

Distribution of HLA Antigens in Bhils and Pawars of Dhadgaon, Maharashtra, India

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ABSTRACT Fifty three unrelated blood samples from Bhils and 38 unrelated blood samples from Pawars, two endogamous tribes residing in Narmada valley, Maharashtra, Western India, were examined for HLA A and B antigen profiles. The Bhil group was characterized by high frequencies of HLA A1, A10, B5, B8, B15 and B27; the frequencies of HLA A11, A28, B7, B12, B35, B37 and B55 were found to be higher in Pawars. Both the tribal populations had higher frequencies of A2, A9 A19, B7, B40 and B53. Two locus haplotype analysis revealed that A10-B8 a common Indian haplotype and A9-B37 were observed in both the tribal populations in positive linkage disequilibrium. Haplotypes A9-B35 and A3-B13 were observed only in Pawars. The haplotype A9-B37 was unique in these two tribal populations and was not reported in the other tribal populations hitherto studied in India. Thus HLA antigen frequencies and haplotypes which are unique for each tribe investigated suggested a genetic isolation.

INTRODUCTION

Tribal groups in India form a significant proportion of the population (8.01% in 1991) and they are believed to be the earliest settlers of the country. There are a total of 427 tribal communities in India (Singh, 1993). The dominant ethnic group among the Indian tribal population is Proto-Australoid, although tribes living in the sub-Himalayan belt have more Asian characteristics. Roy Burman (1971) classified tribal areas on the basis of their geographical, ecological, economic, administrative, ethnic and social factors into 4 geographical regions (*viz.* Himalayan region, Middle Indian region, Western Indian region and South Indian region) along with one distinct sub-region of the Islands (Islands sub-region).

The Bhils are one of the largest tribes concentrated mainly in Western Madhya Pradesh, Rajasthan, Eastern Gujarat and Northern Maharashtra. Racially they were classified as Gondids, Malids (or) Proto-Australoid but their

social history is still a mystery (Bhatia and Rao, 1986). There are several sub-sections of the tribe like the Dhobi Bhil, Dungri Bhil, Mewari Bhil, Rewal Bhil and Tadis Bhil, of which some of the divisions have attained independent tribal status. The Pawars resemble a primitive tribe Koli of western Satpura hills and are concentrated chiefly in Khandesh. Even though migrant admixture have been noticed in tribal groups these tribes hardly perform marriages outside their group even today which makes them unique.

Numerous studies on the genetics of tribal groups in India have been conducted by various workers and literature have been reported on blood groups, red cell enzymes and serum protein polymorphisms (Bhasin et al., 1992, 1994).

The HLA system, the most polymorphic and complex set of genetic markers known in man, is of valuable significance in anthropological studies (Dausset, 1981). Distribution of HLA antigens in various Indian tribal groups from South India *viz.* Irulas, Malayalis, Kotas, Badagas and Koyas have been reported (Selvakumar et al., 1987; Papiha et al., 1983; Pitchappan et al., 1997). However data are rather scanty from other parts of India (Papiha et al., 1983). Therefore we felt it was worthwhile to investigate the distribution of HLA antigens in the Bhils and the Pawars of Dhadgaon in Narmada valley of Dhule district, Maharashtra, Western India.

MATERIALS AND METHODS

An HLA typing laboratory was set up at the Primary Health centre of Dhadgaon, Dhule district and the reagents and equipments were shifted to the field from the Institute in Mumbai. Random blood samples were collected during the morning hours after clinically examining the

individuals. Five milliliters of venous blood was drawn in a sterile tube (in heparin 50IU/ml) from each of the 53 unrelated Bhils and 38 unrelated Pawars and transported at 2-5° to the laboratory within 3 hours.

For HLA typing the heparinised blood was diluted with an equal volume of phosphate buffered saline (PBS), layered on Histopaque-1077 (Sigma H - 8889) and centrifuged at 1,600 rpm for 20 minutes (Boyum, 1968). The interphase lymphocytes were harvested in PBS and washed twice by centrifuging at 1,000 rpm for 10 minutes to remove the contaminating non-lymphocyte cells. The pure lymphocyte suspension was adjusted to 4,000,000 cells/ml. HLA A and HLA B typing was performed by NIH two-stage microlymphocytotoxicity assay (Terasaki and Mccelland, 1964) with a battery of 60 HLA antisera which included 7 commercial sera (Biotest, Germany; Behring, Germany; Pelfreeze, USA), 5 donated sera (NIH, USA) and 46 indigenous sera of local origin (IIH, Mumbai).

The local indigenous sera (r values 0.8 - 1.00) were earlier collected from multiparous women (Mumbai) and first screened by us for HLA antibodies (Shankarkumar et al., 1998). These sera covered all the 11 common specificities of the HLA A locus viz. A1, A2, A3, A9, A24, A10, A26, A11, A19, A33, A28 and all the 20 common specificities of the HLA B locus viz. B5, B51, B7, B8, B12, B44, B13, B14, B15, B62, B17, B18, B22, B55, B27, B35, B37, B40, B61, B53.

The phenotype frequency genotype frequency, standard error of genotype frequency two locus haplotype frequencies co-efficient of linkage disequilibrium (δ) and chi-square with Yate's correction were calculated from 2 x 2 table as described by Mittal (1976) and Baur and Danilovs (1980).

RESULTS AND DISCUSSION

The distribution of HLA A and B antigen phenotypes and their frequencies in Bhils and Pawars are shown in table 1. The study revealed significant higher frequencies in A2, A9, A19, B7, B40 and B53 in both the tribal populations. In the Bhils, one of the largest tribes concentrated in western Madhya Pradesh, increased fre-

quencies of A1, A10, B5, B8, B15 and B27 were observed on the other hand, in Pawars another primitive tribe resembling the Koli tribe of western Satpura hills increased frequencies of A11, A28, B5, B12, B35, B37 and B55 were observed. Although the phenotype frequencies of HLA B22 were more or less equal in both Bhils (5.7%) and Pawars (5.3%), B55 (a split antigen) was identified only among Pawars (2.6%). Also the split antigens A24 (9), B44 (12), B51 (5) and B62 (15) were all identified with low frequencies only among the Bhils and were totally absent among Pawars. Further studies are warranted including all the split antigens and new antigens that have been designated by WHO Committee for Nomenclature of HLA System 1996 (Bodmer et al., 1997).

Table 2 gives the HLA gene frequencies in Pawars and Bhils alongwith the data reported in other Indian tribal populations. Frequencies of A19, B35, B40 and B53 were higher in the present tribes when compared to the other tribal populations. The frequency of A1 was significantly increased in Bhils (16.40%), while B37 was increased in Pawars (4.00%). HLA B14 and B18 were not identified in the present tribes, as was the case in Malayalis, but have been reported in the other tribes (Badagas, Kotas and Koyas) studied. Among the tribal populations reported it is evident that A2 and B7 gene frequencies in Pawars (33.10% and 14.20%) were significantly increased as was in the case of Kotas (45.68% and 15.63%), while it was decreased in the Bhils (24.80% and 9.90%) and Irulas (10.80% and 1.60%). HLA A3 was significantly increased in Badagas (13.79%) and decreased in Irulas (1.20%) whereas it was more or less equal in Bhils (7.9%) and Pawars (8.2). The gene frequencies of A9 among the tribes ranged variably in Kotas (5.34%), Badagas (8.62%), Pawars (14.20%), Bhils (16.40%), Malayalis (19.80%) and Irulas (22.10%). The gene frequency of B5 was found very much reduced in Pawars (4.00%) and increased in Irulas (23.80%). Incidentally, it is also of interest to note that, B51 the split of B5 was increased in Bhils (3.8%) but unidentified in Pawars (0.00%); as was the case in Irulas (9.00%) and Malayalis (0.00%), but occurred variably among other tribes reported (range 0.49 - 2.59%).

Table 1: HLA A and B antigens in Bhils and Pawars, Dhadgaon, India.

HLA antigens	Bhils (n 53)				Pawars (n 38)			
	N+	%PF	%Gf	SEGF	N+	%PF	%Gf	SEGF
A1	16	30.2	16.4	3.9	7	18.4	9.7	3.6
A2	23	43.4	24.8	4.8	21	55.3	33.1	6.6
A3	8	15.1	7.9	2.7	6	15.8	8.2	3.0
A9	16	30.2	16.4	3.9	10	26.3	14.2	4.3
A10	9	17.0	8.9	2.9	5	13.2	6.8	3.0
A11	10	18.9	9.9	3.1	10	26.3	14.2	4.3
A19	19	35.8	19.9	4.3	13	34.2	18.9	5.0
A24	1	1.9	0.9	0.9	0			
A26	1	1.9	0.9	0.9	0			
A 28	4	7.5	3.8	1.9	4	10.5	5.4	2.7
A -	1		0.9		0			
B5	11	20.8	11.0	3.2	3	7.9	4.0	2.3
B7	10	18.9	9.9	3.1	10	26.3	14.2	4.3
B8	9	17.0	8.9	2.9	4	10.5	5.4	2.7
B12	3	5.7	2.9	1.6	3	7.9	4.0	2.3
B13	1	1.9	0.9	0.9	2	5.3	2.7	1.9
B15	5	9.4	4.8	2.1	1	2.6	1.3	1.3
B17	9	17.0	8.9	2.9	6	15.8	8.2	3.3
B22	3	5.7	2.9	1.6	2	5.3	2.7	1.9
B27	4	7.5	3.8	1.9	1	2.6	1.3	1.3
B35	11	20.8	11.0	3.2	12	31.6	17.3	4.8
B37	2	3.8	1.9	1.3	3	7.9	4.0	2.3
B40	21	39.6	22.3	4.6	18	47.4	27.5	6.0
B44	1	1.9	0.9	0.9	0			
B51	4	7.5	3.8	1.9	0			
B53	14	26.4	14.2	3.7	11	28.9	15.7	4.5
B55	0				1	2.6	1.3	1.3
B62	2	3.8	1.9	1.3	0			
B-	3		2.9		0			

N+ = number positive for the allele

%PF = percentage phenotype frequency

%GF = percentage genotype frequency

SEGF = standard error of genotype frequency

Table 2: Gene frequencies (in percentages) of Bhils and pawars compared with Indian tribes

HLA	Pawars (n 38)	Bhils (n 53)	Malayalis@ (n 42)	Irulas@ (n 191)	Koya* (n 94)	Kota# (n 103)	Badagas# (n 58)
A1	9.70	16.40	4.90	6.80	3.7-	7.77	9.48
A2	33.10	24.80	18.40	10.80	23.40	45.68	17.24
A3	8.20	7.90	1.20	5.10	5.30	6.80	13.79
A9	14.20	16.40	19.80	22.10	NR	5.34	8.62
A27	NT	NT	NT	NT	NT	NT	NT
A2	0.00	0.00	NT	NT	13.30	4.94	3.45
A10	6.80	8.90	4.00	10.50	NR	2.91	5.17
A25	NT	NT	NT	NT	0.50	NT	NT
A26	0.00	0.90	NT	NT	4.3-	0.00	0.86
A34	NT	NT	NT	NT	NT	NT	NT
A66	NT	NT	NT	NT	NT	NT	NT
A11	14.20	9.90	22.80	18.60	28.70	16.05	13.79
A19	18.90	19.90	NR	NR	NR	2.91	12.07
A29	NT	NT	0.00	2.70	0.00	NT	NT
A30	NT	NT	NT	NT	0.00	NT	NT
A31	NT	NT	12.70	0.90	0.00	0.00	0.00
A32	NT	NT	0.00	4.00	0.50	0.00	0.00
A33	0.00	0.00	2.40	4.30	NT	0.00	0.00
A74	NT	NT	NT	NT	NT	NT	NT
A28	5.40	3.80	8.70	2.90	1.10	0.49	5.17
A68	NT	NT	NT	NT	NT	NT	NT
A69	NT	NT	NT	NT	NT	NT	NT
A36	NT	NT	NT	NT	NT	NT	NT

Table 2 : Contd.....

HLA	Pawars (n 38)	Bhils (n 53)	Malayalis@ (n 42)	Irulas@ (n 191)	Koya* (n 94)	Kota# (n 103)	Badagas# (n 58)
A43	NT	NT	NT	NT	NT	NT	NT
A80/A-	0.00	0.90	5.50	3.20	13.20	11.16	10.36
B5	4.00	11.00	11.40	23.80	NR	7.28	6.90
B51	0.00	3.80	0.00	9.00	1.60	0.49	2.59
B52	NT	NT	0.00	5.10	3.20	NT	NT
B7	14.20	9.90	11.40	1.60	8.00	15.63	3.45
B8	5.40	8.90	2.40	14.10	4.30	1.46	4.31
B12	4.00	2.90	3.60	1.10	NR	3.40	11.21
B44	0.00	0.90	2.40	0.80	5.30	NT	NT
B45	NT	NT	NT	NT	4.80	NT	NT
B13	2.70	0.90	7.40	5.10	3.20	2.48	7.76
B14	0.00	0.00	0.00	0.30	1.60	2.91	3.45
B64	NT	NT	NT	NT	NT	NT	NT
B65	NT	NT	NT	NT	NT	NT	NT
B15	1.30	4.80	11.40	1.10	1.10	3.88	8.62
B62	0.00	1.90	NT	NT	NT	NT	NT
B63	NT	NT	NT	NT	1.10	NT	NT
B75	NT	NT	NT	NT	NT	NT	NT
B76	NT	NT	NT	NT	NT	NT	NT
B77	NT	NT	NT	NT	NT	NT	NT
B16	NT	NT	NT	NT	1.10	0.00	0.00
B38	NT	NT	NT	NT	NT	NT	NT
B39	NT	NT	NT	NT	NT	NT	NT
B17	8.20	8.90	11.40	8.80	25.50	2.43	3.45
B57	NT	NT	NT	NT	NT	NT	NT
B58	NT	NT	NT	NT	NT	NT	NT
B18	0.00	0.00	NT	NT	2.20	0.49	1.72
B21	NT	NT	NT	NT	0.50	0.00	0.00
B49	NT	NT	NT	NT	NT	NT	NT
B50	NT	NT	NT	NT	NT	NT	NT
B22	2.70	2.90	15.50	2.90	0.50	0.49	9.48
B54	NT	NT	NT	NT	NT	NT	NT
B55	1.30	0.00	0.00	0.30	NT	NT	NT
B56	NT	NT	NT	NT	NT	NT	NT
B27	1.30	3.80	NT	NT	1.60	1.91	4.31
B35	17.30	11.00	1.00	9.90	2.70	3.40	6.03
B37	4.00	1.90	0.00	1.30	0.00	NT	NT
B40	27.50	22.30	15.50	18.50	5.30	11.17	12.07
B60	NT	NT	NT	NT	NT	NT	NT
B61	0.00	0.00	NT	NT	NT	NT	NT
B41	NT	NT	NT	NT	0.5	0.00	0.00
B42	NT	NT	NT	NT	0.5	NT	NT
B46	NT	NT	NT	NT	NT	NT	NT
B47	NT	NT	NT	NT	NT	NT	NT
B48	NT	NT	NT	NT	NT	NT	NT
B53	15.70	26.40	0.00	4.00	1.10	NT	NT
B59	NT	NT	NT	NT	NT	NT	NT
B67	NT	NT	NT	NT	NT	NT	NT
B70	NT	NT	NT	NT	NT	NT	NT
B71	NT	NT	NT	NT	NT	NT	NT
B72	NT	NT	NT	NT	NT	NT	NT
B73	NT	NT	NT	NT	NT	NT	NT
B78	NT	NT	NT	NT	NT	NT	NT
B81	NT	NT	NT	NT	NT	NT	NT
B-	0.00	2.90	9.00	7.50	24.30	12.60	14.65

NT = Not tested ; NR = Not reported

@ : Pitchappan et al., 1997; # : Selvakumar et al., 1987; * : Paphia et al., 1983

Most of the newly identified and defined HLA antigens alongwith their splits (Bodmer et al., 1997) have not been tested previously in these tribal populations. All the antigens which have been studied showed differential gene frequencies in each of the tribal populations *e.g.* HLA B13 had the lowest frequency in Bhils (0.90%), highest frequency in Badagas (7.76%) and variable frequencies in Malayali (7.40%), Irulas (5.10%), Koyas (3.20%) and Kotas (2.48%). This could be attributed to the demic expansion of the population concerned (Cavalli-Sforza et al., 1993).

The two locus haplotype frequencies and the linkage disequilibria figures of Bhils and Pawars alongwith the other reported Indian tribal populations are given in table 3. In Bhils and Pawars significant positive linkage disequilibrium was seen for two haplotypes - A10-B8 ($t = 2.51; 2.52$) and A9-B37 ($t = 1.84; 2.18$). On the contrary, in Pawars A9-B35 ($t = 2.13$) had significant positive linkage disequilibrium and

A3 - B13 ($t = 1.55$) occurred more frequently. Further, the Bhils showed contrast with Pawars in total absence of haplotypes A3 - B13 and A9-B35, the latter being dominantly characteristic of the Pawars.

Haplotype frequencies in Indian populations after HLA typing including A3, A9, A10, B8, B13, B35 and B37 have been reported among Indian Hindus (Mittal et al., 1982), North Indians (Mehra et al., 1986), South Indians (Pitchappan et al., 1984; Selvakumar et al., 1988) and all possess the haplotype A10-B8. Among the tribal populations, Kotas and Badagas (Selvakumar et al., 1987) do not possess this haplotype but Malayalis and Irulas (Pitchappan et al., 1997) do possess it as has also been observed in Bhils and Pawars of the present study. According to Pitchappan (1988) the presence of haplotype A1-B17 and absence of A1-B8 in the Indian population was due to the "Founder Effect". Reports from the tribal population studied viz. Kotas, Badagas, Malay- alis and Irulas

Table 3: Two locus haplotypes identified in Bhils and Pawars and compared with other tribes

Population	Haplotype	HF _a	D _{max}	t*	Ki
Pawars (n 38)	A3-B13	111.00	180.00	1.55	4.712
	A10-B8	296.00	630.00	2.52	13.47
	A9-B35	496.00	1243.00	2.13	4.36
	A9-B37	223.00	267.00	2.18	7.07
Bhils (n 53)	A9-B37	213.00	235.00	1.84	4.45
	A10-B8	376.00	682.00	2.51	13.96
Kotas# (n 103)	A1-B7	463.10	1.00	0.50	NR
	A2-B7	7044.30	2082.50	10.74	NR
	A3-B7	709.40	208.70	3.22	NR
	A9-B40	216.70	146.60	1.55	NR
	A11-B40	197.00	5.80	0.05	NR
	A19-B7	128.10	81.60	0.52	NR
Badagas# (n 58)	A1-B22	28.87	20.17	1.33	NR
	A2-B12	18.37	5.86	0.33	NR
	A2-B15	35.43	17.93	1.06	NR
	A2-B40	15.75	10.17	0.55	NR
	A11-B15	27.56	13.62	0.87	NR
Malayalis@ (n 42)	A10-B8	241.00	232.00	NR	14.60
	A33-B44	241.00	235.00	NR	22.80
	A2-B5	686.00	478.00	NR	4.00
Irulas@ (n 191)	A10-B8	780.00	590.00	NR	41.30
	A2-B5	508.00	252.00	NR	5.00
	A11-B40	813.00	467.00	NR	14.38

a = Haplotype frequency per 10,000

* = $t > 2$ indicates positive linkage disequilibrium

D_{max} = Maximum possible value of Delta per 10,000

= Selvakumar et al., 1987

@ = Pitchappan et al., 1997

revealed that each tribe presented a unique characteristic HLA haplotype. Kotas has A2-B7 while Badagas have A2-B15 more frequent (Selvakumar et al., 1987). The Malayalis had A33-B44 while the Irulas have A11 - B40 more frequent (Pitchappan et al., 1997). Similarly it is observed that Pawars have A9 - B35 while Bhills have A9-B37 more common.

The presence of A10-B8 in all the tribes except Kotas and Badagas suggest a Proto-Australoid descent during the demic expansion of human evolution (Cavalli-Sforza et al., 1993), while the uniqueness observed in the haplotypes of all the tribes hitherto studied suggest genetic isolation, migration caused by geography and culture, selection advantage which supports the Founder-effect hypothesis for the presence and absence of specific haplotypes in a particular tribal population studied. A well planned study using DNA polymorphisms at the sequence level for these HLA alleles and haplotypes will generate more information on the affinity, origin and genetic diversity of these tribal populations.

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