

Evaluation of the gene flow from East to West among various populations of Rajasthan, India

R. K. Kumawat^{1, 2#}, Pankaj Shrivastava^{3*#}, Shivani Dixit³, Divya Shrivastava¹, G.K. Mathur²
and Gyaneshwer Chaubey⁴

¹School of Life Sciences, Jaipur National University, Jaipur-302017, Rajasthan, India

²DNA Division, State Forensic Science Laboratory, Rajasthan, Jaipur -302016, India

³DNA Fingerprinting Unit, State Forensic Science Laboratory, Department of Home (Police),
Govt. of MP, Sagar-470001, India

⁴Cytogenetics Laboratory, Dept. of Zoology, Banaras Hindu University, Varanasi-221005, India

#author with equal credential

*Corresponding Author – pankaj.shrivastava@rediffmail.com

Contact details: Phone No.- +919424371946

Abstract:

Rajasthan is a state located in the north-western part of India and it has been cited as a major route of human migration since ancient times. The present study was conducted to find out the genetic affinity of Rajasthani population with the population living in its east and the west. In particular, we compared them with the population of Pakistan which shares the common geographical boundary with

the Rajasthan while also having a look at their inter and intra population affinities with the population belonging to other Indian states. We investigated the genetic structure and population parameters of Rajasthani populations obtained for twenty polymorphic autosomal STR loci from 669 unrelated individuals belonging to its three population groups including Mina, Gujjar and the admixed population of Rajasthan. The studied populations showed a wide range of genetic diversity and besides the genetic structure of the studied populations, it was found that the average heterozygosity value was highest among the populations of Rajasthan, possibly, because of gene flow from different directions. Various statistical analyses suggested that the Rajasthani populations had a higher affinity with the North Indian populations rather than with the Pakistani population.

Keywords: Genetic affinity; STR; Rajasthan; Heterozygosity; Polymorphic

Introduction:

India is a diverse country which has numerous socio-cultural and linguistic groups. Here, the human diversity is defined by 4693 documented population groups which include 2205 major communities, 589 segmented units and 1900 territorial units spread across the country[1]. Broadly speaking, Indian population is divided into various castes, tribes and religious communities which belong to Austro-Asiatic, Dravidian, Indo-European and Tibeto-Burman linguistic groups[2]. Rajasthan is the largest state by area and seventh largest by population in the country. It lies

on the north-west border of India and its topography varies from world's oldest Aravalli hills to the largest Thar Desert (which covers around 61% of total geographic area of the state). Due to this highly distinctive topography, the climate of Rajasthan varies from scorching summers to chilly winters[3]. Thus, this heterogeneous climate is a major factor in the geographical isolation of Rajasthani populations that has also helped in increasing the level of endogamy along with the social factors. Rajasthan is considered as the major land route of human migration[4]. Prehistoric archeological evidence of Indus valley civilization is also found in this state which reflects the population diversity found in this particular region. On the western side, it shares the geographical boundaries with Pakistan and it comprises the "Thar Desert" which is also known as "Rajasthan or great Indian desert". In the north, east and south, it shares the geographical boundaries with various Indian states viz. Punjab, Haryana, Uttar Pradesh, Madhya Pradesh and Gujarat[5]. The Rajasthani people consider themselves as native inhabitants of Rajasthan state and speaks various dialects of Indo-Aryan linguistic group[6]. As per Census 2011, the population of Rajasthan state was 68.6 million, which was 5.66% of the total population of the country. 57.13% of the total population of Rajasthan lives in the rural areas[7].

Mina community is the dominating tribal population mostly found in western and central Indian states of Rajasthan and Madhya Pradesh. The word Mina is derived

from Sanskrit word ‘Meen’ which means fish, and Minas claim to be mythological descendants of Matsya avatar of lord Vishnu[8] [9]. It is believed that Minas are mixed and impure caste and sometimes it has been supposed that they originated from a branch of Rajputs [10]. The Minas are believed to be a pre-Aryan tribe of Rajputana province in the beginning. The Mina tribe is divided into two branches, “Jamindars”, who adopted agriculture as a profession and “Chowkidars” who worked as watchmen[11] [12].

Gujjar also known as Gurjar, Goojr and Gojar is a middle class community which is mainly spread in north-west India. In India, Gujjars arrived between 5th and 6th century (470-520 AD)[13]. They played diverse historical roles in society. For example, while on one hand, they were founders of many kingdoms, on the other hand many of them were nomads. Now a day, these people are engaged in agriculture along with dairy and livestock farming and this community has its own socio-cultural identity. [5] [13].

Materials and Methods:

Participants and Sample collection

This study was conducted in compliance with ethical standards and was approved by the Institutional Ethical Committee of the Jaipur National University, Jaipur, Rajasthan, India, vide letter no. JNUMSRC/IEC/2018/45 dated 20.07.2018. The

peripheral blood samples were collected from randomly selected 669 unrelated healthy adult individuals from the various populations of Rajasthan, namely; Mina tribe (n=202), Gujjar (n=150) and mixed population of Rajasthan (n=317) after obtaining written informed consent following the declaration of Helsinki. The samples of Mina and Gujjar population were collected from the central and south-eastern parts of Rajasthan. The mixed population of Rajasthan was sampled from almost the whole state [Fig.1].

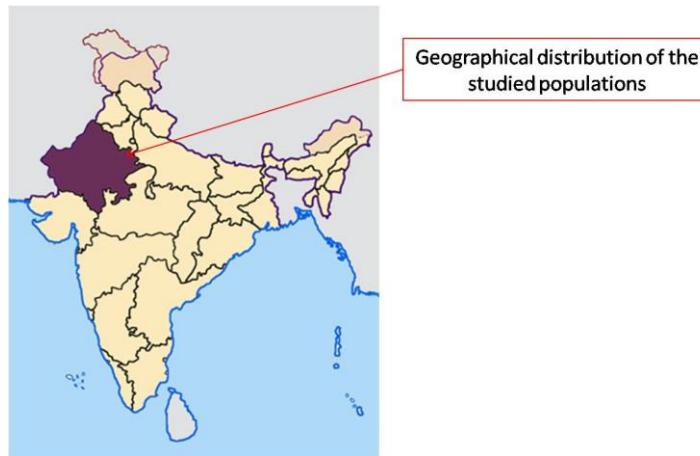


Fig. 1: The geographical distribution of the studied populations

DNA Extraction and Genotyping

The samples were directly amplified without DNA extraction using PowerPlex® 21 multiplex system (Promega, CA, USA) as per recommendations of the manufacturer's except that the 10 μ l reaction volume was used. The direct amplification protocol was initially standardized and the profiles generated by the direct method were compared with the DNA profiles obtained from the

extracted DNA using standard organic extraction method Phenol Chloroform Isoamyl Alcohol (PCIA) as described earlier[17] [18]. The Amplified DNA fragments were analyzed by using ABI 3500XL Genetic Analyzer using 36 cm capillary and POPTM - 4 (Thermo Fisher Scientific, USA-Thermo). Allelic ladder provided along with kit was used to obtain the DNA profiles. Data was analyzed using GeneMapperTM ID-X Software Version 1.5 (Thermo). The peak height threshold was 50 and 200 Relative Fluorescence Unit (RFU) for the heterozygous and homozygous allele respectively.

Statistical analyses

Obtained data was statistically analyzed. Allele frequency and observed heterozygosity were calculated by using GenAlEx 6.5 software [19]. Arlequin v3.5 software[20] was used for calculation of Observed Heterozygosity (H_{obs}), Expected Heterozygosity (H_{exp}) and Hardy Weinberg Equilibrium (HWE) and AMOVA. Further, Arlequin v3.5 software[20] was also used for comparison of allele frequencies of the studied population data with the previously published population data by Fst pairwise distance. The previously reported populations, namely; Kahar Population (Uttar Pradesh) [21], Populations of Uttar Pradesh[18], Khatri (Punjab) [22], Baniya (Punjab)[22], Jatt Sikh (Punjab)[22], Population of Jharkhand[23], Oraon (Chhattishgarh) [24], Gond-1 (Madhya Pradesh) [25], Gond-2 (Madhya Pradesh)[26], Gond (Madhya Pradesh) [27], Central Indian Populations [28], Bhil

(Gujrat) [29], Bhil (Madhya Pradesh) [30], Maheli (Bengal) [31], Kora (Bengal) [31], Lodha (Bengal) [31], Oraon (Chotanagpur) [32], Santhal (Chotanagpur) [32], Munda (Chotanagpur) [32], Yerukula (Andhra Pradesh) [33], Naikpod Gond (Andhra Pradesh) [33], Lambadi (Andhra Pradesh) [33], Chenchu (Andhra Pradesh) [33], Munda (Jharkhand) [25], Kurmans (Tamilnadu)[25], Iyengar (Tamilnadu) [25], Mahadev Koli (Maharashtra) [25], Konkanastha Brahmin (Maharashtra) [25], Sakaldwipi Brahmin (Jharkhand) [25], Balmiki (Punjab) [25], Population of Madhya Pradesh [34], Indian Population [35], Populations of Bangladesh [36], Han population of Southern China[37], Tibetan population (Nepal) [38], Royal Kingdom of Bhutan Population [39], Kashmiri Muslim population living in Pakistan [40], Pakistani population [41], Pakistani groups (Balochi, Brahui, Sindhi, Pathan, and Barusho)[42], were used to explore the genetic diversity and affinity with the studied Mina, Gujjar and mixed population of Rajasthan. Based on Nei's genetic distances [43], Neighbour-joining (NJ) dendrogram was drawn using by using POPTREE2 software[44] with bootstrap value of 1000 replications over loci and viewed in MEGA v6 software [45]. Graphical representation of genetic distances (Dst) of studied populations with other reported populations was also performed based on Principal Component Analysis (PCA) plot generated by PAST 3.02a software[46]. Our studied population data and the data set of previously reported Pakistani population, East Asian population and Indian

populations were used for STRUCTURE software[47], from $k=2$ to $k=10$ performed by 20 iterations after a burn-in of 100000 and selected the k ($k=5$) by evaluation of the Δk and estimated log-probability data statistics[48].

Quality Control:

Quality control standards for this study were followed. The authors have passed the proficiency test of GITAD, Spain (<http://gitad.ugr.es>). This research article follows the guidelines formulated by the journal.

Results:

In this study, we investigated the genetic affinity of studied populations with the reported population groups of Pakistan, East Asian populations and other Indian populations. Among the studied populations, a wide range of heterozygosity was observed which is likely because of gene flow from different directions. Mina population showed a range of heterozygosity with the minimum value of 0.63861 (D5S818) to a maximum value of 0.89604 (Penta-E), Gujjar population showed a range of heterozygosity with the minimum value of 0.62667 (TPOX) to a maximum value of 0.920 (D2S1338) and the mixed population of Rajasthan showed a range of heterozygosity with the minimum value of 0.67192 (D3S1358) to a maximum value of 0.90536 (Penta-E). The exact test for Hardy-Weinberg

Equilibrium (HWE) showed that the most of loci in the studied population were in equilibrium ($p>0.003$) at a significant level of 95% (0.05/20) after Bonferroni Correction [14].

Discussion:

To reveal the genetic relatedness of studied populations with previously reported Pakistani, East Asian and other Indian populations, the phylogenetic analysis based on allele frequencies was performed and NJ (Neighbor-Joining) dendrogram was constructed [Fig. 2].

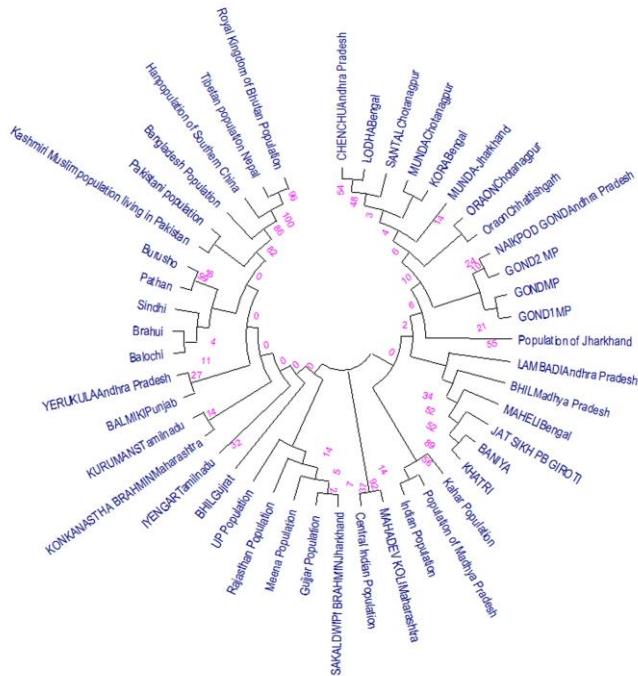


Fig.2. Neighbor Joining Phylogenetic Tree showing relatedness of 46 Asian populations based on 15 autosomal STR data

The NJ (Neighbor-Joining) dendrogram showed two major branches of population clusters with few additional outliers e.g. Mahadev Koli (Maharashtra) and Central

Indian population. Branch 1 had 4 major clusters which consisted mainly of the Indian population belonging to central and north-eastern states and Punjab and Andhra Pradesh. Branch 2 had 3 major clusters which consisted of Rajasthani populations along with populations of Pakistan as well as populations of North India. The studied populations i.e. Mina, Gujjar and mixed populations of Rajasthan were clubbed with population of Uttar Pradesh and Sakaldwipi Brahmins of Jharkhand population in one cluster while Pakistani population showed another cluster which was also reflected in heat map [Fig.4]. Further, our Principal Component Analysis (PCA) plot based on component 1 and component 2 scores which explained 94 % variance. In PCA plot, due to high degree of genetic variations some populations, namely; Jatt Sikh (Punjab), Baniya (Punjab), Khatri (Punjab), population of Bangladesh, Han population of Southern China, Tibetan population (Nepal), Royal Kingdom of Bhutan Population, Kashmiri Muslim population living in Pakistan and Pakistani population showed outlier nature in PCA plot, because of this most of the population clumped at one point [Fig. S1]. To visualize genetic distance among the clumped populations, we removed outlier populations from the PCA analysis and redrew the PCA plot [Fig. 3].

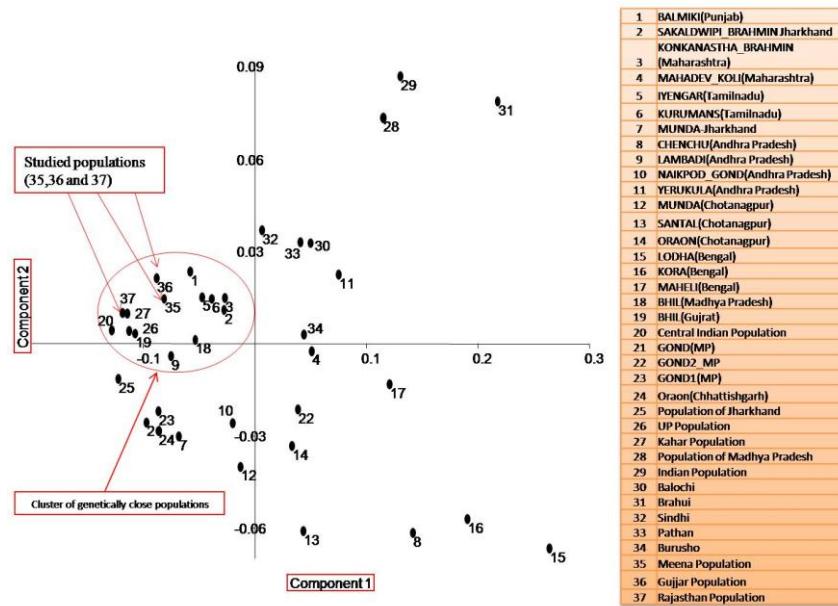


Fig.3. PCA plot indicating the distance and dissimilarity among 37 Asian populations based on 15 autosomal STR data, after removal of outlier populations

The studied population showed genetic closeness with the Central Indian populations, populations of Uttar Pradesh, Kahar population of Uttar Pradesh, and Balmiki's of Punjab. Interestingly, here we see longer distances of the studied populations from the Pakistani groups (Balochi, Brahui, Sindhi, Pathan, and Barusho). This indicates a higher genetic affinity of Rajasthani populations with the North Indian populations rather than Pakistani populations [Fig. 3-4].

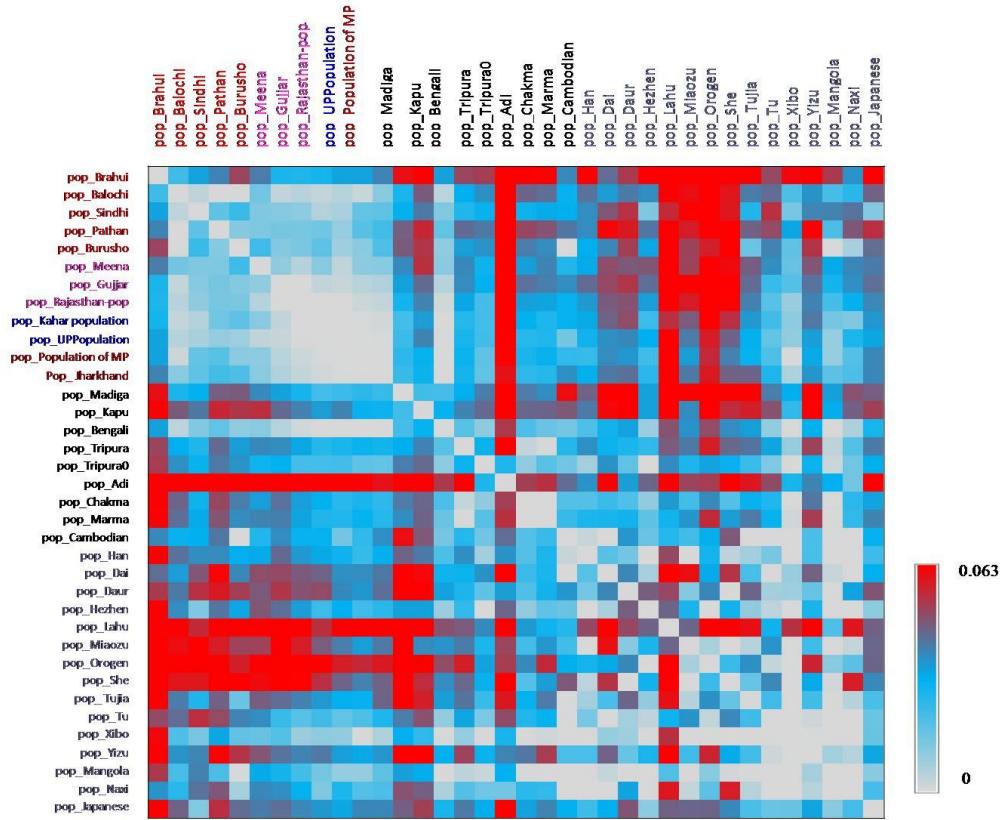


Fig.4. The heat map of Nei's genetic distance values for studied populations and 36 Asian populations

To validate this observation, we have done formal statistical test i.e. Analysis of Molecular of Variance (AMOVA) (Table 1). The North Indian populations had >2 times and Central Indian populations had >1.5 times low Fst values when we compared the Rajasthani populations with that of Pakistan. The AMOVA test suggested a highly significant genetic closeness of Rajasthani populations with the other Indian populations (North and Central Indian), in comparison with the Pakistani populations.

Table 1: Fst values between population of Rajasthan and other ethnic groups. (p values in all the comparisons were <0.0001)

Population groups	variation among the groups	variation among populations	variation within population	Fst
Rajasthan v/s Pakistan	0.25	0.37	99.38	0.0062
Rajasthan v/s Uttar Pradesh	0.05	0.24	99.72	0.00284
Rajasthan v/s Central India	0.20	0.20	99.61	0.00394
Rajasthan v/s East Asia	1.57	0.39	98.05	0.01952

To investigate the genetic similarity and differences among the studied populations and the previously reported Pakistani, East Asian, and other Indian populations, we performed STRUCTURE analysis [Fig. 5].

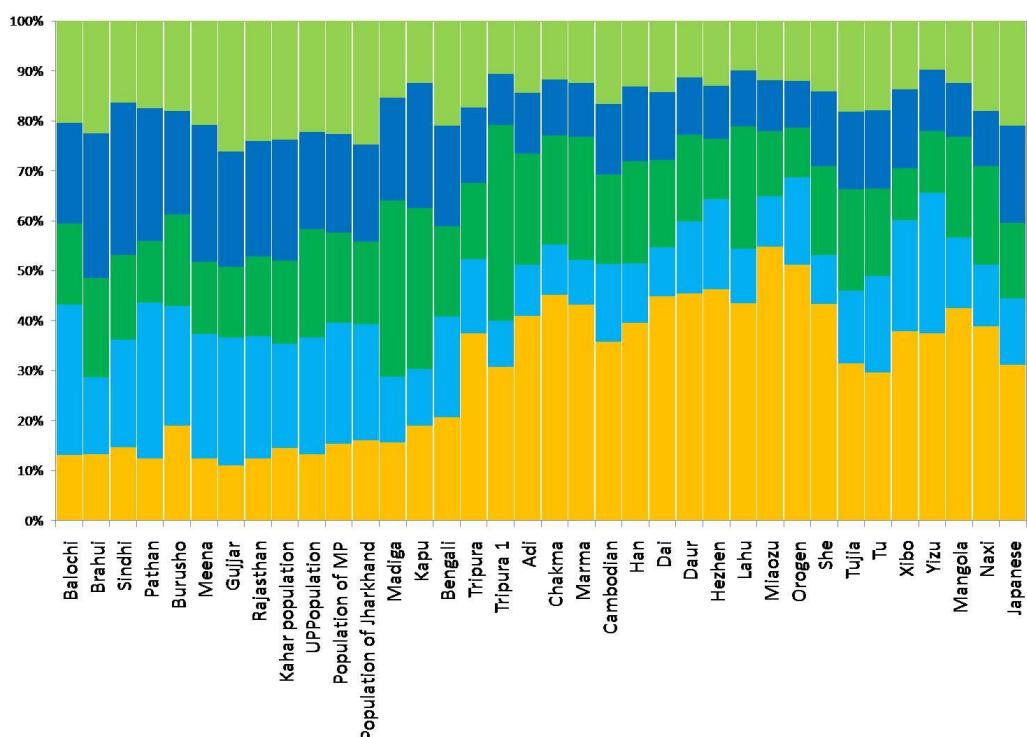


Fig.5. Population genetic structure of 36 Asian (west, south, east, south east) populations based on 15 autosomal STR markers ($k=5$)

The STRUCTURE analysis ($k=5$) suggested sharing of various ancestry components among the studied populations. Most of the ancestry components showed a East-West gradient along the geography. The most distinct component (yellow color) is prevalent in East Asian related populations. The light blue component is more biased towards West Eurasian related populations. The dark green component is prevalent among Dravidian speaking populations and is more similar to the ASI (Ancestral South Indian) component shown elsewhere[15][16]. The dark blue component showed a loose gradient from West to East. The light green component is peaking in North Indian populations and importantly, this is the prominent component which differentiates Rajasthani populations from the Pakistani populations substantially (Fig. 5).

We mostly found homogeneity among the Rajasthani populations in carrying these ancestry components. The sharing of these ancestry components also suggests a mixed ancestry of Rajasthani populations which shares alleles with the other Indian and Pakistani populations.

Conclusion:

In conclusion, we found that studied populations i.e. Mina, Gujjar and the mixed population of Rajasthan showed genetic affinity with populations of North and Central Indian populations rather than Pakistan. In spite of their geographical location, they are more homogeneous when intra-population comparison is done.

The data obtained from the studied populations of Rajasthan enriches the database and can be used for human identification and anthropological studies.

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Declarations:

Ethics approval and consent to participate:

This study was conducted in compliance with ethical standards and was approved by the Institutional Ethical Committee of the Jaipur National University, Jaipur, Rajasthan, India, vide letter no. JNUMSRC/IEC/2018/45 dated 20.07.2018. Written informed consent was taken from the participants, following the declaration of Helsinki.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/S1, Fig S1: PCA plot indicating the distance and dissimilarity among 46 Asian populations based on 15 autosomal STR data.

Author Contributions:

P.S. designed the study, R.K. collected the samples, R.K. and P.S. conducted the experiments, RK and SD analyzed the data, RK wrote the manuscript. P.S., G.C., D.S. and G.K. reviewed the manuscript.

Funding: This research received no external funding.

Conflict of Interest:

The authors declare that they have no conflict of interest in the conduct of the study.

Appendix A:

Fig S1: PCA plot indicating the distance and dissimilarity among 46 Asian populations based on 15 autosomal STR data.

Appendix B:

Fig.1. The geographical distribution of the studied populations

Fig.2. Neighbor Joining Phylogenetic Tree showing relatedness of 46 Asian populations based on 15 autosomal STR data

Fig.3. PCA plot indicating the distance and dissimilarity among 37 Asian populations based on 15 autosomal STR data, after removal of outlier populations

Fig.4. The heat map of Nei's genetic distance values for studied populations and 36 Asian populations

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