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Analysis of VNTR loci, ApoB 3′ HVR and D1S80 in North Indians

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Polymorphic markers like VNTRs at Apolipoprotein B 3′ and locus D1S80 hypervariable region have been used extensively for population studies through out the world. In the present study, the polymorphism data in North Indian population at these VNTR loci was reported. The allele distributions and their genotype frequencies at the VNTR loci, Apo B and D1S80 were reported in 86 (172 chromosomes) and 75 (150 chromosomes) unrelated normal individuals, respectively. Genomic DNA was extracted from blood samples and amplified by polymerase chain reaction. The respective alleles and their sizes were determined, 19 and 24 different alleles making up 51 and 50 genotypes of Apo B and D1S80 respectively were identified in the North Indian population. As per χ^2 analysis, the allele and genotype frequencies for both VNTRs were in Hardy-Weinberg equilibrium. The most frequent allele of Apo B (allele 6) corresponded to 40 repeats, and D1S80 (allele 12) to 28 repeats. The frequencies were 0.087 and 0.17 and observed heterozygosities were 55 and 57% for Apo B and D1S80, respectively. This information may have implications in disease diagnostics, forensics, paternity analysis, and for ruling out maternal contamination in fetal samples during prenatal diagnosis of genetic disorders.

Key words: Apo B, D1S80, hypervariable region, maternal contamination, prenatal diagnosis, variable number of tandem repeats

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Introduction

Each human somatic cell contains 6.4 billion base pairs of DNA. Portions of this DNA encode over 30,000 genes, while the remainders are non-coding. 99.9% percent of human DNA is identical among all individuals, while the remaining 0.1% can differ in several ways. The genome contains hundreds of highly polymorphic segments[1,2], each of which is characterized by allelic variation and attributed to the difference in the copy number of the tandemly repeated DNA sequences[2,3], such as 'Hyper Variable Regions' (HVRs) or "Variable Number of Tandem Repeats" (VNTRs). Although each VNTR locus is usually associated with a large number of different alleles (each allele corresponding to a specific number of repeated sequences) in any population, each individual carries two alleles, one on each of the two homologous chromosomes. VNTRs are the most informative markers for genetic characterization. They are stable across generations for they are inherited in a Mendelian fashion and do not vary in size upon passage from parents to offsprings. The hyper allelism, resulting from the existence of many different hyper variable regions at a single locus within population, has been exploited for linkage studies. One such hyper variable marker of particular interest is sequences situated at the 3' end of the Apolipoprotein B gene[4-6], which is located on the short arm of chromosome 2. The other VNTR marker is the D1S80 locus located at chromosome 1 [7], which has been studied to identify different alleles in the Caucasian population[8,9]. Our laboratory is involved in the DNA diagnosis of various genetic disorders and chorionic villi sample (CVS) is received routinely for prenatal diagnosis. First and foremost, it is important to rule out maternal contamination in the fetal sample. So, in the present study, we have attempted to investigate the allele and genotype frequencies of the above two VNTR markers in unrelated normals from North India in order to find out their utility in determining the maternal contamination in fetal samples prior to prenatal diagnosis and disease diagnostics.

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Abbreviations: CVS, chorionic villi sample; dNTP, deoxynucleoside triphosphate; EDTA, ethylene diamine tetra acetic acid; HVR, hypervariable region; PCR, polymerase chain reaction; VNTR, variable number of tandem repeats

Materials and Methods

Subjects and Blood Collection

Blood samples were collected from 86 and 75 unrelated normal individuals from the North Indian

population for Apo B 3′ and D1S80 VNTR markers respectively. Both males and females were equally represented and the age ranged from 25-45 years. Five milliliters of peripheral blood was collected in EDTA, kept frozen at –20°C and DNA was extracted by using the standard phenol-chloroform method[10].

Polymerase Chain Reaction

All the DNA samples were subjected to PCR[10,11] with slight modifications in a thermocycler (M J Research PTC-200 DNA engine). 100-200 ng of DNA (5 µL) was used for amplification in a final reaction volume of 50 µL containing DNA, PCR buffer (Genetix), 200 µ*M* dNTPs, 25 pmols of each primer and 1.5 U Taq polymerase (MBI Fermentas, Lithuiana). The sequences of forward and reverse Apo B primers (Invitrogen, Gibco BRL) are 5'ATGGAAACGGAGAAATTATG 3' and 5'CCTTCTCACTTGGCAAATAC 3'. The sequences corresponding to the PCR primers CRX51 and CRX66 amplifying the D1S80 locus[8] are 5' CTGGCCTCCAAACACTGCCCGCCG 3' and 5'GTTGGAGATGCACGTGCCCCTTGC 3'. The cycle conditions were as follows: For Apo B, 30 cycles of denaturation at 94°C for 1 min; annealing at 58°C for 1 min; extension at 66°C for 2 min; and for D1S80, 30 cycles of denaturation at 94°C for 1 min; annealing at 65°C for 1 min; extension at 72°C for 1 min. The initial denaturation and final extension was carried out at 94°C for 5 min and 72°C for 10 min for both Apo B and D1S80. The PCR products were resolved on 8-10% polyacrylamide gel electrophoresis along with a 100 bp molecular weight marker (Bangalore Genei, India), stained with ethidium bromide and documented in a gel documentation system (AlphaImager™ 1220, Alpha Innotech Corporation, USA).

Allele Size Determination

The molecular weight of each band was determined using an AlphaImager 1220 v5.5 software programme where the unknown samples were compared with a 100 bp ladder. The alleles were designated according to their respective base pair sizes taking the core repeat sequence as 15 bp for Apo B 3′ VNTR and 16 bp for D1S80 marker.

Results

Polymorphism of the Apo B VNTR

DNA samples of 86 normal individuals (corresponding to 172 chromosomes) from the North Indian population were screened and 19 different alleles were identified. Under the electrophoretic conditions used, separation of alleles representing one-repeat difference (15 bp) was possible. The alleles were designated 1-19 on the basis of increasing repeat numbers. These alleles range in size from ~660-945 bp (Fig. 1). Each allele differs in size from its neighbouring allele by 15 bp (core repeat unit) with a few exceptions. Allele 1 was the smallest allele having 35 repeats and a molecular weight of ~660 bp (core repeat size plus 135bp flanking sequence), while the allele 19 was the largest one having 54 repeats and ~945 bp (Table 1; Fig. 2A). Allele 1 (35 repeats) varies from 2 (36 repeats) by 16 bp, allele 15 (50 repeats) from 16 (51 repeats) by 14 bp, while 14 (48 repeats) from 15 (50 repeats) vary by 30 bp (representing two repeats).

As shown in Figure 2A, allele 6 was most prevalent having 40 repeats, while the second and third most frequent alleles were of 43, 42/48 repeats, respectively. The allele of 49 repeats was absent in the studied population. The 19 alleles found at the Apo B 3′ VNTR showed the presence of a total of 51 different genotypes. The genotypes 41/41 and 43/43 were the most frequent (Table 1) with a frequency of 7%. Chi-square analysis showed that the genotypic distribution at Apo B 3' locus in our population was in Hardy-Weinberg equilibrium. The observed heterozygosity in this population was 55%.

Polymorphism of VNTR at the D1S80 locus

The D1S80 locus of 75 unrelated individuals (corresponding to 150 chromosomes) was amplified and 24 different alleles were observed. Each allele was completely resolved on the basis of increment of the repeat units of the VNTR locus. The length of the core sequence of this VNTR was taken as 16 bp

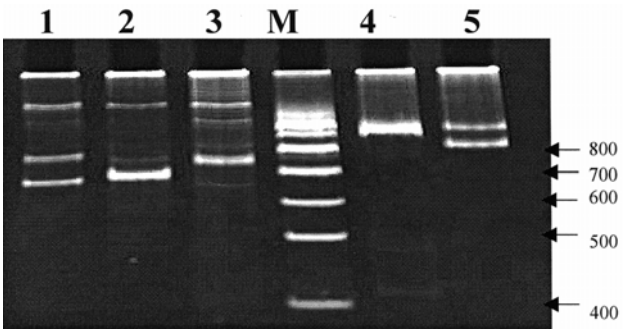

Fig. 1—Representative gel photograph showing the alleles of D1S80 (lanes 1-3) and Apo B 3′ HVR (lanes 4-5) and M, 100 bp ladder

Table 1—Distribution of Apo B 3′ and D1S80 VNTR

Apo B 3′ VNTR		D1S80 VNTR	
Observed genotypes	Frequency (%)	Observed genotypes	Frequency (%)
35/35	3	20/20	4
38/38	2	21/21	6
39/39	2	26/26	4
40/38	3	27/27	4
40/40	4	28/19	4
41/39	2	28/22	10
41/41	6	28/28	12
42/38	2	29/29	6
42/42	3	30/28	6
43/39	2	31/28	4
43/43	6	31/31	4
44/42	2	32/27	4
44/44	2	32/28	4
45/37	2	33/28	4
45/45	5	33/33	6
48/48	2	38/38	4
51/48	2		
50/50	3		

The genotypes having frequencies ≥2.0% in case of Apo B and ≥ 4% in case of D1S80 marker are included in the table.

(Fig. 1). The alleles were designated as 1-24 ranging from 17-40 repeats. Allele 1 was the smallest with 17 repeats and allele 24 had a maximum of 40 repeat units (Table 1).

The frequency of allele 12 (28 repeats, core repeat size ~450 bp) was significantly higher ($p<0.01$), than other alleles (Fig 2B). The alleles with very short (17-20) as well as very long (34-40) repeat units were rare in our study population. A total of fifty different genotypes were obtained, the most frequent was 28/28 with a frequency of 12% (Table 1). The distribution of genotypes at the D1S80 locus in the North Indian population was in Hardy Weinberg equilibrium. The observed heterozygosity was 57%.

Discussion and Conclusion

Tandem repeat minisatellite sequences are scattered throughout the human genome and also occur closely associated with specific genes, such as the hemoglobin cluster[12]. The high degree of variability makes these informative genetic markers useful for pedigree and linkage analysis. The VNTR at the human apolipoprotein B gene is an example of minisatellite region associated with a gene of clinical interest. The hyper allelism of this region has been exploited as a marker for family and population studies. Different numbers of alleles have been identified in different populations. For example, in the French population 12 different alleles were identified

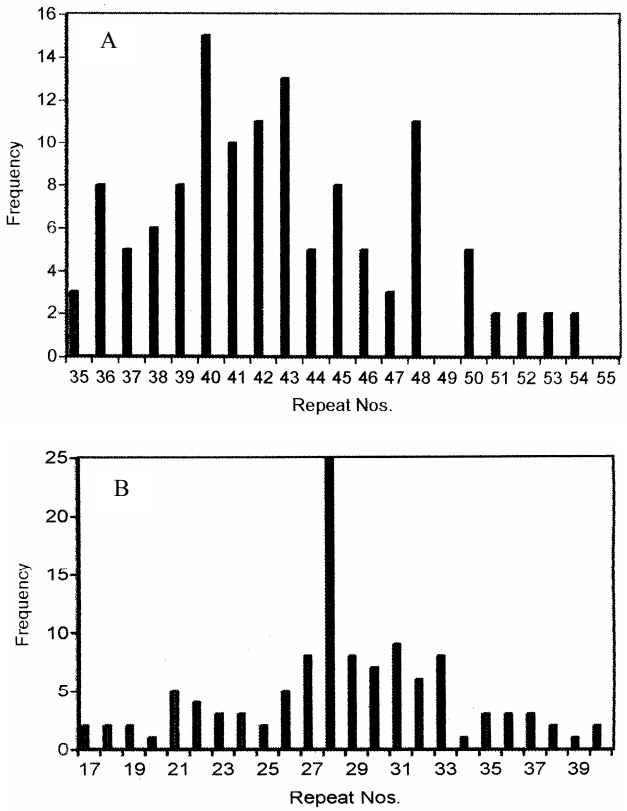

Fig. 2— (A) Genotype frequency distribution of Apo B 3′′ VNTR alleles in normal North Indian population of 86 subjects (172 chromosomes) and (B) Genotype frequency of D1S80 VNTR in normal North Indian population of 75 subjects (150 chromosomes)

at the Apo B 3′ VNTR loci[11], 14 in the Australian population[2] while 18 among the Emirates and 13 allelic variants were identified in 3 groups of Bashkir and Komi populations[13]. Similar to the populations from NW Spain[14], nationals of Abu Dhabi Emirate[15] and Ewondo population[16], we identified 19 alleles indicating once again the highly polymorphic nature in the region of north India. The identified alleles had repeat numbers ranging from 35-54 close to that reported for other populations[13]. The Apo B locus was also studied in some black and Amerindian communities from Columbia[17]. Recently, a study on Apo 3′ HVR in two Indian populations[18] suggested that this locus may be ideal to study the genetic relationships between different ethnic groups of India.

For the D1S80 locus, slight difference was found in the North Indian population in comparison to polymorphism studies from some other populations, such as Caucasians[19], American Indian tribes[20,21], Brahmins and Parsis from Western India[22]. In the present study, 24 alleles ranging from 17-40 repeats

were detected, showing a trimodal distribution (21, 28 and 31-33 repeats). However, allele 12 containing 28 repeats had the highest frequency, which was significantly higher than the other alleles ($p<0.001$). Mastana and Papiha[22] have reported a wide spectrum of variation at the D1S80 locus among the various populations.

The heterozygosity of Apo B and D1S80 VNTRs was found to be 55% and 57%, respectively. However, only 27% of the individuals were heterozygous at both VNTR loci. From these studies, it was observed that 85% of individuals from North India showed heterozygosity at one of the above two markers. The heterozygosity observed for individual VNTR in our study is lower than reported from other populations[2,13-16]. One of the reasons for low value may be that we have used PCR based analysis that gives lower values as compared to Southern blot hybridization[23]. The PCR based analyses for Apo B and D1S80 VNTR were sufficient to provide information about maternal contamination in majority of samples for prenatal diagnosis. In the human genome, VNTRs are not as prevalent as mono and dinucleotide repeats. Therefore, they may not prove as useful markers for DNA mis-match repair system.

In the present analysis, variation at the Apo B 3′ VNTR and D1S80 loci was measured solely on the basis of the number of repeats of the basic core sequence. A number of authors[11,24,25] have reported that the core sequence at the locus is not always faithfully repeated and that the heterogeneity of alleles with the same apparent size is possible. In our study, therefore, sequence based analysis of alleles could potentially have increased the level of polymorphism at this locus, in terms of number of alleles observed.

Due to their highly polymorphic content, VNTRs constitute useful tools as genetic markers for individual identification. The simple PCR based analysis of VNTR can be useful in paternity testing[2,3], monitoring engraftment following bone marrow transplantation and determining maternal contamination in chorionic villus sample for prenatal diagnosis.

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