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Original Article

Phylogenetic analysis of Bangladeshi population with reference to D1S80 VNTR locus

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ABSTRACT: Phylogenetic studies were conducted with reference to a highly polymorphic VNTR locus, D1S80 in a Bangladeshi population sample. Allelic distribution and genotype frequencies were studied for the locus among 146 unrelated Bangladeshi individuals. A total of 22 alleles were detected with repeat units ranging between 14 and 38. D1S80 alleles showed a tri-modal distribution and alleles with 18 and 24 repeats were the most common in Bangladeshi population with frequencies of 0.212 and 0.127, respectively. Seventy different genotypes were detected in the studied individuals of whom 76.7% were heterozygous. Comparison of allele frequency distribution with seventy-one other populations placed Bangladeshi's along with Tamils and Panjabi's in India in a clade separated from the rest.

KEYWORDS: D1S80, Bangladeshi population, allele frequency, genetic relationship

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INTRODUCTION

The largest portion of the human genome is composed of repetitive DNA sequences of various types. Variable number of tandem repeats (VNTRs) belong to this category of sequences in which a core repeat unit is repeated in tandem many times. The length of a VNTR core repeat unit lies in the range of approximately 10–100 bases[1]. VNTR loci usually have large number of alleles, which are co-dominant in nature[2] and considered as the most informative markers for genetic characterization[3]. Polymerase chain reaction (PCR) based VNTR loci amplification has been very useful in forensic tests, paternity analysis, prenatal diagnosis, lineage-specific chimerism quantification after transplantation, disease analysis and population diversity studies[3-7].

D1S80 (GenBank accession number D28507) is a highly informative VNTR locus in the telomeric region of chromosome 1 at 1p36-p35[3,8-10]. Its core unit consists of 16-nucleotides and like an ideal VNTR marker shows a high degree of polymorphism as well as heterozygosity[11], which make this a useful genetic marker in population diversity studies. Lauritzen and Mazumder[12] found D1S80 as the most informative marker among nine (D1S80, APO-B, vWA, D21S11, TH01, COL2A1, F13, MBP and FES). Ruiz *et al.*[13] also found the D1S80 locus as more informative than others (APO-B, DxS52, FVW, YNZ-22 and H-ras). This highly polymorphic locus has no known genetic function[11].

D1S80 has an overall mutation rate of approximately 7.77 × 10^{-5} [14] and its alleles are stably inherited in a Mendelian fashion over generations [3]. Since its discovery by Nakamura *et al.* [15], the D1S80 locus has been widely used in forensic analysis and paternity testing [9,11,16]. D1S80 is also a useful marker in assessing acute graft-versus-host disease after organ transplantation and detection of chimerism after hematopoietic cell transplantation [17,18]. Hypervariable markers like D1S80 extract the maximum information and increase the accuracy of any phylogenetic relationship analysis[9]. Alleles of the D1S80 VNTR locus have been used worldwide to determine origins and genetic relations among and between populations[9,16].

The objective of the study was to determine the allele frequency distribution at D1S80 locus in Bangladeshi individuals and to assess the genetic affinity with other reported world populations. This population specific D1S80 allele frequency data may also find use in forensic and population genetics studies in Bangladesh.

MATERIALS AND METHODS

Sources of DNA samples: Blood samples were collected from 146 unrelated Bangladeshi individuals. Males and females represented 55% and 45%, respectively, of the studied population. DNA was extracted from whole blood using Genomic DNA mini kit (AGB100, ATP Biotech, Taiwan). Purity and concentration was measured using NanoDrop UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc.).

Sequence amplification and detection: D1S80 VNTR region of the DNA samples were amplified by PCR using primers already described by Kloosterman *et al.*[19] in a thermal cycler (Gene Atlas G, Astec Co. Ltd.). 20-50 ng of genomic DNA was used for amplification in a final reaction volume of 25 µl containing PCR buffer (M0273S, NEB, USA), 200 µM dNTPs (110-002, GeneON), 0.4 µM of each primer and 1U of Taq DNA polymerase (M0273S, NEB, USA). The cycle condition was as follows: an initial denaturation step at 94°C for 5 minutes, then 33 cycles-each with denaturation at 94°C for 30 seconds, annealing at 68°C temperature for 30 seconds, and elongation at 72°C for 1 minute followed by a final extension at 72°C for 7

minutes. Amplified products were resolved in 1.8% agarose gel using 0.5x Tris Borate EDTA (TBE) buffer in Agagel Maxi Gel Electrophoresis System (020-300, Biometra, GmbH) along with DNA marker (300003, GeneON). DNA bands were visualized in a gel documentation system (WGD-30, Witeg) following incubation with ethidium bromide in TBE buffer and photographed with WiseCapture II™ software.

Allele size determination and phylogenetic analysis: Sizes of PCR products were determined by comparing their migration relative to a 50 bp DNA ladder (300003, GeneON) in 1.8% (w/v) agarose gels in an electric field. The number of core repeats in the amplified products were calculated using Microsoft Excel following the formula reported by Fujii *et al.* [10]. The alleles were designated according to the number of core repeats. Graph showing the distribution of D1S80 distribution was prepared using GraphPad Prism® software. Phylogenetic tree was generated using POPTREE2 software [20].

Statistical analysis: The frequencies of D1S80 alleles and genotypes of individuals and other statistical analyses (e.g., effective number of alleles, Shannon's information Index, Chi-Square tests for Hardy-Weinberg equilibrium, F-statistics) were determined using GenAlEx 6.5 software[21] and Microsoft Excel programme.

RESULTS AND DISCUSSION

In our study, alleles at the D1S80 VNTR locus was determined based on the amplified fragment length polymorphism (Amp-FLP) and agarose gel electrophoresis. A 1.8% (w/v) agarose gel was good enough to separate the nearby D1S80 alleles (Figure 1). Earlier reports suggested that low DNA template mass (in the range of 0.05 – 0.25 ng) may result in allelic imbalance for D1S80 alleles [18]. In this study 20- 50 ng DNA were used in PCR amplification.

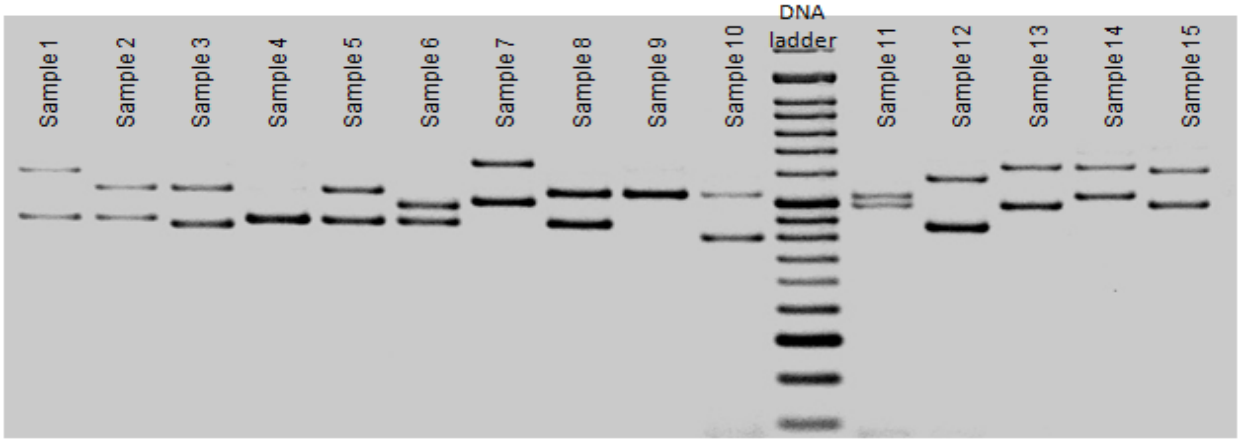

Figure 1: Amplified PCR products were resolved and detected in 1.8% (w/v) agarose gel and 0.5x TBE buffer. Sizes of PCR products were determined by comparing their migration relative to DNA markers.

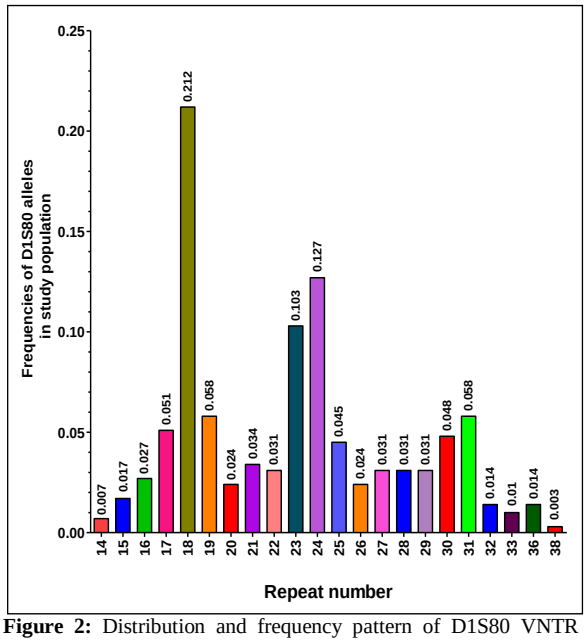

Figure 2: Distribution and frequency pattern of D1S80 VNTR alleles in 146 individuals in Bangladesh. Figure shows a tri-modal distribution of D1S80 alleles with allele 18 (has the highest frequency, F_a=0.212), 24 and 31 at the center of each mode. Alleles with low (14 repeats) and high (38 repeats) repeats numbers have the lowest frequencies.

22 different alleles were detected at the D1S80 locus of 146 unrelated Bangladeshi individuals. Frequencies of D1S80 VNTR alleles are shown in Figure 2. D1S80 allele frequencies showed a tri-modal distribution (Figure 2). Allele with 18 repeats was the most frequent (0.212) in Bangladeshi population. Only 6 of the alleles had a frequency of ≥5%. These 22 alleles constituted 70 different genotypes in the studied population (Table 1). 112 (76.7%) of the individuals among 146 were heterzygous for different alleles. The most frequent genotype was 18,18 followed by 18,24 and 19,25.

Earlier studies [14,16,22-25] also found D1S80 alleles with 18 and 24 repeat units as the most common in majority of the human populations. Allele 24 is relatively more common than allele 18 in African and Caucasian populations [9,11,23,26-28], although some mixed European population had higher frequencies of allele with 18 repeats [29]. A number of studies with Indian sub-populations found that in majority of the subpopulations allele with 18 repeats was the most frequent [2,30,31], while in the others the most prevalent allele had 24 repeats [2,7,32,33]. D1S80 has no known biological function [11]. It is compelling to find that allele with 18 and 24 repeats are the most predominant in mostly all populations on earth despite the absence of any known selection pressure. This might be an indication of either a common ancestry of human race or a yet to be known biological role of these alleles.

Table 1: Genotype frequencies at the D1S80 locus among 146 individuals in Bangladesh. 70 different genotypes were identified among 146 individuals.

Genotype	Frequency, %	Genotype	Frequency, %	Genotype	Frequency, %
32,32	0.013	21,29	0.013	18,22	0.013
31,31	0.013	21,23	0.039	18,21	0.026
29,29	0.013	21,21	0.013	18,18	0.171
28,31	0.053	20,31	0.013	17,38	0.013
27,36	0.013	20,28	0.013	17,30	0.026
27,31	0.013	20,25	0.013	17,27	0.026
26,30	0.013	20,24	0.026	17,24	0.026
26,26	0.026	20,20	0.013	17,23	0.026
25,25	0.026	19,33	0.013	17,21	0.013
24,33	0.013	19,29	0.013	17,19	0.026
24,31	0.053	19,28	0.013	17,17	0.013
24,30	0.039	19,25	0.092	16,32	0.013
24,28	0.026	19,24	0.026	16,26	0.026
24,27	0.013	19,23	0.013	16,25	0.013
24,24	0.039	19,19	0.026	16,23	0.026
23,30	0.053	18,36	0.013	16,18	0.026
23,29	0.013	18,33	0.013	15,36	0.026
23,23	0.066	18,32	0.013	15,30	0.013
22,31	0.026	18,31	0.039	15,24	0.013
22,30	0.013	18,29	0.026	15,17	0.013
22,28	0.026	18,27	0.053	14,30	0.013
22,24	0.026	18,25	0.026	14,29	0.013
22,22	0.013	18,24	0.145		
21,30	0.013	18,23	0.079		

Significant departure from the Hardy-Weinberg equilibrium (HWE) in the allele frequency distribution was observed for the studied locus (***P<0.001). Deviations from expected values may be due to a variety of causes like the occurrence of out-breeding and over-dominant selection. Consanguineous marriage is not much common Bangladesh and this study was conducted with individuals who were randomly selected from Bangladeshi population. Besides, a few D1S80 alleles show higher prevalence than the others in all populations. This might have caused the deviation in D1S80 allelic distribution from HWE in Bangladeshi population. Deviation from the Hardy-Weinberg equilibrium at D1S80 locus was reported in other studies[2,16,25,30]. Study conducted among three VNTR (D1S80, APOB and D4S43) and three STR (vW1, F13A1 and DYS19) loci by Vallinoto *et al*[25] showed that only D1S80 was not in Hardy–Weinberg equilibrium in one Afro-Brazilian population (Pacoval), but it was in equilibrium in the other (Curiau).

The F-statistics (Fixation index) for alleles of D1S80 in Bangladeshi population was -0.103. A negative F means more heterozygotes than expected (out-breeding) compared to HWE expectations. The number of effective D1S80 alleles (N_e) in Bangladeshi population was 10.741. Effective number of alleles (Ne) is the reciprocal of the expected homozygosity in any population. It is a correlated with the genetic diversity in a population which is measured as the expected heterozygosity and allows one to compare the biodiversity with other communities. The Shannon's information index for the D1S80 alleles was 2.702. Shannon's index also provides a relative estimate of the degree of genetic variation within a population.

In concordance with other studies, alleles with repeat numbers at both ends were relatively rare in our population[24,30]. We observed more alleles compared to the studies conducted on small sub-populations or tribes[16,25]. The largest allele that we could detect had 38 core repeat units. However, in a study on Japanese population, the largest allele was detected with 72 repeats[34]. One distinctive feature of the spectrum of D1S80 allele frequencies is its multimodal distribution [28]. We observed a tri-modal distribution of D1S80 alleles in Bangladeshi population. A similar tri-modal distribution was observed in two populations in the United Kingdom (North East England and East Midlands) and the Brahmins and the Parsis from Western India [4]. Tri-modal distribution of D1S80 alleles was observed among Nairs, Muslims and Pulayas in India as well [2].

Distribution of D1S80 allele frequencies were studied in a large number of populations worldwide. In this study, 72 different population data (including the data presented in this study) [7,9,11,22,23,25,27,30,32,33,35-49] were fed in POPTREE2 and a phylogenetic tree was constructed following neighbor-joining (NJ) method (Figure 3). This placed Bangladeshi's along with Tamils and Panjabi's in India in a clade separated from the rest. This finding is consistent with a study conducted by Tania et al using 15 autosomal STR loci in a Bangladeshi population. Although in a study with seven Indian sub-populations, allele 27 was only detected among Muslims and Arayas (at a frequency of 0.007 and 0.025, respectively), and the Muslims had a tri-modal distribution of D1S80 alleles (with allele 18, 24 and 31 at modal points) alike the Bangladeshi population, overall allelic distribution positioned these two Indian ethnic groups in a separate branch in the phylogenetic tree.

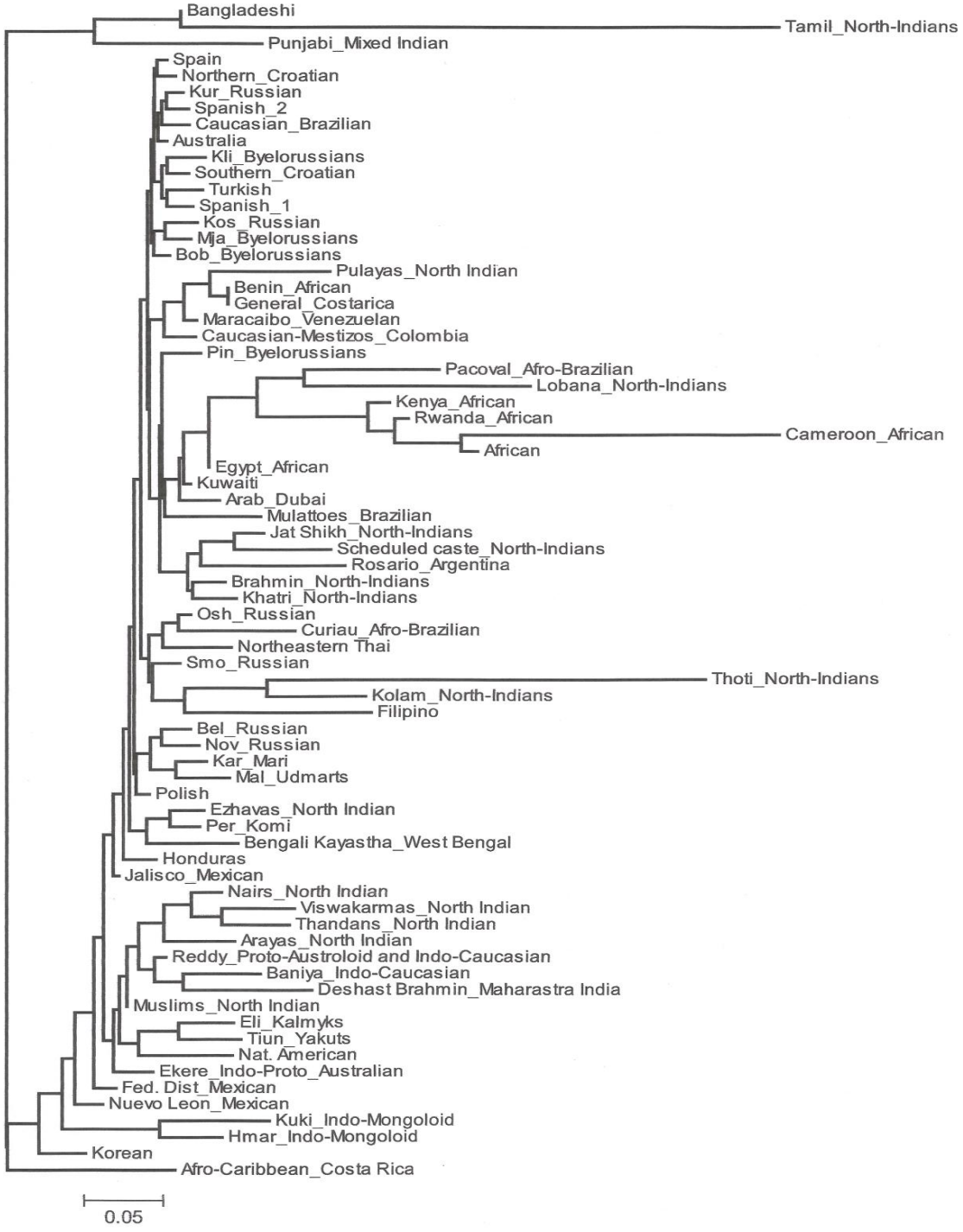

Figure 3: Phylogenetic analysis with D1S80 allele frequency data. 72 different population data were included in this study. This tree was constructed following neighbor-joining (NJ) method using POPTREE2 software.

Evolutionary inference from single-locus data has limitations compared to multi-locus approaches[50]. Therefore, it requires more studies to conclude with confidence the phylogenetic relationship of Bangladeshi population with the others. However, the overall appearance of the phylogenetic tree derived from the

D1S80 allele frequencies (Figure 3) of the world population does not show much deviation from the gross expected outcome. Based on the allele frequency data, POPTREE placed two Indo-Mongoloid populations in adjacent branches. It also placed three different Spanish populations in a single clade, while six different African populations in another one. The European populations were more or less clustered closely in the tree as well. So, based on the phylogenetic tree derived from D1S80 allele frequencies, we may actually have a closer genetic relationship with the Tamils and the Panjabi's in India than the others.

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