD; SNPs; af; gf; pf.

Introduction

The Human Leukocyte Antigens (HLA) was high-quality number popular polymorphic genes defined within the human genome. They were classified as the major histocompatibility complexes (MHCs) because of their main role in allowing the immune organization to identify "self"

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opposed to "non-self" antigens. MHC consists additional there are 200 genes in chromosome 6 and they are divided into three parts-class I HLA-A, HLA-B, HLA-C; phase II consists of genes A and B that mark the α and β chains respectively, the HLA-DR, HLA-DQ, HLA-DP molecules; phase III contains genetically modified compounds (C), hydroxyls, tumor necrosis factors (TNFs) [1]. Molecules of the class of HLAs are expressed over nucleated cells. Class II molecules are expressed in B lymphocytes, antigen-presenting cells (monocytes, macrophages, and dendritic cells), and activated T lymphocyte cells. Many HLA allele have also been identified as being associated with increased drug resistance.

Structure and function of HLA molecules

Class one molecules are composed of a chain of weighted HLA-type genes and Beta2 micro globulin. A heavy chain has three extra-cellular domains (α-1, 2 and 3), a transmembrane region and an intracytoplasmic domain. The domains 1 and α 2 of the heavy chain have a dynamic amino acid sequence and determine the antigenic specification of HLA molecules in class I [2]. α 1 and α 2 domains together form peptide-binding canal and can absorb the peptide process of 8 to 10 amino acid residues.

HLA class II molecules are α and β chain heterodimers. Chains of α and β, both have two outer regions (α 1 and α 2, β 1 and β 2). Alpha 1 and beta 1 combine to form compulsory peptide channels and can contain 12 amino acids or longer peptides [3]. The HLA molecules are highly polymorphic and polymorphism is mainly cluster in the antigen-binding groove. The frequency and distribution of different HLA alleles vary vastly in different ethnic groups.

The main function of class I molecules is to present endogenous peptides to class I restricted T-cells, i.e., CD8+ T-lymphocytes.

The main function was of class II molecules are to represent the exogenous peptides derived from various pathogens to class II restricted B-cells, i.e., CD4+ T-lymphocytes.

Chance of match

The two siblings had a 25% chance of gene typical HLA-like chance, a 50% chance of becoming HLA haploidentical and a 25% chance of not participating in HLA haplo-types species.

The opportunity to find collaborative donors can be enhanced in different ethnic groups. There was a 25% chance that they share no HLA haplo-type and known as haploidentical. The compatible donors may be 25% chance of having HLA enhanced with racial groups of homogenous [4].

Review of literature

HLA typing was done for the organ transplantation. The first approach was developed by the expansion of Molecular Typing methods for second-class loci (Sequence-Specific Primer), straight or moderate PCR SSO (Sequence Specific Oligonucleotide), PCR SSCP, and SBT, and most recently in ICI class , HLA-C person of the last gene you studied with typing techniques [5].

The HLA system is known for its replication but the main role of the HLA is to regulate immune response.

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HLA genetic variation played an important role in shaping the risk of autoimmune diseases and diseases; they were also important in the field of artificial surgery in which donors and recipients must comply with the HLA [6].

Methods of HLA typing and its role in transplantation

Previously, HLA typing of an individual was done by serological method which uses the principle of complement mediated lymphocytotoxicity. In serological method, peripheral blood lymphocytes are used for HLA typing. Further research revealed far more polymorphism in HLA as detected by serological method alone. Cell-based polymerase chain reaction (PCR) methods are designed to differentiate HLA alleles that are not serologically distinct but functional. The molecular methods of HLA typing include specific sequential oligonucleotide (SSO), specific sequence primer or sequential-based typing [7].

Sequence specific oligonucleotide (SSO)

Hybridization DNA was amplified with sso probe is the first cell type typing used to detect HLA Class II allele. This involves PCR-based augmentation of the integrated sequences that are then integrated.

Sequence specific primer (SSP)

The SSP is an alternative molecular technique in which sequence-specific primers are used to amplify the variable sequences. The SSP technique involved the use of primers opposite to HLA allele or set of allele sequence [8]. Primers are designed such that polymorphisms is detected at the end of 3' end of the primer. The SSP method was also used for generic which resolves alleles to at least antigenic stage or for allele level.

Sequence based typing

It is a sequential-based typing. SBT offers very high resolution. It is important to find new allele and their potential impact on transplantation.

Role of HLA alleles

Previously, HLA typing of an individual was done by serological method which uses the principle of complement mediated lymphocytotoxicity [9]. In serological method, peripheral blood lymphocytes are used for HLA typing. HLA typing is done on multiwall plastic trays with each source containing anti-HLA sera for known specification. Further research revealed far more polymorphism in HLA as detected by serological method alone [10]. Molecular mechanisms based on polymerase chain reaction (PCR) were developed to differentiate serologically indistinguishable but functionally distinct HLA alleles [11]. The molecular methods for HLA typing include specific sequential oligonucleotide (SSO), specific sequence primer or sequential-based typing.

- **HLA-A:** It presents peptides for immune recognition. Usually, peptides derived protein from synthesized within in the cell. Known alleles of HLA-A is 2432. Disease related to HLA-A is Carbomoznine-induced hypersensitivity.
- **HLA-BP:** HLA-B presents peptides for immune recognition. Usually, peptides derived protein from synthesized within in the cell. Known

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alleles of HLA-A 322. Disease related to HLA-A is Crown's disease, psoriasis.

- **HLA-C:** Peptides presentation for immune response. Also recognized by some NK receptors. Known alleles of HLA-C 2196. Disease related to HLA-C is control of HIV infection.
- **HLA-DRB1:** Peptide's presentation derived from extracellular proteins. Known alleles of HLA-DRB1 is 1569. Disease related to HLA-DRB1 is rheumatoid, arthritis, multiple sclerosis.
- **HLA-DQA1:** It presents peptides derived from extracellular proteins. Known alleles of HLA-DQA1 33. Disease related to HLA-DQA1 is Bone Marrow Transplantation, kidney transplantation.
- **HLA-DQB1:** It presents peptides derived from extracellular proteins. Known alleles of HLA-DQB1 647. Disease related to HLA-DQB1 is celiac disease, transplantation.

Principle

- The test includes four basic steps
- Separation of DNA
- Enlargement was done by PCR
- Hybridization and detection of sample
- Data was interpreted

Objective of the study

The objective of this study was to report the HLA allele frequencies of the North Indian population using the molecular typing. In this authors assessed allele freq for HLA A, B & DRB1 in 86 north Indian subjects using PCR,-SSOP methodology. In our study, 5 individuals HLA-C and HLA-DQB1 were also typed. The homozygosity and heterozygosity was also observed under this study.

Result

Statistical analysis

A computer program was used to determine gene expression using the formula: gf=1-√1-f, where gf=genetic frequency and f=allele frequency (af)/phenotype frequency (pf) were determined by dividing the number of allele samples specific to the total number of study samples). The program also calculated Hardy-Weinberg equity in standard terms. Genetic frequencies are also calculated manually using the formula n/2N, where n is the number of times the allele was detected, and N is the number of study samples [11]. In homozygous samples the number of alleles was calculated as 2. Both methods produced similar results [13].

Result of HLA allele frequency in north Indian population

HLA-A locus polymorphism

There were 3 locus alleles, i.e., HLA-A, B and C loci were in Hardy Weinberg Equilibrium. Table 1 showed the genetic frequency and frequency phenotype of 18 HLA-A locus alleles found in north Indian subjects in our study. Majority of samples were heterozygous (83/86, 96.51%) and only 4 samples showed homozygosity for A*01;01, A*11;01 and A*68;01. A*11:01 was most common allele in our subjects with a pf of 45.35% followed by A*68:01 (PF-29.07%) and A*01:01 (pf-22%).

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A-locus alleles	Number of alleles	gfa	pfb (%)	A-locus alleles	Number of alleles	gfa	pfb (%)
01:01[c]	22	0.137	25.58	24:02:00	10	0.06	11.63
2:01	6	0.036	6.98	26:01:00	7	0.042	8.14
2:03	3	0.018	3.49	29:01:00	1	0.006	1.16
2:05	2	0.012	2.33	30:01:00	2	0.012	2.33
2:06	2	0.012	2.33	31:01:00	3	0.018	3.49
2:11	13	0.079	15.12	32:01:00	9	0.054	10.47
3:01	18	0.111	20.93	33:03:00	7	0.042	8.14
11:01[c]	39	0.261	45.35	68:01[c]	25	0.158	29.07
23:01	1	0.006	1.16	68:02:00	2	0.012	2.33

Table 1: [a]gf: gene frequency, calculated as n/2N in which n is the quantity of precise alleles and 2N is two times the determine of pattern was studied: b% pf:percent phenotype frequency, calculated as n/N X 100:[C] three samples showed homozygosity of the alleles proven, these alleles have been counted as the two alleles for each samples calculating the gene frequencies.

B-locus alleles	Number of alleles	Af	gfa	pfb (%)	B-locus alleles	Number of alleles	af	gfa	pfb (%)
1:01	2	0.023	0.012	2.33	40:01:00	6	0.07	0.036	6.98
7:02	12	0.14	0.072	13.95	40:02:00	1	0.012	0.006	1.16
7:05	1	0.012	0.006	1.16	40:06[c]	21	0.244	0.131	24.42
8:01	5	0.058	0.03	5.81	41:01:00	3	0.035	0.018	3.49
13:01	1	0.012	0.006	1.16	44:02:00	2	0.023	0.012	2.33
13:02	2	0.023	0.012	2.33	44:03[c]	7	0.081	0.042	8.14
15:01	1	0.012	0.006	1.16	49:01:00	3	0.035	0.018	3.49
15:02	7	0.081	0.042	8.14	50:01:00	1	0.012	0.006	1.16
15:17	1	0.012	0.006	1.16	51:01:00	15	0.174	0.091	17.44
15:18	2	0.023	0.012	2.33	51:08:00	1	0.012	0.006	1.16
18:01	4	0.047	0.024	4.65	52:01:00	21	0.244	0.131	24.42
27:04:00	1	0.012	0.006	1.16	55:01:00	4	0.047	0.024	4.65
27:07:00	1	0.012	0.006	1.16	55:02:00	1	0.012	0.006	1.16
35:01:00	12	0.14	0.072	13.95	56:01:00	1	0.012	0.006	1.16
35:03:00	13	0.151	0.079	15.12	57:01:00	5	0.058	0.03	5.81
35:08:00	2	0.023	0.012	2.33	57:03:00	1	0.012	0.006	1.16
37:01:00	6	0.07	0.036	6.98	58:01:00	4	0.047	0.024	4.65
38:01:00	1	0.012	0.006	1.16	58:02:00	1	0.012	0.006	1.16

Table 2: B*52:01 and [a],[b] as in Table 1;[c] Two samples showing the homozygosity of the exhibited alleles, these alleles are counted as two genetic samples for counting the genes.

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DRB1-locus alleles	Number of alleles	gf[a]	pf[b] (%)	DRB1-locus alleles	Number of alleles	gf[a]	pf[b] (%)
01:01	3	0.018	3.49	11:06	1	0.006	1.16
03:01	9	0.054	10.47	11:11	1	0.006	1.16
04:03	3	0.018	3.49	12:01	2	0.012	2.33
04:05	2	0.012	2.33	12:02	6	0.036	6.98
04:06	3	0.018	3.49	13:01	14	0.085	16.28
04:08	1	0.006	1.16	13:02[c]	5	0.030	5.81
04:10	1	0.006	1.16	13:05	2	0.012	2.33
04:18	1	0.006	1.16	14:01	5	0.030	5.81
04:51	1	0.006	1.16	14:03	1	0.006	1.16
07:01[c]	24	0.151	27.91	14:04	18	0.111	20.93
09:01	2	0.012	2.33	14:54	1	0.006	1.16
10:01	13	0.079	15.12	15:01	10	0.060	11.63
11:01	12	0.072	13.95	15:02[c]	17	0.104	19.77
11:04	9	0.054	1.47	15:03	1	0.006	1.16

Table 3: [a],[b] as in Table 1;[c] Three samples showing the homozygosity of the exhibited alleles, these alleles were counted as two samples in the calculation of gene expression.

HLA-B locus polymorphism

Data table 2 Indicating the freq same as the A. Majority of sample were heterozygous (84/86, 97.67%) and only 2 samples showed homozygosity for B*40:06 and B*44:03.

HLA-DRB1 locus polymorphism

HLA-DRB1 locus alleles frequencies in 86 subjects from north India. Majority samples were heterozygous (83/86, 96.51%) and only 3 samples showed homozygosity for DRB1 07:01, DRB1* 13:02 and DRB1* 15:02. DRB1*07:01 was most common allele in our subjects with a pf of 27.91% followed by DRB1 14:04 (PF-20.93%) and DRB1* 15:02 (pf-19.77%).

HLA-C locus polymorphism: (Table 4).

HLA-DQB1 locus polymorphism: (Table 5).

C-locus alleles	Number of alleles	gfa	pfb(%)
7:01	2	0.012	2.33
7:02	1	0.006	1.16
14:02	1	0.006	1.16
15:02[c]	3	0.018	3.49
15:05	1	0.006	1.16

Table 4: [a],[b] as in Table 1; [c] One sample showed the homozygosity of the indicated alleles, these alleles were counted as two samples in the calculation of gene expression.

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DQB1-locus alleles	Number of alleles	gfa	pfb(%)
2:01	1	0.006	1.16
2:02	1	0.006	1.16
03:01[c]	3	0.018	3.49
5:01	1	0.006	1.16
5:03	1	0.006	1.16
5:05	1	0.006	1.16
6:01	1	0.006	1.16
05;03	1	0.006	1.16

Table 5: [a],[b] as in Table 1;[c] One sample showed the homozygosity of the indicated alleles, these alleles were counted as two samples in the calculation of gene expression.

Conclusion

North Indians HLA allele frequencies have been reported in the literature based on serological and molecular typing methods. Here authors assessed allele freq for HLA A, B and DRB1 in 86 north Indian subjects using PCR-SSOP methodology. In our study, 5 individuals HLA-C and HLA-DQB1 were also typed.

Allele heterozygosity was observed in 96.51% individuals for locus HLA-A, 97.67% individuals for locus HLA-B and 96.51% for HLA-DRB1, overall heterozygosity for all three loci was 89.5% (77/86 individuals). Among individuals showing homozygosity (9 individuals), two showed homozygosity at two loci. Heterozygosity.

The increased heterozygosity for class 1 and class 2 is associated with selective advantage against infectious diseases. It has been reported that individuals with homozygosity at single or additional loci improvement faster to death and AIDS [12]. The phenotypic frequency of B*35 alleles (B*35:01, B*35:03 and B*35:08) in our study is 31.39%. Comparable B*35 allele phenotypic frequency (27.5%) was reported in north Indian patients.

Among HLA-A results, A*11:01 was most common allele in our study followed by A*68:01 and A*01:01. The report also showed comparable results with higher frequency of A*11:01, A*68:01 and A*01:01. In contrast, A*24:01 was a common allele in the report with gf of 0.111 was not seen in our subjects.

In our study B27 was present in only two subjects. The alleles of B 27 in our study are B*27:04 and B*27:07. B*27:07 was present in all three studies, indicating B*27:07 is common to Indians.

Rare alleles, B*81:01, B*18:02, B*41:01 and A*24:03 reported were not detected in our study. The possible reason for this would be the rare nature of these alleles and small sample size of our study.

A*68:01 common allele in our subjects is also frequently reported.

B17 antigens (B*57 and B*58) were noted in 11 individuals accounting to phenotypic frequency of 12.79%. The earlier study from north India reported frequency of 25.3% in

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north Indian subjects. The B*57:01 was most common allele with comparable frequency of B*58:01. Among DRB1 alleles, HLA DRB1*07:01 was the majority familiar.

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