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Genetic Analysis of Y-Chromosome 17 STR in Four Indigenous Populations from Bandarban

Gazi Nurun Nahar Sultana [*, a], ***Rokeya Begum*** [a], ***M. Asaduzzaman*** [a], ***Tanvir Ahmed*** [b], ***Monira Akhtar*** [c]

a Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh. 2. 3.
b Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh
c Dristi, Social welfare Non-Government Organization, Dhaka-1000, Bangladesh

* Corresponding author. E-mail: nngazi@gmail.com

Abstract Despite rapidly growing understandings and dependency on single nucleotide polymorphisms (SNPs), highly variable autosomal 17 Y-chromosome short tandem repeats (STR) are still regarded as the most established method to differentiate individuals. Ethnic and cultural diversity of Bandarban area throughout the Chittagong Hill Tract (CHT) suggests that this hilly range play vital role in genetic evolution of the region. Our previous study suggests that this mountain area acted as a corridor to gene flow across the Indian midland to CHT of Bangladesh. In the present study, we analyzed 17 Ychromosomal short tandem repeat (Y-STR) haplotypes to investigate the Y-STR diversity of four indigenous populations from Bandarban. This study included 55 unrelated male samples from four ethnic populations (Tanchangya, Khumi, Khyang and Mro) were analyzed, among which 41 were unique and 14 Y-STR profiles are shared across the four populations. Khumi and Khyang exhibit relatively high degree of genetic homogeneity lower than 0.5, whereas Tanchangya and Mro represent the other extreme with all loci registering values above 0.5 for the same parameter.

Keywords: Forensic science, CHT indigenous Population, Bandarban, 17 Y-STR, Allelic frequencies, Gene diversity.

1 Introduction

Chittagong Hill Tracts (CHT) is located Southeastern part of Bangladesh and is surrounded by Mizoram state of India and Arakan of Myanmar in the East. The CHT area covers approximately 13.3 thousand km2 of three hill districts (Rangamati, Khagrachori and Bandarban) which indicates about 10% of land area in Bangladesh. Of these three areas, Bandarban is hilly but smaller in land area. There are 11 indigenous populations living in Bandarban (Tanchangya, Khumi, Khyang, Mro, Chak, Baum, Lusai, Pankhua, Chakma, Marma and Tripura).[1] The largest group is Chakma, Tripura, Marma, Tanchangya, and Mro which together make up to 90 percent of the indigenous population of the region.[2] Main tribal populations of Bangladesh Chakma, Marma, and Tripura, Khumi, Mro and Khyang are Tibeto-Burman speakers except Tanchangya which is Indo-European speaking tribe. Rest of the groups is less in percentage which indicates that the smaller groups are, overall, more vulnerable than the larger groups. Therefore it is essential to understand the origin and genetic diversity of these male population using uniparental (Ychromosome STR) markers.

A Y-STR is a short tandem repeat on the Ychromosome. Y-STR are often use for forensic, paternity and genealogical DNA testing. Unlike other chromosomes, Y-chromosomes do not come in pairs. Every human male has only one copy of that chromosome and there is no chance of variations of which copy is inherited, and also for not any shuffling between copies by recombination; so unlike autosomal haplotype, there is effectively not any randomization of the Y-chromosome haplotype between generations.[3] The human male should largely share the same Ychromosome as his father; gives or take few mutations. Thus Y-chromosome tends to pass largely intact from father to son, with limited but accumulated number of mutations that can serve to differentiate male lineage.[4-5]

Arlequin v3.2 and PowerStatV12 were used for calculating haplotype frequencies, matching probabilities,

and performing comparative population genetic analyses.[6-8] The global repository Y-Chromosome Haplotype Reference Database (YHRD) currently supports most frequently used haplotype formats, namely, nine-locus minimal (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS385a/b), SWGDAM-recommended 11-locus extended (minimal + DYS438 and DYS439), 12-locus PowerPlex (SWGDAM + DYS437), and 17-locus Yfiler haplotypes for comparison study.[9]

Our previous studies of Y-chromosomal biallelic[10] and autosomal STR polymorphisms of Tibeto-Burman revealed that these groups arrived in the CHT area during the Neolithic time and strong affinity to Northeast Indian Tibeto-Burman group.[11]

In this study, we have typed four (4) indigenous populations collected from Bandarban of CHT. Tanchangya (19), Khumi (10), Khyang (11), Mro (15), total of fifty five (55) samples were analyzed for 17 Ychromosome short tandem repeat (STR) loci.

2 Materials and Methods

Sample Collection

Blood samples were collected from those healthy male individuals, following procedure Helsinki revised declaration of 1983.[12]

DNA Extraction and PCR Amplification

DNA was extracted[13] and quantified by NanoDrop-100 (Thermo Fisher Scientific, USA). Amplification of the 17 Y-STR loci was performed using AmpFlSTR® Y filer ™ PCR amplification kit. PCR amplification was performed in Bio-Rad C1000 thermal Cycler (Life Science Research, 2000 Alfred Nobel Drive Hercules, CA 94547) according to the manufacturer's recommendations. The PCR amplified products were separated by capillary electrophoresis on ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Data analysis

The results were analyzed using GeneMapper v3.2.1. Alleles were designated according to the recommendations for the DNA commission of the International Society of Forensic Genetics (ISFG) guidelines for forensic STR analysis.[14] Arlequin v3.2 and PowerStat v12 were used for calculating allelic frequency, genetic diversity, haplotype diversity, and discrimination capacity, Neighbor joining (NJ) phylogenetic tree was constructed by Phylip 3.69 by using allelic frequency of studied population and five Indian tribal populations.[15]

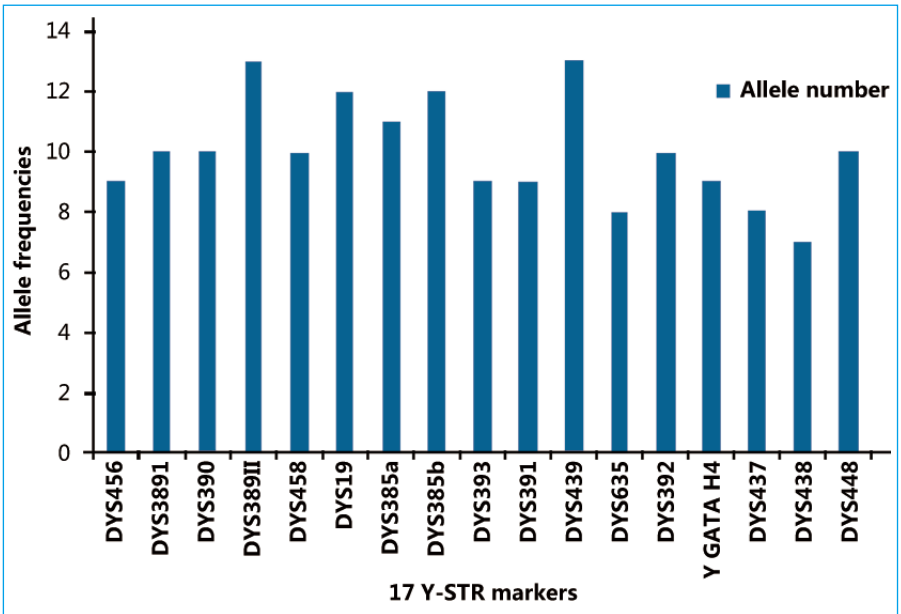

Figure 1. Allele information in four indigenous populations of Bandarban.

3 Results

Gene diversity and allelic frequencies for the 17 YSTR loci analyzed for 4 different Bandarban CHT collections are listed in Supplementary 1 (S1-S4). Markers DSY389II and DYS439 both are equally the most informative loci. The least discriminating and informative locus is the DSY438 (Figure 1). As expected, Mro possesses the highest average gene diversity (0.6325) followed by Tanchangya (0.5890), Khyang (0.2103) and Khumi (0.1266) respectively.

A total of 48 haplotypes for the

Table 1. Parameters of forensic interest in Bandarban populations using 17 Y filer Haplotypes.

Haplotypes	Tanchangya	Mro	Khyang	Khumi	Total
Sample size	19	15	11	10	55
No. of unique haplotypes (n=1)	13	15	7	6	41
No. of different haplotypes (n=2)	3	0	2	2	7
Genetic Diversity	0.5890	0.6325	0.2102	0.1266	0.3895
Discrimination Capacity	0.5692	0.5733	0.2281	0.2101	0.8727
Haplotype Diversity	0.9625	0.00	0.9166	0.8928	0.99069
Matching probability	0.0833	0.00	0.0375	0.1071	0.009309

17 Y-STR markers were identified in 55 unrelated four Bandarban tribal populations – Tanchangya, Mro, Khyang and Khumi. Among 41 (75.53%) haplotypes were unique and 7 were found in 2 individuals (Table 1). Two null alleles were detected at loci DYS439, one in Khumi and other in Khyang.

The haplotype diversity determined in Bandarban collections at 17 Y-STR loci was 0.99069 while the corresponding higher and minimal haplotypes were 0.9625 and 0.8928 in Tanchangya and Khumi respectively. The genetic homogeneity in Khumi is also reflected in their reduced average gene diversity (0.1266). In addition discrimination capacities in Khumi and Khyang are lower than that of Tanchangya and Mro (Table 1). The haplotype matching probability in all four groups determined was 0.009309. The Khumi shows the highest maximum match probability (0.1071) followed by Khyang 0.0833 and Tanchangya 0.0375, whereas Mro shows the unique match probability respectively.

Phylogenetic relationship between the four Bandarban populations and other neighboring populations were assessed using NJ tree (Figure 2). The genetic similarities observed in three populations Mro, Khyang and Khumi based on their Y-STR loci and are reflected in the high frequencies of Y haplogroup (O3a3c) as observed in our previous study.[11]

4 Discussion

DNA samples from 55 healthy unrelated male individuals of four indigenous community of Bandarban were analyzed. A total of 41 unique haplotypes were identified among 55 individuals. From this study it is found that in Bandarban populations' marker DSY389II and DYS439 both are equally the most informative loci. The least discriminating and informative locus is the DSY438. This finding is different from other study done with mainstream Bengali population[16] of Bangladesh. Therefore, this data reveals that 17 Y-STR might differentiate the indigenous people from mainstream population. The values of combined Matching Probability (MP), probability of discrimination (PD) and exclusion indicates that these results have enriched the databases of 17 Y-STR loci for four indigenous populations of Bandarban and exposed as an excellent tool for male human identification tests and population genetic analysis.

The significance increase in the proportion of unique haplotypes using 17 Y-STR markers compared to the minimal haplotype reflects the power of discrimination at different loci. The overall diversity of Bandarban population was 0.9906 while the corresponding values for the extended

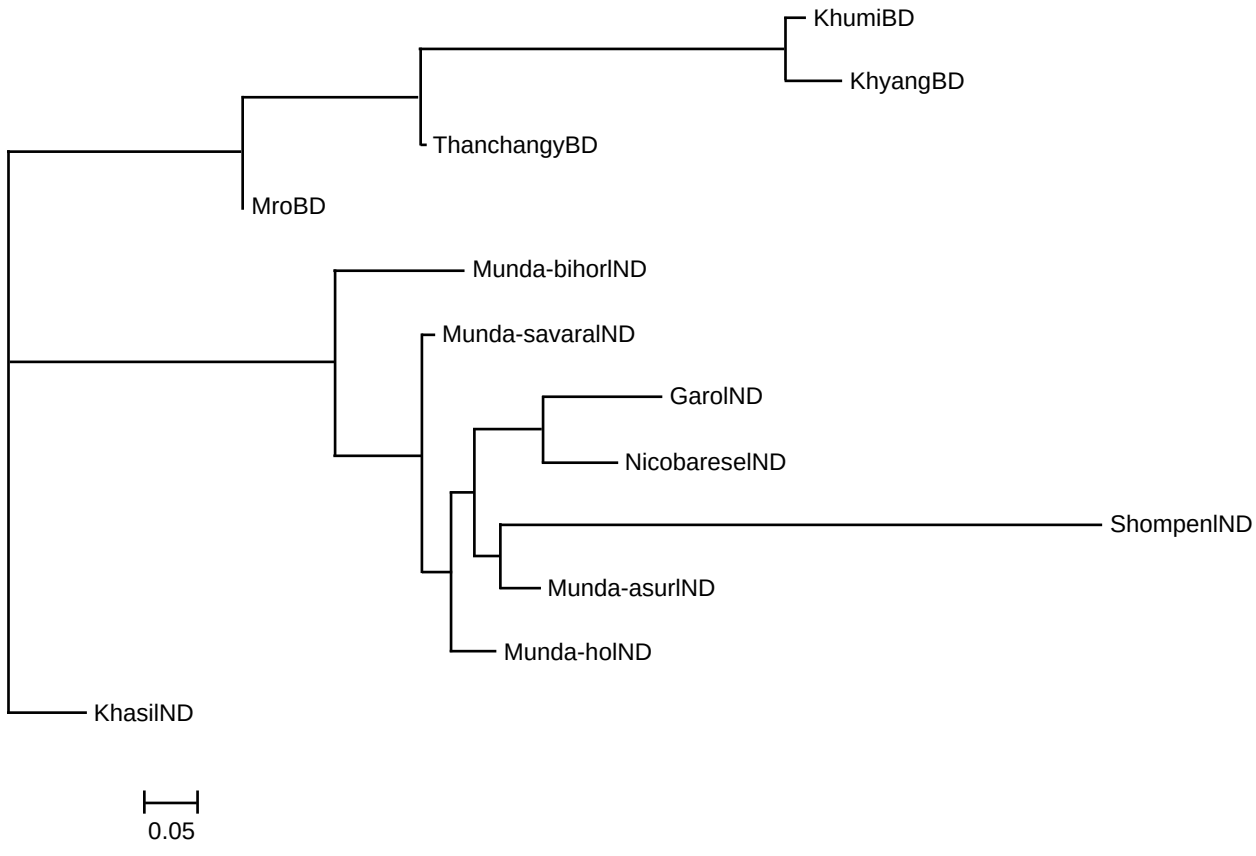

Figure 2. The NJ tree of four Bandarban collection compared with Indian population..

and minimal haplotypes were 0.9625 for Tanchangya and 0.8928 for khumi respectively (Table 1). The relatively lower diversity for Khyang (0.9166) and Khumi (0.8928) may be attributed to the reduced heterogeneity observed in these populations, which in turn may be the results of founder effects in this case. The only Indo-European speaking tribe Tanchangya has higher haplotype diversity than the Tibeto-Burman collection due to higher heterogeneity. The haplotype diversity for Mro is found zero (0.000) due to unique haplotype for each studied individual.

The NJ tree revealed that the Bandarban tribes (Khyang and Khumi) are more close within the populations but distant from Mro and Tanchangya but are distant from South and North Indian tribes[17] (Munda, Khasi, Shompen, Garo and Nicobarese). The unpublished data on autosomal STR depicted that these tribes are more related to East and Southeast Asian population and it is supported by their phenotypic traits thus implying their recent dispersal from Southeast Asia followed by admixture with local Tibeto-Burman populations. However Tanchangya is especially different in terms of language and autosomal STR reported in previous study.

5 Conclusion

In conclusion this study, 17 Y-STR database along with other database have been enlarged for population of Bangladesh and this can contribute considerably for individual identification and further research in population genetics. This is so far the first report on Bandarban indigenous population based on Y-STR analysis.

From the result of this study it can be concluded that, 17 Y-STR might be capable of differentiate tribal male from mainstream male of Bangladeshi populations. This 17 Y-STR also differentiate Indo-European speaking tribes from Tibeto-Burman tribes, which was previously found by autosomal STR data. Therefore, it can be concluded that this 17 Y-STR might play an important role to understand the genetic structure of indigenous population of Bandarban as well as CHT of Bangladesh. To confirm this hypothesis, increasing substantial number of samples and huge information needs to be gathered.

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