

Molecular Diversity of HLA-A, HLA-B, HLA-DRB1 and HLA-DQB1 Alleles from Mumbai India

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ABSTRACT Indian population exhibits not only a wide variety of ethnic but also great cultural and linguistic diversities. In the present study, 296 unrelated individuals belonging to different linguistic groups from Maharashtra are studied for HLA A, B, Cw, DRB1 and DQB1 gene frequencies using commercial PCR-SSOP kits. The results revealed that A*02 (19.25%), A*11 (13.17%), A*24 (15.70%), A*33 (11.82%), B*35 (15.37%), B*40 (12.66%), DRB1*15 (19.25%) DRB1*07 (12.83%) DQB1*02 (20.17%), DQB1*03 (20.85%), DQB1*06 (21.52%), and DQB1*06 (32.73%) are the common alleles. The common subtypes among these alleles are A*02:01:01:01, A*02:03:02, A*02:06:01, A*02:11, A*02:22:01 out of 160 A*02 alleles, A*11:01:01, A*11:03 out of 78 A*11 alleles, A*24:02:01:01, A*24:06, A*24:07 out of 128 A*24 alleles, A*33:03:01 out of 34 A*33 alleles, B*35:01:01, B*35:03:01, B*35:20:01 out of 135 B*35 alleles, B*40:06:01:01 only allele out of 120 B*40 alleles, DRB1*07:01:01:01, DRB1*07:06 out of 19 DRB1*07 alleles, DRB1*15:01:01:01, DRB1*15:01:02, DRB1*15:02:02 out of 63 DRB1*15 alleles, DQB1*02:01:01, DQB1*02:02, out of 6 DQB1*06 alleles, DQB1*03:01:01, DQB1*03:02:01, DQB1*03:03:02 out of 36 DQB1*03 alleles, DQB1*05:01:01, DQB1*05:02:01, DQB1*05:03:01 out of 8 DQB1*05 alleles, DQB1*06:01:01, DQB1*06:01:02, DQB1*06:02:01, DQB1*06:03:01 out of 50 DQB1*06 alleles, existing alleles are identified as per the recent HLA 2010 Nomenclature. When compared with other reported studies on Indian population differential frequencies are observed suggesting an influence of a genetic drift caused by selection geography. The Indian population may not be considered as a single panmictic population.

INTRODUCTION

India with 1,189,700,000 billion people, having 3824 castes and 461 tribes is one of the Mega diversity countries in the world. It is the second continent after Africa habituated by man for the past 60,000 years. Many more migrations have settled in various parts of India and huge expansion of Mankind has occurred. As a result, different marriage practices and social customs were developed. The basic pattern of the society and value system seems to have been laid down well before the origin and spread of Dravidian and Aryan languages in India. India is an ancient land of immigration / spread of many different streams of people, whereas Africa is a land of origin, expansion and divergence of the same gene pool for the past 0.2 million years. Comparing these gene pools has already thrown

newer insights into various evolutionary principles (Cavalizaforza et al. 2003; Riech et al. 2009; Norman et al. 2007). One of the greatest experiments of Nature was the Caste system in India. (Dobzhansky 1973). A caste or tribe is an isolated breeding unit and drifts away from one another as time passes. A tribe is primitive in mode of subsistence, economy and living conditions, mostly living in isolation in hilly terrains sigh of the modern developments. On the contrary, castes live in plains and are capable of articulation and egging their income in more modern interdependent civilizations. Most of the castes and tribes in India are inbred and endogamous, with a variable degree from one region to another. Each caste / tribe contains many clans, mostly patriliney, though matriliney is an ancient custom and is practiced even today in a few castes / tribes. Usually the clans of tribes are Naturalistic and animistic, whereas, Castes are derived from seven *Rishis*, temples or other modern surrogates. Each caste / tribe is a social unit and a social security system defined by his or her own characteristics, territory, job and interdependency. Different castes living in the same region, sharing the environment and

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epidemiology as on date are sympatrically isolated in terms of their gene pool. This has great significance in terms of epidemiology and infectious disease transmission and susceptibility. It is known that not all the infected develop the disease (Pitchappan 2002). People having two different genetic makeup may not be equally susceptible to a given disease, however, the Nature Nurture interaction plays a dominant role in the incidence and prevalence of various diseases.

Earlier studies on HLA allelic and haplotype diversity have been amply confirmed and their implications discussed (Pitchappan 2002). Distribution of HLA antigens in various ethnic groups of India by molecular methods has been reported (Shanmugalaksmi et al. 2003; Shankarkumar et al. 2003). A study on the "Biology of the people of Tamil Nadu", has also brought out the differences in anthropometric, and other genetic polymorphisms: the nose shape and skin color are the most discriminatory characters and the authors have proposed three to four waves of migrations into Tamil Nadu / India (Sangvi 1981). The waves of migration have been seen in tribal population, Scheduled Caste population, people on the plains, and the Vellala related Brahmin, and priestly classes (Malhotra et al. 1981). They have estimated 20,000 years to 3,000 years as the dates of migration based on morphological characteristics. Using PCR based DNA technologies; we have demonstrated the genetic diversity of HLA alleles in the population group from Mumbai

MATERIALS AND METHODS

Study Population: A total of 296 unrelated normal healthy individuals from Mumbai were included in this study. EDTA Blood sample (5 ml) was drawn by vein puncture and the genomic DNA was isolated from the total PBMC using standard salting out procedure (Miller et al. 1988). Molecular Typing: HLA A, HLA B, HLA DRB1 and HLA DQB1 alleles were identified using polymerase chain reaction reverse line strip sequence-specific oligonucleotide hybridization (PCR-RLS-SSOP) strips as per the manufacturer's protocol (Dynal-Reli-SSO : Dynal Biotech Ltd UK, Innogenetics, Inno-Lipa Belgium kits) and the software. The alleles were reassigned as per the current 2010 Nomenclature

using ANTT software ver.0.5.0 (Mack and Hollenbach 2010).

Statistical Analysis: The allele frequencies were estimated from the number of positive typing reactions divided by the 2 x total number of individuals studied.

RESULTS

The distribution of HLA A allele subtypes (Table 1) HLA B allele subtypes (Table 2) HLA DRB1 allele subtypes (Table 3) and HLA DQB1 allele subtypes (Table 4) among the Mumbai population studied is presented. Further the comparative distribution HLA alleles Class I (Table 5) and Class II (Table 6) have been given. The study showed a higher frequency of A*02 (19.25%), A*11 (13.17%), A*24 (15.70%), A*33 (11.82%), B*35 (15.37%), B*40 (12.66%), DRB1*15 (19.25%) DRB1*07 (12.83%) DQB1*02 (20.17%), DQB1*03 (20.85%), DQB1*05 (21.52%), and DQB1*06 (32.73%) alleles. The common subtypes of these alleles were A*02:01:01:01, A*02:03:02,

Table 1: HLA A allele distribution among Mumbai Maharashtrian population

<i>HLA old before April 2010</i>	<i>HLA new April 2010</i>	<i>N+</i>	<i>%AF</i>
<i>Nomenclature</i>	<i>Nomenclature</i>		
<i>N= 296</i>	<i>N= 296</i>		
HLAA*	HLAA*		
A*01010101	A*01:01:01:01	51	8.61
A*02010101	A*02:01:01:01	36	6.08
A*020301	A*02:03:01	3	0.506
A*020601	A*02:06:01	2	0.337
A*0211	A*02:11	72	12.162
A*022201	A*02:22:01	1	0.168
A*03010101	A*03:01:01:01	52	8.783
A*03010103	A*03:01:01:03	5	0.844
A*0302	A*03:02	1	0.168
A*110101	A*11:01:01	76	12.837
A*1103	A*11:03	2	0.337
A*24020101	A*24:02:01:01	86	14.527
A*2406	A*24:06	4	0.675
A*2407	A*24:07	3	0.506
A*260101	A*26:01:01	28	4.729
A*290101	A*29:01:01	13	2.195
A*300101	A*30:01:01	15	2.533
A*310102	A*31:01:02	23	3.885
A*320101	A*32:01:01	13	2.195
A*330301	A*33:03:01	70	11.824
A*3601	A*36:01	2	0.337
A*6601	A*66:01	1	0.168
A*680101	A*68:01:01	24	4.054
A*680102	A*68:01:02	6	1.013
A*7401	A*74:01	3	0.506
		592	

Table 2: HLA B allele distribution among Mumbai Maharashtrian population

<i>HLA old before April 2010</i> <i>Nomenclature</i> <i>N= 296</i>	<i>HLA new April 2010</i> <i>Nomenclature</i> <i>N= 296</i>	<i>N+</i>	<i>%AF</i>
B*070201	B*07:02:01	30	5.067
B*0706	B*07:06	14	2.364
B*080101	B*08:01:01	28	4.729
B*130101	B*13:01:01	12	2.027
B*1401	B*14:01	1	0.168
B*15010101	B*15:01:01:01	27	4.56
B*150201	B*15:02:01	7	1.182
B*180101	B*18:01:01	22	3.716
B*270401	B*27:04:01	2	0.337
B*270502	B*27:05:02	8	1.351
B*2708	B*27:08	2	0.337
B*350101	B*35:01:01	53	8.952
B*350301	B*35:03:01	35	5.912
B*352001	B*35:20:01	6	1.013
B*370101	B*37:01:01	13	2.195
B*380101	B*38:01:01	11	1.858
B*39010101	B*39:01:01:01	3	0.506
B*40060101	B*40:06:01:01	75	12.668
B*4101	B*41:01	3	0.506
B*44020101	B*44:02:01:01	6	1.013
B*440302	B*44:03:02	39	6.587
B*4701	B*47:01	1	0.168
B*480101	B*48:01:01	4	0.675
B*490101	B*49:01:01	2	0.337
B*500101	B*50:01:01	5	0.844
B*510101	B*51:01:01	52	8.783
B*520101	B*52:01:01	33	5.574
B*5202	B*52:02	7	1.182
B*530101	B*53:01:01	3	0.506
B*550101	B*55:01:01	5	0.844
B*560101	B*56:01:01	7	1.182
B*570101	B*57:01:01	35	5.912
B*5708	B*57:08	4	0.675
B*580101	B*58:01:01	25	4.222
B*670101	B*67:01:01	1	0.168
B*7301	B*73:01	8	1.351
B*8101	B*81:01	3	0.506

A*02:06:01, A*02:11, A*02:22:01 out of 160 A*02 alleles, A*11:01:01, A*11:03 out of 78 A*11 alleles, A*24:02:01:01, A*24:06, A*24:07 out of 128 A*24 alleles, A*33:03:01 out of 34 A*33 alleles, B*35:01:01, B*35:03:01, B*35:20:01 out of 135 B*35 alleles, B*40:06:01:01 only allele out of 120 B*40 alleles, DRB1*07:01:01:01, DRB1*07:06 out of 19 DRB1*07 alleles, DRB1*15:01:01:01:01, DRB1*15:01:02, DRB1*15:02:02 out of 63 DRB1*15 alleles, DQB1*02:01:01, DQB1*02:02, out of 6 DQB1*06 alleles, DQB1*03:01:01, DQB1*03:02:01, DQB1*03:03:02 out of 36 DQB1*03 alleles, DQB1*05:01:01, DQB1*05:02:01, DQB1*05:03:01 out of 8 DQB1*05 alleles, DQB1*06:01:01, DQB1

Table 3: HLA DRB1 allele distribution among Mumbai Maharashtrian population

<i>HLA old before April 2010</i> <i>Nomenclature</i> <i>N= 296</i>	<i>HLA new April 2010</i> <i>Nomenclature</i> <i>N= 296</i>	<i>N+</i>	<i>%AF</i>
HLA DRB1*	HLA DRB1*		
DRB1*010101	DRB1*01:01:01	23	3.885
DRB1*03010101	DRB1*03:01:01:01	49	8.277
DRB1*03010102	DRB1*03:01:01:02	4	0.675
DRB1*030202	DRB1*03:02:02	3	0.506
DRB1*040101	DRB1*04:01:01	36	6.081
DRB1*040301	DRB1*04:03:01	13	2.195
DRB1*040701	DRB1*04:07:01	5	0.844
DRB1*07010101	DRB1*07:01:01:01	74	12.5
DRB1*0706	DRB1*07:06	2	0.337
DRB1*080101	DRB1*08:01:01	12	2.027
DRB1*090102	DRB1*09:01:02	12	2.027
DRB1*100101	DRB1*10:01:01	59	9.966
DRB1*110101	DRB1*11:01:01	32	5.405
DRB1*120201	DRB1*12:02:01	8	1.351
DRB1*130101	DRB1*13:01:01	37	6.25
DRB1*130102	DRB1*13:01:02	4	0.675
DRB1*130201	DRB1*13:02:01	3	0.506
DRB1*130701	DRB1*13:07:01	2	0.337
DRB1*140101	DRB1*14:01:01	44	7.432
DRB1*140103	DRB1*14:01:03	6	1.013
DRB1*140301	DRB1*14:03:01	3	0.506
DRB1*1404	DRB1*14:04	9	1.52
DRB1*140501	DRB1*14:05:01	2	0.337
DRB1*150101	DRB1*15:01:01	125	21.114
DRB1*150102	DRB1*15:01:02	4	0.675
DRB1*150202	DRB1*15:02:02	15	2.533
DRB1*160101	DRB1*16:01:01	4	0.675
DRB1*1602	DRB1*16:02	2	0.337
		592	

Table 4: HLA DQB1 allele distribution among Mumbai Maharashtrian population

<i>HLA old before April 2010</i> <i>Nomenclature</i> <i>N= 223</i>	<i>HLA new April 2010</i> <i>Nomenclature</i> <i>N= 223</i>	<i>N+</i>	<i>%AF</i>
HLA DQB1*	HLA DQB1*		
DQB1*020101	DQB1*02:01:01	35	7.847
DQB1*0202	DQB1*02:02	52	11.659
DQB1*0203	DQB1*02:03	3	0.672
DQB1*030101	DQB1*03:01:01	69	15.47
DQB1*030201	DQB1*03:02:01	14	3.139
DQB1*030302	DQB1*03:03:02	10	2.242
DQB1*040101	DQB1*04:01:01	18	4.035
DQB1*0402	DQB1*04:02	3	0.672
DQB1*050101	DQB1*05:01:01	63	14.125
DQB1*050201	DQB1*05:02:01	3	0.672
DQB1*050301	DQB1*05:03:01	30	6.726
DQB1*060101	DQB1*06:01:01	116	26.008
DQB1*060102	DQB1*06:01:02	18	4.035
DQB1*060201	DQB1*06:02:01	9	2.017
DQB1*060301	DQB1*06:03:01	3	0.672
		446	

06:01:02, DQB1*06:02:01, DQB1*06:03:01 out of 50 DQB1*06 alleles, were identified as per

Table 5: Selected high frequency HLA class I allele frequencies in different Indian populations reported

Population	Sample size	A*02:11	A*11:01:01	A*24:02:01:01	A*33:03:01	B*40:06:01:01
Gola (AP)	111	9.70	11.90	14.20	6.30	13.00
Pawra Tribe	50	16.00	21.80	16.00	3.00	17.00
Bhil Tribe	50	4.00	0.00	0.00	18.00	19.00
Parsi	50	0.00	9.00	0.00	14.00	0.00
Maratha	91	8.60	12.30	16.70	13.00	17.90
New Delhi	71	6.80	23.50	11.40	6.10	10.60
North Delhi	90	1.70	9.40	1.70	0.00	6.60
North Hindus	72	6.70	12.50	19.20	8.70	15.40
Nadars	61	0.00	9.80	15.60	6.60	9.00
Lucknow	125	NT	NT	NT	NT	NT
Bombay	59	NT	NT	NT	NT	NT
Mumbai*	296	12.16	12.84	14.53	11.82	12.67

AP = Andhra Pradesh *Present study

Table 6: Selected high frequency HLA class II allele frequencies in different Indian populations reported

Population	Sample size	DRB1* 07:01:01	DRB1* 15:01:01	DQB1* 02:02	DQB1* 03:01:01	DQB1* 05:01:01	DQB1* 06:01:01
Gola (AP)	111	6.90	6.00	NT	NT	NT	NT
Bhramin (AP)	98	26.50	0.00	NT	NT	NT	NT
Sunni (AP)	100	14.00	0.00	NT	NT	NT	NT
North India	85	9.20	11.20	NT	NT	NT	NT
Chennai (TN)	137	15.30	8.75	NT	NT	NT	NT
Dravidian (TN)	156	24.30	16.00	NT	NT	NT	NT
Nadars	61	28.57	10.71	NT	NT	NT	NT
New Delhi	102	8.80	0.00	0.00	0.00	0.00	25.00
New Delhi	112	17.50	5.90	2.25	12.50	7.50	8.95
Maratha	91	12.00	19.40	12.00	13.60	11.60	28.30
New Delhi	71	17.50	5.90	2.25	12.25	7.12	8.92
North Hindus	72	9.30	14.80	0.00	13.40	8.20	18.70
Lucknow	125	19.80	11.20	0.00	12.90	12.50	12.90
Bombay	59	NT	NT	0.00	10.20	7.60	26.30
Kayastha (NE)	190	21.10	9.70	0.00	13.70	9.70	15.50
Lachung (NE)	58	13.80	9.50	0.00	23.30	17.20	13.80
Mathur (NE)	155	23.90	8.40	0.00	9.40	14.50	19.30
Mech (NE)	63	11.90	13.50	0.00	26.20	16.70	15.10
Rajbanshi (NE)	98	13.90	13.70	0.00	23.40	29.10	19.00
Rastogi (NE)	196	19.60	9.90	0.00	9.70	12.20	16.10
Shia (NE)	190	14.70	4.70	0.00	14.20	15.20	11.60
Sunni(NE)	188	15.90	3.40	0.00	13.30	17.30	13.50
Vaish (NE)	198	25.70	8.60	0.00	15.20	17.90	10.40
Hindus (UP)	202	18.80	9.00	0.00	14.60	11.90	15.10
Mumbai*	296	12.50	21.11	11.66	15.47	14.13	26.01

AP = Andhra Pradesh, TN + Tamil Nadu, NE = North East, UP = Uttar Pradesh *Present study

current HLA Nomenclature. When the results were compared with other reported Indian studies, it was observed that Pawra tribe (Maharashtra, western India) had the highest allele frequency of HLA A*02:11 (16%) whereas New Delhi general population showed HLA A*11:01:01(23.50%) and A24:02:01:01 (19.20%) from North India, the Bhil tribe (Maharashtra western India) had highest frequency of A*33:03:01 (18%) and HLA B*40:06:01:01 of the Class I loci. The HLA DRB1*07:01:01(28.57%) showed the highest frequency among Nadars,

South Indian Caste; while DRB1*15:01:01:01 (19.4%), DQB1*02:02 and DQB1*06:01:01 were high in Marathas, Western Indian caste; HLA DQB1*03:01:01 (36.20%) was high among the Métis and DQB1*05:01:01(29.10%) among Rajbanshis, both caste groups from the Northeast India

DISCUSSION

In India 3824 castes groups are living as breeding isolates, natural habitats and are

evolved morphogenetic characteristics in their ecological niches. The net effect has created multiple genetic isolates, unique accentuation of polymorphisms mutations leading to population heterogeneity and genetic diversity. The present study on western India has revealed unique HLA allele profile. These differences can be attributed to migration and expansion of communities and also the other contributing factors, heterozygote advantage and balancing selection is still operating in these loci has been evident from other Indian studies. The migration, isolation and expansion as a cause of HLA diversity is supported by the observation that two different subtypes of the same allele were exclusively distributed in a gradient: one in Eastern Europe and Mediterranean regions and another one in India and South East Asia. HLA DRB1*15 (2) is found to be common in South East Asia and South Indian populations, but

DRB*16 (2) is common in Eastern European populations. HLA DRB1*10 and DRB1*07 are common in South Indian populations whereas DRB1*01 allele, characteristic of European populations are distributed less frequently in India (Mehra 2010). The distribution of DRB1*01 is found to be increased among the Maratha caste (Shankarkumar et al. 2003) similar to that of Caucasoid population but, although this allele is rare in Nadars and other caste groups studied. Some HLA alleles identified among western Indians in the present study are reported earlier among New Delhi population (North India), Nadar caste group (South India), and Maratha caste group (western India) (Shankarkumar et al. 2003). However, Pawra tribe has the highest allele frequency of HLA A*02:11 (16%) as reported earlier, New Delhi general population shows HLA A*11:01:01 (23.50%) and A24:02:01:01 (19.20%) from North India, Bhil tribe had highest frequency of Class I loci A*33:03:01 (18%) and HLA B*40:06:01:01. The HLA DRB1*07:01:01 (28.57%) shows the highest frequency among Nadars from Tamil Nadu, HLA DRB1*15:01:01:01 (19.4%), DQB1*02:02 and DQB1*06:01:01 are high in Marathas from Maharashtra, HLA DQB1*03:01:01 (36.20%) is the highest among the Mechs and DQB1*05:01:01 (29.10%) among Rajbanshis from the Northeast India. The diversification of these alleles has occurred in different directions. The migration, isolation in addition, expansion of human colo-

nies subjected to various bottlenecks may thus be the major cause of the present day HLA distribution around the globe (Shankarkumar et al. 2010).

In the present study, we have identified specific HLA allele subtypes not reported earlier among the Western Indians. This could be as a result of evolutionary history, endogamy and consanguinity with high genetic differentiation as suggested recently (Klitz et al. 2010). The observed existence of these HLA alleles could have also been as a consequence of founder effect, racial admixture and geographical or socioeconomic barriers contributing to the genetic drift (Shankarkumar 2010). More extensive HLA molecular genotyping studies in other Indian caste/population groups are essential to resolve their evolutionary history. Such HLA resource data will have potential implications in anthropological investigations, paternity testing, disease associations and stem-cell transplantation studies.

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