

Genetic variation of ApoB 3' hyper variable region polymorphism among Brahmins of North India

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ApoB 3' hyper variable region (ApoB 3'HVR) is highly polymorphic and hence an informative marker. It could be an ideal candidate to study the genetic heterogeneity among different population groups of the Indian sub-continent. It is one of the markers for which population data are available. This makes the ApoB 3'HVR an ideal locus for a pilot study to investigate the relationships between different populations and the micro-evolutionary processes leading to their present-day distribution. In the present investigation, we have studied ApoB 3'HVR in three endogamous groups of North India and have compared these populations on the basis of inter- and intra-group diversity. The sub-populations chosen were Bhargavas, Chaturvedis, and non-Bhargava non-Chaturvedi Brahmins of Uttar Pradesh. Nineteen segregating alleles were detected in our population groups. The average observed heterozygosity was quite high (0.717), suggesting high diversity at the ApoB 3'HVR locus. Low value of average G_{ST} (0.0126) and F_{ST} (0.002) reflects non-significant deviation of heterozygosity between the three subgroups. On comparing the three study groups with ApoB 3'HVR of other Indian and world populations, it was clear that greater diversity was observed for Africans followed by Europeans and Asians. There was relative homogeneity among the continental groups. In our study it was observed that there was high heterozygosity, an extended range of allele size, a quasi unimodal allele size distribution, centred on HVE 37. These findings indicate that our populations may be characterized as ancestral, since similar features are observed in the African population. ApoB 3'HVR polymorphism suggests that despite practising restricted marital patterns, these groups or castes do not significantly differ from each other at the genetic level. This may be because of the fact that divergence time may not be enough to cause genetic variation in these groups. However, it may not be ruled out that the ApoB 3'HVR polymorphism probably predates the divergence of these sub-castes. We are further testing this observation, using mt-DNA for maternal lineages and Y-chromosome markers for paternal lineages.

MINISATELLITES – the tandem repeats of sequences ranging from 10 to 70 base pairs, are amongst the most polymorphic markers reported till date¹. They exhibit substantial

allelic variability in the number of repeat units as a consequence of high rate of germline mutations leading to new allelic states².

Among numerous minisatellites known so far, the one located about 75 bp downstream from the 3' end of the apolipoprotein-coding gene is a hyper variable region, designated as ApoB 3'HVR³. This locus is highly polymorphic and until now about 23 alleles have been reported. ApoB 3'HVR consists of an AT-rich core repeat sequence of 15 bp. Two basic types of 15-bp repeats (X and Y) have been identified^{4,5}. Presence of high allelic variability at ApoB 3'HVR is due to the complex mutational pattern. Earlier studies have reported that stepwise mutational model (SMM), which reflects gain or loss of one or few repeat units probably due to replication slippage, is responsible for creating high polymorphism at ApoB 3'HVR².

All these features make ApoB 3'HVR a useful marker for population studies at the genetic level. The availability of numerous population data makes the ApoB 3'HVR an ideal locus for a pilot study to find out the relationships between different populations on the basis of allele frequency distribution and also the micro-evolutionary processes leading to their present-day distribution.

As ApoB 3'HVR is a highly polymorphic and informative marker, it is ideal to study the genetic heterogeneity among different groups in India. Our previous studies⁶⁻⁹ indicated that the North Indians of Uttar Pradesh (UP) occupy an intermediary zone which has Caucasoid, Negroid, Australoid and Mongoloid elements. Another important feature of Indian populations is caste system, which is the basic element of the Indian social structure¹⁰.

The major caste groups in India are Kshatriyas, Brahmins and Vaisyas; they are subdivided into several subgroups (identified by 'gotras' and surnames) and marriage within the same gotra is generally not preferred/permitted. In North India, a further degree of endogamy is seen in some sects/groups where marriages are restricted within the same surname. Two such groups are Bhargavas and Chaturvedis. They belong to the broad caste group of Brahmins, but they do not marry outside their own surnames. Thus, Bhargavas marry within Bhargavas and Chaturvedis within Chaturvedis, and not with other Brahmins.

In the present investigation, we have studied ApoB 3'HVR in three endogamous groups of North India and have compared these populations on the basis of within group diversity (in terms of heterozygosity, number of alleles, and allele size distribution), between group diversity (in terms of total genomic diversity and coefficient of gene differentiation) and Wright's F -statistics (in terms of fixation index). The sub-populations chosen for the study were Bhargavas, Chaturvedis and non-Bhargava non-Chaturvedi Brahmins of UP, where the former two are the subsets of Brahmins. They follow endogamy within same subcaste and have a well-defined pattern of exogamy within the same gotra. Thus, there is no consanguinity in these subpopulations in spite of surname endogamy.

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The study is further enlarged by comparing these subgroups with five Indian populations from Central India and 29 world populations on the basis of allele numbers, heterozygosities and finally by computing genetic distances between these populations. The purpose of the study was to obtain information regarding ApoB 3'HVR in North Indian populations and to place these groups in the context of other world populations and also to find out whether the allele frequency distribution of ApoB 3'HVR reveals information about genetic differentiation resulting due to restricted marital patterns among these subgroups.

Two hundred unrelated individuals were randomly selected from each of the three sub-populations, viz. Bhargavas, Chaturvedis and (non-Bhargavas and non-Chaturvedis) Brahmins. Before sample collection, regional directories of these populations were obtained, detailed computerized lists were prepared, random numbers were generated with the help of a computer and samples were collected from Lucknow, Kanpur and Agra. Three-generation pedigree charts were prepared to assure (i) endogamy within surname for Bhargavas and Chaturvedis and (ii) un-relatedness in all the three groups. The ethical committee of the Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow approved the study and blood samples were taken after obtaining informed consent from the subjects.

Genomic DNA was prepared from 2 ml of EDTA blood by high salting-out technique¹¹. ApoB 3'HVR was amplified using primers described by Boerwinkle *et al.*¹². The amplified product was then electrophoresed on 5% PAGE and allele scoring was done with the help of ApoB 3'HVR allelic ladder and commercial ladder (Invitrogen Ltd.), as shown in Figure 1.

Allele frequencies were calculated by a simple gene count method¹³. Calculations of heterozygosity, H_T , H_S , G_{ST} ,

F_{IS} and F_{ST} and genetic distances, phylogenetic relationship and principal components (PC)-plot analysis were done with the help of software, including POPGENE (v32), CERVUS (v1.6), ARLEQUIN (v1.1), PHYLIP (v22) and POPSTR (v3).

In the case of within group diversity, as a first step, we calculated and compared gene diversity in terms of allele number, allele size distribution and heterozygosity in the three study groups. Among the 23 alleles reported in the literature, only 19 segregating alleles were detected in our population groups. Eighteen alleles were present in Brahmins and Chaturvedis, while only 16 alleles were found in Bhargavas. Allele frequency distribution at ApoB 3'HVR in these populations is shown in Figure 2. All the populations under study were in Hardy-Weinberg equilibrium; we calculated this by comparing observed vs expected gene frequencies. The allele frequency distribution shows no significant difference among the three groups. Allele size distribution in all the three populations resembles a bell-shaped and unimodal curve. The mode is centred at 35 HVE. The other two alleles with relatively high frequencies are 37 HVE and 33 HVE.

The observed heterozygosities were quite high (average, 0.717), suggesting high diversity at ApoB 3'HVR locus. Brahmins reveal highest heterozygosity (0.745) and Bhargavas were least heterozygous (0.682); the heterozygosity at ApoB 3'HVR locus among Chaturvedis was 0.724. The observed heterozygosity further confirms that all the three populations are almost similar to one another at this locus.

In the case of between group diversity, the total genomic diversity (H_T) among populations was found to be high (0.877). However, most of the genomic diversity was because of diversity between individuals within a subpopulation ($H_S = 0.866$). The percentage of genomic diversity attributable to between populations relative to the total genomic diversity (G_{ST}) was low (0.0126). Further, the

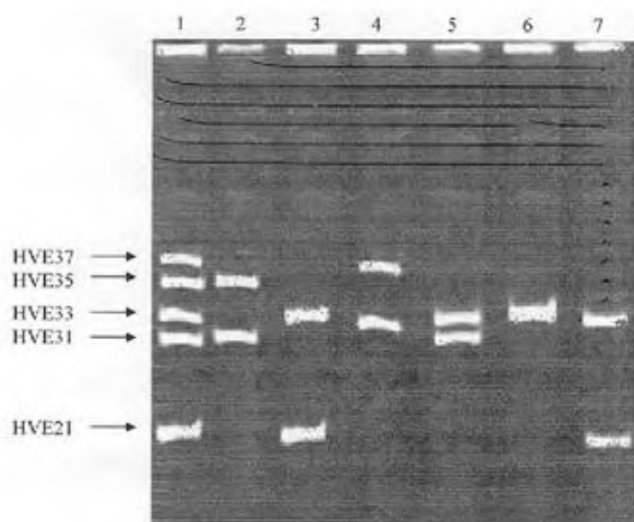


Figure 1. Different alleles at ApoB 3'HVR with sizing ladder. Lane 1, Allelic ladder for ApoB 3'HVR; lanes 2–7, Different samples showing various alleles of ApoB 3'HVR.

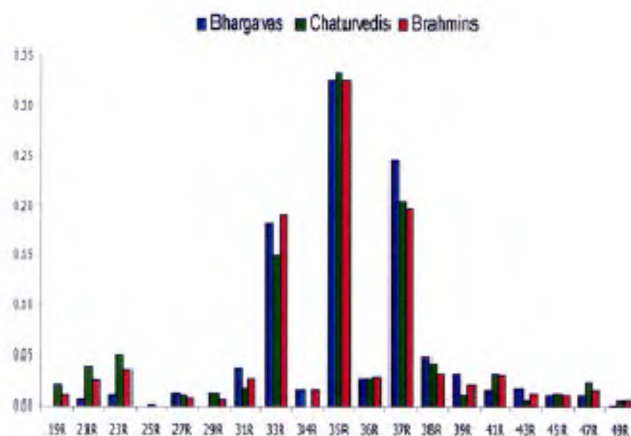


Figure 2. Allele frequency distribution of ApoB 3'HVR in three North Indian populations.

low value of the fixation index, F_{ST} (0.002) reflects non-significant deviation of heterozygosities between the three subgroups.

The second step of our analysis was to compare the three study groups with other Indian and world populations at the ApoB 3'HVR. Table 1 gives the source of different populations used in the analysis. We have compared our populations with other populations in terms of allele numbers, allele size distribution and heterozygosity (Table 2). We used a four-allele system as described by Alvantic *et al.*¹⁴, in which ApoB 3'HVR alleles are categorized into four classes: HVE < 35, HVE35, HVE37 and HVE > 37. This analysis clearly indicates greater diversity of Africans followed by Europeans and Asians. Our population reflects more diversity in terms of more allele numbers (average, 17.6) and high heterozygosity (0.717) compared to other Indian and Asian populations. However, when we compared our population with the Africans, we found that the African populations show high diversity in comparison to our population.

Further, inter-population relationships were analysed using Nei's pairwise distance, and Reynolds's F_{ST} -based distance methods. The obtained distance matrices were used to build UPGMA trees. African, European and Asian populations formed separate clusters. Our population clustered with other Indian populations; however, Indian tribal groups revealed slight deviations from other Indian popu-

lations, including our study groups. Our populations clustered near South Asian, Serbians and UAE populations.

Further, we have also computed PCs of the population gene differences and plotted the first two PCs. PC-plot analysis revealed no difference among the three study groups (Figure 3). It also shows clustering of different populations in different groups relative to their ethnicity and geographical locations.

The allele frequency distribution at ApoB 3'HVR in three North Indian populations suggests that ApoB 3'HVR may be an ideal locus to study the genetic relationships between different ethnic groups of India. The large number of segregating alleles and high value of heterozygosity further support the utility of ApoB 3'HVR in the context of Indian populations for carrying out population studies at the genetic level, linkage analysis and forensic purposes.

The two subpopulations selected for the present study, viz. Bhargavas and Chaturvedis follow strict surname endogamy. However, within the group exogamy is followed at gotra level among Chaturvedis and at Kuldevi level among Bhargavas. Our analysis at ApoB 3'HVR in these three subgroups reveals no variation in the allele frequencies in the three subpopulations. This is also reflected by Wright's F statistics. F_{ST} score over all loci, which was 0.013, implying that there is no significant deviation in the heterozygosities. Further, gene diversity analysis shows that genomic diversity among populations is high ($H_T = 0.877$); but most of it is contributed by the diversity between individuals within a subpopulation ($H_S = 0.866$), while the genomic diversity among the population contributed a negligible amount ($G_{ST} = 0.0126$). The phylogenetic tree constructed using distant methods and PC analysis also confirms the similarity between the three subgroups. The results of the present study also confirm earlier findings based on other genetic markers¹⁵ that although Indian populations follow a unique marital pattern, various caste groups are not significantly different from one another, which reflects that initially there was a common ancestral popu-

Table 1. Source of ApoB 3'HVR population data

Population group	Reference
Ewondo (Cameroon)	27
Tunisian	28
American Blacks	29
Germany	30
Caucasians (Germany)	22
Austria	31
Dogrib (Brazil)	22
Pehunche (Chile)	22
Greece	32
Central Italy	33
Southern Italy	32
Calabria (Italy)	32
Bologna (Italy)	34
New Guinea Highlanders	22
Serbia	14
Sweden	35
US Mexicans	36
US Whites	29
Ukraine	37
Catolina (Spain)	38
Nancy (France)	39
Caucasoid (France)	12
Han Chinese	26
Taiuaan	25
United Arab Emirates	23
Kacharies (India)	40
Khatris (India)	40
Brahmins (India)	40
Gonds (India)	40
Sotho (Africa)	41

Table 2. Comparison of study groups with world populations

Population	No. of alleles	Observed heterozygosity (%)
Ewondo (Cameroon)	17	89.2
Sotho (Africa)	15	88
Tunisian	16	80
American Blacks	21	89.7
Calabria (Italy)	11	78.4
Sweden	13	77.1
German	11	74.3
Catolina (Spain)	16	80.84
Han Chinese	13	57.52
United Arab Emirates	18	80.17
Pehunche (Chile)	8	71.7
Dogrib (Brazil)	5	65.3
New Guinea	4	67.7
North Indian Bhargavas	17	69.85
North Indian Brahmins	18	74.58
North Indian Chaturvedis	18	72.45

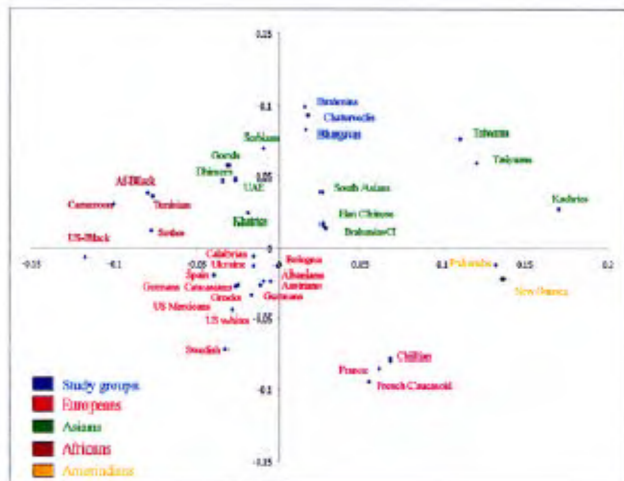


Figure 3. Comparison of study populations with other world populations based on frequency of ApoB 3'HVR alleles.

lation from which present-day populations got isolated, but time of divergence is too small to reflect the genetic differences between them. However, the three populations show significant variation from other Indian tribal populations. Dutta *et al.*¹⁶ have demonstrated that there is a genetic diversity among nine Indian populations of diverse ethnic, linguistic and geographical background. They have taken both upper caste groups and some of the tribal populations and have found significant differences between them.

Comparing our populations with other human populations at the ApoB 3'HVR, provides evidence for a higher genetic diversity in Africans than in non-Africans. Our results are consistent with those of numerous other genetic studies^{17,18}, which suggest that the founder non-African population arose as a subset of a large African population^{19–21}. In the case of ApoB 3'HVR, it is speculated that the ancestral population was characterized by high heterozygosity, an extended range of allele size, and a quasi unimodal allele size distribution, which is centred on 37HVE. The high level of gene diversity and allele size variance observed in four African populations of diverse geographic origin suggest that most of the present-day ApoB 3'HVR variation was present in the ancestral African population¹⁷. When the ancestral population underwent large expansion, it moved out of the continent and inhabited the rest of the world; it underwent random genetic drift with a consequent decrease of gene diversity and a reduction of allele size range in the founding populations of non-African groups. Further, sampling processes, perhaps bottlenecks that occurred in more recent times, could be responsible for the marked reduction in the number of alleles found in some of the Asians, Amerindians, and European populations^{17,22}.

This reconstruction suggests that the 37 HVE was predominant in the ancestral population and probably the

oldest²². This is suggested by its predominant frequency in almost all the populations examined. In our study population there was a high frequency of 35 HVE (average, 0.33) followed by 37 HVE (average, 0.22), which is in agreement with other Indian and Asian populations^{23–26}. It may be deduced from these results that in the Asian continent, especially in the south Asian regions, there is relative uniformity in the ApoB 3'HVR polymorphism with the presence or absence of certain alleles of low frequency. Moreover, the clustering of different populations in UPGMA tree and PC-plot further supports relative homogeneity between continental and/or ethnic groups.

All the inferences drawn are based on the present study of ApoB 3'HVR polymorphism suggest that although Indian social hierarchy pattern creates isolated groups based on restricted marital patterns, these groups or castes do not significantly differ from each other at the genetic level. This suggests that the ApoB 3'HVR polymorphism probably predates the divergence of Indian populations. Our study also supports higher genomic diversity in African than non-African groups as well as relative homogeneity among the Continental groups at the ApoB 3'HVR. However, consideration of other markers like mt-DNA and Y-chromosome markers and incorporation of more realistic demographic models could be of help in the exact dating of the different divergences that took place in the Indian subcontinent.

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