Genomic Insight Into 21 Hypervariable Molecular Markers in The Population of Lower Himalayan Geographical Province Himachal Pradesh, India

Arun Sharma

State Forensic Science Laboratory ,Junga,Shimla,Himanchal Pradesh

Ankit Srivastava

Institute of Forensic Science and Criminology, Bundelkhand University, Jhansi - UP, India

Vivek Sahajpal

State Forensic Science Laboratory ,Junga,Shimla,Himanchal Pradesh

R Kumawat

State Forensic Science Laboratory, Rajasthan, Jaipur

Shivani Dixit

State Forensic Science Laboratory, Sagar (MP)

Gyaneshwer Chaubey

Banaras Hindu University

Pankaj Shrivastava (✉ pankaj.shrivastava@rediffmail.com)

State Forensic Science Laboratory, Sagar (MP)

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Abstract

A natural geographical barrier between Tibetan plateau and south Asian countries, the Himalayan Mountain range, serves as a natural barrier between these regions for genetic flow. This has led to vast genomic divergence among the population residing in Indian Himalayan region. This study was designed with an aim to decipher the genomic diversity and molecular characterization of 21 hypervariable molecular markers in the population of geographical province of Himachal Pradesh in the lower Himalayan region. Randomly selected 401 unrelated individuals native of the lower Himalayan geographical region were included for this study. 21 hypervariable molecular markers included in the PowerPlex® 21 system were amplified and genotyped. A total of 246 alleles and 12.3 (SE 0.927) private alleles per locus were observed. Population differentiation analysis revealed that the studied population showed a genetic affinity with the population of North India, North-west India, Central India, and Uttar Pradesh rather than the population of East India, South India, East Asia, and West Asia. Heterozygosity at the studied loci was found in the range of 0.686 to 0.920. The unite discrimination power (PD) and exclusion power (PE) was found to be 1 and 0.99999998073765 respectively. The unite value of matching probability and typical paternity index was found to be 9.33×10^{-26} and 5.05×10^8 respectively for the studied population. All the tested loci were found in Hardy Weinberg Equilibrium (HWE) expectations. Overall the studied population exhibited a great extent of genomic diversity and had a greater genetic affinity with Indo-European speakers rather than Dravidian and Tibeto-Burman speakers.

Introduction:

The Himalayan Mountain range which runs from west-northwest (Pakistan) to east-southeast (Burma) in an arch of 2400 km¹ acts as a geographical barrier for gene flow between the Tibetan plateau and the South Asian subcontinent². These geographical conditions of the Himalayan landscape lead to unique genetic characteristics in that region. The North and North-east geographical region of India lies in the foothills of the Himalayan Mountains and harbors genomic diversity in the populations of this regions⁶. Genomic^{3,4}, and lingual studies⁵ have revealed initial settlement of modern men in the Tibetan plateau about 25000–30000 years ago². It has been well established that India is a highly diverse country aligned in the hierarchy of caste and tribal populations and various linguistic groups^{7,8}. Indian population's is broadly divided into *Australoid*, *Indo-Caucasoid*, *Indo-Mongoloid* and *Negrito* ethnics groups along with *Indo European*, *Dravidian*, *Astro-Asiatic* and *Sino-Tibetan* speakers⁹. The eastern Himalayan belt has the majority of Tibeto-Burman speakers whereas west and north-west belt has the majority of Indo European lingual family¹⁰. The genetic structure of the Indian population is broadly categorized into Ancestral North Indian (ANI) and Ancestral South Indian (ASI) population. ANI is genetically closer to the Middle East, Central Asian, and Europeans¹¹. Several migration waves into India during the history reflected the complex genetic structure of the Indian populations. Around 4000 years ago, the last major migration wave of Indo-European speakers attributed to the high degree of genetic complex¹². Several genetic studies based on the Short tandem repeats (STRs) molecular markers have been reported with reference to Indian population to understand the diversity in terms of ethnicity, cultural, lingual, and geographical affinity^{9,8,13}.

STRs are the highly acceptable and gold standard markers in the forensic application due to their highly polymorphic nature, heterozygosity, short sequence length along with wide distribution throughout the human genome^{14,15}. However, mutation rates in the STR markers are higher than the single nucleotide polymorphisms (SNPs)¹⁶, but the STR markers are very useful and potential markers for the genealogical studies as well as forensic application¹⁷.

To understand the genetic diversity in the lower Himalayan geographical province Himachal Pradesh, 15 autosomal STR markers¹⁸ and 17 Y-STR marker¹⁹ have been used but it is not rationale in the terms of molecular markers and effective sample size. Therefore, the present study was undertaken to explore the genetic structure of the admixed population of the North Indian Himalayan region, Himachal Pradesh using 21 hypervariable STRs.

Material And Methods:

Samples: 401 unrelated healthy individual volunteers residing in the geographical province of Himachal Pradesh (Fig.1) were selected randomly for this study. About 1 ml of peripheral blood was collected from these volunteers in the EDTA vials following the guideline described under the declaration of Helsinki²⁰ and stored at -20°C till further processing.

DNA Isolation: Genomic DNA was isolated from the selected samples using Phenol Chloroform Isoamyl Alcohol (PCIA) organic extraction method as described by Sambrook et al., 1989²¹.

DNA Quantitation: Isolated DNA was quantified with Quantifiler® Duo DNA Quantification Kit (Thermo Fisher Scientific, CA, USA) using RT-PCR 7500 platform as per recommendations of the manufacturer.

Amplification: 1 ng of genomic DNA was used to amplify the 21 STR markers included in the PowerPlex® 21 system (Promega, CA, USA) using 9700 thermal cycler (Thermo Fisher Scientific, CA, USA) as per manufacturer's protocol except for 10 µl reaction volume.

Genotyping: The amplified fragments were subjected to size based separation through capillary electrophoresis using Genetic Analyzer 3500XL (Thermo Fisher Scientific, CA, USA). 1 µl amplicon was diluted in the 10 µl mixture volume of HiDi Formamide (Thermo Fisher Scientific, CA, USA) and WEN ILS 500 (Provided along with kit) and denatured as per recommended protocol. POP™-4 and 36 cm capillary array was used while running the samples on Genetic Analyzer. Allelic ladder (provided along with kit) was used to assign allele number in the DNA profile. GeneMapper™ IDX software v1.5 was used to analyze the genotyped data.

Quality control: To monitor quality parameter during the experiments, positive control (with DNA template of 2800M) and negative control (without DNA template) was used. The authors also qualified international DNA proficiency test conducted by GITAD, Spain (<http://gitad.ugr.es/principal.htm>).

Statistical analysis: Allele frequency and forensic interest parameters viz., Power of discrimination (PD), Power of exclusion (PE), Polymorphic Information Contents (PIC), Matching Probability (Pm), Typical Paternity index (TPI), Observed Heterozygosity (H_{obs}), Expected Heterozygosity (H_{exp}), Gene Diversity (GD) and Hardy Weinberg equilibrium (HWE) test were performed using GenaAlex6²² and STRAF statistical tool²³. Population differentiation test *Fst* was calculated using Arlequin 3.5 software²⁴. Phylogenetic analysis was done with the help of POPTREE²⁵, MEGA-6²⁶, and Structure2.3.4²⁷. Principal Coordinate Analysis (PCoA) was executed using PAST3.26 software²⁸.

Genetic data of reported populations for comparison:

The genetic dataset of present study was compared with the dataset of reported indigenous populations viz., Balmiki (Punjab)²⁹, Sakaldwipi Brahmin (Jharkhand)²⁹, Konkanastha_Brahmin (Maharashtra)²⁹, Iyengar (Tamilnadu)²⁹, Kurumans (Tamilnadu)²⁹, Munda (Jharkhand)²⁹, Chenchu (Andhra Pradesh)³⁰, Lambadi (Andhra Pradesh)³⁰, Naikpod_Gond (Andhra Pradesh)³⁰, Yerukula (Andhra Pradesh)³⁰, Munda (Chotanagpur)³¹, Santal (Chotanagpur)³¹, Oraon (Chotanagpur)³¹, Lodha (Bengal)³², Kora (Bengal)³², Maheli (Bengal)³², Central Indian Population³³, Gond (MP)³⁴, Gond_2 (MP)³⁵, Gond_1 (MP)²⁹, Oraon (Chhattishgarh)³⁶, Population of Jharkhand³⁷, Jat Sikh (Punjab)³⁸, Baniya (Punjab)³⁸, Khatri (Punjab)³⁸, Population of Uttar Pradesh³⁹, Kahar population (UP)⁴⁰, Himachal Pradesh Population¹⁸, Gorkhas⁴¹, Tamil Population (Tamil Nadu)⁴², Population of Rajasthan⁴³, Population of Odisha⁴⁴ and Bhuiyan Population (Jharkhand)⁴⁵; and global populations namely; Manchu Population of China⁴⁶, Han Population of Southern China⁴⁷, Tibetan Population (Nepal)⁴⁸, Kathmandu⁴⁹, Tibet⁴⁹, Newar⁴⁹, Nepalese⁵⁰, Korean Population⁵¹, Royal Kingdom of Bhutan Population⁵², Punjabi-Pakistan⁵³, Pashtun-Pakistan⁵³, Sindhi-Pakistan⁵³, Saraiki-Pakistan⁵³, Baloch-Pakistan⁵³ from published sources (Table 1).

Ethical approval: Written informed consent was obtained from all the participants. The study was conducted under the ethical guidelines and it was approved by the Institute Ethics Committee (Human Studies) of Maharani Laxmi Bai Medical College, Jhansi, vide letter no. 4648 dated 05.09.2020. Maharani Laxmi Bai Medical College, Jhansi is part of Bundelkhamd University, Jhansi. One of the co-author of this manuscript is posted in the same university and we took the ethical clearance on our proposal from the same Institute which is part of Bundelkhamd University, Jhansi, UP, India.

Results And Discussion:

Allelic frequencies and forensic parameters:

A total of 246 alleles were found in the studied population at all the tested loci. A total of 19 alleles were observed at the loci D1S1656 and FGA and these were the highest number of the observed alleles. At the locus TH01, lowest number of alleles were observed i.e. 6 alleles. In the currently studied highly diverse population, the mean number of alleles observed were 12.3 (SE 0.927) and mean number of effective alleles were 5.911 (SE 0.073) per locus. Allele frequencies ranged from 0.001 to 0.389 and allele 8 of locus TPOX was found to be the most frequent allele among all the studied loci (Table 2). All the loci were found under the Hardy Weinberg Equilibrium (HWE) ($p < 0.05$). The observed heterozygosity (H_{obs}) ranged from 0.686 (CSF1PO) to 0.920 (D1S1656) and gene diversity (GD) varied from 0.698 (TPOX) to 0.913 (Penta E). The power of discrimination (PD) and exclusion (PE) were found in range of 0.850 (TPOX) and 0.407 (CSF1PO) to 0.984 (Penta E) and 0.837 (D1S1656) respectively. The cumulative value of discrimination (CPD) and exclusion CPE) was found to be 1 and 0.99999998073765 respectively. The matching probability (Pm) and typical paternity index (TPI) were found in a range of 0.016 (Penta E) and 1.591 (CSF1PO) to 0.150 (TPOX) and 6.266 (D1S1656) respectively. The combined value of matching probability (coPm) and typical paternity index (coTPI) was observed as 9.33×10^{-26} and 5.05×10^8 respectively for all the tested loci. An autosomal marker is considered as highly polymorphic when its discrimination power (PD) value is greater than 0.80 ($PD > 0.80$), power of exclusion value is greater than 0.50 ($PE > 0.50$) and PIC value is greater than 0.5 ($PIC > 0.5$)^{54,55,56}. In the present study, all the loci were highly polymorphic for the studied population because the value of $PIC > 0.5$. Noticeably, the locus Penta E was found to be most informative in the terms of highest GD, PD, and PIC and lowest value of Pm. Locus TPOX was found to be least informative in the terms of lowest GD, PD, and PIC and the highest value of Pm among all the tested loci (Table 3). Findings of the present study hold a prominent forensic importance as other previously reported population studies of Indo European speakers^{43, 57, 40, 39, 58}. The data observed regarding the locus Penta E is highly informative among all the studied loci.

Interpopulation comparison:

Allele frequencies at the common 15 STR markers of the studied population were compared with 47 populations using Nei's Da distance to draw a Phylogenetic tree. Population genetic distance *Fst* was calculated between studied population and other population groups based on the geographical distance as shown in Table 4. The *Fst* revealed higher genetic affinity between the population of Himachal Pradesh and geographically closer population groups of Uttar Pradesh, Central India, Northwest India rather than East and South India. When compared with West and East Asian populations, the population of Himachal Pradesh had 2.5 times higher genetic affinity with the West Asian than the East Asian population. The studied population had 7 times higher genetic distances with the South Indian populations in comparison with the population of central and north India (Fig. 2). This finding observed in the present study corroborates with the earlier published study wherein Indian genetic structure was broadly divided into ANI and ASI¹³. Moreover, the present study also indicated the presence of a small genetic pool in East India which showed a degree of genetic distance from the South, North, North-west and Central Indian genetic pool^{59,60,61}.

Phylogenetic Analysis:

The allelic data of the studied population was compared with the 47 reported populations of India, West and East Asia at common 15 genetic markers. The Da distance was calculated by the Neighbor-Joining method using PopTree2 software. The distance was reflected in the Phylogenetic tree using MEGA-6 software. The Phylogenetic tree revealed that the population of Himachal Pradesh was most closely related with the Rajasthani and Punjabi population of Pakistan, followed by Pashtun, Sindhi, Baloch, and Saraiki populations of Pakistan, which might be results of shared ancestry (Fig. 3). In the Phylogenetic tree,

East Indian, East Asian, and South Indian populations are depicted into the separate branches. This Phylogenetic relationship indicates the similar genetic constituents among the North, North-west, Central India, and West Asia^{62,53}. However geographically close North and Northeast populations viz., Nepalese, Gorkhas, Kathmandu, Tibet, Newar, Tibetan population (Nepal) showed a higher genetic affinity with the East Asian populations viz., Manchu population of China, Korean population, Royal Kingdom of Bhutan Population and Han population of Southern China rather than the population of Himachal Pradesh. The possible reason for this genetic variation could be the geographical barrier created by the Himalayan range for the genetic flow².

Principal Coordinate Analysis (PCoA) and Heat map :

The PCoA plot was drawn using Nei's Da distance matrix based on the allele frequencies of studied and compared 47 populations on common 15 STR markers. Euclidean similarity index was used to analyze principal coordinates, in which Coordinate 1 and 2 covered 96.301% of the variations. Due to a higher level of genetic variations, few populations viz., Chenchu (Andhra Pradesh)³⁰, Central Indian Population³³, Jat Sikh (Punjab)³⁸ and Kathmandu⁶⁰ depicted in the outlier and resulted in clumping of most of the populations at one part of the plot (Fig. S1). To visualize genetic distance among the clumped populations, outlier populations were removed from the analysis, and the PCoA plot was redrawn (Fig. 4). Studied population clustered with the population of Himachal Pradesh¹⁸, Rajasthan followed by the populations of Pakistan viz., Punjabi⁵³, Pashtun⁵³, Sindhi⁵³, Baloch⁵³, and Saraiki⁵³. The studied population was depicted with relative higher distance in PCoA plot with the Gorkhas, Newar, Han population of Southern China, Royal Kingdom of Bhutan Population, Tibet, Korean population, Nepalese, Tibetan population (Nepal) and Manchu population of China. The studied population had a relatively higher genetic distance with the Lodha, Kora, Maheli populations of East Indian geographical province Bengal than the populations of close geographical state Uttar Pradesh, Madhya Pradesh, Punjab, Jharkhand.

The genetic distances between the studied and compared 47 Indian and Asian populations were exemplified in the heat map (Fig. 5), which revealed a similar fashion of genetic relatedness as illustrated in Phylogenetic tree and Principal Coordinate Analysis.

Structure Analysis:

To examine the genetic similarity and differentiation between the studied population and 5 populations of Pakistan, 16 populations of East and South-East Asia, 3 populations of Bangladesh along with 12 populations of India were subjected to structure analysis at the common 15 autosomal STR markers. The inferred cluster *k* ranged from 2 to 10 with 20 repetitions after a 100,000 burn in period and 100,000 MCMC simulations for each *k*. Δk and estimated log probability data statistics were evaluated using Structure Harvester⁶³ for the selection of most suitable configured *K*. Cluster 4 (*K*=4) was found in the informative shared ancestry component among the studied and compared populations. The ancestry component (Yellow color) was found most prevalent in the North-west Indian populations viz., Gujjar⁶⁴, Meena⁶⁴, Kahar population of Uttar Pradesh, admixed populations of Uttar Pradesh, Rajasthan⁶⁴, Madhya Pradesh, Jharkhand similar to ANI^{61,65}; and Pakistani populations (Balochi, Brahui, Sindhi, Pathan, and Burusho)(Fig. 6). The ancestry component (Green color) was found concentrated among the East Asian related populations (Cambodian, Han, Dai, Daur, Hezhen, Lahu, Miao, Orogon, She, Tujia, Tu, Xibo, Yizu, Mangola, Nexi, Japanese, Chakma, Marma, Tripura, and Bengali). The dark blue color depicted that the ancestry component was most prevalent in the Dravidian speakers (Madiga, Adi, kapu) similar to ASI^{61,65}. The light blue color depicted an ancestry component found in a loose gradient from west to east Asian populations. Overall most of the ancestry component was found in an east-west gradient along with geography. The ancestry component of the studied population was found in homogeneity with the geographically close populations of North, Northwest, Central India and population of Pakistan.

Conclusion

This study provides a genetic evidence for an unique gene pool among the North, North-west, Central Indian populations which significantly differed from the East and South Indian genetic pool. The findings of the population differentiations, Phylogenetic relatedness, PCoA, a Heat map, and structure analysis revealed that the studied population showed gradient gene flow towards West rather than South and East Indian populations. The studied 21 STR markers were found to be highly useful for the population genetics as well as population migration study. The dataset of this study will be of wide use in the preview of the recently introduced "The DNA technology (use and application) regulation bill 2019. It will enrich the existence of an autosomal STR Indian DNA database.

Declarations

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Author contributions

Shrivastava P. designed the study. Sharma A and Srivastava A. collected samples and Shrivastava P. did experimental work. Kumawat R.K. performed data evaluation, statistical analysis, and wrote the manuscript. Sharma A., Sahajpal V., Dixit S. and Chaubey G. reviewed the manuscript. All authors reviewed and approved the manuscript.

Competing interests

The authors declared that they have no competing interests.

Additional information

Supplementary information is available for this paper at—————

Correspondence and requests for materials should be addressed to P.S.

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Tables

Table 1. Datasets of various populations used for various analyses in this study.

Sr. No.	Analysis	Population	Geographical region	Sample (n)
1	Population differentiation test, phylogenetic tree, PCoA	Balmiki (Punjab)	India	62
		Sakaldwipi Brahmin (Jharkhand)		68
		Konkanastha_Brahmin (Maharashtra)		71
		Iyengar (Tamilnadu)		67
		Kurumans (Tamilnadu)		67
		Munda (Jharkhand)		68
		Chenchu (Andhra Pradesh)		100
		Lambadi (Andhra Pradesh)		107
		Naikpod_Gond (Andhra Pradesh)		104
		Yerukula (Andhra Pradesh)		101
		Munda (Chotanagpur)		128
		Santal (Chotanagpur)		122
		Oraon (Chotanagpur)		120
		Lodha (Bengal)		198
		Kora (Bengal)		118
		Maheli (Bengal)		98
		Central Indian Population		582
		Gond (MP)		117
		Gond_2 (MP)		89
		Gond_1 (MP)		75
		Oraon (Chhattishgarh)		103
		Population of Jharkhand		200
		Jat Sikh (Punjab)		52
		Baniya (Punjab)		40
		Khatri (Punjab)		40
		Population of Uttar Pradesh		168
		Kahar Population (UP)		147
		Himachal Pradesh Population		275
		Gorkhas		98
		Tamil Population (Tamil Nadu)		272
		Population of Rajasthan		317
		Population of Odisha		508
		Bhuiyan Population (Jharkhand)		182
		Population of Himachal Pradesh		401
		Manchu Population of China	China	150
		Han Population of Southern China		1161
		Tibetan Population (Nepal)	Nepal	122
		Kathmandu		77
		Tibet		153
		Newar		66
		Nepalese	Korea	953
		Korean Population		1805

2	Structure	Royal Kingdom of Bhutan Population	Bhutan	936
		Punjabi-Pakistan	Pakistan	212
		Pashtun-Pakistan		214
		Sindhi- Pakistan		206
		Saraiki-Pakistan		208
		Baloch-Pakistan		218
		Balochi	Pakistan	19
		Brahui		18
		Sindhi		24
		Pathan		24
		Burusho		25
		Madiga	India	43
		Kapu		37
		Adi		30
		Chakma	Bangladesh	113
		Marma		83
		Tripura		83
		Cambodian	East and South east asia	10
		Han		43
		Dai		10
		Daur		9
		Hezhen		7
		Lahu		8
		Miaozu		10
		Orogen		9
		She		10
		Tujia		10
		Tu		10
		Xibo		9
		Yizu		10
		Mangola		10
		Naxi		7
		Japanese		28
		Bengali	India	223
		Population of Uttar Pradesh		100
		Tripura		87
		Meena		202
		Gujjar		150
		Population of Rajasthan		317
		Kahar Population		147
		Population of MP		374
		Population of Jharkhand		198
		Population of Himachal Pradesh		401

Table 2: Allele frequency observed at autosomal STR loci of PowerPlex® 21 system in the population of Himachal Pradesh, India (n=401)													
Allele	D3S1358	D1S1656	D6S1043	D13S317	PENTA-E	D16S539	D18S51	D2S1338	CSF1PO	PENTA-D	TH01	vWA	D21S11
5					0.042					0.001			
6						0.001				0.002	0.248		
7				0.010	0.094				0.001	0.022	0.145		
8		0.021		0.186	0.011	0.070			0.001	0.021	0.151		
9		0.005	0.002	0.079	0.021	0.142	0.001		0.022	0.217	0.293		
9.3											0.152		
10		0.011	0.017	0.110	0.046	0.081	0.004		0.203	0.198	0.011	0.002	
11		0.185	0.387	0.241	0.142	0.360	0.025		0.305	0.204			
12		0.096	0.186	0.296	0.107	0.234	0.099		0.379	0.130			
12.2													
13	0.001	0.130	0.082	0.062	0.075	0.096	0.137		0.075	0.122		0.006	
13.2													
14	0.041	0.086	0.079	0.017	0.079	0.014	0.241		0.011	0.060		0.116	
14.2													
15	0.289	0.161	0.007		0.076	0.001	0.150	0.001	0.001	0.016		0.071	
15.2		0.001											
15.3		0.030											
16	0.302	0.132	0.005		0.127		0.126	0.007		0.004		0.226	
16.2		0.001											
16.3		0.027											
17	0.233	0.055	0.035		0.076		0.084	0.076		0.001		0.259	
17.3		0.037											
18	0.122	0.006	0.081		0.055		0.061	0.148				0.209	
18.2													
18.3		0.011											
19	0.010	0.001	0.072		0.019		0.050	0.153				0.090	
19.3		0.002											
20	0.001		0.037		0.019		0.010	0.092				0.020	
20.2													
21			0.006		0.006		0.007	0.052					
21.2													
22			0.002		0.005		0.005	0.074					
22.2													
23								0.175					
23.2													
24							0.001	0.116					
24.2													
25								0.086					
26								0.015					0.007
26.2													
27								0.002					0.010

28	0.001	0.147
28.2		0.004
29		0.176
29.2		0.005
29.3		0.001
30		0.191
30.2		0.040
31		0.024
31.2		0.135
32		0.005
32.2		0.186
33		0.001
33.2		0.060
34.2		0.006
35		0.001
38		0.001

Table 3: Forensic interest and population genetic parameters for the population of Himachal Pradesh, India (N=401)									
	PD	PE	PIC	Pm	TPI	GD	Hobs	Hexp	pHW
D3S1358	0.902	0.453	0.712	0.098	1.759	0.755	0.716	0.754	0.326
D1S1656	0.971	0.837	0.871	0.029	6.266	0.883	0.920	0.882	0.419
D6S1043	0.934	0.577	0.767	0.066	2.359	0.789	0.788	0.788	0.263
D13S317	0.932	0.541	0.769	0.068	2.156	0.799	0.768	0.798	0.323
PENTA-E	0.984	0.781	0.905	0.016	4.663	0.913	0.893	0.912	0.622
D16S539	0.918	0.546	0.744	0.082	2.179	0.775	0.771	0.774	0.687
D18S51	0.964	0.696	0.847	0.036	3.342	0.862	0.850	0.861	0.456
D2S1338	0.973	0.691	0.868	0.027	3.287	0.881	0.848	0.880	0.379
CSF1PO	0.870	0.407	0.666	0.130	1.591	0.716	0.686	0.715	0.088
PENTA-D	0.949	0.676	0.814	0.051	3.133	0.836	0.840	0.835	0.551
TH01	0.921	0.528	0.752	0.079	2.089	0.787	0.761	0.786	0.556
vWA	0.935	0.642	0.784	0.065	2.824	0.812	0.823	0.811	0.559
D21S11	0.957	0.755	0.835	0.043	4.177	0.853	0.880	0.852	0.303
D7S820	0.931	0.541	0.773	0.069	2.156	0.803	0.768	0.802	0.022
D5S818	0.884	0.486	0.694	0.116	1.892	0.738	0.736	0.737	0.502
TPOX	0.850	0.461	0.645	0.150	1.790	0.698	0.721	0.697	0.824
D8S1179	0.957	0.647	0.828	0.043	2.864	0.848	0.825	0.847	0.216
D12S391	0.961	0.657	0.833	0.039	2.949	0.850	0.830	0.849	0.838
D19S433	0.942	0.671	0.795	0.058	3.085	0.818	0.838	0.817	0.289
FGA	0.965	0.705	0.853	0.035	3.457	0.869	0.855	0.868	0.064
PD – power of discrimination; PE- Power of Exclusion; PIC- Polymorphic Information Content; Pm – Matching Probability; TPI – typical paternity index; GD- Gene Diversity; Hobs - observed heterozygosity; Hexp - expected heterozygosity; pHW - Hardy-Weinberg equilibrium p value.									

Table 4: Fst values between population of Himachal Pradesh and other geographical population group				
Population groups	Among group variation	Among population variation	within population variation	FST
Himachal Pradesh v/s Uttar Pradesh	0.06	0.18	99.76	0.00236
Himachal Pradesh v/s Central India	0.06	0.18	99.76	0.00242
Himachal Pradesh v/s North-west India	-0.02	0.29	99.73	0.00271
Himachal Pradesh v/s East India	-0.01	1.01	99	0.01
Himachal Pradesh v/s South India	0.57	1.05	98.38	0.01619
Himachal Pradesh v/s East Asia	0.82	0.91	98.27	0.01727
Himachal Pradesh v/s West Asia	-0.24	0.93	99.31	0.00686

Figures

Figure 1

Geographical location of the studied population. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Fst values between the population of Himachal Pradesh and other ethnic groups.

Figure 3

Phylogenetic tree constructed using POPTREE based on Nei's DA distance of 15 autosomal STRs in the population of Himachal Pradesh and 47 other populations.

Figure 4

Principal Coordinate Analysis (PCoA) plot constructed from Nei's DA distance of 15 autosomal STRs after removal of outlier populations

Figure 5

Graphical presentation of comparative Fst values of the studied and compared 47 Asian populations.

Figure 6

Population genetic structure of 37 Asian (western, southern, eastern, south-eastern) populations based on 15 autosomal STR markers (k=4).

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