HLA A*02 allele and B-associated haplotype diversity in Indians

U. SHANKARKUMAR

HLA Department, Institute of Immunohaematology (ICMR), 13th Floor, K.E.M. Hospital, Parel, Mumbai – 400012, India

Accepted: 14 December 2002

ABSTRACT

Human leucocyte antigen (HLA) A2 is the most heterogeneous allele at the HLA A locus, with approximately 56 different subtypes. Substantial heterogeneity in A*02 distribution has been observed in populations worldwide. HLA A2 allele distribution varies (4-25%) in different Indian populations and castes. HLA B40 is the most common allele associated with A2 haplotypes. In this study, molecular A*02 subtyping in Maratha (western Indian – Aryan) and Nadar (south Indian – Dravidian) caste groups using high-resolution polymerase chain reaction/reverse line strip/sequence-specific oligonucleotide hybridisation (PCR-RLS-SSOP) indicates the presence of 10 subtypes (A*02011, A*0203, A*0205, A*0206, A*0207, A*0208, A*0209, A*0211, A*0222 and A*0236) in variable frequencies. Moreover, A*0211-B*4006 was commonly observed among the north Indian Maratha caste and A*02011-B*4006 among the south Indian Nadar caste groups. HLA A*0211 is found frequently in this population and in Asian Indians, and has been reported with low frequencies in many other populations worldwide. A*0222 has been observed among Hispanics, while A*0236 has been observed exclusively among members of the Maratha caste. The findings demonstrate a substantial heterogeneity among the groups studied that may be a consequence of founder effect, racial admixture or selection pressure due to environmental factors.

KEY WORDS: Founder effect. Haplotypes. HLA-A2 antigens. HLA-B antigens

Introduction

Human leucocyte antigen (HLA) A*02 is the most heterogeneous allele family at the HLA A locus, comprising some 56 different alleles. Most of the nucleotide differences in subtypes occur in the α1 and α2 domains responsible for peptide binding and HLA-restricted recognition by the T-cell receptor. This is consistent with the view that positive selection for polymorphism is a major factor in the fixation of this allele.[1] Furthermore, HLA A2 has also been implicated in a number of diseases including Alzheimer's,[2] psoriatic arthritis,[3] vitiligo[4] and pulmonary tuberculosis[5].

Substantial heterogeneity in A2 subtype distribution has been observed in populations worldwide. HLA A2 occurs with varying frequency in north Indians (17.33%),[6] south Indians (15.06%),[7] Marathi-speaking Hindus (14.4%),[8] Gujarathi Hindus (12.43%)[9] and Uttar Pradesh Indians (10.99%).[10] Distribution of A2 among the different caste groups also shows heterogeneity.[11,12] In addition, substantial A*02 subtype distribution has been observed in populations worldwide,[6,13] with HLA A*02011 showing the highest frequency in most populations.[14-18]

A*0211 is the most frequent allele reported among north Indians but it is a relatively rare allele in populations elsewhere around the world.[14,18-21] A*0206 and A*0203 are common alleles in most Asian populations, while A*0205 occurs in Caucasians and Negroes. Recently, A*0209 has been reported in north Indians.[13]

In view of the these observations, and the A19 allelic diversity among Western populations,[22] this study aims to determine the molecular diversity of the A*02 allele and its B* allele haplotype associations in the western Indian Maratha caste (Aryan descent) from the Maharastra state and the south Indian Nadar caste (originally Dravidian) from the Tamil Nadu state, using a high-resolution polymerase chain reaction (PCR)-based reverse blot sequence-specific oligonucleotide hybridisation technique.

Materials and methods

Subjects

A total of 2908 unrelated healthy individuals belonging to different population and caste groups (Table 1) were studied and the A2 allele was determined by serology. In order to study the different A*02 subtypes, 61 healthy individuals from the Nadar caste group in the southern Indian state of Tamil Nadu,[23] and 93 healthy individuals from the Maratha caste group in the western Indian state of Maharastra[24] were studied. A total of 51 A*02-positive samples belonging to the Maratha (n=38) and Nadar (n=13) castes were studied for molecular subtypes and their B* allele-associated haplotypes.

*HLA A*02 and B*40 molecular typing*

DNA was obtained from EDTA-anticoagulated peripheral mononuclear cells using standard methods.[25] Genomic DNA from 51 A2-positive samples was selected and genotyped for A*02 and B* allele subtypes by PCR/reverse line strip/sequence-specific oligonucleotide hybridisation (PCR-RLS-SSOP) strips (Roche Molecular, Oakland CA, USA). Each HLA A typing strip contained 57 immobilised sequence-specific oligos (SSOs), while the HLA B typing strip

Email: shankarkumar16@hotmail.com

Table 1. HLA A2 - and B-associated haplotyope distribution among Indians

Population	Number studied	Pos	AF(%) haplotype	Associated	HF(%)	LD(%)	χ^2	Reference
North Indians	225	78	17.33					6
South Indians	385	116	15.06	A2-B40	3.00	1.68		7
Marathi Hindus	392	113	14.41	A2-B40	3.93	1.86	12.45	8
Gujarathi Hindus	414	103	12.43	A2-B40	3.23	1.42	4.86	9
Utter Pradesh	382	84	10.99	A2-B7, A2-B5	0.34,	1.67		10
Caste (North India)								
Brahmins Maharastra	55	14	12.72					11
Kunbi	26	13	25					11
Mahars	32	13	20.31					11
CKP	50	15	15					11
Patels	112	35	15.62	A2-B5	6.37	3.72	6.16	12
Prajapathis	50	15	15	A2-B73	6.62	4.52	4.39	12
Parsis	67	6	4.47					12
Marathas	289	89	15.39	A2-B12	1.10	-1.54	5.43	24
Jains	161	36	11.18					35
Caste (South India)								
Lyers	74	10	6.75					34
Nadars	101	18	8.91	A2-B40	2.70	0.10		23
Kallars	36	6	8.33					23
Naidus	57	23	20.17	A2-B40	2.50	0.10		23
Total	2908	787	13.53					

AF(%) = allele frequency in percentage; HF= haplotype frequency;
LD=linkage disequilibrium; CKP= Chandinya Kayastha Prabu

Table 2. HLA A*02 subtype distribution among studied caste groups compared with other Indians

HLA	Maratha caste		Nadar caste		North India *		North India**	
	Pos	AF(%)	Pos	AF(%)	Pos	AF(%)	Pos	AF(%)
A*02011	4	5.26	13	50.00	3	1.92	6	5.77
A*0203	7	9.21	0	0.00	2	1.28	0	0.00
A*0205	1	1.31	0	0.00	12	7.69	1	0.96
A*0206	0	0.00	0	0.00	6	3.84	3	2.88
A*0207	0	0.00	0	0.00	25	16.02	0	0.00
A*0208	0	0.00	0	0.00	2	1.28	0	0.00
A*0209	0	0.00	0	0.00	2	1.28	1	0.96
A*0211	21	27.63	0	0.00	26	16.66	7	6.73
A*0222	1	1.31	0	0.00	0	0.00	0	0.00
A*0236	4	5.26	0	0.00	0	0.00	0	0.00

* data from ref. 6
** data from ref. 13

contained 84 immobilised SSOs.

Genomic DNA was amplified using HLA A or HLA B locus-specific biotinylated primers and hybridised with the SSO strips. Alkaline phosphatase-conjugated streptavidin was responsible for the positive colour development, using bromochloroindolyl phosphate/nitro blue tetrazolium

(BICP/NBT) in dimethylformamide (DMF) as substrate.

Alleles were determined using the pattern interpretation software supplied with the kit.

Results

Of the 56 A*02 alleles known, 10 subtypes (A*02011, A*0203, A*0205, A*0206, A*0207, A*0208, A*0209, A*0211, A*0222 and A*0236) were identified in the Indian populations studied (Table 2). A high frequency of A*0211 was found among members of the Maratha caste, which corroborates earlier observations among north Indians (Aryans), while the high frequency of A*02011 in the south Indian Nadar caste (Dravidian) was observed for the first time.

HLA B*4006 was the most frequent allele among the B*40 subtypes, and the one most frequently associated with A*0211 and A*02011 in members of the Maratha and Nadar castes, respectively. A*02-B*13, A*02-B*35, A*02-B*07, A*02011-B*5701 and A*02011-B*5603 haplotypes were observed in both caste groups studied (Table 3).

Discussion

Distribution of the A*02 allele in the Indian population reveals a high level of gene diversity, as the gene pool in the subcontinent can best be described as 'a melting pot', having experienced foreign invasion from both the east and west. The data presented in this study shows a clear picture of the inherent diversity in the A*02 family and its associated B* loci allele haplotypes among western Indians.

HLA diversity within population groups is due to the founder effect, selection or random genetic drift, intergeneic recombination and/or population admixture.[26] Furthermore, HLA A19 diversity and a high frequency of A*3303 among western Indians has been reported recently.[22] Analysis of A*02 subtype frequencies among north Indians reveals the predominance of A*0211,[6] despite its low frequency in many other populations worldwide.[14,18-21] A*0206 and A*0203 are common Asian alleles also found among members of the Maratha caste, while the less-frequent A*0205 allele occurs in Caucasians and Negroes.

The A*0222 allele identified in the present study has also been reported in Hispanic and American Indians, and only differs from A*02011 by one nucleotide at position 196 (G-T) in exon 3. Interestingly, the same nucleotide substitution is present in the A*68 allele, and it has been shown that differences in this residue may elicit allele recognition by cytotoxic T-lymphocytes.[27] A*0236 observed among members of the Maratha caste has been reported only infrequently.[15]

HLA A*0211 differs from A*02011 by just two residues, at 290 (C-T) and 292 (C-G), leading to amino acid changes in the α1 domain of the corresponding gene products.[28] HLA A*02 subtypes are functionally distinct, both in terms of peptide binding and in functional presentation of the peptide epitope to cytotoxic T-lymphocytes.[29] Furthermore, the clustering of certain A*02 alleles in different ethnic populations supports the view that these alleles have mutated and been selected in response to pathogens prevalent locally. Thus, A*02 diversification driven by selection could have favoured the high frequencies of A*0211 and A*02011 among members of the Maratha and Nadar castes, respectively.

Table 3. HLA A*02 alleles showing associations with HLA B* alleles in the caste groups

Halpotype	HF(%) Possible origin
Maratha caste	
A*02-B*40	14.47 Oriental [@]
A*0211-B*4006	9.21
A*0203-B*4006	2.63
A*0222-B*4006	1.31
A*0205-B*4006	1.31
A*02-B*07	13.15
A*0203-B*0705	3.94
A*0236-B*0702	2.63
A*02011-B*0702	2.63
A*02-B*44	2.63 Mediterranean [#]
A*0211-B*4403	1.31
A*0211-B*4406	1.31
A*02-B*35	7.89
A*0211-B*3503	5.26
A*0203-B*3503	1.31
A*0211-B*3520	1.31
A*0236-B*1302	2.63
A*02011-B*5701	2.63
A*0211-B*5103	2.63 Mediterranean [$]
A*0211-B*5801	2.63 Oriental *
Nadar caste	
A*02011-B*4006	42.3 Oriental [@]
A*02011-B*5603	7.92

[@] also Japanese (HF=1.2), Koreans (HF=1.2) North Indian (HF=6.1) and Golla Karnam subcaste (HF=5.9)

[#] also Koreans (HF=3.8),Thais (HF=3.1),Vietnamese (HF=1.8) and Golla Puja subcaste (HF=2.3)

[$] only present in Spaniards (HF=1.0), USA (HF=0.9) and Golla Karnam subcaste (HF=11.8)

[*] only present in Li (HF=3.5) and Golla Doddi subcaste (HF=5.0)

Despite the well-known ethnic differences in A*02 allele distribution, haplotype associations of A*02 alleles with other HLA loci have been studied only rarely in different populations, with a few described in Singapore Chinese,[30] Japanese[31] and Koreans.[32] In the present study, five of the eight most common A*02-B* allele haplotypes were variably associated with two or more A*02 subtypes (Table 3) – for example, A*02-B*40 with A*0211, A*0203, A*0205 and A*0222. However, a few haplotypes showed exclusive associations with a single A*02 subtype in members of the Maratha and Nadar castes.

Many of the haplotypes observed in the present study are found in Mediterranean, Asian Indian and Oriental populations, while some are unique to members of the Maratha and Nadar castes.[33] The observed haplotypes demonstrate A*02 allele variability among Indians, and

further studies are indicated in other Indian caste and tribal groups in order to understand this A*02 allele diversity and its clinical implications.

References

1 Holmes N, Ennis P, Wan AM *et al*. Multiple genetic mechanisms have contributed to the generation of HLA A2/28 family of class I MHC molecules. *J Immunol* 1987; **139**: 936-41.

2 Combarros O, Escribano J, Sanchez-Velasco P *et al*. Association of HLA A2 allele with an earlier age of onset of Alzheimer's disease. *Acta Neurol Scand* 1998; **98**: 140 –1.

3 Muto M, date Y, Ichimiya M, Moriwaki Y *et al*. Significance of antibodies to streptococcal M protein in psoriatic arthritis and their association with HLA A*0207. *Tissue Antigens* 1996; **48**: 645-50.

4 Buc M, Busova B, Hegyi E, Kolibasova K. Vitiligo is associated with HLA A2 and Dw7 in the Slovak population. *Folia Biol (Prala)* 1996; **42**: 23-5.

5 Rajalingam R, Mehra NK, Mehra RD *et al*. HLA class I profile in Asian Indian patients with pulmonary tuberculosis. *Indian J Exp Biol* 1997; **35**: 1055-9.

6 Mehra NK, Jain R, Rajalingam R *et al*. Molecular diversity of HLA A*02 in Asian Indians: predominance of A*0211. *Tissue Antigens* 2001; **57**: 502-7.

7 Pitchappan RM, Kakkanniah VN, Rajasekar R *et al*. HLA antigens in South India: I. Major groups of Tamil Nadu. *Tissue Antigens* 1984; **24**: 190-6.

8 Shankarkumar U, Pednakar SV, Gupte S *et al*. HLA antigen distribution in Marathi-speaking Hindu population from Mumbai, Maharastra, India. *J Hum Ecol* 1999; **10**: 367-72.

9 Chayya SU, Panthaki M, Shankarkumar U. HLA antigen distribution in Gujarathi- speaking Hindu population from Mumbai, Maharastra, India. *Anthropologist* 2000; **2**: 7-12.

10 Agrawal S, Singh AK, Sharma RK. HLA gene and haplotype frequency in renal transplant recipients and donors of Uttar Pradesh (North India). *Indian J Nephrol* 2002; July/Sup: 88-97.

11 Shankarkumar U, Ghosh K, Mohanty D. HLA Class I antigen profile among Brahmins and related caste groups from Mumbai, Maharastra, India. *Indian J Hum Genet* 2000; **6**: 21-8.

12 Shankarkumar U, Ghosh K, Colah RB *et al*. HLA antigen distribution in selected caste groups from Mumbai, Maharastra, India. *J Hum Ecol* 2002; **13**: 209-15.

13 Rajalingam R, Krausa P, Shilling HG *et al*. Distinctive KIR and HLA diversity in a panel of North Indian Hindus. *Immunogenetics* 2002; **53**: 1009-19.

14 Browning M, Krausa P. Genetic diversity of HLA A2: evolutionary and functional significance. Immuol *Today* 1996; **17**: 165-70.

15 Ellis JM, Henson V, Slack R *et al*. Frequencies of HLA A2 alleles in five US population groups. Predominance of A*02011 and identification of HLA A*0231. *Hum Immunol* 2000; **61**: 334-40.

16 Gao X, Jakobsen IB, Serjeantson SW. Characterization of HLA A polymorphism by locus- specific polymerase chain reaction amplification and oligonucleotide hybridization *Hum Immunol* 1994; **41**: 267-79.

17 Hammond MG, Toil ED du, Sanchez Mazas A *et al*. HLA in sub-Saharan Africa: 12th International Histocompatibility Workshop SSAF report. In: Charran D, ed. *HLA genetic diversity of HLA functional and medical implications*. Sevres: EDK Publishers, 1997: 345-53.

18 Ishikawa Y, Tanaka H, Semana G *et al*. AHS#2: HLA A2, A28, A9, A203, A210. In: Charran D, ed. *HLA genetic diversity of HLA functional and medical implications*. Sevres: EDK Publishers, 1997: 35-41.

19 Belich MP, Madrigal TA, Hildebrand WH *et al*. Unusual HLA B allele in two tribes of Brazilian Indians. *Nature* 1992; 357: 326-9.

20 Fan L, Chen D, Guo S *et al*. 12th International Histocompatibiltiy Workshop anthropology regional report: Asia-China. In: Charran D, ed. HLA genetic diversity of *HLA functional and medical implications*. Sevres: EDK Publishers, 1997: 292-4.

21 Watkins DI, McAdams SN, Liu X *et al*. New recombinant HLA B alleles in a tribe of South American Amerindians indicate rapid evolution of MHC class I loci. *Nature* 1992; **357**: 329-33.

22 Shankarkumar U, Ghosh K, Mohanty D. Defining the allelic variants of HLA A19 in the Western Indian population *Hum Immunol* 2002; **63**: 779-82.

23 Rajasekar R, Kakkanniah VN, Pitchappan RM. HLA antigens in South India: II. Selected caste groups of Tamil Nadu. *Tissue Antigens* 1987; **30**: 113-8.

24 Shankarkumar U, Ghosh K, Mohanty D. HLA antigen distribution in Maratha community from Mumbai, Maharastra India. *Int J Hum Genet* 2001; **1**: 173-7.

25 Miller SA, Dikes DED, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.

26 Awdeh ZL, Raum D, Yunis EJ, Alper CA. Extended HLA/complement allele haplotypes: evidence for T/t like complex in man. *Proc Natl Acad Sci USA* 1983; **80**: 259-63.

27 Marcos CY, Frenandez-Vina MA, Lazaro AM *et al*. Novel HLA A and HLA B alleles in South American Indians. *Tissue Antigens* 1999; **53**: 476-85.

28 Mason PM, Parham P. HLA class I region sequences 1998. *Tissue Antigens* 1998; **51**: 417-66.

29 Fleischhaur K, Tanzarella S, Russo V *et al*. Functional heterogeneity of A*02 subtypes revealed by presentation of MAGE-3-encoded peptide to cytotoxic T-cell clones. *J Immunol* 1997; **159**: 2513-21.

30 Krausa P, Brywka H M, Savage D *et al*. Genetic polymorphism within HLA A*02: significant allelic variation revealed in different populations. *Tissue Antigens* 1995; **45**: 223-31.

31 Tokunaga K, Ishikawa Y, Ogawa A *et al*. Sequence-based association analysis of HLA class I and class II alleles in Japanese supports conservation of common haplotypes. *Immunogenetics* 1997; **46**: 199-205.

32 Park MH, Whang DH, Kong SJ, Han KS. HLA A*02 allele frequencies and haplotypic associations in Koreans. *Tissue Antigens* 2000; **55**: 250-6.

33 Crawford MH, Mohan Reddy B *et al*. Genetic variation among the Golla Pastoral caste subdivision of Andhra Pradesh according to the HLA system. *Hum Immunol* 2001; **62**: 1031-41.