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HLA DRB1 Gene Study in Different Population Groups From Mumbai, Maharashtra, India

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ABSTRACT Indian population exhibits not only a wide variety of ethnic but also great culture and linguistic diversity. In the present study 483 unrelated individuals belonging to different linguistic groups from Maharashtra were studied for their HLA DRB1* allele gene frequencies using commercially procured PCR-SSOP kits. The results revealed that > 0.1 allele frequencies observed for HLA DRB1*03 among Marathi, Gujarathi and Punjabi linguistic groups; for HLA DRB1*06 among Marathi, Gujarathi, South Indian, Muslim and Christians; for HLA DRB1*05 among Gujarathi, Punjabi, Muslim and Christian; for HLA DRB1*07 among Gujarathi, Punjabi and Muslim. HLA DRB1*02 was observed > 0.1 allele frequencies among all the Marathi, Gujarathi, Punjabi, South Indian, Muslim, Christian and the random Western Indians studied. Further HLA DRB1*01, DRB1*08, DRB1*09 and DRB1*10 were < 0.1 allele frequencies in all the population groups studied. When compared with other reported Indian populations HLA DRB1*0305, DRB1*0308, DRB1*0309, DRB1*1107, DRB1*1111, DRB1*1123, DRB1*1128, DRB1*1307, DRB1*1315, DRB1*1334, DRB1*1402, DRB1*1410, DRB1*1411 and DRB1*1416 were identified in our study. Our results suggest the influence of genetic drift caused by selection geography and culture among different population groups studied and the Indian population cannot be considered as a single panmictic population.

INTRODUCTION

India occupies a center stage in Human evolution. It has served as a major corridor for dispersal of modern humans that started from Africa about 100,000 years ago (Cann 2001). Various evolutionary forces particularly natural selection has acted on during the period of evolution of modern humans from its most recent common ancestor. The study of human genomic variation among individuals can help us understand the nature and intensity of actions of various forces that have modulated our evolutionary course. It can also provide valuable data for understanding of various diseases that afflict us. With the second largest population in the world, India is known for its vast ethnic diversity and cultural traits. Anthropological and historical evidence classifies Indians broadly as Dravidians and Aryans. The Dravidians as the earliest settlers who were driven southwards following invasion by Aryans from the Northwest during 2000-3000BC.

Being the most highly polymorphic genetic system, HLA has been used to great advantage for the definition of various racial groups, their migration pattern, possible admixture etc. The distribution of HLA antigen frequencies among populations showed marked differences. The presence or lack of specific alleles for some ethnic groups is characteristic (Shankarkumar and Sridharan 2004). Distribution of HLA antigens by molecular methods in various ethnic groups of India have been reported (Shankarkumar and Sridharan 2004; Shankarkumar et al. 2003; Rajalingam et al. 2002; Shanmugalakshmi et al. 2003) Using PCR based DNA technologies, we have demonstrated the genetic diversity of HLA DRB1 alleles among the selected population groups from Mumbai.

MATERIALS AND METHODS

Study Population: Four hundred and eighty-three healthy unrelated individuals belonging to different population groups from western India were included in this study. EDTA blood samples (5 ml) were drawn and the genomic DNA was isolated from mononuclear cells using the standard salting out procedure and quantified

by spectrophotometer and gel analysis (Miller et al. 1988).

HLA Molecular Typing: The HLA DRB1 and DQB1 alleles were identified using the commercial (Dynal RELI SSO; Dynal Biotech Ltd., UK, Innogenetics, Inno-Lipa Belgium) kits by PCR-SSP and/or PCR-SSOP techniques following the manufacturer's protocol. The alleles were determined using the Pattern interpretation software supplied along with the kit that defined the alleles until October 2002 HLA Nomenclature.

Statistical Analysis: Allele frequencies were estimated from the number of positive typing reactions divided by the total number of haplotypes tested (number of individuals studied $\div^2$).

RESULTS

We characterized the HLA diversity in 483 unrelated individuals belonging to different linguistic groups from Mumbai using DNA based molecular high-resolution method. The expected and observed genotype frequency values did not differ significantly and were consistent within the population groups being in Hardy-Weinberg equilibrium.

Distribution of HLA Alleles: Table 1 presents a comparison of the allele frequencies of DRB1 in different population groups studied from Western India. The comparative distribution of allele frequencies reported in other Indian populations and/or caste groups are given in Table 2. Our results revealed that > 0.1 allele frequencies observed for HLA DRB1*03 among Marathi, Gujarathi and Punjabi linguistic groups; for HLA DRB1*06 among Marathi, Gujarathi, South Indian, Muslim and Christians; for HLA DRB1*05 among Gujarathi, Punjabi, Muslim and Christian; for HLA DRB1*07 among Gujarathi, Punjabi and Muslim. HLA DRB1*02 was observed > 0.1 allele frequencies among all the Marathi, Gujarathi, Punjabi, South Indian, Muslim, Christian and the random Western Indians studied. Further HLA DRB1*01, DRB1*08, DRB1*09 and DRB1*10 were < 0.1 allele frequencies in all the population groups studied. When compared with other reported Indian populations HLA DRB1*0305, DRB1*0308, DRB1*0309, DRB1*1107, DRB1*1111, DRB1*1123, DRB1*1128, DRB1*1307, DRB1*1315, DRB1*1334, DRB1*1402, DRB1*1410, DRB1*1411 and DRB1*1416 were identified in our study.

DISCUSSION

The present study on different population groups of western India has revealed their unique HLA DRB1* allele profiles. These differences can be attributed to migration and expansion of human communities but still evidences of heterozygote advantage and balancing selection still operating in these loci have been evident from other Indian studies. The migration, isolation, expansion and dispersal as a cause of HLA diversity is supported by the observation that two different subtypes of the same allele were distributed mutually exclusive gradient in two different directions in Eastern Europe and Mediterranean regions on the one hand and in India and South East Asia on the other. HLA DRB1*15 (2) was found to be common in South East Asia and South Indian populations, but DRB*16 (2) was common in Eastern European populations. HLA DRB1*10 and DRB1*07 are common in South Indian populations while DRB1*01 a characteristic of European populations is distributed less frequently in India. HLA DRB1*0305, DRB1*0308, DRB1*0309, DRB1*1107, DRB1*1111, DRB1*1123, DRB1*1128, DRB1*1307, DRB1*1315, DRB1*1334, DRB1*1402, DRB1*1410, DRB1*1411 and DRB1*1416 were identified among western Indians in the present study. Recently HLA DRB1*0302, DRB1*0317, DRB1*1317, DRB1*1322, DRB1*1405, DRB1*1434, DQB1*0307, DQB1*0609 and DQB1*0614 were reported in Nadars (Shankarkumar and Sridharan 2004). Further DRB1*0401, DRB1*1504, DRB1*1202, DQB1*0610 and DQB1*0402 have been identified among North Indians; DRB1*1105, DRB1*1108, DRB1*1117 and DQB1* 0203 identified exclusively among Maratha caste groups. The most frequent DR alleles observed in the Indian populations are DRB1*15/16(2) and DRB1*07 with DRB1*09 occurring with the least frequency (Mehra 1998). The distribution of DRB1*01 was found to be increased among the Maratha caste group (Shankarkumar et al. 2003) similar to that of Western Caucasoid although this allele is generally low in Nadars and other Indian caste and population groups studied. A complete absence of DRB1*1601 among Indian population has been reported (Shankarkumar et al. 2003). Incidentally 1601 allele is also absent among Chinese and population of Oceania.

Table: 1 Percentage DRB1* allele frequencies among different population groups from India.

*HLA DRB1**	*Marathi N = 96*	*Gujarathi N = 59*	*Punjabi N = 31*	*S.Indian N = 29*	*Muslim N = 24*	*Christian N = 34*	*Random N = 210*
*01	5.2	0.8	3.2	5.2	2	4.3	4.27
*02	26.04	12.7	16.1	24.1	14.5	17.6	21.65
*15011	25	11.1	16.1	21.7	0	13.2	18.58
*1502	1.04	0	0	1.6	0	0	1.42
*1503	0	0.8	0	0	0	4.4	0.23
*1601	0	0	0	0	0	0	0.47
*16021	0	0.8	0	0.8	4.1	0	0.95
*03	11.45	11.8	16.1	6.8	6.2	7.3	10.71
*03011	9.89	2.5	1.6	1.7	2	0	10.25
*03021	0.52	0	0	0	0	1.4	0
*0304	0	1.6	0	0	0	2.9	0
*0305	1.04	0	0	0	0	0	0
*0308	0	0	0	0	0	0	0.23
*0309	0	0	0	0	0	0	0.23
*04	8.33	5	8.1	5.1	6.2	8.8	14.04
*0401	8	4.2	0	0	0	0	12.2
*0402	0.33	0	0	0	0	0	1.73
*0403	0	0	0	0	0	0.01	0.47
*0404	0	0.8	0	0	0	0	0
*05	7.28	18.5	11.2	5.1	12.5	17.5	6.17
*11(5)	5.2	13.5	11.2	5.1	12.5	11.7	5.23
*1101	5.2	13.5	8	3.4	12	5.9	3.84
*1103	0	0	0	1.7	0	0	0
*1105	0	0	0	0	0	1.4	0
*1107	0	0	0	0	0	0	0.23
*1109	0	0	1.6	0	0	4.4	0.23
*1111	0	0	0	0	0	0	0.23
*1123	0	0	0	0	0	0	0.23
*1128	0	0	0	0	0	0	0.23
*1132	0	0	1.6	0	0.5	0	0
*12(5)	2.08	5	0	0	0	5.8	0.94
*1201	0.52	0.8	0	0	0	1.4	0.23
*12021	1.56	4.2	0	0	0	4.4	0.71
*06	17.73	21.1	8	20.5	29.1	20.5	16.16
*13(6)	9.89	16.1	6.4	13.7	12.5	16.1	8.57
*1301	8.33	14.5	4.8	0	8.5	14.7	7.15
*13031	0	0	0	0	0	1.4	0
*1305	0	0.8	1.6	0	0	0	0
*13071	0	0	0	0	2	0	0
*1309	0	0.8	0	0	2	0	0.95
*1315	1.56	0	0	0	0	0	0
*1334	0	0	0	0	0	0	0.47
*14(6)	7.84	5	1.6	6.8	16.6	4.4	7.59
*1401	2.08	4.2	0	3.3	4.1	3	0.71
*1402	1.56	0	0	0	0	0	0
*1404	4.2	0.8	1.6	3.5	12.5	1.4	6.19
*1410	0	0	0	0	0	0	0.23
*1411	0	0	0	0	0	0	0.23
*1416	0	0	0	0	0	0	0.23
*07	8.33	13.5	19.3	5.1	12.5	7.3	14.04
*0701	8.33	13.5	19.3	5.1	12.5	7.3	14.04
*08	1.56	0	0	5.1	2	0	0.94
*0901	0	0	1.6	1.7	2	2.9	1.42
*1001	8.85	5.9	4.8	8.6	0	8.8	1.9

However we have identified among random western Indians DRB1*1601 (0.47%) in the present study. Similarly occurrence of DRB1*1502 is relatively infrequent among Western Caucasians but is a predominant subtype in both Indians and as well as Orientals. Among Indians, several unique alleles and haplotypes were encountered that have not been reported in any

Table: 2 HLA DRB1* allele frequencies (x100) in Nadars of south India compared with other Indians reported.

HLA alleles Number	*South India*					*North India*				*West India*
		Tamil Nadu				*Lucknow*	*Delhi (NK)*	*Delhi (RR)*	*Kashmir*	*Mumbai*
	Nadars	*Kallars*	*Yadava*	*Vanniyar*	*Random*	*Random*	*Random*	*Random*	*Brahmin*	*Maratha*
Number	*84*	*202*	*233*	*132*	*84*	*123*	*308*	*47*		*113*
DRB1*										
*01	1.19	3.76	3.07	2.9	1.7	1.7	3.08		1.3	7.96
*02	10.71	26.71	14.33	13.6	23.12	19.8	21.42	13.8	12	23.45
*15011	10.71	22.51	6.47	9.5	8.82	11.2		8.5		18.61
*1502		3.5	6.4	4.1	12.6	7		5.3		2.7
*1504						1.2				
*16					1.1	0.4				3.09
*03	11.9	11.6	14.34	13.8	6.89	9.1	6.98	7.45	3.9	3.09
*0301	4.76	10.22	12.52		5.19	9.1		7.45		0.44
*0302	2.38									
*0317	4.76									
*04	11.9	10.78	18.5	7.9	11.33	7.4	7.79	12.75	12	5.75
*0401								1.05		
*0402	1.19					0.8				
*0403	4.76					4.6		6.4		
*0404	5.95					1.2		5.3		
*0406						0.8				
*11(5)	3.57	0.92	4.33	2.9	5.19	9	8.6	10.7	10.6	11.94
*1101	3.57					7		3.2		
*1103						0.8		1.05		
*1104						0.8		5.2		
*1105										7.96
*1106										0.88
*1108						0.4				
*1117										3.09
*12(5)		3.04	1.83	0.3	4.01	4.1	3.24	2.1	3	0.88
*1201						1.2				
*1202						2.5		2.15		
*1203						0.4				
*06	20.23	8.26	4.93	7.6	5.13	17.8	13.95	28.8	15.8	7.07
*13(6)	4.76			2.2		11.2	6.65	12.8	5.2	1.32
*1301	1.19					7.9				
*1302						2.9				
*1308	1.19					0.4				
*1317	1.19									
*1322	1.19									
*14(6)	15.47	2.57	0.81	5.4	0.56	6.6	7.3	16	10.6	5.75
*1401	1.19	2.57	0.81		0.56	4.1				
*1402						0.4				
*1403						0.4				
*1404	10.71					1.7				
*1405	1.19									
*1434	2.38									
*07	28.57	8.18	13.69	12.4	20.07	19.8	13.96	14.9	27.5	13.27
*0701	28.57	8.18	13.69	12.4	20.07	19.8	13.96	14.9	27.5	13.27
*08		4.67	5.73	5.1	5.73	0.4	0.64	1.1		2.65
*09		0.46	1.63	1.9	0.56	1.2	0.64	1.1		3.09
*1001	11.9	9.97	8.22	7.2	9.44	9.1	5.51	3.2	3.9	7.96

of the populations tested so far (Shanmughalakshmi et al. 2003; Jaini et al. 2002). The diversification of these alleles thus must have occurred in different directions that must have contributed to the observed HLA gradients.

The migration, isolation and expansion of human colonies subjected to various bottlenecks may thus be the major cause of the present day HLA distribution around the globe (Shankarkumar 2002). Further analyses imply that selection

still operates in these south Indian caste groups and populations. Further, it has been suggested that selection is a possible cause of driving the allelic divergence and expansion at DRB1 locus (Chen et al. 1999; Simoni et al. 1999). The enormous diversity identified in the second exon and introns of DRB1* has been suggested to be of the recent origin (Wells et al. 2001). Many studies consider infectious diseases as a major cause of selection and divergence (Huges and Nei 1986; Takahata and Nei 1990; Hill 1998). In conclusion, the present study reveals, the heterogeneous nature of the Indian population suggested that the population as such or even a linguistic or regional population within it could not be considered as a panmictic pool; only a caste group might be considered as a homogenous gene pool with its diverse haplotype combinations and high rates of consanguinity.

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