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HLA Antigen Distribution in Sikhs from Punjab, India

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ABSTRACT HLA class I antigen distribution of Sikh population from Punjab is presented. A total of 404 individual of Sikh community who were either donors for renal transplant recipients or staff in PGIMER Chandigarh were selected. HLA class I antigens (A and B) were identified using the standard National Institute of Health two stage micro lymphocytotoxicity assay. The phenotypic frequencies of HLA-A10 ,B5 ,B8 were found to be increased while the frequencies of HLA-A19, A28, B35 and B40 were found to be decreased when compared to another north Indian population. The two-locus haplotype analysis revealed highly significant positive linkage disequilibrium for A2-B21, A10-B8. Significant negative linkage disequilibrium was also seen A9-B8, A10-B5, A10-B7 and A9-B24 and positive linkage for A11-B5. Haplotype A2-B21 appears to be unique to Sikhs.

INTRODUCTION

Punjab lies at the north west of India. It was the first seat of the Indus valley civilization of Mohanjodaro and Harappa replaced later by the Nomadic Aryans from Central Asia. The Aryans who were tall and fair, drove out dark skinned inhabitants and occupied east of Northern Hindustan. Punjab, by virtue of its geographic location has been battle point and first homes to all the conquests. Persians, Greeks Scythian tribes, Mongoloids, Afghans, followed Aryans. Out of this mixture were born the Punjabi people. The ethnic pattern of Punjab has changed with every new conquest. The people of Punjab were united by the development of a new religious faith- SIKHISM. Wearing turbans and keeping beards recognizes Sikhs. The Sikhs refer to the themselves as Jat Sikhs Mazhabi Sikhs, Ramgarhia Sikhs etc. Jat Sikhs have surnames like Chauhans, Dhillon, Arora, Oberoi, Saini that display caste background. Ramgarhian and Mazhabis have a generally no surname as Sikh tradition recommends. Males suffix Singh to their name and females as Kaur (Singh 1999).

Human leucocyte antigen or HLA is a highly polymorphic system provides useful information to study population Reports are available from various parts of the India regarding HLA profile of those population (Mehra et al. 1986; Selvakumar et al. 1988; Balakrishanan et al. 1996; Agarwal et al. 1999; Chhaya and Shankarkumar 2001). However none is available from Punjab, the home to 98% Sikhs of the country.

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MATERIALS AND METHODS

Samples: The data was collected from the files of the department of Immunopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India. Donors of live related renal transplant recipients for whom HLA typing for class I antigens was done and healthy volunteers from the staff and students were the subjects of this study. The study population was selected from the file based on their surnames, which belong to Sikh community, and those with Singh or Kaur suffixed to their names as explained in the introduction. A total of 404 people were included in the study.

HLA Serological Typing: 10-15 ml of venous blood collected in clean heparinised tube was used for lymphocyte isolation using density gradient centrifugation. Alleles of HLA-A and B antigens were identified using National Institute of Health two stage microlymphocytotoxicity assay (Terasaki and McClleland 1964) using commercial antisera (Biotest, Germany; BAG, Germany; Pelfreez, USA). A total of 120-140 sera were used to type major specificity's of HLA-A and -B. Typing trays included a minimum of three

antisera for each superspecificity.

Statistical Analysis: The phenotypic frequency (PF), genotype frequency (GF), standard error of genotype frequency (SEGF), Chi square with Yate's correction, coefficient of linkage disequilibrium (delta D) were calculated following the method described by Baur and Danilovs (1980).

RESULTS

The results of HLA-A and -B antigen frequency, genotype frequency, standard error of genotype frequency are shown in table 1. Since this data is over a long period (1983-2000) and composition of trays, source and availability of sera has changed, the splits have been collapsed into major antigens for most. HLA A9 was mainly represented by A24, B15 by B62 and B17 by B57. The antigen frequencies of HLA-A10 and B5 were found to be increased ($p< .001$) while the frequencies of HLA-A19, B35 and B40 were found to be decreased ($p<0.001$) when compared to another north Indian population. A28, A32, A 33 we also found to be low in our population and B8 increased. Antigen frequency of B14 was very low and we did not detect B16 in our population. The observed haplotype frequency and linkage disequilibrium (delta) (per 1000 population) for HLA -A and -B loci are shown in table 2. The 2 locus haplotype analysis revealed highly significant positive linkage disequilibrium for A2-B21, A10-B8 ($p<0.001$). Significant negative linkage disequibrium was also seen A9-B8, A10-B5,A10-B7 and A9-B24 and positive linkage for A11-B5 ($p<0.05$) Most of the newly identified and defined HLA antigen have not been tested in this population (Bodmer 1999).

Table 1: HLA-A and B antigen distribution in Sikh population from India

Antigen	*PF%*	*GF%*	*SEGF%*
A1	22.77	12.12	1.14
A2	26.38	14.2	2.97
A3	22.77	12.12	1.14
A9	25.24	13.54	1.2
A10	17.32	9.08	1.01
A11	20.04	10.58	1.08
A!9	5.69	2.63	0.56
A28	8.91	4.56	0.73
A32	0.74	0.38	0.32
A33	0.24	0.13	0.12
B5	40.09	22.6	1.47
B7	12.12	6.26	0.85
B8	13.61	7.06	0.9
B12	12.62	6.53	0.85
B13	4.95	2.51	0.54
B14	0.24	0.13	0.12
B15	11.13	5.73	0.81
B17	10.89	5.61	0.8
B18	2.72	1.37	0.4
B21	4.2	2.13	0.5
B22	3.71	1.88	0.47
B27	5.69	2.89	0.58
B35	12.87	6.66	0.87
B37	3.46	1.75	0.46
B40	11.63	6	0.83
B41	0.49	0.25	0.17

Table 2: Haplotypes showing significant linkage disequilibrium in Sikh population from India

Haplotype	*HF*	*Delta*	*Chi*
Positive Linkage Disequilibrium			
A10-B8	27.4	21	30.76
A2-B21	15.5	12.5	23.13
A9-B27	10	6.1	4.28
A11-B5	37.7	13.8	4.33
Negative Linkage Disequilibrium			
A9-B8	-3.7	-13.3	5.27
A10-B5	2.6	-17.9	7.29
A10-B7	-2.2	-7.9	4.92

HF,haplotype frequency per 1000, Delta, linkage disequilibrium per 1000

DISCUSSION

Sikhs form a unique group, characterized by their physical appearance, language, and religion. the HLA profile of Sikhs resembles Caucasians in generals (Imanashi 1992) and is almost similar to the other Indian populations (Mehra et al. 1986; Selvakumar et al. 1988; Balakrishanan et al. 1996; Agarwal et al 1999; Chhaya and Shankarkumar 2001). There are some notable differences though. The phenotypic frequencies of HLA A10, B5 and B8 are increased and those of HLA A19 ,B35 and B40 decreased as compared to north Indian Hindus (Mehra et al. 1986; Rajalingam et al. 1997). However it may be pointed out that the discrepancy in antigen frequency of HLA-B5 and B35 may be due to failure to differentiate the two due to lack of monospecific antisera.

Highly significant linkage disequilibrium was seen for haplotype HLA A10-B8. Indeed it has been reported as characteristic of Indian population as reported by previously (Mehra et al. 1986; Selvakumar et al. 1988; Balakrishanan et al. 1996; Agarwal et al. 1999; Chhaya and Shankarkumar 2001). None of the other ethnic groups show any significant linkage disequilibrium for HLA A10-B8. Highly significant linkage disequilibrium was also found for haplotype HLA A2-B21. This haplotype has not

been described in any population studied so far and appears to be unique to Sikhs. The other haplotypes showing significant negative linkage disequilibrium were A9-B8, A10-B7 and A10-B5 and A9-B27. Thus based on HLA profile of class I antigens, it appears that Sikhs are essentially Caucasoid in origin as other Indian groups with unique haplotype.

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