

Study of Genetic Relationships of Indian Gurkha Population on the Basis of HLA - A and B Loci Antigens

Monojit Debnath and Tapas K. Chaudhuri*

*Cellular Immunology Laboratory, Department of Zoology, University of North Bengal
Siliguri 734 430, West Bengal, India*

KEYWORDS Population genetics; HLA antigens; genetic relationship; Gurkha population

ABSTRACT Indian population is well known for its genetic diversity. Among the numerous endogamous ethnic groups of North Bengal, we have collected blood samples from 50 unrelated Nepali speaking individuals, belonging to Gurkha community. In the present investigation, the frequency of HLA-A and B loci antigens has been analyzed serologically and compared with few major world populations. The frequency of A2, A11, A33, B18 and B51 showed increased frequency and several haplotypes including A3-B35, A33-B44, A68-B35 have significant linkage disequilibrium values. The standard genetic distances of the Gurkha population were calculated on the basis of gene frequency data of HLA antigens and when compared with other populations, it has revealed that Gurkha population is genetically closer to the population of Mongoloid origin. It can be considered that the Gurkha population may have been originated from Mongolians and/or Tibetan stocks.

INTRODUCTION

Paleoanthropologists Nicholas Rolland hypothesized that early humans first ventured out of Africa via Middle East close to 2 million years ago, but were prevented by climate and geography from turning west. Instead they dispersed east, taking a southern route to China and even Java. They then turned north and started west again across more central reaches of Asia and Europe (Balter and Gibbons 2000). However, evidences from Y chromosome biallelic polymorphism (Wells et al. 2001) and mtDNA haplotype have clearly demonstrated that the ancestor modern humans must have come from three major waves of human migrations leading into Europe, the Americas and India from Africa (Majumder 2001).

India is a land of enormous cultural and linguistic diversity, which has led to genetic stratification of various population groups. The population of India has been subjected to successive waves of immigration and invasion from the Middle East, Central Asia and Mongolia, contributing to the present day gene pool (Bhasin et al. 1994). Studies on genetic diversity among contemporary human populations are useful in reconstructing population diversity and

origin of the population from a particular geographical region. Recent study on the molecular diversity of HLA-A*02 among Asian Indians support the idea that populations in the Indian subcontinent represent 'a melting pot of races' (Mehra et al. 2001).

The Sub-Himalayan part of India represents a hotspot of social, cultural and ethnic heterogeneity with a number of unique caste and primitive tribal populations. Amongst them, Gurkhas form the major population group in Darjeeling district of West Bengal and Sikkim. These groups are endogamous (Chatterjee 1974). Gurkhas are grouped into a large number of tribes, the chief of which are Mangar, Rai, Limbu, Sunar and Tamang (Coon 1983; Subba 1985). It appears from the evidence that in ancient times people of the Tibeto-Burman family spread themselves over the high plateau of the Himalayan and the country round about the mouth of the Ganges (Northey and Morris 1987). Gurkha population is supposed to belong the above-mentioned race. The ancestors of Gurkha population are also thought to be migrants from the neighboring Mongolian region.

The HLA complex is the most diverse and polymorphic genetic system with major functional and medical implication (Charron 1997) and acts as an excellent marker for population genetic analyses, paternity determination and disease association studies. Analysis of the HLA system has also been extensively applied in anthro-

*Address all correspondence to: Dr. T.K. Chaudhuri
Phone: +91-353-2582124(O)
Fax: +91-353-2581546
E-mail: dr_tkc_nbu@rediffmail.com

pological investigations of genetic relatedness and migrations of different world populations (Charron 2000). There exist an exhaustive data regarding distribution of HLA antigens in different world populations including Indian subcontinent (Imanishi et al. 1992; Pitchappan et al. 1984; Mehra et al. 1986a, b; Shankarkumar et al. 2002, 2003; Thomas et al. 2004). In the present investigation, the frequency of HLA –Class I antigens was studied in the Nepali speaking Indian Gurkha population to understand its genetic and evolutionary relationships.

MATERIALS AND METHODS

A total number of 50 healthy unrelated individuals belonging to Nepali speaking Indian Gurkha population residing in Siliguri subdivision of Darjeeling district were considered for the present investigation. About 10ml. of blood samples were collected in the sterile tube containing heparin (50IU/ml) from each individuals. All the individuals were studied for HLA-Class I antigen profiles by NIH-two stage microlymphocytotoxicity assay (Terasaki and McClelland 1964). A total number of 72 antiserum were used for defining 15 specificities for HLA-A locus and 23 specificities for HLA-B locus. The antisera were obtained from One Lambda, USA and BAG, Germany. The phenotype

frequencies (PF) were calculated by direct count and gene frequencies (GF) were calculated from them. Genetic distances of the populations were calculated by standard genetic distance measures of Nei (1972) and phylogenetic tree was constructed by the Neighbor Joining (Saitou et al. 1987) method.

RESULTS

The results of HLA-Class I gene frequencies of Gurkha population have been represented in Table 1. The gene frequencies of several antigens like A2, A11, A33, B18, B44 and B51 were higher in Gurkhas. Table 2 represents the observed two-locus haplotype frequencies and positive linkage disequilibrium values among Gurkhas. Though several haplotypes showed positive linkage disequilibrium values, haplotype A3-B35 and A33-B44 showed very significant values. The frequency of different antigens and pattern of haplotype distribution of Gurkha was compared with various Indian castes and tribal populations as well as with other major world populations. The frequency distribution of HLA antigens in different populations was used to construct phylogenetic tree based on genetic distances. The genetic distance values of Gurkha populations has been calculated and compared with the genetic distances of nine other major

Table 1: Gene frequencies of HLA-A & -B antigens of Gurkha population (N=50).

<i>Antigens</i>	<i>Gene frequency</i>	<i>GF%</i>	<i>Antigens</i>	<i>Gene frequency</i>	<i>GF%</i>
A1	0.01	1.00	B5	0.0304	3.04
A2	0.14	14.00	B7	0.041	4.1
A3	0.062	6.2	B8	0.0304	3.04
A11	0.1633	16.33	B13	0.0202	2.02
A23	0.01	1.00	B17	0.01	1.00
A24	0.02	2.00	B18	0.073	7.3
A25	0.0202	2.02	B22	0.000	0
A26	0.04	4.00	B27	0.041	4.1
A28	0.0513	5.13	B35	0.0513	5.13
A29	0.000	0	B37	0.041	4.1
A30	0.01	1.00	B39	0.0304	3.04
A31	0.0513	5.13	B44	0.073	7.3
A32	0.01	1.00	B49	0.01	1.0
A33	0.0835	8.35	B50	0.01	1.0
A36	0.0202	2.02	B51	0.0945	9.45
			B52	0.041	4.1
			B54	0.01	1.0
			B55	0.02	2.0
			B56	0.01	1.0
			B57	0.01	1.0
			B58	0.01	1.0
			B60	0.0305	3.05
			B61	0.0305	3.05
			B62	0.062	6.2
			B76	0.01	1.0

Table 2: Linkage disequilibria ($\Delta X 10^3$) and haplotype frequencies (HF/1000) in HLA-A and-B haplotypes in Indian Gurkhas

Haplotype	HF/1000	$\Delta X 10^3$	X^2
A3 – B35	406	374	24.327
A26 – B8	199	187	14.924
A68 – B35	202	191	18.75
A31 – B27	197	180	10.421
A33 – B44	304	289	28.723
A33 – B60	198	183	11.387

world populations. A neighbour-joining tree based on the genetic distances derived from HLA-A and B loci antigen frequencies comparing with the major world populations is shown in figure1.

DISCUSSION

Indians can be divided into several groups based on physiographical region (north, south, east and west). Anthropological and historical evidence classifies Indians broadly as Dravidians and Aryans. The Dravidians are considered as the earliest settlers who were driven southwards following invasion by Aryans from the Northwest during 2000-3000BC. Between the 12th and 16th centuries AD waves of Arabics and Moghuls invaded the Indian subcontinent followed by Mongols and Tibeto-Burmans (Bhasin et al. 1994).

Tribes of Mongoloid stocks are found along the whole length of the Himalayas with languages closely akin to those of the Tibetans on the Northern side, while further east, similar peoples have driven the older Austro-Asiatic

speaking populations out of most of Burma and Siam. The Aryan speakers from India entered Nepal probably both from West, along the hills, and from the plains on the South, although certainly much less in number than the Mongolian element (Northey and Morris 1987).

It has already been suggested that the languages of those tribes belong to at least three distinct families-a) Munda, a division of an Austro-Asiatic b) Tibeto-Burman and c) Indo-Aryan. It may be noted that the identity of languages does not necessarily connote identity of the races and in Nepal itself the aborigines, of whatsoever race, largely changed their language for that of Mongoloid invaders, while today the same process as the language of the latter is gradually replaced by the Aryans.

In the present investigation, HLA-A and B loci antigen frequencies were studied to resolve the evolutionary history of Indian Gurkhas. When the frequency of these antigens were compared with other Indian tribe and caste populations, it has been observed that the frequency of A2 and A11 is uniformly high in all the populations like Sikh (Babita and Usha 2004), Gujarathi, Maharastrian (Kankonkar et al. 2004), different caste group of Western India (Shankarkumar 2004) and North India (Mehra et al. 1997). However, when the frequency profile of Gurkhas were compared with other major world populations it was found that HLA-A2 and A11 were also higher in Greeks (Pachoula-Papasteriadiis et al. 1989), Ukrainian, Tibetan (Imanishi et al. 1992), Chinese, Korean, Mongolian and Japanese (Tanaka et al. 1997).

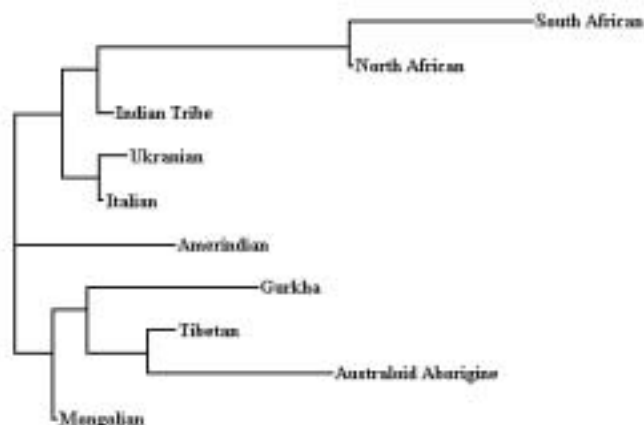


Fig. 1. Neighbor joining tree showing the relationship between Indian Gurkha population and various world populations.

Several HLA-A and B haplotypes were significant in Gurkhas. Amongst them only A33-B44 which is characteristic haplotype of Korean and Japanese populations (Tanaka et al. 1997) occur with a significant positive linkage disequilibrium among Gurkhas. The distribution of these haplotypes supports a strong genetic affinity of Gurkhas with Koreans and Japanese populations.

It has been reported earlier that Gurkhas may have originated on the course of evolution from the Mongolian population directly or, from the stock of Mongoloid origin. It can be assumed from the phylogenetic tree constructed by NJ method that Gurkha has originated from Mongoloid stock directly or Tibetan stock or there may be one or more intermediate populations between Gurkha and Monogolians/ Tibetans from which Gurkhas might have originated.

REFERENCES

- Babita K, Usha D 2004. HLA antigen distribution in Sikhs from Punjab, India. *International Journal of Human Genetics*, **4(2)**: 111-113.
- Balter M, Gibbons A 2000. A glimpse of Human's first journey out of Africa. *Science*, **288**: 948-950.
- Bhasin MK, Walter H, Danker-Hopfe H 1994. *People of India: An Investigation of Biological Variability in Ecological, Ethno-Economic and Linguistic Groups*. Delhi: Kamla Raj Enterprises, pp.1-21.
- Charron D 1997. Genetic diversity of HLA. Functional and Medical implications. *Proceedings of 12th International Histocompatibility Workshop and Conference*. France: EDK.
- Charron D 2000. HLA 2000. *Proceedings of 26th Congress of International Society for Blood Transfusion*. Vox Sang, **78(supple2)**: 27-28.
- Chatterjee SK 1974. *Kirata-Jana-Krti, The Indo-Mongoloids: Their Contribution to the History and Culture of India*. 2nd Edition, Calcutta: Asiatic Society.
- Coon CS 1983. Human Populations. In: *The New Encyclopaedia Britannica*. vol. 14. USA: Encyclopaedia Britannica, Inc., pp. 839.
- Imanishi T, Akaza A, Kimura A, Tokunaga K, Gojabori T 1992. Allele and haplotype frequencies of HLA and complement loci in various ethnic groups. In: K Tsuji, M Aizawa, T Sasazuki (Eds.): *HLA 1991. Proceedings of the 11th International Histocompatibility Workshop and Conference*. Oxford: Oxford University Press, Vol. 1, pp. 1064-74.
- Kankonkar S, Sangita R, Sonal T 2004. HLA antigen distribution in selected population groups from Maharashtra. *International Journal of Human Genetics*, **4(2)**: 115-118.
- Majumder PP 2001. Ethnic population of India as seen from evolutionary perspective. *J Biosci*, **26**: 533-45.
- Mehra NK, Taneja V, Kailash S, Chaudhuri TK, Vaidya MC, Balakrishnan K, Contractor N, Hammond MG, Undevia JN, Khan R 1986a. North Indian: Ethnic study. In: M Aizawa (Ed.): *HLA in Asia-Oceania*. Japan: Hokkaido University Press, pp. 271-276.
- Mehra NK, Taneja V, Kailash S, Raizada N, Vaidya MC 1986b. Distribution of HLA antigens in a sample of North Indian Hindu population. *Tissue Antigens*, **27**: 64-74.
- Mehra NK, Rajalingam R, Kanga U et al. 1997. Genetic diversity in the populations of India, Sri Lanka and Iran. *Proceedings of 12th International Histocompatibility Workshop and Conference*. France: EDK, pp.314-320.
- Mehra NK, Jaini R, Rajalingam R et al. 2001. Molecular diversity of HLA-A*02 in Asian Indians: Predominance of A*0211. *Tissue Antigens*, **57**: 502-507.
- Nei M 1972. Genetic distance between populations. *American Nature*, **106**: 283-292.
- Northey WB, Morris CJ 1987. *The Gurkhas*. New Delhi: Cosmo Publication, pp.63-237.
- Pachoula-Papasteriadis C, Ollier W, Cutbush S, Economidou J, Festenstein H. 1989. HLA antigens and haplotype frequencies in Greeks. *Tissue Antigens*, **33**: 488-490.
- Pitchappan RM, Kakkaniiah VN, Rajasekar R, Arulraj N, Muthukaruppan VR 1984. HLA antigens in South India: I Major groups of Tamil Nadu. *Tissue Antigens*, **24**: 190-196.
- Saitou N, Nei M 1987. The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**: 406-425.
- Shankarkumar U, Ghosh K, Colah RB, Gorakshakar AC, Gupte SC, Mohanty D 2002. HLA antigen distribution in selected caste groups from Mumbai, Maharashtra, India. *J Hum Ecol*, **13**: 209-215.
- Shankarkumar U, Sridharan B, Pitchappan RM. 2003. HLA diversity among Nadars, a primitive Dravidian caste of South India. *Tissue Antigens*, **62**: 542-547.
- Shankarkumar U 2004. Diversity among selected caste and tribal groups of Western India: origin, HLA and disease associations. *International Journal of Human Genetics*, **4(2)**: 105-110.
- Subba TB 1985. Caste and adaptation: the case of the Nepalis in Darjeeling. In: SK Chaube (Ed.): *The Himalayas: Profiles of Modernization and Adaptation*. New Delhi: Sterling Publishers Pvt. Ltd., pp.72-90.
- Tanaka H, Tokunaga K, Inoko H, Tsuji K et al. 1997. Distribution of HLA-A, B and DRB1 alleles and haplotypes in Northeast Asia. *Proceedings of 12th International Histocompatibility Workshop and Conference*. France: EDK, pp.285-291.
- Terasaki PI, McClelland JD 1964. Microdroplet assay of human serum cytotoxins. *Nature*, **204**: 998-1000.
- Thomas R, Nair SB, Banerjee M. 2004. HLA-B and HLA-C alleles and haplotypes in the Dravidian tribal populations of Southern India. *Tissue Antigens*, **64**: 58-65.
- Wells RS, Yuldasheva N et al. 2001. The Eurasian Heartland; A continental perspective on Y-chromosome diversity. *Proc Natl Acad Sci USA*, **98**:10244-9.