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Distribution of HLA Class II Antigens in Three North Indian Populations

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ABSTRACT The distribution of HLA-DRB1 alleles and DQB1 alleles in 583 individuals of three different caste groups of North India was analyzed by using the polymerase chain reaction with sequence specific oligonucleotide probes (SSOP) method. The populations selected were Bhargavas, Chaturvedi and Brahmins. The gene frequency and haplotype analysis revealed that these populations are distinct from one another. The phylogenetic analysis shows that there is a evolutionary relationship among the three groups. Bhargavas and Chaturvedis emerged from a common ancestral population (presumably the original Brahmanical priest population). Chaturvedis diverged first from the original group and at a later date Bhargavas and Brahmins separated from one another.

INTRODUCTION

India occupies an area of 32,87,263 kms and is located between latitudes 8º4' to 37º6'north and longitudes 68º7'and 97º25'east and measures about 3,214 km from North to South and about 2,933 km from east to west. In India, majority of Hindus follow endogamous marital pattern from last several centuries. Besides the consideration of socio-cultural status, restriction of marriage within the caste is an important requirement of such marriages. However, within the caste there is a sanction against marriage between relations even up to seven generations. The major castes viz, Khatriyas Brahmins and Vaish are subdivided into several *subgroups* (identified by gotras and surnames) and marriage within the same *gotra* is generally not preferred, hence consanguinity is avoided (Bhasin et al. 1994; Bhasin and Walter 2001).

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In North India, a further degree of endogamy is seen in some sects where marriages are restricted within the same surname. Two such groups are Bhargavas and Chaturvedis. They belong to the broad caste group of Brahmins but they do not marry outside their own surname. Thus, Bhargavas marry within Bhargavas and Chaturvedis within Chaturvedis and not with other Brahmins.

Brahmin population is one of the major caste groups, which follow group endogamy. They are broadly divided into seven groups, which are further subdivided into gotras and surnames (Sharma1994). For each of these subdivisions endogamy is practiced at the group level and exogamy at the *gotra* level. Bhargavas are a subdivision of Brahmins having a different origin. They are subdivided into six gotras and further into thirty-six kuldevies, based on the deity of worship. They practice endogamy at surname as well as at gotra level but exogamy at the level of kuldevies (Sherring 1974). Chaturvedis are a subgroup having an origin from the main stock of Brahmins but have formed a separate group due to practice of endogamy. They are further divided into seven *gotras* and sixty-four als depending upon either the founder guru or the place of origin. They practise endogamy at surname level but exogamy at level of gotra's and als (Sherring 1974).

In past, the inter group genetic variation studies have mostly utilized blood groups, and electrophoretic pattern of serum proteins and red cell enzyme polymorphisms (Lanchbury et al. 1996). These studies have shown that various castes and sub castes within one geographic region are much more similar to one another than the same caste and sub castes from different geographic regions. However, these studies have a limitation that expressed phenotypes may be subject to selection. Further genetic differentiation within the region as an effect of socio-cultural barriers is not documented. In recent

years a large number of DNA polymorphisms, especially in the non-coding regions of the human genome, have been discovered which are selectively neutral. These markers have not been adequately explored in Indian populations.

Human leukocyte antigens or HLA is a highly polymorphic system and provide useful information to study populations (Harpending 1998). HLA are a group of cell membrane proteins encoded by the HLA gene complex on the short arm of chromosome six, with a high level of genetic polymorphism, which regulate the immune response by self / non-self discrimination (Hughes and Nei 1992; Little and Parham 1999). HLA genetic polymorphisms and linkage disequilibria of alleles at distinct HLA loci show considerable ethnic differences (Djoulah et al. 1994). The presence of HLA haplotypes as well as HLA alleles allows inferring the evolutionary relationships among ethnic groups (Monslave et al. 1999). Due to the sociocultural separation among the groups coupled with the effect of random genetic drift, gene and haplotype frequencies in the isolated populations begin to change gradually with time. Thus we can estimate the genetic distances between populations from the gene frequencies and reconstruct the phylogenetic trees for human populations (Rogers 1999; Blasczyk et al. 1998).

In the present study, the allele frequency distribution of HLA class II genes DRB1, DQA1, and DQB1 were analysed by PCR-SSOP method in three endogamous caste groups of Uttar Pradesh, Bhargavas, Chaturvedis, and other Brahmins. The phylogenetic relationships among the three populations have been constructed.

MATERIALS AND METHODS

Peripheral blood cells (PBMC's) form 583 individuals from three caste groups of North India (Uttar Pradesh) were collected from Bhargavas (196), Chaturvedis (191) and Brahmins (196). Before sample collection regional directories were prepared, random numbers were generated with the help of computer. An informed consent of the individuals participating in the study was obtained.

HLA typing was done using primers and sequence specific oligonucleotide probes (SSOP) according to the 12th International Histocompatibility Workshop.

HLA gene and phenotype frequencies were estimated by direct counting. The two and three locus haplotype frequencies for the HLA system were computed by using maximum likelihood method. Phylip and POPGENE statistical packages were used for analyzing allele frequency, test of Hardy-Weinberg equlibrium, F_{IS} (intra-population inbreeding coefficient), F_{ST} (inter population variance), genomic diversity (H_S and H_T),relationship among populations (Nei's standard genetic distance and phylogenetic trees).

RESULTS

Allele frequencies at HLA DRB1 locus in three populations are shown in Table 1. At DRB1 locus a total of 25 different alleles have been detected in the three populations.

In Bhargavas most common alleles were DRB1*1501 and DRB1*0701 (8.5%) followed by DRB1*140x (11.4%), DRB1*130x (11.1%), and DRB1*040x (8.5%). Alleles DRB1*1410, DRB1* 1502 and DRB1*1301 were less common and occurred with a frequency of 0.3 to 0.8%. Some of the other alleles like DRB1*0103, DRB1*0406, DRB1*090x and DRB1*160x were not at all represented.

In Chaturvedis the most frequent alleles were DRB1*0701 (21.5%), followed by DRB1*040X (11.4%), DRB1*030X (11.4%), DRB1*150X (8.7%), DRB1*010X (8.4%) and DRB1*1001

Table 1: Allele frequency distribution at DRB1 locus

Allele	*Bharagavas*	*Chaturvedis*	*Brahmins*
DRB1*0103	0.000	0.003	0.000
DRB1*010X	0.064	0.084	0.051
DRB1*0301	0.003	0.003	0.000
DRB1*03011	0.016	0.000	0.005
DRB1*03012	0.003	0.000	0.000
DRB1*030X	0.050	0.114	0.080
DRB1*0402	0.013	0.014	0.000
DRB1*0406	0.000	0.003	0.000
DRB1*040X	0.085	0.114	0.019
DRB1*0701	0.165	0.215	0.188
DRB1*080X	0.013	0.003	0.016
DRB1*090X	0.000	0.005	0.011
DRB1*1001	0.037	0.071	0.048
DRB1*110X	0.042	0.033	0.091
DRB1*120X	0.019	0.014	0.024
DRB1*1301	0.003	0.003	0.000
DRB1*1302	0.005	0.003	0.000
DRB1*1305	0.011	0.000	0.000
DRB1*130X	0.111	0.133	0.085
DRB1*1410	0.005	0.000	0.000
DRB1*140X	0.114	0.057	0.125
DRB1*1501	0.164	0.035	0.069
DRB1*1502	0.008	0.008	0.064
DRB1*150X	0.069	0.087	0.123
DRB1*160X	0.000	0.000	0.003

(7.1%). Rare alleles were DRB1*0406, DRB1*080X, DRB1*1301, DRB1*1302, DRB1*1502 and DRB1*090X, these alleles occurred with a frequency of 0.3% to 0.8%. Some of the alleles like DRB1*160X, DRB1*1410, DRB1*1305, DRB1*03011 and DRB1* 03012 were totally absent.

Among Brahmins the most frequent alleles were DRB1*0701 (18.8%) followed by DRB1*140X (12.5%), DRB1*150x (12.3%), DRB1*110X (9.1%), DRB1*130X (8.5%) and DRB1*030X (8.0%). Alleles like DRB1*0103, DRB1*0301, DRB1*03012, DRB1*0402, DRB1* 0406, DRB1*1301, DRB1*1302, DRB1*1305 and DRB1*1410 were completely absent. Other less common alleles were DRB1*090X, DRB1*080X, DRB1*040X, DRB1*120X, DRB1*010X, DRB1*1502 and DRB1*1501. These alleles occurred with a frequency of 1.1% to 6.9%. DRB1*160X and DRB1*03011 occurred with a very low frequency i.e. 0.3 and 0.5% respectively.

Allele Frequency Distribution at DQA1 Locus: Allele frequencies at DQA1 locus among Bhargavas Chaturvedis and Brahmins are shown in Table 2. Among Bhargavas the most common allele at DQA1* locus is DQA1*010x (24.0%) followed by DQA1*030X (20.1%), DQA1*050X (17.5%), DQA1*0201 (16.3%), DQA1* 0103 (16.0%), DQA1*0401 (3.6%). Frequency of other alleles like DQA1*040X, DQA1*0102, DQA1*0601, DQA1*0501 ranged between 0.3 %-1.2%.

Table 2: Allele frequency distribution at DQA1 locus

Allele	*Bharagavas*	*Chaturvedis*	*Brahmins*
DQA1*0102	0.003	0.004	0.000
DQA1*0103	0.160	0.162	0.149
DQA1*010X	0.240	0.417	0.183
DQA1*0201	0.163	0.000	0.209
DQA1*020X	0.000	0.004	0.000
DQA1*030X	0.201	0.102	0.289
DQA1*0401	0.036	0.011	0.013
DQA1*040X	0.012	0.004	0.013
DQA1*0501	0.003	0.000	0.000
DQA1*050X	0.175	0.075	0.145
DQA1*0601	0.009	0.000	0.000

In Chaturvedis allele DQA1*010X (41.7%) was the most common allele. Other alleles included DQA1*0201 (22.2%), DQA1*0103 (16.2%), DQA1*030X (10.2%), DQA1*050X (7.5%). the frequency of rare alleles ranged from 1.1% to 0.4% and these were DQA1*0401, DQA1*0102, DQA1*040X, DQA1*020x. Alleles DQA1*0501and DQA1*0601were completely absent among Chaturvedis.

In Brahmins DQA1*030X (28.9%) was most common allele, followed by DQA1*0201 (20.9%), DQA1*010x (18.3%), DQA1*0103 (14.9%), DQA1*050X (14.5%). Rare alleles with a frequency of 1.3% were DQA1*0401and DQA1*040X. Alleles DQA1*0102, DQA1*0601, DQA1*020X and DQA1*0501 were completely absent in Brahmins.

Allele Frequency Distribution at DQB1 Locus:* It is evident from the Table 3 that most common allele among Bhargavas was DQB1*0601 (24.9%) followed by DQB1*0201 (15.5%), DQB1*0304 (9.9%), DQB1*0301 (7.5%) and DQB1*060X (7.5%). Rare alleles included DQB1*0603 and DQB1*0504. These alleles occurred with a frequency of 0.6 to 0.8%. Uncommon alleles included DQB1*0302, DQB1*0303, DQB1*0503, DQB1*0604, DQB1* 0401, DQB1*0402, DQB1*050X, DQB1*0502 and DQB1*0602. Their frequencies ranged between 1.1 to 6.4%. Alleles DQB1*0103 and DQB1*0305 were totally absent among Bhargavas.

Table 3: Allele frequencies at DQB1 locus

Allele	*Bharagavas*	*Chaturvedis*	*Brahmins*
DQB1*0103	0.000	0.003	0.000
DQB1*0201	0.155	0.207	0.159
DQB1*020X	0.050	0.076	0.044
DQB1*0301	0.075	0.036	0.103
DQB1*0302	0.022	0.014	0.003
DQB1*0303	0.030	0.073	0.041
DQB1*0304	0.014	0.003	0.000
DQB1*0305	0.000	0.000	0.006
DQB1*030X	0.099	0.146	0.065
DQB1*0401	0.036	0.011	0.006
DQB1*0402	0.017	0.008	0.003
DQB1*0501	0.006	0.034	0.056
DQB1*0502	0.011	0.000	0.003
DQB1*0503	0.030	0.008	0.050
DQB1*0504	0.008	0.003	0.000
DQB1*050X	0.064	0.076	0.168
DQB1*0601	0.249	0.176	0.174
DQB1*0602	0.025	0.011	0.000
DQB1*0603	0.006	0.003	0.003
DQB1*0604	0.030	0.006	0.003
DQBI*060X	0.075	0.106	0.115

In Chaturvedis the most common allele at DQB1 locus were DQB1*0201 (20.7%) followed by DQB1*0601 (17.6%), DQB1*030X (14.6%), DQB1*060X (10.6%), DQB1*020X (7.6%), DQB1*050X (7.6%), DQB1*0303 (7.3%), DQB1*03013 (3.6%), DQB1*0501 (3.4%) and DQB1*0302 (1.4%). Rare alleles included DQB1*0103, DQB1*0304, DQB1*0504, DQB1* 0602, DQB1*0604, DQB1*0402, DQB1*0503.The

frequency of these alleles ranged between 0.3 to 0.8%. Allele DQB1*0305 was completely absent.

Among Brahmins the most common allele was DQB1*0601 (17.4%), followed by DQB1*050X (16.8%), DQB1*0201 (15.9%), DQB1*060X (11.5%), DQB1*0301 (10.3%) and DQB1*030X (6.5%). Rare alleles occurred with a frequency of 0.3-0.6%, these alleles were DQB1*0401, DQB1*0302, DQB1*0402, DQB1*0502, DQB1* 0603 and DQB1*0604. Completely absent alleles included DQB1*0103, DQB1*0303, DQB1*0304, DQB1*0503, DQB1*0504 and DQB1*0602.

It is evident from tables 1 and 3 that various alleles at these loci are not equally distributed in the study populations. Chi square analysis was done to compare these populations and it was observed that the frequencies of DRB1*010X, DRB1*040X, DRB1*080X, DRB1*110X, DRB1* 130X, DRB1*140X, DRB1*1501 and DRB1*1502 were significantly different in Chaturvedis and Brahmins. The Brahmins and Bhargavas were different at the alleles DRB1*0402, DRB1*040X DRB1*090X, DRB1*1210X, DRB1*1501, DRB1*1502, DRB1*150X (Table 4). Cumulative Chi square at this locus revealed maximum differences between Chaturvedis and Brahmins.

Table 4: c^2 Matrix for DRB1 locus

Locus	*Bh x Ch*	*Ch x Br*	*Br x Bh*
DRB1*0103	1.34	0.25	-
DRB1*010X	2.63	8.13**	1.33
DRB1*0301	0.00	1.34	1.34
DRB1*03011	14.18***	3.21	4.81
DRB1*03012	1.34	-	1.34
DRB1*030X	26.36***	6.22	6.92
DRB1*0402	0.00	12.16***	11.15***
DRB1*0406	1.34	1.34	-
DRB1*040X	4.38	71.17***	42.85***
DRB1*0701	7.21***	2.12	1.38
DRB1*080X	5.10	7.65**	0.14
DRB1*090X	3.21	1.58	9.14
DRB1*1001	10.66	4.32	1.23
DRB1*110X	0.89	27.93***	18.56***
DRB1*120X	0.49	2.17	0.38
DRB1*1301	0.00	1.34	1.34
DRB1*1302	0.13	1.34	3.21
DRB1*1305	9.14**	-	9.14
DRB1*130X	2.06	11.37***	3.54
DRB1*1410	0.07	-	3.21
DRB1*140X	20.05***	27.13***	0.48
DRB1*1501	91.43***	11.05***	42.92***
DRB1*1502	0.000	43.58***	43.58***
DRB1*150X	0.16	6.52	16.18***
DRB1*160X	-	0.25	1.34
Total c^2	202.16	252.17	225.51

At DQA1* locus, analysis (Table 5) revealed that Bhargavas and Chaturvedis differ significantly from one another at DQA1* 010X, DQA1* 0201, DQA1* 020X, DQA1* 030X, DQA1* 0501 and DQA1* 050X alleles. Cumulative Chi square at this locus revealed maximum differences between Chaturvedis and Brahmins.

At DQB1* locus (Table 6) significant differences were observed between Bhargavas and Chaturvedis at alleles DQB1* 0201, DQB1* 0301, DQB1* 0303, DQB1* 030X, DQB1* 04012, DQB1* 0501, DQB1* 0502, DQB1* 0503, DQB1* 0601and DQB1* 0604 and DQB1*060X alleles. Cumulative Chi square at this locus revealed

Table 5: c^2 Matrix for DQA1 locus

Locus	*Bh x Ch*	*Ch x Br*	*Br x Bh*
DQA1*0102	0.00	2.25	1.34
DQA1*0103	0.00	0.55	0.38
DQA1*010X	71.18***	129.51***	9.12**
DQA1*0201	10.82**	0.43	6.69
DQA1*020X	37.36***	109.98***	20.46***
DQA1*030X	12.55***	201.90***	10.13**
DQA1*0401	3.09	3.80	0.00
DQA1*040X	1.34	-	1.34
DQA1*0501	44.80***	24.32***	3.13
DQA1*050X	7.14**	-	7.14**
DQA1*0601	2.25	2.25	-
Total c^2	190.53	474.99	59.73

Table 6: c^2 matrix for DQB1 locus

*Allele DQB1**	*Bh x Ch*	*Ch x Br*	*Br x Bh*
0103	1.34	1.34	-
0201	8.77**	7.39	0.06
020X	5.29	8.52**	0.28
0301	13.77***	33.68***	4.50
0302	1.39	5.93	13.12***
0303	18.06***	8.94**	1.46
0304	5.93	1.34	12.16***
0305	-	4.18	4.18
030X	9.84**	33.91***	7.23
0401	12.55***	0.95	20.45***
0502	2.59	1.46	8.54**
0501	18.60***	5.13	39.96***
0502	9.14**	1.34	3.52
0503	11.83***	29.85***	4.70
0504	1.46	3.00	6.15
050X	0.93	38.65***	51.73***
0601	14.69***	0.03	16.53***
0602	4.78	9.14**	23.33***
0603	0.45	0.00	0.45
0604	14.96***	0.45	20.83***
060X	5.47	0.33	8.85**
Total c^2	161.84	195.56	248.03

maximum differences between Bhargavas and Brahmins.

When Chi-square values at DRB1, DQA1 and DQB1 loci in the three populations were combined (Table 7) and pair wise differences were calculated maximum differences were observed between Chaturvedis and Brahmins.

Table 7: c^2 matrix over all loci

*Allele DQB1**	*Bh x Ch*	*Ch x Br*	*Br x Bh*
DRB1	202.16	252.17	225.51
DQA1	190.53	407.99	59.73
DQB1	161.84	195.56	248.03
Total c^2	554.53***	855.72***	533.27***

Bh = *Bhargavas*, Ch = *Chaturvedis*, Br = *Brahmins*

Test of Hardy-Weinberg Equilibrium: The distributions of various allele frequencies at DRB1, DQA1 and DQB1 loci were tested to see whether these populations were in Hardy-Weinberg equilibrium or not. Our results revealed that the observed frequencies obey the null hypothesis.

Genetic Variation in Three Populations: The observed heterozygosities in three populations revealed that the heterozygosities at the DRB1 locus in the three populations were almost equal with an average of 0.6622. At DQA1 locus, Chaturvedis were having the lowest frequency of heterozygotes (0.5083) as compared to the other two groups i.e. Bhargavas (0.7290) and Brahmins (0.7723). At DQB1 locus, Brahmins showed the highest heterozygosity i.e.0.7129. When mean observed heterozygosity was calculated over all loci, Brahmins showed the highest value of 0.7162 as compared to the other two populations.

Genomic Diversity Between Populations: Genomic Diversity among populations is shown in Table 8 for each locus and as well as for all loci combined. The total genomic diversity (H_T) among populations is quite high (ranging from 0.7150-0.8681), average being 0.7759.However, most of the diversity was that between individuals

Table 8: Genomic diversity in three populations (HLA data)

Marker	H_T	H_S	G_{ST}
DRB1	0.8681	0.8567	0.0131
DQA1	0.7150	0.6974	0.0246
DQB1	0.7446	0.7369	0.0103
Mean	0.7759	0.7637	0.0157

within the populations (average H_S=0.7637). The total genomic diversity between populations (G_{ST}) in relation to total diversity was generally low (range 1.03-2.46%), the average over three loci being 1.57%.

Levels of Inter- Population and Intra-Population Variation: Our results show that intra-population inbreeding coefficient (F_{IS}) is generally the main component of the total variation in all the three populations at DRB1 and DQB1 loci and also when averaged over all the three loci. The inter-population variation as indicated by F_{ST} was found to be low (Table 9).

Table 9: Intra and inter population variation at DRB1, DQA1, and DQB1 loci in three populations

Locus	F_{IS}	F_{IT}	F_{ST}
DRB1	0.2240	0.2365	0.0161
DQA1	0.0356	0.0617	0.0270
DQB1	0.1056	0.1192	0.0152
Mean	0.1286	0.1453	0.0192

Relationship Among Populations: From the genetic distance (Table10) data we constructed dendrogram according to UPGMA method. In the dendrogram, Bhargavas and Brahmins cluster together, it is clear that the distance between Brahmins and Chaturvedis is highest while between Bhargavas and Brahmins it is lowest.

Table 10: Genetic distance based on allele frequency data at DRB1, DQA1 and DQB1 loci (Nei, Original, 1972)

Allele	*Bharagavas*	*Chaturvedis*	*Brahmins*
Bhargavas	-	0.9196	0.9486
Chaturvedis	0.0838	-	0.8627
Brahmins	0.0528	0.1477	-

Haplotype Analysis: Two and three locus haplotype analysis was done to further study the differences and relationships among three populations. Two locus haplotype analysis (Table 11) revealed that there were certain DRB1*-DQB1* haplotype which were shared by Bhargavas, Chaturvedis and Brahmins whereas there were some haplotypes which were unique to a particular population. The total numbers of significant haplotypes found were nine in Bhargavas and five each in Chaturvedis and Brahmins. The most common haplotype i.e.DRB1*0701-DQB1*0201 was universally present in all the three populations at a higher frequency. Another haplotype i.e. DRB1*0301-

Table 11: Two-locus haplotypes (DRB1 - DQB1) among three populations

Bhargavas		*Chaturvedis*		*Brahmins*	
DRB1-DQB1	*Hf*	*DRB1-DQB1*	*Hf*	*DRB1-DQB1*	*Hf*
03011-0301	0.009677	0701-0201	0.091146	0701-0201	0.103680
0701-0201	0.041140	0701-0601	0.030205	0701-0303	0.029702
0701-0301	0.015336	1001-0501	0.012500	0701-0503	0.024752
0701-0401	0.011200	1001-0601	0.028954	1102-0601	0.064028
0701-0601	0.030356	1501-0601	0.016666	1501-0601	0.059208
1001-0601	0.017408				
1501-0201	0.036808				
1501-0301	0.017361				
1501-0502	0.009677				
1501-0601	0.105210				

Hf = Haplotype frequency

Table 12: Three locus haplotypes (DRB1-DQA1-DQB1) in three populations

	Hf		
Haplotype DRB1-DQA1-DQB1	*Bhargavas*	*Chaturvedis*	*Brahmins*
0701-0103-0601	0.012491	0.02500	
0701-0201-0201	0.025806	0.060029	0.079207
1501-0103-0601	0.052024	0.011496	0.0198801
1501-0201-0201	0.012903		
1501-0201-0303	0.0096774		
1501-0201-0601	0.0096774		0.014851
0701-0201-0303		0.037500	0.014851
1502-0103-0601			0.029702
1502-0201-0601			0.014851

Hf : Haplotype frequency

DQB1*0601 was found with a higher frequency in Bhargavas and Brahmins. However, it was less frequent in Chaturvedis.

Three-locus haplotype (Table 12) analysis was carried out in all the groups. There were six significant haplotypes in Bhargavas and Brahmins and four in Chaturvedis.The haplotypes common among all the three populations were DRB1*0701-DQA1*0201-DQB1*0201 and DRB1*1501-DQA1*0103-DQB1*0601. Other than these haplotype DRB1*0701-DQA1*0103-DQB1*0601 was shared between Bhargavas and Chaturvedis and haplotype DRB1*0701-DQA1*0201-DQB1*0303

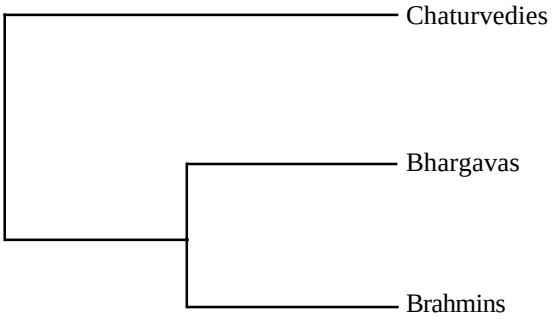

Fig.1. UPGMA tree depicting genomic affinities among three populations based on HLA class II loci polymorphism

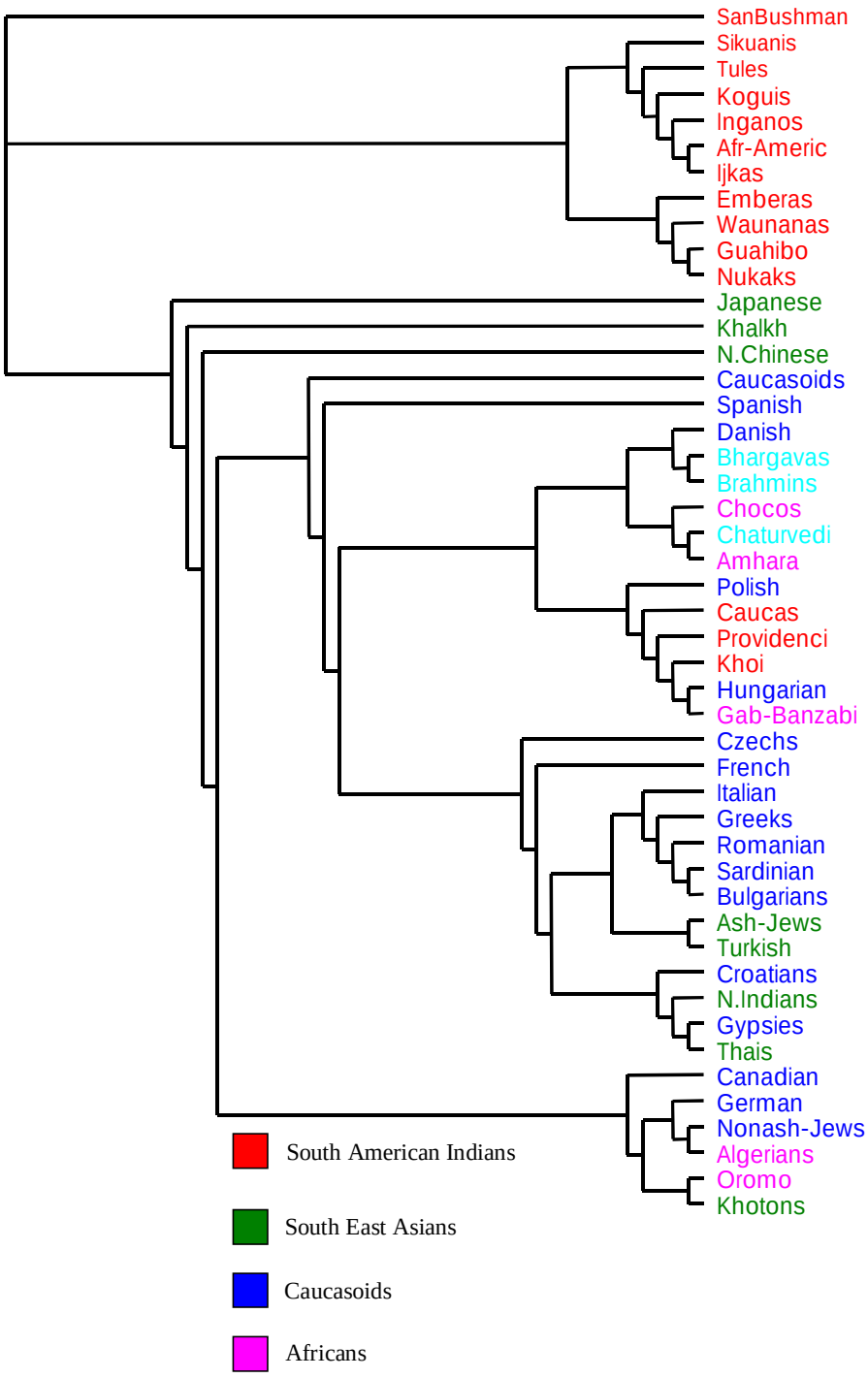

Fig. 2. Neighbour-joining tree depicting genomic affinities among 47 populations based on HLA class II loci polymorphisms (Rectangular Cladogram)

was common between Chaturvedis and Brahmins.

DISCUSSION

In this study, the polymorphism of HLA class II DRB1* and DQB1* genes was examined in three populations of Uttar Pradesh. No data is so far available about gene polymorphism at the DNA level in these populations. In this study an attempt has been made to compare the allele frequency data of our populations with one another and also with the other world populations. Bhargava, Chaturvedis and Brahmins differ from one another at DRB1 and DQB1 loci. However, the differences are less marked between Bhargavas and Brahmins.

We have compared our results with other 44 world populations. We have constructed the dendrograme using neighbor-joining (NJ) method of Saitou and Nei (1987). The first cluster formed in the tree (Fig. 2) can be labled as the South American cluster under which populations like Skiuanis, Tules, Koguis, Inganos, African-Americans and Ijkas fall (Trachtenberg et al. 1996). Trachtenberg et al. (1996) has suggested that these populations underwent little or no admixture with other neighboring populations in last 300 hundred years and hence are distinct and form the separate cluster have. The other populations, which are placed together, are Japanese, Khalkhs and North Chinese (Munkhabat et al. 1997). These populations have been described as Mongoloid populations. Our populations form a separate cluster where Bhargavas and Brahmins are nearer to one another and are placed in the branch near to Danish. Chaturvedis cluster with Amharas. Amhara is a population, which constitutes 38% of Ethiopian component and 62% of Caucasoid component. Our results indicate that the main element in all the three groups under study is Caucasoid element, however, Chaturvedis have 38% of Ethopian component.

We have also used haplotype analysis for our comparisons. Haplotype in HLA system are unique organization of HLA genes that have been well conserved through thousand of years and also characteristic haplotype show a limited regional distribution. Therefore, the HLA haplotype is a powerful marker and is useful for surveys among closely related ethnic groups. We have compared our populations with one another and also with other world populations at two locus and three locus haplotypes

Two locus haplotype analysis revealed that the haplotypes DRB1*1501-DQB1*0601 and DRB1*0701-DQB1*0201 were significantly represented in all the three populations. When we compared our data with the other North Indian population (Rani 1998) it was evident that the common haplotypes found in our population were also represented in North Indians from Delhi (Rani 1998). The haplotype DRB1*1501-DQB1*0601 was the most frequent haplotype found in Bhargavas and its frequency was lowest among Chaturvedis. Brahmins had a frequency intermediate between the two. This haplotype may be considered as unique for this region as it was not found in other North Indians from Delhi. In Buyis (Chen et al. 1991) this haplotype is represented significantly this points towards African admixture in our populations.

Other unique haplotypes of our data were DRB1*1001-DQB1*0601, DRB1*03011-DQB1*0301, DRB1*0701-DQB1*0401, DRB1*0701-DQB1*0601, DRB1*0701-DQB1*0503, DRB1*1501-DQB1*0201, DRB1*1501-DQB1*0301, DRB1*1102-DQB1*0601. These haplotypes were present in one or the other population and not in the entire population group under study. Hence some of the haplotypes may be considered as marker haplotype for a particular population where they are occurring with a significant frequency.

When the three locus haplotype comparison in our population was done it was evident that the number of haplotypes shared by the three populations was only two i.e. DRB1*0701-DQA1*0201-DQB1*0201 and DRB1*1501-DQA1*0103-DQB1*0601.However the frequency of DRB1*0701-DQA1*0201-DQB1*0201 was higher in Chaturvedis and Brahmins while the second haplotype i.e. DRB1*1501-DQA1*0103-DQB1*0601 was higher in Bhargavas, but it was similar in Chaturvedis and Brahmins. The haplotype DRB1 * 0701- DQA1 *0201 - DQB1* 0201 was the most common haplotype. It is seen in most of the other world populations so far reported in the literature. This may be considered as an ancient haplotype. Both allele frequency and haplotype comparison of our data with other world populations revealed that our populations differ from one another and are unique. When we compared these groups with other world populations we found that our population have a major Caucasian element in their genetic constitution.

REFERENCES

Bhasin MK, Walter H 2001. *Genetics of Castes and Tribes of India.* Delhi: Kamla-Raj Enterprises.

Bhasin MK, Walter H, Danker-Hopfe H 1994. *People of India: An Investigation of Biological Variability in Ecological, Ethno-economic and Linguistic Groups*. Delhi: Kamla-Raj Enterprises.

Blasczyk R, Kotsch K, Wehling J 1998. The nature of polymorphism of the HLA-DRB intron sequences is lineage specific. *Tissue Antigens*, **52**: 19-26.

Chen RB, Ye GH, Geng ZhCh, Wang ZH, Kong IH, Tian D, Bao PY, Liu RY, Liu J, Song FJ, Fan LA, Zhang GL, Guo Sh Sh, Lu LM, Xu XP, Cheng Z Zh, Zhao X Zh 1991. In: K Tsuji, M Aizawa, T Sasazuki, (Eds.): *HLA 1991*. Vol 1. Oxford: Oxford University Press.

Chen YS, Torroni A, Excoffier L, Santachiara-Benerecetti AS, Wallace DC 1995. Analysis of mtDNA variation in African populations reveals the most ancient of all human continent-specific haplogroups. *Am J Hum Genet*, **57**: 133-149.

Djoulah S, Sanchez-Mazas A, Khalil I, Benhamamouch S, Degos L, Deschamps I, Hors J 1994. HLA-DRB1, DQA1 and DQB1 DNA polymorphisms in healthy Algerian and genetic relationships with other populations. *Tissue Antigens*, **43**: 102-109.

Felesentein J 1989. PHYLIP- Phylogeny Inference Package (version 3.2). *Cladistics*, **5:** 164-166.

Harpending HC, Batzer MA, Gurven M, Jorde LB, Rogers AR, Sherry ST 1998. Genetic traces of ancient demography. *Proc Natl Acad Sci USA*, **17**: 1961-1967.

Hughes AL, Nei M 1992. Maintenance of MHC polymorphism. *Nature*, **355**: 402-403.

Lanchbury JS, Agarwal SS, Papiha SS 1996. Genetic differentiation and population structure of some occupational caste groups in Uttar Pradesh, India. *Hum Biol*, **68**: 655-678.

Levene H 1949. On a matching problem in genetics. *Ann Math Stat*, **20**: 91-94.

Little AM, Parham P 1999. Polymorphism and evolution of HLA class I and II genes and molecules. *Reviews in Immunogenetics*, **1**: 105-123.

Monsalve MV, Booth PB, Cavalli-Sforza LL 1999. Languages, Geography and HLA haplotypes in native American and Asian Populations. *Proc R Soc Lond B Biol Sci*, **266**: 2209-2216.

Nei M 1973. Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA*, **70**: 3321-3323.

Rani R, Fernandez-Vina MA, Stastny P 1998. Associations between HLA class II alleles in a North Indian population. *Tissue Antigens*, **52**: 37-43.

Rogers J, Comuzzie AG 1995. When is ancient polymorphism a potential problem for molecular phylogenetics. *Am J Phys Anthropol*, **98**: 216-218.

Saitou, N, Nei M 1987. The neighbour-joining method: a new method for reconstructing phylo-genetic trees. *Mol Biol Evol*, **4:** 406-25.

Sharma BR 1974. A comparative study of genetic variation at five VNTR loci in three ethnic groups of Houston, Texas. *J Forensic Sci*, **40**: 933-42.

Sherring MA. 1974. In: *Hindu Tribes and Castes- as represented in Banares* Vol.1, Delhi: Cosmo Publications.

Trachtenberg EA, Keyeux G, Bernal JE, Rhodas MC, Erlich HA 1996. Results of Expedition Humana I Analysis of HLA class II (DRB1-DQA1-DQB1-DPB1) alleles and DR-DQ haplotypes in nine Amerindian populations from Colombia. *Tissue Antigens*, **84:** 174-181.

Trowsdale J, Powis SH 1992. The MHC: relationship between linkage and function. *Curr Opin Genet Dev*, **2**: 492-497.