RESEARCH ARTICLE

GENETIC ANALYSIS AND EVOLUTIONARY RELATIONSHIP OF JAMMU & KASHMIR MUSLIM POPULATION WITH SHORT TANDEM REPEAT LOCI

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ABSTRACT

In this study, the allele frequencies and statistical parameters of forensic interest were determined for the fifteen short tandem repeat loci included in AmpFlSTR Identifiler (Applied Biosystems) genotyping kit in Jammu & Kashmir (J&K) Muslim population. A total of 150 samples were analyzed for the above said study. All the analyzed loci met Hardy-Weinberg equilibrium expectations. A high combined power of discrimination and high combined power of exclusion was observed for all the loci, indicating them to be a group of excellent markers for paternity testing, identification of individuals and also for other forensic applications. The genetic relatedness analysis revealed the similarity between studied population and previously studied J&K Muslim population.

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INTRODUCTION

Polymorphic Short Tandem Repeat (STR) loci are intensively used as markers for personal identification and paternity testing. The scientific forensic community is interested in compiling data in different populations in order to establish ethnic and geographic allele frequency distributions needed for the forensic casework (Buttler, 2001, Gill *et al*., 1985, Naidu, 2005). Extensive population studies are carried out on STRs in various Indian population groups but only limited data on STR markers in Jammu and Kashmir (J&K) Muslim population is available in the literature so far. The region we casually refer to as Kashmir is fairly large and diverse; it is the Northwestern region of the Indian subcontinent. Geographically, the area has number of natural barriers, and it includes mountains, plains, glaciers, lakes, forested foothills and high mountainous ranges such as the Himalayas and the Karakoram, stretching up to Mount K2, the second highest peak in the world. The people of J&K have proved to be an important subject for forensic research and evolution as well as human migration studies. The state of J&K exhibits huge ethnic and cultural diversities. Kashmiri Muslims, mainly marry within their relations. Yadav *et al* carried out the genetic diversity analysis on J& K

Saraswat Brahmins (Yadav *et al*., 2010). To study the genetic diversity of Muslim population of the state, the study was carried out with 150 unrelated individuals from J&K population. The aim of the study was to report allele frequency distributions for the D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA loci in J&K population samples, as well as statistical evaluations including the possible divergence from Hardy-Weinberg expectations and other forensic parameters of interest. Also, the analysis of genetic diversity of this population was major objective of the study.

MATERIALS AND METHODS

The study was carried out at 150 samples, either in form of liquid blood samples collected in EDTA tubes or blood stains on sterile gauze taken from unrelated individuals involved in criminal and paternity cases submitted in the laboratory for DNA profiling from time to time. DNA isolation from the samples was carried out via organic extraction method (Sambrook *et al*., 1989) and Qiagen DNA extraction kit method. Amplification was done according to the AmpFlSTR Identifiler Kit protocol using GeneAmp 9700 thermal cycler (Applied Biosystems, 2001). Amplified products were analysed by capillary electrophoresis on the 3100 Avant Genetic

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Analyzer using LIZ 500 as Internal Lane DNA standard and employing Applied Biosystems software (Applied Biosystems, 2001). Forensically relevant statistical analyses like Power of Discrimination or Power of Exclusion were calculated using the Powerstats 1.2 worksheet (2009). Exact tests for checking the Hardy-Weinberg expectations were performed using DNATYPE computer program (DNATYPE computer program (Windows 95/NT version. Y. Zhong, CHG, University of Texas)) and it was compared with the previous date. The genetic distance from previously studied populations (Shrivastava *et al*., 2015, Allah Rakha *et al*, 2009, Maity *et al*., 2003, Dubey *et al*., 2009, Ashma and Kashyap, 2002, Gaikwad and Kashyap, 2002, Tandon *et al*., 2002) was calculated by DISPAN software (Ota, 1993) and the Neighbor joining tree was constructed by the Mega 6 software (Tamura *et al*., 2013).

RESULTS AND DISCUSSION

The allelic frequency data at 15 STR loci for the studied population are presented in Table 1. The allele frequencies did not show significant differences in comparison with the other major Indian population data (Chattopadhyay *et al*., 2000, Dutta and Kashyap, 2001, Gaikwad and Kashyap, 2002, Kashyap *et al*., 2002b, Kushwaha *et al*., 2007, Gaikwad and Kashyap, 2003). The results of the statistical tests are shown in Table 1 and Table 2. The Heterozygosity observed in the Muslim Population of state of J&K indicates a high degree of polymorphism for the studied loci. The values vary from 0.607 to 0.882.

Table 1. Allele frequencies of AmpFlSTR loci in Jammu & Kashmir Muslim Population samples (n = 300)

|	CSF1PO	D13S317	D16S539	D18S51	D19S433	D21S11	D2S1338	D3S1358	D5S818	D7S820	D8S1179	FGA	THO1	TPOX	vWA
5	--	--	--	0.006	--	--	--	--	--	--	--	--	--	--	--
6	--	--	--	--	--	--	--	--	--	--	--	--	0.250	--	--
7	--	--	--	--	--	--	--	--	--	--	--	--	0.173	0.006	--
8	--	0.112	0.059	--	--	--	--	--	0.006	0.224		--	0.155	0.298	--
9	0.018	0.065	0.206	--	--	--	--	--	0.060	0.088	0.006	--	0.244	0.125	--
9.3	--	--	--	--	--	--	--	--	--	--	--	--	0.179	--	--
10	0.229	0.082	0.065	0.018	0.006	--	--	--	0.101	0.159	0.167	--	--	0.083	--
11	0.312	0.265	0.265	0.018	--	--	--	--	0.292	0.247	0.107	--	--	0.464	--
12	0.365	0.312	0.229	0.065	0.048	--	--	--	0.351	0.235	0.077	--	--	0.024	--
13	0.059	0.112	0.159	0.161	0.185	--	--	--	0.173	0.047	0.256	--	--	--	--
14	0.018	0.035	0.012	0.256	0.357	--	--	0.042	0.018	--	0.173	--	--	--	0.113
14.2	--	--	--	--	0.065	--	--	--	--	--	--	--	--	--	--
15	--	0.018	0.006	0.137	0.137	--	--	0.333	--	--	0.149	--	--	--	0.077
15.2	--	--	--	--	0.071	--	--	--	--	--	--	--	--	--	--
16	--	--	--	0.137	0.036	--	0.006	0.286	--	--	0.048	--	--	--	0.214
16.2	--	--	--	--	0.054	--	--	--	--	--	--	--	--	--	--
17	--	--	--	0.077	0.042	--	0.088	0.161	--	--	0.018	--	--	--	0.286
18	--	--	--	0.054	--	--	0.141	0.161	--	--	--	0.012	--	--	0.226
19	--	--	--	0.048	--	--	0.141	0.018	--	--	--	0.035	--	--	0.077
20	--	--	--	0.036	--	--	0.176	--	--	--	--	0.153	--	--	0.006
21	--	--	--	--	--	--	0.012	--	--	--	--	0.147	--	--	--
21.2	--	--	--	--	--	--	--	--	--	--	--	0.012	--	--	--
22	--	--	--	--	--	--	0.029	--	--	--	--	0.124	--	--	--
22.2	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--
23	--	--	--	--	--	--	0.188	--	--	--	--	0.224	--	--	--
23.2	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--
24	--	--	--	--	--	--	0.129	--	--	--	--	0.082	--	--	--
24.2	--	--	--	--	--	--	--	--	--	--	--	0.012	--	--	--
25	--	--	--	--	--	--	0.071	--	--	--	--	0.129	--	--	--
26	--	--	--	--	--	--	0.018	--	--	--	--	0.035	--	--	--
27	--	--	--	--	--	0.006	--	--	--	--	--	0.024	--	--	--
28	--	--	--	--	--	0.129	--	--	--	--	--	--	--	--	--
28.2	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--	--
29	--	--	--	--	--	0.206	--	--	--	--	--	--	--	--	--
29.2	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--	--
30	--	--	--	--	--	0.206	--	--	--	--	--	--	--	--	--
30.2	--	--	--	--	--	0.035	--	--	--	--	--	--	--	--	--
31	--	--	--	--	--	0.018	--	--	--	--	--	--	--	--	--
31.2	--	--	--	--	--	0.106	--	--	--	--	--	--	--	--	--
32	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--	--
32.2	--	--	--	--	--	0.218	--	--	--	--	--	--	--	--	--
33.2	--	--	--	--	--	0.059	--	--	--	--	--	--	--	--	--

Table 2. Statistical analysis for HWE and forensic suitability of AmpFlSTR loci in Jammu & Kashmir Muslim Population samples (n = 300)

	CSF1PO	D13S317	D16S539	D18S51	D19S433	D21S11	D2S1338	D3S1358	D5S818	D7S820	D8S1179	FGA	THO1	TPOX	vWA
H_{obs}	0.624	0.847	0.824	0.857	0.774	0.824	0.882	0.738	0.798	0.811	0.774	0.824	0.833	0.607	0.750
H_{exp}	0.717	0.800	0.807	0.861	0.807	0.840	0.868	0.758	0.752	0.803	0.840	0.869	0.797	0.677	0.801
HT	0.057	0.246	0.787	1.000	0.449	0.781	0.751	0.691	0.348	0.883	0.093	0.256	0.429	0.143	0.193
ET	0.433	0.517	**0.003**	0.450	**0.025**	0.125	0.154	0.849	0.732	0.775	**0.040**	0.366	0.421	0.190	0.480
LR	0.541	0.722	**0.001**	0.546	**0.009**	0.131	0.124	0.782	0.635	0.800	0.088	0.419	0.299	0.410	0.574
PM	0.126	0.091	0.087	0.042	0.069	0.058	0.046	0.101	0.115	0.078	0.060	0.040	0.087	0.162	0.073
PD	0.874	0.909	0.913	0.958	0.931	0.942	0.954	0.899	0.885	0.922	0.940	0.960	0.913	0.838	0.927
PIC	0.66	0.77	0.77	0.84	0.78	0.81	0.85	0.71	0.71	0.77	0.81	0.85	0.76	0.62	0.77
PE	0.320	0.689	0.643	0.709	0.551	0.643	0.760	0.490	0.595	0.621	0.551	0.666	0.663	0.300	0.510
TPI	1.33	3.27	2.83	3.50	2.21	2.83	4.25	1.91	2.47	2.66	2.21	3.04	3.00	1.27	2.00

Hobs = observed heterozygosity; Hexp = expected heterozygosity (Unbiased); HT = homozygosity test; ET = exact test; LR = likelihood ratio test; PM = matching probability; PD = power of discrimination; PIC = polymorphism information content; PE = power of exclusion; TPI = typical paternity index

Table 3. Average heterozygosity and its standard deviation for Jammu & Kashmir Muslim Population (n = 300) and other previously studied populations

Sr. No.	Community	Average heterozygosity	Standard deviation	Reference no.
1	Muslim (JK)*	0.794623	0.015449	Present study
2	Central India	0.793635	0.014620	16
3	Desasht	0.809182	0.015248	21
4	Chitpavan	0.800839	0.015551	21
5	Bhumihar (Bihar)	0.798874	0.015908	20
6	Oraon	0.786491	0.020511	18
7	Gond (MP)	0.801261	0.017575	19
8	Tribal Hmar	0.766011	0.032199	18
9	Tribal Mara	0.784958	0.025247	18
10	UP. Jat	0.808622	0.013437	22
11	KS. Muslim	0.792789	0.017735	17
12	UP-Kurmi	0.807754	0.018337	22
13	Thakur	0.854153	0.012907	22
14	Khatri	0.842568	0.012406	22
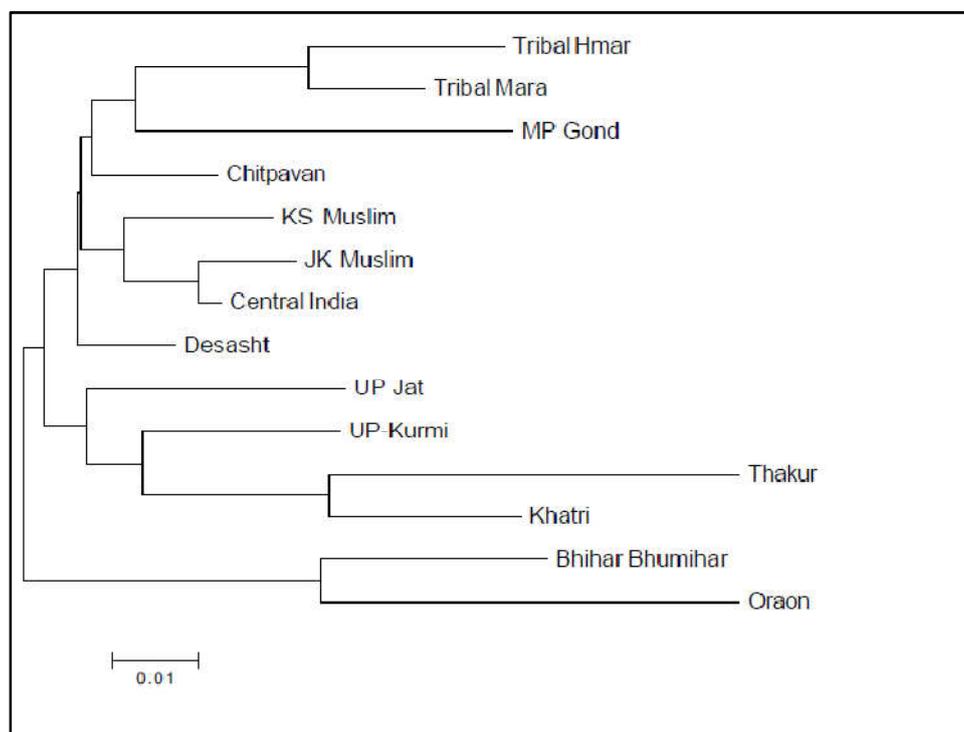
Figure 1. Evolutionary relationships of J&K Muslim population with other Indian populations

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.49478242 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were provided by the user. Evolutionary analyses were conducted in MEGA6

The Power of discrimination (PD) calculated for the studied population displayed high discriminatory power of the analyzed markers. The PD value at 15 STR loci was observed to be greater than 0.838. The Power of exclusion (PE) exhibited the expected values ranging from 0.300 to 0.760. The average heterozygosity and its standard deviation were also found to be falling within the acceptable limits as evident from Table 3. The genotype frequencies of the 15 STR loci showed no significant deviations from HWE expectations. The J&K Muslim population showed a considerable genetic distance with other published Indian population which were used for comparison (Maity *et al.*, 2003, Dubey *et al.*, 2009, Ashma and Kashyap, 2002, Gaikwad and Kashyap, 2002, Tandon *et al.*, 2002). In the Neighbour- Joining tree (Figure 1), the studied

population showed no significant variation from previously studied Kashmiri Muslim data (Allah Rakha *et al.*, 2009). It is interesting to note that the J&K population was clustered with Central Indian population (Shrivastava *et al.*, 2015) and revealed genetic relatedness with this population. The population under consideration displayed much variation from the Saraswat Brahmin population of J&K. The current population study will add to the DNA databank of various studies conducted on Indian populations. Thus, it is concluded that the 15 STR markers selected for the study are ideal for human identification as they are substantially polymorphic. The genetic distance analysis gave the genetic relatedness of this population with the other previously studied populations.

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