

*Article*

**Genetic polymorphism study at D1S80 VNTR in four major tribal populations (Garo, Santal, Khasia and Monipuri) of Bangladesh**

Abu Ashfaque Sajib<sup>1</sup>, Md. Mahamud Hasan<sup>2</sup>, Md. Mahmudul Hasan<sup>1</sup>, Tarana Sharmin<sup>1</sup> and Sharif Akhteruzzaman<sup>1,\*</sup>

<sup>1</sup>Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh

<sup>2</sup>National Forensic DNA Profiling Laboratory, Dhaka Medical College Campus, Dhaka-1000, Bangladesh

\*Corresponding author: Professor Dr. Sharif Akhteruzzaman, Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh. E-mail: sharif\_akhteruzzaman@yahoo.com

Received: 07 August 2016/Accepted: 25 August 2016/ Published: 31 August 2016

**Abstract:** D1S80 is a highly informative variable number of tandem repeat (VNTR) marker located in the telomeric region of chromosome 1 at 1p36-p35. Since its discovery, the D1S80 locus has been used widely in determining the origins and genetic relations among and between various populations worldwide. Here, we studied the allele frequencies and other population genetic parameters at the D1S80 locus among individuals from four major tribal populations in Bangladesh (Santal, Garo, Monipuri and Khasia). The data was then compared with the other populations including the mainstream Bengali population. A total of 31 different alleles were detected with repeat unit numbers ranging between 14 and 50. D1S80 allele with 18 repeats was the most frequent in three populations except the Santals, in which allele with 19 repeats was the most common. Observed heterozygosity was less than the expected in all four populations. Pair-wise observed genetic distances were, in general, more between the tribal populations and the mixed mainstream Bangladeshis compared to the distances between the tribal populations. Comparison of D1S80 allelic frequency distribution among seventy-six different populations placed the tribal populations along with the mainstream mixed Bangladeshis, Tamils and mixed Panjabi Indians in a clade separated from the rest.

**Keywords:** D1S80; genetic relationship; Garo; Santal; Khasia; Monipuri

**1. Introduction**

Bangladesh is a South Asian country with a large population of more than 160 million and houses over 40 indigenous groups (Abdullah, 2011; Hannan, 2015). The mainstream Bengalis are, however, by far the largest and comprise about 98% of the population. The remainder is mostly indigenous tribal populations living in the southeastern, north-central and northeastern region of the country. Santal, Garo, Monipuri and Khasia (Khasi) are four among these socially and culturally distinct indigenous populations which along with the other ethnic groups have significantly enriched the culture of Bangladesh with their distinct and colourful lifestyles (Abdullah, 2011; Das and Islam, 2005). The migration history of these groups on this land is not exactly known. Genetic data is also scarce to portray the genetic structure of these populations.

D1S80 is variable number of tandem repeat (VNTR) locus positioned in the telomeric region of chromosome 1 at 1p36-p35 with a core repeat unit of 16-nucleotides (Choong *et al.*, 2011; Fujii *et al.*, 2004; Herrera *et al.*, 2004; Mukherjee *et al.*, 2005; Verbenko *et al.*, 2006). Like an ideal VNTR marker the D1S80 locus shows a high degree of polymorphism as well as heterozygosity (Herrera *et al.*, 2004). This makes D1S80 a highly useful genetic marker in population diversity studies (Choong *et al.*, 2011; Fujii *et al.*, 2004; Herrera *et al.*, 2004; Mukherjee *et al.*, 2005; Verbenko *et al.*, 2006). D1S80 locus has no known genetic function, and therefore, is supposed to be under no selection pressure (Herrera *et al.*, 2004). Like the other VNTRs, its alleles are co-dominant in nature and stably inherited in a Mendelian fashion over generations (Mukherjee *et al.*, 2005).

Since its discovery by Nakamura *et al.* (Nakamura *et al.*, 1988), the D1S80 locus has been widely used in forensic analysis, paternity testing, assessing chimerism after organ and hematopoietic cell transplantation, and determining the origins and genetic relations among and between various populations worldwide (Herrera *et al.*, 2004; Thin *et al.*, 2009; Verbenko *et al.*, 2006; Walsh and Eckhoff, 2007; Waterhouse *et al.*, 2013).

The objective of the study was to determine the allelic profiles at the D1S80 locus among individuals of the Santal, Garo, Monipuri and Khasia (Khasi) tribes in Bangladesh and compare with the other populations, including the mainstream Bangladeshis. This population specific D1S80 allele frequency data may find use in forensic and clinical applications as well. This study may be extended to other indigenous tribes in Bangladesh.

## 2. Materials and Methods

### 2.1. Sources of DNA samples

Liquid blood and buccal swabs were collected from randomly selected individuals from the Garo, Santal, Khasia and Monipuri populations. Genomic DNA was extracted by using Chelex<sup>®</sup>-100 method (Singer-Sam *et al.*, 1989; Walsh *et al.*, 1991). Concentration and purity of the extracted DNA was measured using a spectrophotometer (NanoDrop<sup>™</sup>, Thermo Fisher Scientific Inc.).

### 2.2. PCR amplification and detection

D1S80 VNTR region of the DNA samples was amplified by polymerase chain reaction (PCR) using primers already described by Kloosterman *et al.* (Kloosterman *et al.*, 1993) in a thermal cycler (Gene Atlas G, Astec Co. Ltd.). 10-50 ng of genomic DNA was used for amplification in a final reaction volume of 25 µl containing GeneAmp PCR Gold Buffer (4311806, Thermo Fisher Scientific), 1.5mM MgCl<sub>2</sub>, 200 µM dNTPs (110-002, GeneON), 0.4 µM of each primer and 1 U of AmpliTaq Gold<sup>®</sup> DNA Polymerase (4311806, Thermo Fisher Scientific). The reaction cycle condition was as follows: an initial denaturation step at 95°C for 9 minutes, then 33 cycles- each with denaturation at 94°C for 20 seconds, annealing at 68°C temperature for 30 seconds, and elongation at 72°C for 70 seconds 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 observed in a gel documentation system (WGD-30, Witeg) following incubation with ethidium bromide (0492, Amresco<sup>®</sup>) in TBE buffer and photographed with WiseCapture II<sup>™</sup> software.

### 2.3. Allele size determination and phylogenetic analysis

Sizes of PCR products were determined using the “GelAnalyzer” software (Lazar and Lazar, 2010) by comparing their migration relative to DNA size markers (300003, GeneON) in 1.8% (w/v) agarose gels in an electric field. The number of 16-bp core repeats in the amplicons were calculated using Microsoft Excel following the formula reported by Fujii *et al.* (Fujii *et al.*, 2004). The alleles were designated according to the number of core repeats. The phylogenetic tree was generated using POPTREE2 software (Takezak *et al.*, 2010).

### 2.4. 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, observed heterozygosity, Chi-Square tests for Hardy-Weinberg equilibrium, F-statistics, Nei genetic distance and identity) were determined using GenAlEx 6.5 software (Peakall and Smouse, 2012) and Microsoft Excel programme.

## 3. Results and Discussion

In this study allelic composition at D1S80 locus among individuals of four indigenous populations in Bangladesh was studied using amplified fragment length polymorphism (Amp-FLP) technique. The allele frequency and other population genetic parameters, are shown in Table 1. The number of different alleles in the studied populations ranged between 17 and 24. D1S80 allele frequencies showed multi-modal distribution in all four populations. Multimodal distribution is a distinctive feature of the spectrum of D1S80 allele frequencies (Das and Seshadri, 2004; Limborska *et al.*, 2011; Mastana and Papiha, 2001). Allele with 18 repeats was the most frequent in three populations (Garo, Khasia and Monipuri), while in Santals allele with 19 repeat was the most common. In the majority of the studied human populations (Balamurugan *et al.*, 2012; Çakir *et al.*, 2001; Hutz *et al.*, 1997; Soares-Vieira *et al.*, 2000; Vallinoto *et al.*, 2003; Walsh and Eckhoff, 2007), D1S80 alleles with 18 and/or 24 repeat units were the most common. Allele with 24 repeats is relatively more common in the African and the Caucasian populations (Bernal *et al.*, 2000; Herrera *et al.*, 2004; Koseler *et al.*, 2012; Limborska *et al.*, 2011; Soares-Vieira *et al.*, 2000; Verbenko *et al.*, 2006). In the majority of Indian sub-populations allele

with 18 repeats was found to be the most prevalent one (Das *et al.*, 2002; Das and Seshadri, 2004; Trivedi *et al.*, 2002). Some mixed European populations also had higher frequencies of allele with 18 repeats (Verbenko *et al.*, 2003). The fact that D1S80 alleles with 18 and 24 repeats are the most prevalent in almost all populations on earth despite having no known biological function and, therefore, the absence of selection pressure (Herrera *et al.*, 2004), might be an indication of the common ancestry of human race.

The number of observed heterozygosity was lower than the expected ones in all populations (Table 1). Monipuri population showed the maximum deviation from the expected heterozygosity. Lower than the expected heterozygosity may be attributed to inbreeding, which is kind of true for the tribal populations. The observed heterozygosity was, however, higher than the expected heterozygosity in the mixed Bangladeshi population (Sajib *et al.*, 2016). Chi-square test showed significant deviation (\*\*\*)  $P < 0.001$  in D1S80 allelic frequencies from the Hardy-Weinberg Equilibrium (HWE) in all four populations. Similar observations at D1S80 locus were reported earlier (Das and Seshadri, 2004; Trivedi *et al.*, 2002; Vallinoto *et al.*, 2003; Walsh and Eckhoff, 2007). This deviation might root from the more frequent presence of certain D1S80 alleles in populations as well as low representation of alleles at the bond ends of D1S80 allelic spectrum (Hutz *et al.*, 1997; Trivedi *et al.*, 2002). The reason for such distribution of D1S80 alleles calls for further investigation.

Large number of alleles along with its high allelic heterozygosity among individuals in population has made D1S80 a useful genetic marker in population genetics study (Herrera *et al.*, 2004; Verbenko *et al.*, 2003). D1S80 locus provides more information about a population compared to other VNTRs and STRs (Lauritzen and Mazumder, 2008; Ruiz *et al.*, 2009). The number of effective D1S80 alleles ( $N_e$ ) was the highest (15.313) in the Monipuri population and the least (8.817) in Khasias (Table 1). Effective number of alleles ( $N_e$ ) is the reciprocal of the expected homozygosity in any population and correlated with the genetic diversity in a population, which allows one to compare the biodiversity with other communities. More genetic diversity in Monipuri population may result from their mixed genetic architecture (Sanajaoba, 2005). Pair-wise genetic distances among the tribal populations, in general, were less than the genetic distances between mixed mainstream Bangladeshis and the tribal populations (Table 2). Garo and Santal appears to be genetically more related compared to the other tribes (Table 2).

D1S80 allele frequencies were studied in a large number of populations worldwide. In this study, 76 different population data (including the data presented in this study) (Al-Nassar *et al.*, 1996; Alkhayat *et al.*, 1996; Balamurugan *et al.*, 2001; Bernal *et al.*, 2000; Cabrero *et al.*, 1995; Çakir *et al.*, 2001; Cerda-Flores *et al.*, 2002; Ciesielka *et al.*, 1996; Das and Seshadri, 2004; Gutowski *et al.*, 1995; Halos *et al.*, 1999; Herrera *et al.*, 2004; Keys *et al.*, 1996; Lorentea *et al.*, 1997; Mastana, 1999; Matamoros *et al.*, 2004; Morales *et al.*, 2001; Ruangjirachuporn *et al.*, 2006; Sachdeva *et al.*, 2004; Sajib *et al.*, 2016; Soares-Vieira *et al.*, 2000; Tenaglia *et al.*, 2004; Trivedi *et al.*, 2002; Vallinoto *et al.*, 2003; Verbenko *et al.*, 2006; Yunis *et al.*, 2001) were fed into POPTREE2 and a phylogenetic tree was constructed following neighbor-joining (NJ) method (Figure 1). Phylogenetic analysis based on the D1S80 allelic profiles placed the four indigenous populations along with the mainstream Bangladeshis, the north Indian Tamils and the mixed Panjabi Indians in a clade separated from the rest. The Garo is an ethnic group belonging to the Mongolian human race which had the aboriginal dwelling in areas adjacent to mid-Asia (Das and Islam, 2005). Their ancestors probably moved from northwestern side of China to the northern side of 'Tibet' and then to the northeastern area of India through Bhutan (Das and Islam, 2005). Garos established an empire near the Garohill in northern area of Bangladesh during the 9th century (Das and Islam, 2005). There is also an opinion that the original home of the Garo was in the Assam of India, and they may be the successors to the Khasias, Nagas and Monipuris of Assam (Das and Islam, 2005). The Monipuris may have a mixed origin from different ethnic groups belonging to the Mongoloids, the Tibeto-Burman, Indo-Aryans and the Tais (Shans) (Sanajaoba, 2005). History of the origin and migration of the Khasia is still a mystery and different opinions exist. Some have described the Khasias as Mongoloid people who speak an Austric tongue (Mou Khmer) (Ahmmed, 2005). The Santals also speak in a language of with Austric (Mou Khmer) origin (Bappy, 2012). On the other hand, Garos and Khasias have similar matrilineal hierarchy in family in which daughter inherits mother's property and inherit descent from mothers side (Bappy, 2012). Similar social norms may indicate that these populations originated from the same root.

It was reported earlier that low DNA template mass (in the range of 0.05 – 0.25 ng) may result in allelic imbalance/drop out for D1S80 alleles (Waterhouse *et al.*, 2013). In this investigation, 10- 50 ng DNA was used in PCR amplification to avoid such events. Amplified samples from different populations were resolved together in agarose gels in batches to minimize any possible biasness in the calculation of product sizes resulting from gel-to-gel relative migration.

**Table 1. Frequency of D1S80 alleles in four major tribal populations (Garo, Khasia, Monipuri and Santal) in Bangladesh.**

D1S80 Allele	Garo	Khasia	Monipuri	Santal
14	0.000	0.000	0.000	0.008
15	0.000	0.000	0.014	0.008
16	0.007	0.013	0.000	0.038
17	0.063	0.000	0.014	0.083
18	0.120	0.256	0.114	0.114
19	0.106	0.128	0.086	0.167
20	0.099	0.038	0.071	0.068
21	0.035	0.051	0.057	0.030
22	0.042	0.000	0.043	0.015
23	0.021	0.000	0.014	0.045
24	0.035	0.038	0.086	0.023
25	0.063	0.077	0.029	0.023
26	0.028	0.064	0.086	0.038
27	0.035	0.077	0.043	0.061
28	0.021	0.051	0.043	0.045
29	0.014	0.051	0.057	0.038
30	0.049	0.026	0.029	0.008
31	0.042	0.000	0.000	0.030
32	0.077	0.026	0.086	0.030
33	0.063	0.013	0.043	0.030
34	0.028	0.038	0.014	0.045
35	0.014	0.000	0.014	0.030
36	0.007	0.026	0.000	0.000
37	0.000	0.026	0.029	0.000
39	0.014	0.000	0.000	0.015
41	0.007	0.000	0.000	0.000
43	0.000	0.000	0.000	0.008
44	0.007	0.000	0.000	0.000
47	0.000	0.000	0.014	0.000
50	0.000	0.000	0.014	0.000
HWE	*** (p<0.001)	*** (p<0.001)	*** (p<0.001)	*** (p<0.001)
N	71	39	35	66
Na	24	17	22	24
I	2.896	2.503	2.875	2.876
Ho	0.704	0.487	0.457	0.682
He	0.934	0.887	0.935	0.927
F	0.246	0.451	0.511	0.265

HWE= *Hardy-Weinberg equilibrium*

N= Number of samples

Na = No. of different alleles

I = Shannon's Information Index =  $-1 * \sum (\pi * \ln(\pi))$

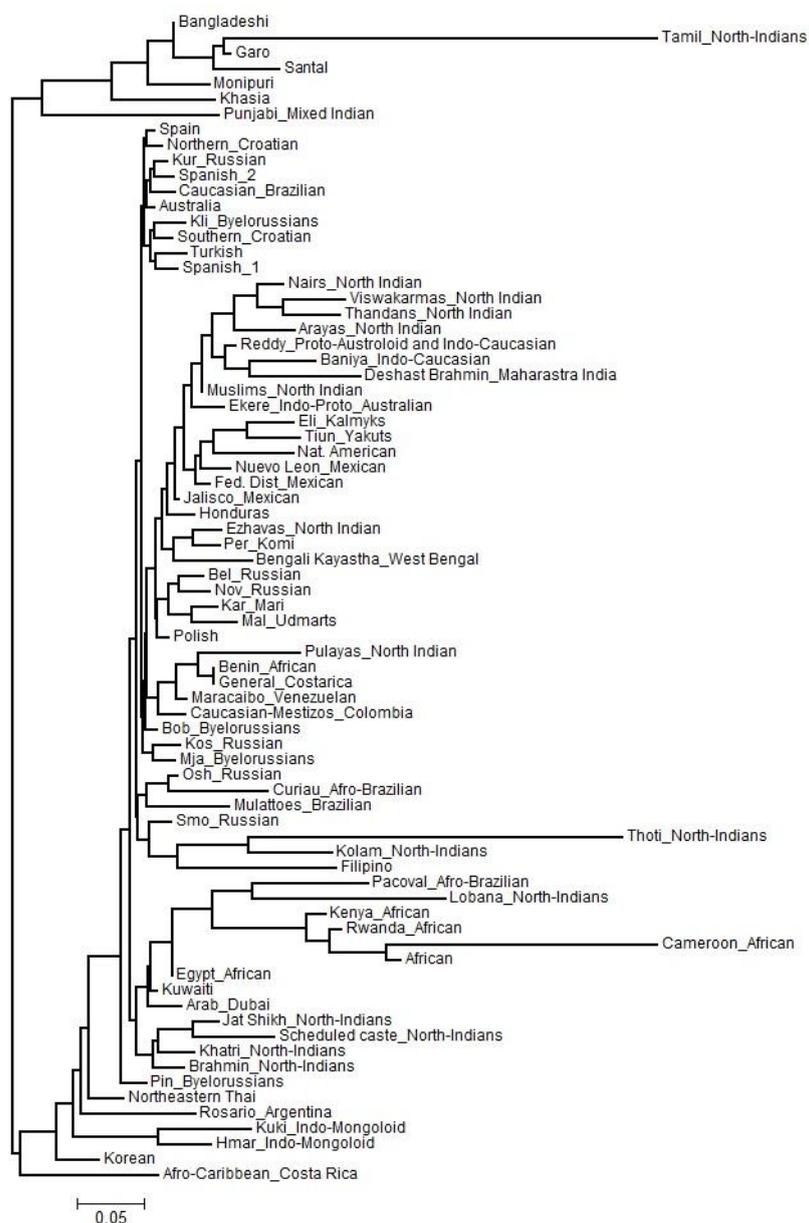
Ho = Observed Heterozygosity = No. of Heterozygous / N

He = Expected Heterozygosity =  $1 - \sum \pi^2$

F = Fixation Index =  $(He - Ho) / He = 1 - (Ho / He)$ , where  $\pi$  is the frequency of the *i*th allele for the population &  $\sum \pi^2$  is the sum of the squared population allele frequencies.

**Table 2. Genetic relatedness among four major tribal populations (Garo, Khasia, Monipuri and Santal) in Bangladesh.**

Pair-wise Population Matrix of Nei Genetic Distance				
Garo	Khasia	Monipuri	Santal	Mixed_Bangladeshi
0.000				
0.246	0.000			
0.150	0.204	0.000		
0.131	0.229	0.240	0.000	
0.289	0.221	0.291	0.329	0.000



**Figure 1. Phylogenetic analysis with D1S80 allele frequency data. 76 different population data were included in this analysis. This tree was constructed following neighbor-joining (NJ) method using POPTREE2 software (Takezak *et al.*, 2010).**

**4. Conclusions**

Comparison of D1S80 allelic frequency distribution among seventy-six different populations placed the four tribal populations (Garo, Santal, Khasia and Monipuri) along with the mainstream mixed Bangladeshis, Tamils and mixed Panjabi Indians in a clade separated from the rest. Inference of genetic relationship from single-locus

data has limitations (Fujisawa and Barraclough, 2013). Investigation at multiple highly polymorphic loci may add more confidence to such genetic relationship studies. However, the gross appearance of the POPTREE generated (only the D1S80 allele frequency based) phylogenetic tree of the world population does not show much deviation from the overall expected outcome. Based on the allele frequency data, POPTREE grouped six different African populations in one clade, while placed three different Spanish populations together in a separate one. Different European populations were clustered closely in the tree as well. So, this study may add new dimension to investigations on the actual origin of the major indigenous populations of Bangladesh. This population specific D1S80 allele frequency data may also find use in forensic and clinical applications.

### Acknowledgements

This study was conducted with the Biotechnology Research Center (BRC), University of Dhaka grant-2016 to SAZ. The authors thank the BRC, DU for its support.

### Conflict of interest

None to declare.

### References

- Abdullah N, 2011. Survey on Socio-Demographic and Health Status of Tribal Community of Bangladesh: Santals. Dissertation, East West University, Bangladesh.
- Ahmed MF, 2005. The Khasis in Bangladesh. *Journal of Ethnic Affairs* 1: 18-26.
- Al-Nassar KE, J Mathew, J Thomas and HR Fatania, 1996. Analysis of the D1s80 (Pmct118) Vntr locus polymorphism in a native Kuwaiti population by the polymerase chain reaction. *Forensic Science International* 78: 131-38.
- Alkhayat A, F Alshamali and B Budowle, 1996. Population data on the PCR-based loci Ldlr, Gypa, Hbga, D7s8, Gc, Hla-Dqa1, and D1s80 from Arabs from Dubai. *Forensic Science International*, 81: 29-34
- Balamurugan K, N Prabakaran, G Duncan, B Budowle, M Tahir and M Tracey, 2001. Allele frequencies of 13 STR loci and the D1S80 locus in a Tamil population from Madras, India. *J. Forensic Sci.*, 46: 1515-17.
- Balamurugan K, ML Tracey, U Heine, GC Maha and GT Duncan, 2012. Mutation at the human D1S80 minisatellite locus. *ScientificWorldJournal*, 2012: 917235.
- Bappy AKMAA, 2012. Indigenous people in greater Mymensingh area: an economic study on their livelihood and food security. Dissertation, Bangladesh Agricultural University, Mymensingh, Bangladesh.
- Bernal LP, D Castro-Guerra, MG Villasmil and L Borjas-Fajardo, 2000. VNTR locus D1S80: application to the study of a mixed Venezuelan sample. *Am. J. Hum. Biol.*, 12: 616-22.
- Cabrero C, A Diez, E Valverde, A Carracedo and J Alemany, 1995. Allele frequency distribution of four PCR-amplified loci in the Spanish population. *Forensic Science International*, 71: 153-64.
- Çakir AH, L Açık and T Kesici, 2001. Distribution of amplified fragment length polymorphism D1S80 alleles in Turkish population. *J. Forensic Sci.*, 46: 1002.
- Cerda-Flores RM, MC Villalobos-Torres, HA Barrera-Saldana, LM Cortes-Prieto, LO Barajas, F Rivas, A Carracedo, Y Zhong, SA Barton and R Chakraborty, 2002. Genetic admixture in three Mexican Mestizo populations based on D1S80 and HLA-DQA1 loci. *Am. J. Hum. Biol.*, 14: 257-63.
- Choong SS, S Rosmanizam, K Ibrahim, GG Gan and H Ariffin, 2011. Development of an algorithm of satellite markers for monitoring chimerism status in post-allogeneic haematopoietic stem cell transplantation patients. *Int. J. Lab Hematol.*, 33: 182-6.
- Ciesielka M, P Kozioł and A Krajczak, 1996. Allele frequency distributions of D1S80 in the Polish population. *Forensic Science International*, 81: 141-47.
- Das B, A Ghosh, PS Chauhan and M Seshadri, 2002. Polymorphism study at four minisatellite loci (D1S80, D17S5, D19S20, and APOB) among five Indian population groups. *Human Biology*, 74: 345-61.
- Das B and M Seshadri, 2004. Genetic variability at the D1S80 minisatellite: predominance of allele 18 among some Indian populations. *Annals of Human Biology*, 31: 541-53.
- Das TK and SMHZ Islam, 2005. Psycho-Social Dimensions of Ethnicity: The Situation of Garo Community in Bangladesh. *Asian Affairs*, 27: 45-54.
- Fujii K, K Sekiguchi, K Shimizu and K Kasai, 2004. A new sequenced allelic ladder marker for D1S80 typing. *J. Hum. Genet.*, 49: 169-171.
- Fujisawa T and TG Barraclough, 2013. Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. *Syst. Biol.*, 62: 707-24.

- Gutowski S, B Budowle, J Auer and R Oorschot, 1995. Statistical analysis of an Australian population for the loci Gc, HLA-DQA1, D1S80 and HUMTH01. *Forensic Science International*, 76: 1-6.
- Halos SC, JY Chu, ACM Ferreón and MCF Magno, 1999. Philippine population database at nine microsatellite loci for forensic and paternity applications. *Forensic Science International*, 101: 27-32.
- Hannan MA, 2015. Human rights of the aboriginals in the context of Bangladesh. *OIDA International Journal of Sustainable Development*, 8: 45-68.
- Herrera RJ, LR Adrien, LM Ruiz, NY Sanabria and G Dunca, 2004. D1S80 single-locus discrimination among African populations. *Human Biology*, 76: 87-108.
- Hutz MH, VS Mattev, SM Callegari-Jacques, FM Salzano, JrCEA Coimbra, RV Santos, RF Carnese, AS Goicoechea and CB Dejean, 1997. D1S80 locus variability in south American Indians. *Annals of Human Biology*, 24: 249- 55.
- Keys KM, B Budowle, S Andelinovic, M Definis-Gojanovic, I Drmic, M Mladen and M Primorac, 1996. Northern and southern Croatian population data on seven PCR-based loci. *Forensic Science International*, 81: 191-99.
- Kloosterman AD, B Budowle and P Daselaar, 1993. PCR-amplification and detection of the human D1S80 VNTR locus- amplification conditions, population genetics and application in forensic analysis. *Int. J. Leg. Med.*, 105: 257-64.
- Koseler A, O Ozturk, A Atalay and EO Atalay, 2012. Allele frequency of VNTR locus D1S80 observed in Hb D-Los Angeles carriers. *Mol. Biol. Rep.*, 39: 10747-50.
- Lauritzen SL and A Mazumder, 2008. Informativeness of genetic markers for forensic inference—an information theoretic approach. *Forensic Science International, Genetics Supplement Series 1*: 652-53.
- Lazar I and I Lazar, 2010. Gel Analyzer 2010a: freeware 1D gel electrophoresis image analysis software. <http://www.gelanalyzer.com>, accessed on July 09, 2016 (2010):
- Limborska SA, AV Khrunin, OV Flegontova, VA Tasitz and DA Verbenko, 2011. Specificity of genetic diversity in D1S80 revealed by SNP-VNTR haplotyping. *Ann. Hum. Biol.*, 38: 564-569.
- Lorentea M, JA Lorentea, MR Wilson, B Budowle and E Villanueva, 1997. panish population data on seven loci: D1S80, D17S5, HUMTH01, HUMVWA, ACTBP2, D21S11 and HLA-DQA1. *Forensic Science International*, 86: 163-171.
- Mastana S, 1999. Genetic analysis of the D1S80 locus in five north Indian populations. *Annals of Human Biology*, 26: 405-11.
- Mastana SS and SS Papiha, 2001. D1S80 distribution in world populations with new data from the UK and the Indian sub-continent. *Ann. Hum. Biol.*, 28: 308-318.
- Matamoros M, S Silva and O Garcí'a, 2004. Population data on nine short tandem repeat loci and D1S80 in a sample from Honduras. *Forensic Science International*, 141: 39-40.
- Morales AI, B Morera, G Jimenez-Arce, G Sanchez-Rivera, F Calafell and R Barrantes, 2001. Allele frequencies of markers LDLR, GYPA, D7S8, HBG, GC, HLA-DQA1 and D1S80 in the general and minority populations of Costa Rica. *Forensic Science International*, 124: 1-4.
- Mukherjee M, A Srivastava, A Kesari and B Mittal, 2005. Analysis of VNTR loci, APOB 3' HVR and D1S80 in north Indians. *Indian Journal of Biotechnology*, 4: 358-362.
- Nakamura Y, M Carlson, K Krapcho and R White, 1988. Isolation and mapping of a polymorphic DNA sequence (pMCT118) on chromosome 1p [D1S80]. *Nucleic Acids Research*, 16: 9364.
- Peakall R and PE Smouse, 2012. GenAIEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, 28: 2537-2539.
- Ruangjirachuporn W, O Japa, M Tanratanavijit and S Nanakorn, 2006. Population genetic data on D1S80, D17S5, ApoB, COL2A1 and Ig-JH in Northeastern Thais. *Legal Medicine*, 8: 308-309.
- Ruiz ADS, MDLF Chauffaille, STA Alves and JSRD Oliveira, 2009. Prevalence of chimerism after non-myeloablative hematopoietic stem cell transplantation. *Sao Paulo Med. J.*, 127: 251-258.
- Sachdeva MP, SS Mastana, KN Saraswathy, AM Elizabeth, R Chaudhary and AK Kalla, 2004. Genetic variation at three VNTR loci (D1S80, APOB, and D17S5) in two tribal populations of Andhra Pradesh, India. *Ann. Hum. Biol.*, 31: 95-102.
- Sajib AA, S Yeasmin, M Akter, MA Uddin and S Akhteruzzaman, 2016. Phylogenetic analysis of Bangladeshi population with reference to D1S80 VNTR locus. *Biores Comm*, 2: 146-51.
- Sanajaoba N, 2005. Manipur- past and present, the ordeals and heritage of a civilization. Mittal publications, New Delhi, India:
- Singer-Sam J, RL Tanguay and AO Rjggs, 1989. Use of Chelex to improve PCR signal from a small number of cells. *Amplifications: A Forum for PCR Users*, 3: 11.

- Soares-Vieira JA, AEC Billerbeck, EM Iwamura, DR Muñoz and PA Otto, 2000. Allele and genotype frequencies for D1S80 locus in a Brazilian population sample. *J. Forensic Sci.*, 45: 696-697.
- Takezak N, M Nei and K Tamura, 2010. POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. *Mol. Biol. Evol.*, 27: 747-752.
- Tenaglia M, A Scollo, R Tripaldi, I Grappiolo and AM Perichón, 2004. Genetic data on nine STRs (CSF1PO, TPOX, THO1, F13AO1, FESFPS, vWA, D16S539, D7S820 and D13S317) and two VNTRs (D1S80 and D17S5) in Rosario population, Santa Fe Argentine. *Forensic Science International*, 141: 185-87.
- Thin L, G Macquillan, L Adams, G Garas, C Seow, P Cannell, B Augustson, A Mitchell, L Delriveire and G Jeffrey, 2009. Acute graft-versus-host disease after liver transplant: novel use of etanercept and the role of tumor necrosis factor alpha inhibitors. *Liver Transpl*, 15: 421-426.
- Trivedi R, P Chattopadhyay, R Dutta, N Sarkar, AK Sharma and VK Kashayp, 2002. D1S80 population data in eight predominant populations of India. *J. Forensic Sci.*, 47: 676-677.
- Vallinoto IMVC, ACR Vallinoto, CMD Valente and JF Guerreiro, 2003. Allele frequency distributions of six hypervariable loci (D1S80, APOB, D4S43, vW1, F13A and DYS19) in two African-Brazilian communities from the Amazon region. *Genetics and Molecular Biology*, 26: 235-240.
- Verbenko DA, TV Kekeeva, TV Pogoda, EK Khusnutdinova, AI Mikulich, SA Kravchenko, LA Livshits, NA Bebyakova and SA Limborska, 2003. Allele frequencies for D1S80 (pMCT118) locus in some East European populations. *J. Forensic Sci.*, 48: 207-208.
- Verbenko DA, PA Slominsky, VA Spitsyn, NA Bebyakova, EK Khusnutdinova, AI Mikulich, LA Tarskaia, MV Sorensen, VP Ivanov, LV Bets and SA Limborska, 2006. Polymorphisms at locus D1S80 and other hypervariable regions in the analysis of Eastern European ethnic group relationships. *Annals of Human Biology*, 33: 570-584.
- Walsh PS, DA Metzger and R Higuchi, 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, 10: 506-513.
- Walsh SJ and C Eckhoff, 2007. Australian Aboriginal population genetics at the D1S80 VNTR locus. *Ann. Hum. Biol.*, 34: 557-565.
- Waterhouse M, R Kunzmann, M Torres, H Bertz and J Finke, 2013. An internal validation approach and quality control on hematopoietic chimerism testing after allogeneic hematopoietic cell transplantation. *Clin. Chem. Lab. Med.*, 51: 363-369.
- Yunis JJ, O Garcia, I Uriarte and EJ Yunis, 2001. Population data on D16S539, D7S820, D13S317, LPL, F13B and D1S80 loci in a sample of Caucasian-Mestizos from Colombia. *Forensic Science International*, 115: 117-118.