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Original Article

STR Data for the AmpFlSTR SGM Plus Loci From Three Ethnic Populations of Bangladesh and Their Genetic Affinities With Other Populations

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ABSTRACT: Allele frequency distribution of 10 autosomal STR loci, D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, and FGA was studied in three ethnic populations such as, Garo, Marma and Rakhains living in different parts of Bangladesh. There were no significant deviations from Hardy–Weinberg equilibrium in any of the loci after Bonferroni correction ($p < 0.005$) in the studied populations. Forensic efficiency parameters like observed and expected heterozygosity (Ho and He), power of discrimination (PD), power of exclusion (PE), probability of match (PM) and typical paternity index (TPI) were studied for these loci. The combined power of discrimination and combined power of exclusion were >0.9999999999 and >0.9995 respectively in all the populations. A neighbor-joining tree constructed based on pair-wise Nei's genetic distance by comparing allele frequencies for the 10 STR loci with two other ethnic populations and mainstream Bangladeshi population is also presented.

Key words: Short tandem repeats, Allele frequency, Heterozygosity, Power of discrimination, Neighbor-joining tree.

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INTRODUCTION

For centuries, Bangladesh has been the dwelling place of different ethnic groups. The mainstream Bengalis are ethnically homogeneous and comprise 98% of the total Bangladeshi population. The remainders are mostly ethnic minorities living in different pockets of the hilly zones and some areas of the plain lands of the country. The Garos are distributed in the north-eastern districts of Bangladesh such as, Mymensingh, Tangail, Netrokona, Sherpur, Jamalpur, and Sylhet. Both Murmas and Rakhains are distributed in different areas of Chittagong hill tracts and descendants of Arakanese (Figure 1). They are believed to have migrated from Arakan kingdom of Burma (Myanmar) to Bangladesh during 14th to 17th century [1].

Microsatellites, or short tandem repeats (STRs) have been widely used for inferring human population history [2, 3], determining relationships among continental as well as between geographically contiguous populations by many researchers [4-6]. The relative ease of determination, shorter amplicon size, multiplexing capability and high discriminatory power have made these loci a valuable tools for both personal identification and parentage testing [7]. Besides forensic applications differentiation among closely related populations

or, even, subpopulations has been facilitated by the high mutation rate of these microsatellite markers [8]. In this study, we report the allele frequency data from three ethnic populations such as, Garo, Marma and Rakhain and their genetic affinities with the mainstream Bengali and two other ethnic population of Bangladesh based on 10 autosomal STR loci included in AmpF*l*STR® SGM Plus™ PCR amplification kit.

Figure 1. The map of Bangladesh showing the locations of various populations is included in this study (shown in red circles).

MATERIALS AND METHODS

Samples and DNA extraction

Liquid blood samples were collected from randomly selected 75 Garo, 104 Marma and 85 Rakhain individuals living in different parts of Bangladesh, following procedures that are in accordance with Helsinki declaration revised in 1983. Genomic DNA was extracted using Chelex-100 method [9]. Extracted DNA was quantified by NanoDrop-1000 (NanoDrop Technologies, Inc, Wilmington DE 19810, USA).

PCR amplification

Approximately 1-2 ng of template DNA was used for each PCR amplification process. Ten autsomal STR loci (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433 TH01, and FGA) were coamplified by using AmpF*l*STR® SGM Plus™ PCR amplification kit. The PCR reaction was carried out in a GenAmp PCR sysem 2720 (Applied Biosystems, USA). Thermal cycling parameters were setup according to the manufacturer's protocol.

STR typing

The PCR amplified products were separated on ABI Prism 3100-avnt Genetic Analyzer (Applied Biosystems) using POP-4 polymer and data collection software v3.7. Data were sized using GeneScan software v3.7 and ROX-500 internal size standard. Genotypes were assigned by Genotyper software v3.7 NT after comparison with allelic ladder.

Data analysis

Allele frequencies at each locus and other statistical parameters of forensic efficiency were calculated by using PowerStat Microsoft Excel Workbook v1.2 [10]. The hardy-Weinberg equilibrium check and calculation of observed and expected heterozygosity were performed by Arlequin software v3.11 [11]. Pairwise genetic distance between populations was calculated using Nei's formula in PHYLIP software v3.6 [12].

Quality control

Positive control DNA and allelic ladder provided in AmpF*l*STR® SGM Plus™ PCR amplification kit served as a means of quality control. Approximately 10% of samples from each population were genotyped twice to further ensure the reproducibility and accuracy of the result. All genotypes were in full concordance.

RESULTS AND DISCUSSION

The allele frequency distribution of Garo, Marma and Rakhain populations are presented in Tables 1-3. All the loci were in Hardy-Weinberg equilibrium except D3S1358 in both Garo and Rakhain, D21S11 in Marma, and, D2S1338 and TH01 in Rakhain. However, after Bonferroni's correction for the number of loci analyzed no departure was observed ($p = <0.05/10 = 0.005$) [13]. A number of variant alleles were observed at FGA locus in Garo (24.2), Rakhain (22.2 and 23.2) and Marma (22.2, 23.2, 24.2 and 25.2) population. Only one variant allele 23 was observed at D21S11 locus.

The forensic efficiency parameters calculated for all the populations are summarized in Table 4. Among all the populations studied both Garo and Murma showed least polymorphism at TH01 locus (PIC <0.6). Lack of admixture and inbreeding, which is a characteristic feature of these populations, might be the reason. The most frequent allele was allele 9 at TH01 locus in all the population studied. This finding was concordant with a study conducted on Chakma, Marma and Tripura by a different group [13]. The TH01 locus is one of the most widely used forensic DNA

Table 1. Allele frequency distribution of 10 autosomal STR loci in Garo population (n = 75)

Allele	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
6									0.0400	
7									0.2733	
8			0.0600						0.0267	
9			0.1400					0.0133	0.5467	
9.3									0.0933	
10			0.1533		0.0800				0.0200	
11			0.3133		0.0733			0.0133		
12			0.1733		0.0733		0.0333	0.0400		
12.2								0.0067		
13		0.0067	0.1400		0.1200		0.2133	0.3400		
13.2								0.0133		
14	0.0400	0.1067	0.0200		0.2667		0.1667	0.1933		
14.2								0.1000		
15	0.2333	0.0067			0.2533		0.2133	0.0067		
15.2								0.2400		
16	0.4467	0.2000		0.0067	0.1267		0.1867			
16.2								0.0333		
17	0.2467	0.3933		0.1133	0.0067		0.0200			
18	0.0333	0.1867		0.1200			0.0200			0.0733
19		0.0933		0.2067			0.0933			0.0800
20		0.0067		0.0333			0.0333			0.0733
21				0.0067						0.1600
22				0.0400			0.0200			0.1667
22.2										
23				0.1267						0.0800
23.2										
24				0.2733						0.1533
24.2										0.0133
25				0.0467						0.0733
26				0.0067						0.0267
26.2										0.0267
27				0.0200		0.0067				0.0533
28						0.0600				0.0133
29						0.3533				
30						0.1933				0.0067
30.2						0.0200				
31						0.0600				
31.2						0.1067				
32						0.0267				
32.2						0.1133				
33.2						0.0600				

markers and is the mandatory locus in many forensic databases (e.g. CODIS in the US, DAD in Germany and UK Forensic Database in Great Britain) [14]. The tetrameric STR at the TH01 locus located in the intron 1 of the *tyrosine hydroxylase* (*TH*) gene acts as the binding motif for regulatory factors involved in the expression of the gene. Besides being a forensically important genetic marker, TH01 has also been shown to be associated with psychiatric disorders, hypertension and infant death syndrome [15-18]. Conclusion about the association of this maker with a particular disease however will require further studies including clinical data of these population subjects.

The combined power of exclusion (PE) for Garo, Marma and Rakhain were 0.99996, 0.99992 and 0.9995 respectively. The combined probability of match (PM) for all the three population were between 1.0×10^{-11} - 1.0×10^{-12}. The 10 loci therefore showed a very high forensic efficiency and proved to be valuable for differentiating individuals in these populations. Although all the populations achieved a PM in the order of 10^{11} to 10^{12} a 'θ' correction would be reasonable while using this data in forensic situations [19].

A neighbor-joining tree was constructed based on pairwise Nei's genetic distance by comparing the allele frequencies for the 10 autosomal STR loci from the populations under study, and data from two other ethnic populations living in Bangladesh and mainstream Bengali population data published elsewhere from this laboratory [20 21 22]. Our results from the pair-wise F_{ST} suggest that "Garo" and

Table 2. Allele frequency distribution of 10 autosomal STR loci in Marma population (n = 104)

Allele	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
5										
6									0.0769	
7									0.1346	
8					0.0144				0.0385	
9			0.2067					0.0048	0.6875	
9.3									0.0337	
10			0.1298		0.0913				0.0288	
11			0.2740		0.0481					
12			0.2788		0.0577		0.1202	0.0433		
12.2								0.0048		
13			0.1058		0.2740		0.1250	0.2308		
13.2								0.0096		
14	0.1779	0.2115	0.0048		0.1923		0.2356	0.2260		
14.2								0.2356		
15	0.2404	0.0192			0.2356		0.1538	0.0962		
15.2								0.1010		
16	0.3413	0.1827			0.0769		0.0962	0.0337		
16.2								0.0048		
17	0.1490	0.2596		0.0144			0.1154	0.0096		
18	0.0817	0.1731		0.0769	0.0048		0.0048			
19	0.0096	0.1298		0.2115	0.0048		0.0529			0.0433
20		0.0240		0.1875			0.0337			0.0433
21				0.0337			0.0481			0.1250
21.2										
22				0.0337			0.0048			0.1587
22.2										0.0192
23				0.3173		0.0048	0.0048			0.1587
23.2										0.0048
24				0.1154			0.0048			0.2644
24.2										0.0288
25				0.0096						0.0769
25.2										0.0048
26										0.0673
26.2										0.0048
28						0.0192				
28.2						0.0048				
29						0.1875				
29.2										
30						0.3462				
30.2						0.0288				
31						0.0769				
31.2						0.0817				
32						0.0096				
32.2						0.1683				
33										
33.2						0.0721				

"Murma" are most distantly related populations (F_{ST} = 0.02366). Mainstream Bengalis and "Garo" are diverging populations having an F_{ST} value of 0.02165 (Figure 2). Bengali and Murma populations are also diverging populations with an F_{ST} value of 0.02022. Among the entire query populations in this case study, "Garo" population is mostly diverse populations having very higher F_{ST} values on an average. The pair-wise F_{ST} values also suggest that "Tripura and Chakma" populations are most closely related (F_{ST} = 0.00529) or less diverged populations.

To visualize the relationships among the six populations, the MDS plot, derived from a distance matrix of Fst, is shown in Figure 3. The Chakma and Tripura populations are clustered

Table 3. Allele frequency distribution of 10 autosomal STR loci in Rakhain population (n = 85)

Allele	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
5									0.0059	
6									0.1235	
7									0.1529	
8			0.0353		0.0059				0.1000	
9			0.1941		0.0235				0.4412	
9.3									0.1412	
10			0.2294		0.1176		0.0118		0.0353	
11			0.2353		0.0529					
12			0.2059		0.0765		0.0765	0.0706		
13	0.0059		0.0941		0.3118		0.1059	0.3176		
13.2								0.0235		
14	0.0529	0.1706	0.0059		0.2353		0.2118	0.2706		
14.2								0.1235		
15	0.3294	0.0353			0.0941		0.2235	0.1000		
15.2								0.0647		
16	0.3471	0.1882			0.0824		0.1529	0.0118		
16.2								0.0118		
17	0.1588	0.2471		0.0706			0.0706			
17.2								0.0059		
18	0.1059	0.2529		0.1706			0.0412			0.0118
19		0.1059		0.1294			0.0412			0.0647
20				0.2000			0.0235			0.0706
21				0.0294			0.0176			0.1294
22				0.0529			0.0176			0.2176
22.2										0.0059
23				0.1765			0.0059			0.1353
23.2										0.0294
24				0.1294						0.2000
25				0.0412						0.0824
26										0.0471
28						0.0824				0.0059
29						0.2412				
29.2						0.0059				
30						0.2941				
30.2						0.0294				
31						0.0353				
31.2						0.0765				
32						0.0059				
32.2						0.1647				
33.2						0.0647				
34										

Table 4. Forensic efficiency parameters of 10 autosomal STR loci in Garo, Marma and Rakhain populations

	Garo (n = 75)										
	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	TH01	FGA	Overall
Ho	0.85915	0.78873	0.78873	0.84507	0.85915	0.8169	0.83099	0.78873	0.59155	0.91549	-
He	0.69583	0.76186	0.81271	0.83928	0.8222	0.8178	0.83988	0.78234	0.62601	0.89571	-
PIC	0.6369	0.7214	0.7807	0.8139	0.7928	0.7906	0.8126	0.7454	0.5701	0.8793	-
TPI	3.550	2.367	2.367	3.227	3.550	2.731	2.958	2.367	1.224	5.917	
PM	0.2192	0.1022	0.0744	0.0542	0.0649	0.0676	0.0538	0.0915	0.1760	0.0331	1.13×10^{-11}
PD	0.7808	0.8978	0.9256	0.9458	0.9351	0.9324	0.9462	0.9085	0.8240	0.9669	0.99999999998
PE	0.7130	0.5783	0.5783	0.6852	0.7130	0.6308	0.6578	0.5783	0.2809	0.8272	0.99996
p-value	0.0207	0.7165	0.2458	0.7386	0.8118	0.3964	0.7699	0.4803	0.8475	0.6546	-

**Garo population

Murma (n = 104)											
	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	TH01	FGA	Overall
Ho	0.7596	0.7885	0.7885	0.8173	0.7981	0.7596	0.8654	0.8654	0.5000	0.8942	-
He	0.7688	0.8106	0.7801	0.8015	0.8162	0.8014	0.8660	0.8214	0.5023	0.8528	-
PIC	0.7283	0.7782	0.7403	0.7702	0.7872	0.7731	0.8472	0.7929	0.4735	0.8322	-
TPI	2.080	2.364	2.364	2.737	2.476	2.080	3.714	3.714	1.000	4.727	-
PM	0.0954	0.0756	0.0919	0.0751	0.0632	0.0719	0.0407	0.0660	0.2814	0.0503	8.60×10^{-12}
PD	0.9046	0.9244	0.9081	0.9249	0.9368	0.9281	0.9593	0.9340	0.7186	0.9497	0.999999999991
PE	0.5264	0.5778	0.5778	0.6315	0.5955	0.5264	0.7254	0.7254	0.1875	0.7836	0.99992
p-value	0.3843	0.0923	0.8729	0.4109	0.7059	0.0421	0.7826	0.8950	0.4513	0.4618	-

**Murma population

Rakhain (n = 85)											
	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	TH01	FGA	Overall
Ho	0.6235	0.7412	0.8353	0.7647	0.8000	0.8118	0.7765	0.6588	0.6353	0.8588	-
He	0.7361	0.8027	0.8065	0.8610	0.8135	0.8140	0.8601	0.7953	0.7399	0.8634	-
PIC	0.6869	0.7672	0.7716	0.8392	0.7850	0.7845	0.8392	0.7627	0.7047	0.8428	-
TPI	1.328	1.932	3.036	2.125	2.500	2.656	2.237	1.466	1.371	3.542	-
PM	0.1161	0.0754	0.0788	0.0453	0.0641	0.0660	0.0436	0.0718	0.1109	0.0455	2.08×10^{-12}
PD	0.8839	0.9246	0.9212	0.9547	0.9359	0.9340	0.9564	0.9282	0.8891	0.9545	0.999999999997
PE	0.3201	0.4948	0.6661	0.5353	0.5990	0.6211	0.5561	0.3675	0.3354	0.7123	0.99956
p-value	0.0147	0.1402	0.7208	0.0475	0.7296	0.8887	0.0968	0.0684	0.0264	0.1826	-

**Rakhain population

together in the lower left corner of the MDS, indicating a close genetic relationship among these two populations. On the other hand Garo and Murma populations are presented at the mostly distant regions of the MDS plot indicating that

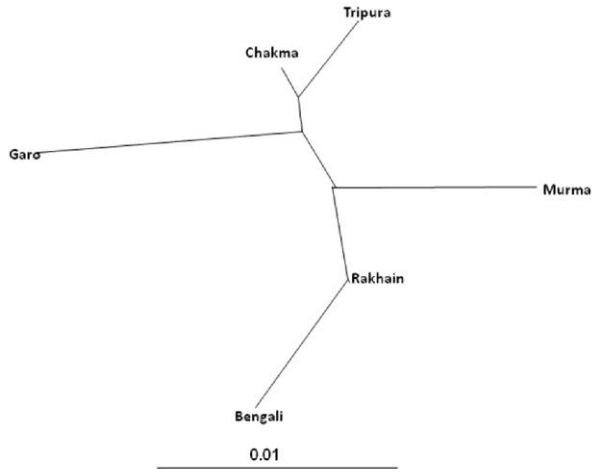

Figure 2. Phylogenetic analysis of the six populations based on genetic distance for the 10 autosomal STR loci in six populations. The GENDIST option of thePHYLIP software was used to create branch distances. This program computes genetic distance from a set of gene frequencies in different populations (or species). We used Nei's genetic distance (Nei, 1972) to formulate the phylogenetic tree.

these two populations are completely separate and do not share any genetic relationship. The ethnic group Rakhain is placed in the upper right side box of the MDS plot with the mainstream Bengali population, but is not clustered together in a close relationship. The stress value is within the limits identified by Sturrock and Rocha [23].

Among the populations studied, the Chakma, Marma and Rakahins believed to have migrated to Bangladesh from the Arakan kingdom of Myanmar [1]. The Tripura and Garos on the other hand live in different pockets of both in Bangladesh and India and believed to have descended from western and northeastern China [24].

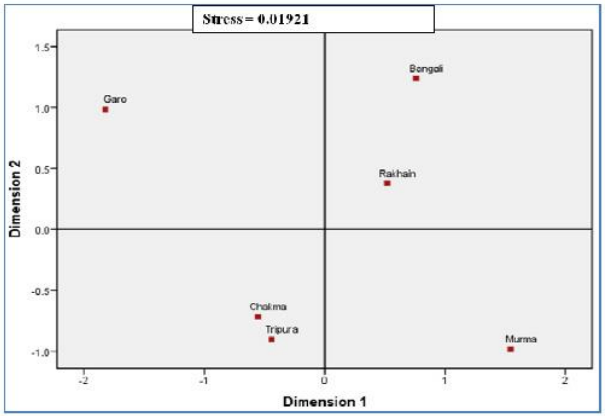

Figure 3. Multidimensional scaling plot of studied populations based on genetic distances among populations (Fst) [Stress value: 0.01921].

Interestingly, all the five populations except the mainstream Bengalis speak branches of Tibeto-Burman language family originated in China [24]. However this study failed to reveal any strong genetic grouping attributable to geographic, linguistic, or socio-cultural affiliation using these ten STR markers. This may be due to genetic drift, lack of gene flow and isolation experienced by the populations that follow highly endogamous practices. Selection bias of highly polymorphic forensic microsatellite markers might be another reason. Hatzikotoulas et al describes the

application of GWAS in achieving population attributable grouping genetic markers for isolated populations [25]. Further studies employing large number of microsatellite or SNPs may provide a better resolution to explain the genetic structure of these populations.

In conclusion, this 10 autosomal STR system offers a considerable number of polymorphic patterns among different populations from Bangladesh. The distributions of the allelic frequencies at certain loci differ between different population groups. The genetic distance between populations estimated and the phylogenetic tree constructed according to this system are very informative. It may be useful in forensic identification analysis and ethno-geographic research.

Conflict of Interest

The authors declare that they have no conflicts of interest in publishing this manuscript.

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