Genetic diversity at 15 microsatellite loci among the Adi Pasi population of Adi tribal cluster in Arunachal Pradesh, India

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Abstract

Genetic polymorphisms at 15 tetrameric short tandem repeat (STR) loci were studied in 203 healthy individuals of Adi Pasi population from Arunachal Pradesh, India. All the loci analyzed were highly polymorphic and there was no significant deviation from the Hardy–Weinberg equilibrium (HWE) excepting D8S1179 and D18S51. Other forensic useful statistical parameters were also calculated and the 15 microsatellite markers selected for this study were found to be suitable for human identification and population genetic studies.

Keywords: Short tandem repeats (STR); Arunachal Pradesh; AmpF*l* STR® Identifiler™; Allele frequency; Forensic science; Population genetics

1. Population

The present study reports the allele frequencies of 15 microsatellite loci among Adi Pasi, one of the sub-tribes of Adi tribe of Arunachal Pradesh, located in the northeastern parts of India bordering with Bhutan to the west, China to the north and northeast and Myanmar to the east [1]. Adi is one of the major tribe that belongs to Mongoloid ethnicity and Tibeto-Burman linguistic family [2,3]. The total population of this tribe is estimated to be 0.236 million [4]. The ethno history suggests their origin from Tibet (China) and the migration and settlement of their ancestors at different time periods at different locations at about 5th–7th century AD [5]. There are about 12 sub-tribes of Adi tribal cluster categorized under two major groups. These are distinguished based on their linguistic diversity, culture, ethno-historical migration and distribution on the Siang river valley. One cluster consists of the sub-tribes: Minyong, Padam, Shimong, Milan, Pasi, Panggi and Komkar and the other cluster includes: Gallong, Ramo, Bokar, Pailobo and Bori sub-tribes [5]. Of these 12 sub-tribes, Minyong and Padam are the largest, the rest being small (in thousands).

The studied population, Adi Pasi is one such small sub-tribe of Adi tribe, numbering about a 3–4 thousand individuals and are mostly confined to about 12 villages located at two major locations at different altitudes (lower and higher) on the mountain ranges on either side of Siang river in East Siang and Upper Siang districts of the state. This population offers significant opportunity for genetic studies due to their relative isolation, small population size, and transitional stage of hunting-gathering population structure. However, there were hardly any biological study [6,7], although some anthropological investigations and monographic works have been attempted [8,9]. These studies were sporadic and only a few monographic studies are about a particular Adi sub-tribe [1]. This is perhaps the first attempt to report on the microsatellite diversity among the Adi Pasi sub-tribe.

2. DNA isolation and quantitation

Blood samples were collected with prior informed consent from 203 healthy voluntary donors belonging to the Adi Pasi sub-tribe from seven villages located at lower

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Table 1
Allele frequencies of 15 STR loci in Adi Pasi population of Arunachal Pradesh ($N=406$)

	D5S818	FGA	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	Vwa	TPOX	D18S51
5								0.003							
6						0.003		0.01						0.005	
7					0.008	0.003		0.183						0.028	
8	0.092		0.002		0.179			0.048	0.143	0.02			0.002	0.479	
9	0.188				0.12	0.078		0.6	0.146	0.17		0.005		0.119	
9.2					0.005							0.002			
9.3								0.132							
10	0.088		0.025		0.155	0.179		0.012	0.125	0.381		0.01		0.056	
10.2															0.006
11	0.292		0.02		0.431	0.263			0.339	0.164		0.007	0.005	0.298	
11.2												0.025			
12	0.128		0.103		0.075	0.436		0.012	0.188	0.207		0.035	0.002	0.015	0.023
12.2												0.045			0.003
13	0.205		0.197		0.027	0.035	0.005		0.043	0.045	0.003	0.125			0.392
13.2												0.06			0.036
14	0.002		0.249			0.003	0.01		0.008	0.01		0.23	0.136		0.088
14.2											0.003	0.2			0.003
15	0.005		0.249				0.293		0.008			0.065	0.01		0.196
15.2							0.015				0.006	0.16			
16			0.131				0.415			0.003	0.008	0.005	0.261		0.08
16.2							0.007					0.015			
17			0.005				0.075				0.106	0.002	0.256		0.042
18		0.038	0.005				0.162				0.172	0.003	0.127		0.062
18.2													0.002		
19		0.097	0.012				0.015				0.1	0.003	0.122		0.042
19.2												0.003			
20		0.064	0.002	0.005			0.003				0.092		0.06		0.006
20.2		0.012													
21		0.076									0.089		0.002		0.006
21.2		0.009													
22		0.137									0.014		0.015		0.003
22.2		0.015													
23		0.155									0.136				0.006
23.2		0.032													
24		0.155									0.241				
24.2		0.02													
25		0.143		0.002							0.03				0.003
25.2		0.003													
26		0.029		0.002											0.003
26.2		0.003													
27		0.003		0.069											
28		0.003		0.01											
28.2				0.017											
29				0.251											
29.2				0.01											

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Table 1 (*continued*)

	D5S818	FGA	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	Vwa	TPOX	D18S51
30				0.286											
30.2				0.035											
31				0.06											
31.2				0.037											
32				0.035											
32.2		0.006		0.115											
33				0.022											
33.2				0.037											
34				0.005											
36				0.002											

N, number of chromosomes.

altitude mountain ranges around Pasighat. This study was carried out with the approval of the ethics committee and in accordance with the ethical guidelines of the institutions involved. High molecular weight DNA was isolated using the standard phenol/chloroform method [10]. The extracted DNA was then quantitated using the Quantiblot® Human DNA Quantification Kit (Applied Biosystems, Foster City, CA).

3. PCR and microsatellite typing

Individual DNA samples were amplified for the 15 microsatellite loci: D5S818, FGA, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWa, TPOX, D18S51 using AmpF*l* STR® Identifiler™ Multiplex system (Applied Biosystems, Foster City, CA USA). The amplified products were then separated on a 4% polyacrylamide gel using the ABI Prism 377 automated DNA sequencer (Applied Biosystems, USA). The resultant data analysis was carried out by the Gene scan analysis software (Version 3.7) and the allele designation was done with the Genotyper DNA fragment analysis (Version 3.7) software (Applied Biosystems, Foster City, USA).

4. Results

Allele frequencies of the analyzed markers are represented in Table 1. Other statistical estimates of genetic and forensic interest are shown in Table 2.

5. Statistical analysis

The allele frequencies were calculated using the DNA TYPE software [11]. The proportion of heterozygous individuals at each locus and also the average heterozygosity were estimated to assess the extent and magnitude of diversity. In addition, likelihood ratio test (LR) and the exact test (ET) were performed to test the possible divergence from the HWE [12,13]. The Power of discrimination (PD) was calculated for each locus for validation of the markers and human identification.

6. Remarks

All the loci are highly polymorphic. FGA and D19S433 were both represented by 19 alleles and hence more polymorphic, whereas TPOX (seven alleles) was found to be the least polymorphic of all the loci analyzed. When compared to 11 Indian and two global Mongoloid populations, Adi Pasi exhibits about 16 unique alleles distributed over 10 different STR loci (for example, allele

Table 2
Statistical parameters of genetic and forensic importance deduced from the allele frequencies of 15 STR loci in Adi Pasi population of Arunachal Pradesh, India

Locus	Alleles	*h*	*P*	ET	LR	PD
D5S818	8	0.765	0.156	0.485	0.53	0.8047
FGA	19	0.795	0.000095	0.433	0.717	0.8892
D8S1179	12	0.827	0.488	0.002	0	0.8082
D21S11	18	0.803	0.355	0.643	0.855	0.8274
D7S820	8	0.62	0.0002	0.53	0.754	0.7373
CSF1PO	8	0.67	0.382	0.197	0.18	0.7013
D3S1358	10	0.696	0.698	0.255	0.409	0.7094
THO1	8	0.6	0.697	0.428	0.005	0.5864
D13S317	8	0.799	0.754	0.743	0.839	0.7903
D16S539	8	0.727	0.39	0.032	0.071	0.7536
D2S1338	13	0.755	0.00016	0.177	0.31	0.855
D19S433	19	0.795	0.0187	0.001	0.306	0.8537
Vwa	13	0.815	0.91	0.282	0.066	0.8129
TPOX	7	0.555	0.0014	0.283	0.065	0.6634
D18S51	18	0.68	0.0015	0.001	0.001	0.7844

h, Heterozygosity; *P*, probability of homozygosity; ET, exact test; LR, likelihood ratio test, PD, power of discrimination.

12 at THO1 locus, allele 9.2 at D7S820 locus, allele 20 at D8S1179 locus) [14]. Unique alleles have also been reported in other Indian populations as in Dheria Gond, an Australoid tribe of Madhya Pradesh (allele 27.2 at D21S11 locus) and in Mongoloid populations especially, Buddhist tribes from Ladakh and Lepcha and Bhutia tribes from Sikkim (allele 23.2 at D21S11 and Penta E loci) [15].

The locus TPOX was least heterozygous (55.5%) and the locus D8S1179 was most heterozygous (82.7%) among the studied markers. This range of observed heterozygosity values indicates a high degree of polymorphism for the studied loci and hence their significance in analysis of genetic variations among the populations. The average heterozygosity based on 15 STR loci for the tribe is 0.727. In comparison to other mongoloid populations, Adi Pasi show lower average heterozygosity except with Lepcha and Bhutia of Sikkim (0.680 and 0.713 based on 12 STR loci, respectively) [14]. This lower average heterozygosity of Adi Pasi suggests the influence of population structure variables, especially high rate of endogamy and least admixture.

The power of discrimination calculated for the population displayed high discriminatory power of these markers. The PD values were found to range from 0.5864 (THO1) to 0.8892 (FGA). High PD values thereby facilitate the validation of the used markers and the utility of these markers in human identification.

The exact test and likelihood test values indicate that almost all the loci were in HWE, except D8S1179 ($P=0.002$) and D18S51 ($P=0.001$). Small populations are characterized by high degree of endogamy which might result in excessive homozygosity of a few loci that might in turn lead to departure from HWE. In case of Adi Pasi, due to their remote location and extreme isolation they practice endogamy which might be one of the reasons for the 2 loci to deviate from HWE. Such deviations from HWE have also been observed in other Indian populations as in some caste populations: Gowda of Karnataka (vWA and Penta E loci); Dhangar of Maharashtra, Satnami of Madhya Pradesh and Gounder of Tamil Nadu (D8S1179 locus); and in other tribes: Hmar of Mizoram (FGA locus); Kuki and Balti of Manipur and Ladakh regions, respectively (vWA) [15].

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References

[1] Paul AK. Arunachal—the land of lovely dawn. Kolkata: Good Book Distributors Publishers; 2004.
[2] Grierson GA. Linguistic survey of India.: Registrar General of India; 1927.
[3] Malhotra KC, Vasulu TS. Structure of human populations in India. In: Majumder PP, editor. Human population genetics. New York: Plenum; 1993. p. 207–33.
[4] Census 1991. Scheduled tribes—primary, census abstracts. Part II–B (III), New Delhi; 1996.
[5] Nath J. Cultural heritage of tribal societies. vol. 1 (The Adis). New Delhi: Omsons Publications; 2000.
[6] Adak DK. Variation in anthropometric characters and their relationship with climatic factors: a study on the peoples of Arunachal Pradesh. Anthropol Anz (Germany) 2004;62:445–61.
[7] Chaturvedi HK, Mahanta J. Sociocultural diversity and substance use pattern in Arunachal Pradesh, India. Drug alcohol Depend 2004;74:97–104.
[8] Chowdhury JN. The tribal culture and history of Arunachal Pradesh. New Delhi: Daya Publishing House; 1990.

[9] Roy S. Aspects of Padam Minyong culture. Guwahati: M/S Purbadesh Mudran; 1997.
[10] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor; 1989.
[11] Chakraborty R, King A, Stivers D, Zhong Y. DNATYPE [Windows 95/NT version], CHG. Houston, TX: University of Texas; 1998.
[12] Chakraborty R, Zhong Y. Statistical power of an exact test of Hardy–Weinberg proportions of genotype data at a multi-allelic locus. Hum Hered 1994;44:1–9.
[13] Guo SW, Thompson EA. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 1992;48:361–72.
[14] Krithika S, Trivedi R, Kashyap VK, Bharati P, Vasulu TS. Antiquity, geographic contiguity and genetic affinity among the Mongoloid populations of India: a microsatellite study. Ann Hum Biol. 2005 (in press).
[15] Kashyap VK, Ashma R, Gaikwad S, Sarkar BN, Trivedi R. Deciphering diversity in populations of various linguistic and ethnic affiliations of different geographical regions of India: analysis based on 15 microsatellite markers. J Genet 2004;83:49–63.