Original Article

Genetic diversity of 15 autosomal short tandem repeats loci using the AmpFLSTR® Identifiler™ kit in a Bhil Tribe Population from Gujarat state, India

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MATERIALS AND METHODS: The genetic diversity and forensic parameters based on 15 autosomal short tandem repeats (STR) loci; D8S1179,D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317,D16S539, D2S1338, D19S433, vWA, TPOX, D18S51,D5S818, and FGA in AmpFLSTR® Identifiler™ kit from Applied Biosystems, Foster City, CA, USA were evaluated in saliva samples of 297 unrelated individuals from the Bhil Tribe population of Gujarat state, India to study genetic diversities and relatedness of this population with other national and international populations.
RESUITS: Statistical analysis of the data revealed all loci were within Hardy-Weinberg Equilibrium expectations with the exception of the locus vWA (0.019) and locus D18S51 (0.016). The neighbour joining phylogeny tree and Principal Co-ordinate Analysis plot constructed based on Fst distances from autosomal STRs allele frequencies of the present study and other national as well as international populations show clustering of all the South Asian populations in one branch of the tree, while Middle Eastern and African populations cluster in a separate branch.
CONCLUSION: Our findings reveal strong genetic affinities seen between the Indo-European (IE) speaking Bhil Tribe of Gujarat and Dravidian groups of South India.

Key words: Allelic frequencies, Bhil Tribe, genetic distance, genetic diversity, short tandem repeats

Introduction

The Bhil Tribe is one of the largest tribal communities in India and spread over continuous covering four large Indian states, namely Gujarat, Madhya Pradesh, Rajasthan, and Maharashtra. Mostly, The Bhil Tribe resides in the mountains of central western part of India [Map 1]. In Gujarat, the Bhil Tribe represents 46% of total Tribe's population.[1] The aims of study on the Bhil Tribe in Gujarat have still remained isolated and less focused. Therefore, genetic study will provide a valuable source of information to understand their genetic variability and affiliation.[2]

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Materials and Methods

Sampling

Saliva samples were collected on the indicating FTA® classic card (Cat # WB120206, Whatman®, UK) by using sterile foam tipped applicators (Cat # WB 100032, Whatman®, UK) from 297 unrelated individuals form the Bhil Tribe population of the Gujarat state, India. Samples were collected in accordance with the ethical guidelines stipulated by the institutions involved in this study.

Quality control

The research study was conducted in accordance with quality control measures as well as successfully participated in the fifth Asian American-Indonesian Cultural and Educational Foundation DNA proficiency test. A positive and negative control as specified in the Identifiler® kit user's manual.

DNA extraction

Genomic DNA was extracted from the saliva

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samples using saliva FTA® card application note[3] and QIAamp® DNA mini kit as per manufacturer's recommendations (Qiagen, Germany).

Polymerase chain reaction amplification

Multiplex polymerase chain reaction (PCR) reaction was carried out for each DNA sample, and amplified 15 autosomal short tandem repeats (STR) loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) using AmpFlSTR® Identyfiler™ PCR Amplification kit following the manufacturer's protocol and GeneAmp® PCR System 9700 (Applied Biosystems [ABI], Foster City, CA, USA).

Electrophoresis and typing

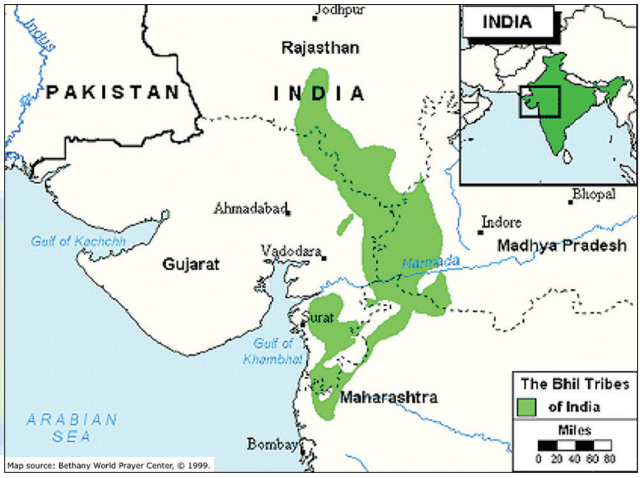
Genotyping was performed by capillary electrophoresis; 1.5 μL of the amplified PCR product was combined with 12 μL of formamide and 0.5 μL of GeneScan 500 LIZ™ internal size standard. Detection of PCR products and genotyping were carried out on the ABI 3130 Genetic analyzer (ABI, Foster City, CA, USA) using the data collection software® v3.0 and GeneMapper® ID v3.2 analysis software (ABI, Foster City, CA, USA).

Statistical analysis

An estimation of allele frequencies distribution and other forensic parameters, including power of discrimination (PD), power of exclusion (PE), and matching probability (MP), polymorphism information content (PIC), and typical paternity index (TPI), were calculated using PowerStats software version 1.2.[4] (Available on http://www.promega.com/geneticidtools/powerstats) Population's genetic structure deviation from Hardy-Weinberg equilibrium (HWE), observed heterozygosity (HO) and expected heterozygosity (HE) were calculated using Arlequin v3.1.[5] Allelic frequencies from samples of the present study and other Indian's as well as International populations shown in Table 1 were employed to generate the neighbor-joining (NJ) phylogeny tree based on Fst distances using POPTREE2 software: Naoko Takezaki, Masatoshi Nei, and Koichiro Tamura (Available on http://www.med.kagawa-u.ac.jp/~genomelb/takezaki/poptree2/index.html).[6] These Fst distance were utilized to generate a NJ phylogeny tree using the same software. The robustness of the phylogenetic relationship calculated by the NJ tree was assessed using 1000 replications bootstrap analysis. Graphical representation of genetic distance (Fst) of the population in this study along with seven national and 10 international populations were performed based on Principal Coordinate Analysis (PCA) plot using GenAlEx software v6.3.[7]

Results and Discussion

The observed allele frequencies and statistical parameters based on the 15 autosomal STR markers in Bhil Tribe population are summarized in Table 2.



Map 1: Green color in map of central western part of India where the Bhil Tribe highly concentrated

Table 1: The national and international population data used for analysis using NJ tree and PCA plot from genetic distances

Population	Abbreviation	Number of loci	References
Bhil, West India	BHI	15	Present study
Gujarati, West India	GUJ	13	Clark *et al.*[8]
Tamil, south India	TAM	15	Balamurugan *et al.*[9]
Punjabi, North India	PUN	15	Shepard and Herrera[10]
Gond, Central India	GON	15	Dubey *et al.*[11]
Dhimal, East India	DHI	15	Roy *et al.*[12]
Paliya, East India	PAL	15	Roy *et al.*[12]
Pakistani, South Asia	PAK	15	Shepard and Herrera[10]
Nepalese, South Asia	NEP	15	Jha *et al.*[13]
Yemen, Middle East	YEM	15	Shepard and Herrera[10]
Egypt, Africa	EGY	15	Shepard and Herrera[10]
Rwandan (Hutu), Africa	RWH	15	Shepard and Herrera[10]
Kenya, Africa	KEN	15	Shepard and Herrera[10]
Sudan, Africa	SUD	15	Shepard and Herrera[10]
Bahrain, Middle East	BAH	15	Shepard and Herrera[10]
Jordan, Middle East	JOR	15	Shepard and Herrera[10]
Oman, Middle East	OMN	15	Shepard and Herrera[10]

Table 2: Allele frequencies and associated statistical parameters of AmpFLSTR® Identifiler™ Kit PCR amplification kit loci in Bhil Tribe population (*n*=297 samples)

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	THO1	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
4						0.002									
5						0.002									
5.2			0.002												
5.3						0.003									
6						0.253									
7			0.027			0.123	0.010								
8	0.010		0.242			0.130	0.209	0.067			0.003	0.303			
9	0.007		0.068	0.039		0.365	0.077	0.180				0.178		0.025	
9.3						0.123									
10	0.126		0.231	0.172			0.128	0.086				0.054	0.002	0.166	
10.2													0.005		
11	0.091		0.258	0.296			0.261	0.273				0.441	0.012	0.311	
11.2												0.002	0.003		
12	0.111		0.164	0.390			0.226	0.227	0.002	0.090		0.022	0.094	0.318	
12.2										0.002			0.002		
13	0.170		0.008	0.086			0.062	0.143		0.304	0.002		0.104	0.171	
13.2										0.015					
14	0.157			0.012	0.049		0.027	0.024		0.241	0.118		0.334	0.008	0.002
14.2										0.065					
15	0.214			0.003	0.330					0.113	0.084		0.160	0.002	
15.2										0.046					
16	0.103			0.002	0.263				0.005	0.067	0.208		0.109		0.002
16.2										0.046					
17	0.010				0.227				0.047	0.009	0.299		0.075		
17.2										0.002					
18	0.002				0.103				0.138	0.002	0.188		0.044		0.002
19					0.024				0.202		0.093		0.022		0.080
20					0.005				0.106		0.005		0.014		0.134
20.2															0.005
21									0.067				0.012		0.120
21.2															0.002
22									0.051				0.005		0.137
22.2															0.008
23									0.180						0.146
23.2															0.003
24									0.106						0.198
24.2															0.003
25		0.002							0.074						0.098
25.2															0.002
26		0.002							0.015				0.002		0.044
27		0.012							0.007						0.012
28		0.135													0.002
28.2															
29		0.190													
29.2		0.002													
30		0.160													
30.2		0.042													
31		0.042													
31.2		0.133													
32		0.008													
32.2		0.210													
33															
33.2		0.054													
34.2		0.008													
MP	0.041	0.041	0.080	0.120	0.100	0.104	0.065	0.066	0.031	0.055	0.068	0.159	0.054	0.107	0.033
PD	0.959	0.959	0.920	0.880	0.900	0.896	0.935	0.934	0.969	0.945	0.932	0.841	0.946	0.893	0.967
PIC	0.840	0.830	0.760	0.680	0.720	0.720	0.780	0.780	0.860	0.790	0.770	0.620	0.800	0.700	0.860
PE	0.679	0.711	0.582	0.427	0.546	0.557	0.595	0.602	0.705	0.541	0.527	0.343	0.578	0.443	0.751
TPI	3.16	3.52	2.39	1.66	2.18	2.36	2.48	2.52	3.45	2.15	2.08	1.39	2.36	1.72	4.10
Ho	0.842	0.838	0.791	0.699	0.771	0.700	0.798	0.801	0.855	0.710	0.760	0.641	0.788	0.709	0.878
He	0.855	0.824	0.790	0.723	0.758	0.667	0.812	0.810	0.872	0.760	0.804	0.678	0.823	0.747	0.865
HWE	0.229	0.950	0.681	0.749	0.639	0.504	0.071	0.033	0.904	0.354	0.019	0.256	0.016	0.554	0.895

MP: Matching probability, PD: Power of discrimination, PIC: Polymorphism information content, PE: Probability exclusion, TPI: Typical paternity index, Ho: Observed heterozygosity, He: Expected heterozygosity, HWE: Haedy–Wenberg equilibrium

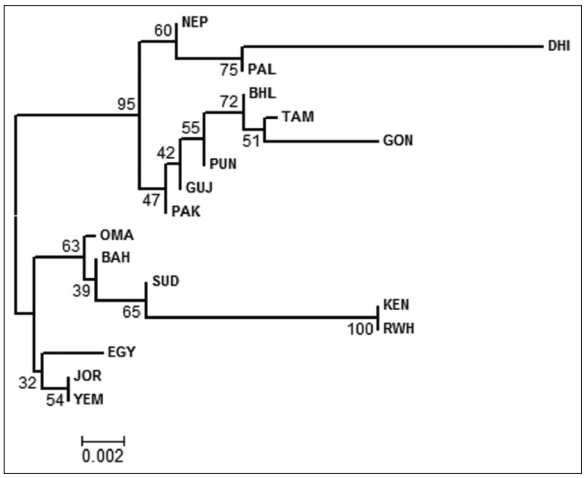


Figure 1: Neighbor- Joining tree based on Fst distances from allele frequencies of the autosomal short tandem repeat loci. The numbers at the nodes represent bootstrap values estimated from 1000 replications. The population codes and descriptions are given in Table 1

A total 155 alleles at these 15 STR loci were found with corresponding allelic frequencies ranging 0.002-0.441 in the Bhil Tribe population. These STR loci were found to be highly polymorphic, and statistical analysis of these data revealed all loci met HWE expectations with the exception of vWA (0.019) and D18S51 (0.016). The combined PD and for all loci were >0.99982, while the combined MP is 2.53×10^{17} and the average heterozygosity is 0.7720.

Fst distance was used to compare the seven national and ten global populations in relation to the allele frequencies of 15 autosomal STR loci and generate the NJ phylogeny tree shown in Figure 1. Examination of the NJ phylogeny tree shows the cluster of all the South Asian population as one group and other Middle Eastern with African population as other group. Nearest affinity can be seen among the Middle Eastern populations, while the three African populations branched away from the Middle Eastern group. South Asian populations cluster shows nearest affinities among the Indian population with Pakistani population (except Dhimal and Paliya). Cluster branch of Indian population of Dhimal and Paliya show nearest affinity seen with Nepali population. A PCA plots were constructed [Figure 2], the grouping of the population in the PCA plot is consistent with the clustering pattern of the NJ phylogeny tree. Moreover, The Bhil Tribe is genetically and linguistic affiliated to Indo-European (IE) group of India.[14] This

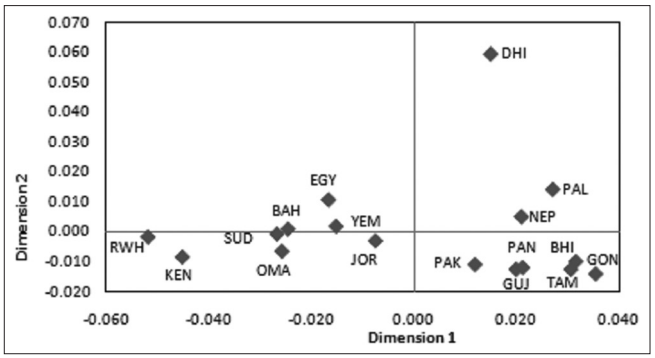


Figure 2: Principal Coordinate Analysis of Fst distances derived from the population from the present study and National and International populations. The population codes and descriptions are given in Table 1

is in contrast to the findings in the present study, found strong genetic similarities between the Bhil Tribe of Gujarat and Dravidian affiliated group of Gond Tribe and Tamil population,[15] whereas closely genetic affinity seen among Gujarati and Punjabi populations.

Conclusion

Clustering pattern of NJ phylogeny tree, PCA plot, genetic study and hypothetical evidence supporting to the IE specking Bhil Tribe of Gujarat is genetically affiliated with Dravidian group of South India.[15-17]

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