



Alu Indel Polymorphisms as a Tool for Genomic Diversity Study in Naga Tribes

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ABSTRACT The current study aims to find out the genomic relationship and affinities within and between the tribes and to compare the genomic of Nagas with that of the other populations. Four Naga tribes namely Angami, Lotha, Tikhir and Chakhesang were studied. Eight autosomal DNA markers of the Aluindel that is, ACE, CD4, PLAT, PV92, TPA25, FXIIIB, APO and D1 are used as a tool to find the diversity. Genotypes were determined for several Alu insertion/deletion loci after DNA was obtained, amplified by PCR, processed on agarose gel electrophoresis, and isolated DNA was amplified. The average heterozygosity is found to be moderated in all the four studied populations. At AluCD4 (GST=24.8%), the four study groups appear to differ the most, while Alu PLAT (GST=0.2%) shows the least variation. The GST value, which may be attributed to population variance, was 7.5 percent when all the markers are taken into account together. The heat map indicates the affinities of the studied population with the Irula tribe of the South India population. According to this study, the Naga population groupings are more genetically related to Southeast Asia than to either Africa or Europe.

INTRODUCTION

More than 1 billion people live on the Indian subcontinent, making up around one fifth of all people on earth. Australoid (central and southern region), Negritos, Mongoloids (sub-Himalayan and north eastern region), and Caucasoids (scattered throughout India) are four distinct morphological groups that can be found in India. However, India exhibits high levels of endogamy because of its rigid social borders and high rates of genetic drift as a result of isolation over a long period of time. These factors, along with India's extremely complicated history, make genetic research on Indian people difficult. 8.6 percent of the overall population is made up of modern Indian tribal populations. Only 10 percent of them reside in cities, with most of them living in rural areas. Scheduled Tribes (STs) mostly live in Central India and North-eastern India, two separate geographic regions. The majority of Nagaland

State is a hilly region with rich forest soil, an abundance of wildlife, and favourable agro-climatic conditions that range from sub-tropical to temperate at an elevation of 300 MSL to 3000 MSL. It is situated in India's far north-eastern corner. Its western border is with the Assam state, its northern and eastern borders are with Arunachal Pradesh and Assam, and its southern border is with Manipur. It is one among the smallest states in India. There are 16 tribes living in the state. Except for the regions bordering the Assam valley, which make up 9 percent of the state's total land, the territory is primarily mountainous. The research study has been severely constrained by Nagaland's terrain, lack of access to the sea, and limited communication infrastructure. The Naga tribes are believed to have moved to the western hills of Manipur from southwest China, most possibly in the later part of the 13th century A.D. However, no definite conclusion is made.

Objectives

The objectives of this study were:

1. To quantify the genomic diversity within and between the tribes of Nagaland.
2. To find out the genomic relationship and affinities within and between the tribes.

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- To study and compare the genomics of Nagas with that of the other populations.

MATERIAL AND METHODS

In the present study four Naga tribe communities were studied which are from the Angami, Lotha, Tikhir, and Chakhesang groups. Because of their predominate distribution in these districts, the four study population groups were precisely chosen from the Dimapur, Chumoukedima, Kohima, and Kipheri districts. Biography details were collected from participants in a proforma. Ethical permission was sought from the Institutional Ethical Committee wide letter number SJU/ZOO/IHEC/2018/01 for this study. Intravenous blood samples (2 ml) was taken from 137 unrelated individuals from the four studied population, including 33 Angami, 30 Tikhir, 40 Lotha, and 34 Chakhesang people of both male and female with the age range of 18–60 years, groupings, had. The method for choosing the subjects was a simple random sampling procedure. The blood samples were drawn by a trained medical expert.

Using a conventional salting-out, non-enzymatic method, the DNA samples were isolated from peripheral blood cells. Blood samples were used to extract genomic DNA using the conventional non-enzymatic salting out extraction method. A total of 137 blood samples were collected in centrifuge-tubes from healthy persons and unrelated persons. 2 ml of blood sample was added with EDTA with equal volume of TKM-I. Then they were added with 100 μ l of NP40 or Triton X and mixed well by inversion direction. It was then centrifuges at 2200 or 3000 rpm for 10 minutes. After that, the removal of supernatant was done. Another 5ml of TKM-I was added and the process was repeated up to 2- 4 times till white pellets were attained. After that 800 μ l of TKM-II was added to white pellets and mixed well. Then 125 μ l of 10 percent SDS was added and mixed well to incubate at 55 C in a water bath for 10 minutes. The contents were transferred to sterile eppendorf 1.5ml tubes, and added with 300 μ l of 6 μ NaCl and mixed well by inversion method and centrifuged at 10,000 or 12,000 RPM for 5 minutes in microcentrifuge. Then the supernatant was transferred to a 1.5ml centrifuge tube and double the volume of 100 percent alcohol was added. Finally, the DNA is preserved with 70 percent of alco-

hol. With the usage of locus-specific primers for the eight autosomal DNA markers each DNA sample was amplified by polymerase chain reaction (PCR). The markers include Alu ACE, Alu TPA25, Alu FXIIB, Alu APO, Alu D1, Alu PV92, Alu PLAT and Alu CD4 deletion. The procedures for these markers have previously been detailed (Stoneking et al. 1997). PCR products that had been amplified were run on an agarose gel, observed under a UV light, and recorded.

Statistical Analysis

For each population, the allele frequencies were calculated using direct counting. Using the allele frequencies for each group, heterozygosities at specific loci and the overall average heterozygosity were estimated. The 2 goodness of fit test was used to examine the Hardy-Weinberg equilibrium. The dendrograms were created by the neighbour joining (NJ) method (Nei 1973). Using the raw data of allele frequencies, Principal Coordinates (PCoA) Analysis was used to calculate the distance between populations.

Population Genetic Analysis software (POPGENE, Version 1.3) and Paleontological Statistics (Version 3) were used to analyse allele frequency, heterozygosity (H_T), observed heterozygosity (H_S) and genomic diversity (G_{ST}) and the Hardy Weinberg equilibrium was also by calculated using the software POPGENE. Past 3 software was used for the construction of NJ trees and principal coordinate analysis.

RESULTS

The results of the individual allele frequencies are shown in Table 1 for studied four Naga tribes of Nagaland. All the four populations showed polymorphism in their respective loci, the phenotype and genotype frequencies. When tested through the chi-square goodness of fit test I order to check their accuracy from Hardy-Weinberg proportions it was found that for most of the loci the phenotype and genotype frequencies showed their accuracy with their own Hardy-Weinberg outcomes and fail to show substantial difference from predictable frequencies. It shows that the maximum allele frequency (0.75) was in the Tikhir tribe for CD4 and the minimum was

Table 1: Allele frequencies at eight Alu Insertion / Deletion Polymorphic loci in four tribal populations

Loci	Angami			Tikhir			Lotha			Chakhesang		
	N	P(+)	χ^2 p -vate	N	P(+)	χ^2 p -vate	N	P(+)	χ^2 p -vate	N	P(+)	χ^2 p -vate
ACE	33	0.50	2.18 0.13	30	0.53	0.08 0.765	40	0.443	3.52 0.060	34	0.662	8.43* 0.003*
CD4	33	0.69	4.5 0.045*	30	0.75	1.52 1.52	40	0.212	0.008 0.92	40	0.241	2.69 0.10
PLAT	33	0.24	0.658 0.417	30	0.28	4.36 0.036*	40	0.263	3.71 0.053	40	0.221	0.191 0.66
PV92	33	0.27	4.34 0.037*	30	0.185	1.24 0.27	40	0.095	0.341 0.558	40	0.233	2.55 0.110
TPA25	33	0.15	10.38* 0.001*	30	0.333	0.414 0.52	40	0.075	3.942 0.047*	40	0.220	2.101 0.147
FXIIIIB	33	0.12	0.542 0.461	30	0.333	5.26* 0.02*	40	0.250	1.428 0.231	40	0.220	2.514 0.112
APO	33	0.36	0.034 0.852	30	0.166	0.945 0.33	40	0.378	0.015 0.900	40	0.349	2.628 0.104
D1	33	0.51	9.61* 0.001*	30	0.066	8.82* 0.002*	40	0.513	8.54* 0.003*	40	0.205	0.468 0.493

Note: χ^2 - Chi-square test for Hardy-Weinberg equilibrium; Degree of freedom : 1P(+)-frequency of '+' allele p-value (* significant d⁷⁰0.05)

(0.066) for D1 recorded in Tikhir tribe. D1 locus displays maximum difference through the populations reaching from 0.66 percent among Tikhir tribe to 51.3 percent among the Lotha tribe and CD4, which ranges from 21.2 percent in Lotha tribe to 75 percent in Tikhir tribe. On the other hand, PLAT locus shows minimum variation through the populations reaching from 22.1 percent among Chakhesang tribe to 28 percent among Tikhir tribe.

Table 2 displays the heterozygosity at respective locus and the overall average heterozygosity for the studied populations. The average heterozygosity is found to be mediocre in all the four studied populations for the eight markers with lowest at Alu TPA with average heterozygosity at 0.295 and the maximum heterozygosity in the Alu ACE with the average heterozygosity at 0.484 (Table 2), which is near to the highest potential heterozygosity of 0.5 for a biallelic locus. The four study populations' total average heterozygosity ranges from 0.367 in the Lotha tribe to 0.370 in the Angami tribe.

The genomic diversity analysis (Table 3) displays the estimated genetic diversity for every locus and for all loci together among the four Nagaland research groups. All of the studied populations showed significant levels of genetic variety, as seen in Table 3. At AluCD4 (GST=24.8%), the four study groups appear to differ the most, while Alu PLAT (GST=0.2%) shows the least variation. The GST value, which may be attributed to population variance, was 7.5 percent when all the markers are taken into account together. Overall moderate levels of heterozygosity were found among the subpopulations, with HT ranging from a low of 0.0163 at the Alu APO locus to a high of 0.2911 at the Alu ACE locus.

A neighbour joining tree was created to find the genomic similarities of the four Naga tribe study populations with that of the various Indian tribal groups by using available data (Mukherjee et al. 2000; Majumder et al. 1999; Basu et al. 2003; Vishwanathan et al. 2004; Saraswathy et al. 2008; Yadav and Arora 2010; Kshatriya et al. 2011; Panjalya et al. 2012) and the tree was illustrated in Figure 1. The tree shows that the four Naga tribe study populations have closer affinities with other Indian tribal populations such as Mota Chaudhary.

The information of Krishnaveni and Prabhakaran (2015), Kshatriya et al. (2011), Saraswathy et al.

Table 2: Average heterozygosity based on 8 Alu loci in Naga tribal populations from North East India

Name of the population	Alu ACE	Alu CD4	Alu PLAT	Alu PV92	Alu TPA25	Alu FXIIB	Alu APO	Alu D1	All loci
Angami	0.491	0.370	0.370	0.310	0.290	0.341	0.420	0.370	0.370
Tikhir	0.481	0.380	0.380	0.300	0.300	0.340	0.410	0.360	0.368
Lotha	0.484	0.360	0.370	0.306	0.296	0.344	0.415	0.362	0.367
Chakesang	0.480	0.374	0.376	0.306	0.296	0.344	0.416	0.361	0.369
Ave.Het	0.484	0.371	0.374	0.305	0.295	0.342	0.415	0.363	0.368

Note: ACE: Angiotensin Converting Enzyme; TPA 25: Tissue Plasminogen activator 25; FxIIB: Improved Coagulation Factor XIIIb; CD4: Cluster of Differentiation 4; APO: Apolipoprotein; D1: Alu insertion D1; PV92: Alu insertion PV92; PLAT: Tissue Plasminogen Activator

Table 3: Analysis of gene diversity for individual loci considered jointly between populations

Alu locus	Ht	Hs	Gst
Alu ACE	0.2911	0.2576	0.0259
Alu CD4	0.2932	0.0287	0.2489
Alu PLAT	0.0565	0.0534	0.0029
Alu PV92	0.2803	0.2445	0.0280
Alu TPA25	0.2519	0.2948	0.0574
Alu FXIIB	0.0174	0.0155	0.0323
Alu APO	0.0163	0.0499	0.0342
Alu D1	0.3043	0.0772	0.1741
All loci	0.18887	0.1277	0.0754

Note: Ht–Total genomic diversity among the populations
 Hs–Diversity between individuals within population
 Gst–Genetic diversity between population

(2008), Dada et al. (2011) and Rathika (2016) which are mutual with the current study were utilised to construct NJ trees in order to find out the genetic associations of the research of the study groups with various tribal groups of India (Fig. 1).

Another neighbour joining tree was created to find the genomic similarities of the four current study groups with various Indian as well as the world populations by utilising accessible sources and the tree was shown in Figure 1 and Figure 2. This tree illustrates that the four studied Naga tribes are closer with several Indian as well as the world tribal populations. Principal coordinate analysis (PCoA) is a technique to find the status and position of the population and to check and determine its closeness and distance from other populations. The analysis using principal coordinate analysis on the present study (Fig. 3) showed that the studied Naga population are close to the Siddis tribe of Andhra Pradesh and the Mota Chaudhary population of India, however are distant from various other Indian populations. G_{ST}

analysis was done and the result shows that the studied population has a closer relationship with that of Southeast Asia and Africa.

A plot of heterozygosity in contradiction of distance from the centroid of four study tribal group in addition to the world populations founded on allele occurrence data of eight loci (Fig. 4) showed that the studied Naga population showed closer association with that of the Southeast Asian population.

The results of the heat map on the other hand show the affinities of the four tribal populations with that of the Irula tribe of Tamil Nadu and vaiphe whereby the Chakesang tribe have a closer affinity to both the groups. The Lotha tribe on the other hand shows closer affinity with that of the Irula tribe.

DISCUSSION

Distribution of Allele frequency analysis at 8 autosomal DNA loci particularly Aluindels were among the currently studied four populations of Nagaland were carried out, which showed that there were differences in the occurrence pattern at most of the loci between four populations.

The current four groups of study, allele frequencies of Alu loci polymorphism as tools were compared to those of other Indian populations and the global population, and they revealed striking allele frequencies similarities to Siddis tribe of Andhra Pradesh and Mota Chaudhary population of Indian population and Southeast Asian populations globally, signifying their genetic closeness with the study groups.

Through this present study the average heterozygosity using the 8 markers varies from 0.367 in Lotha tribe to 0.370 in Angami tribe. When com-

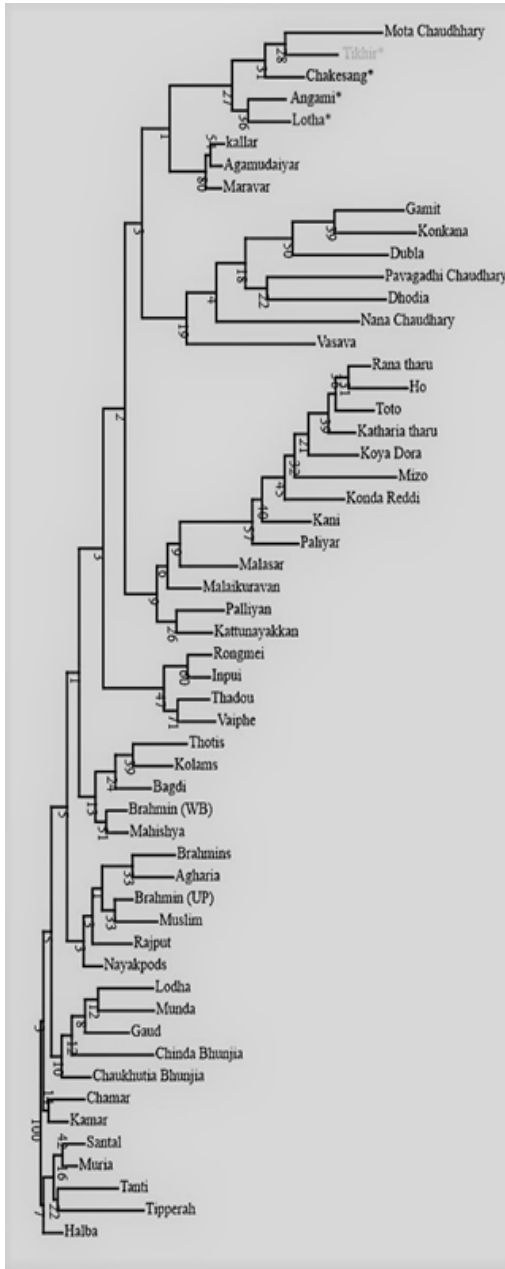


Fig. 1. Neighbour joining tree depicting genomic affinities of study populations with other Indian tribal populations based on allele frequencies at seven *Alu* indel loci

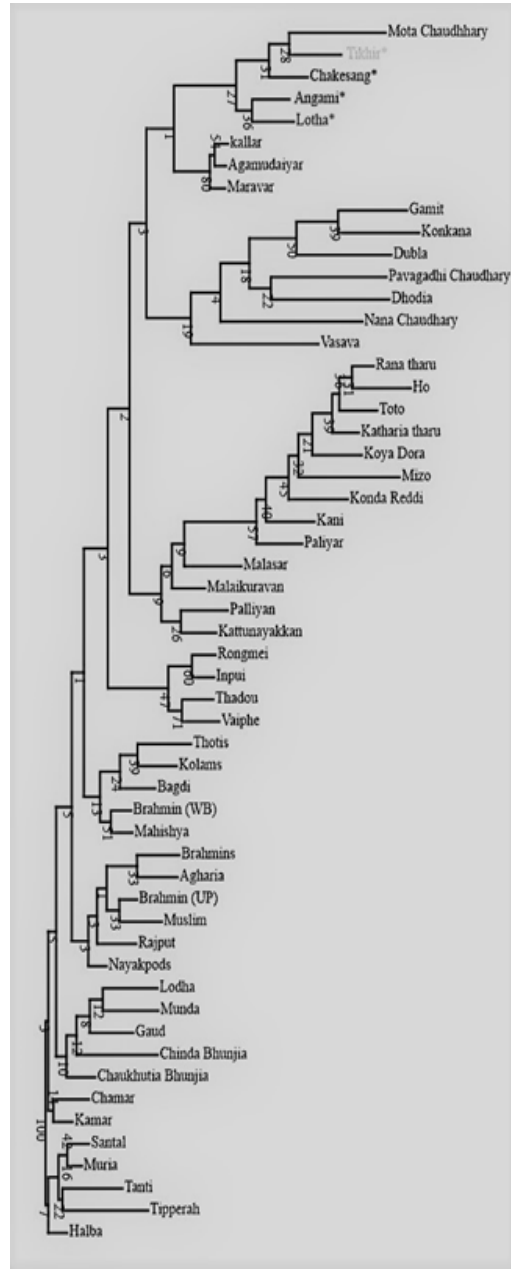


Fig. 2. NJ tree depicting genomic affinities of four study populations with 89 other global populations based on allele frequencies at six *Alu* insertion loci

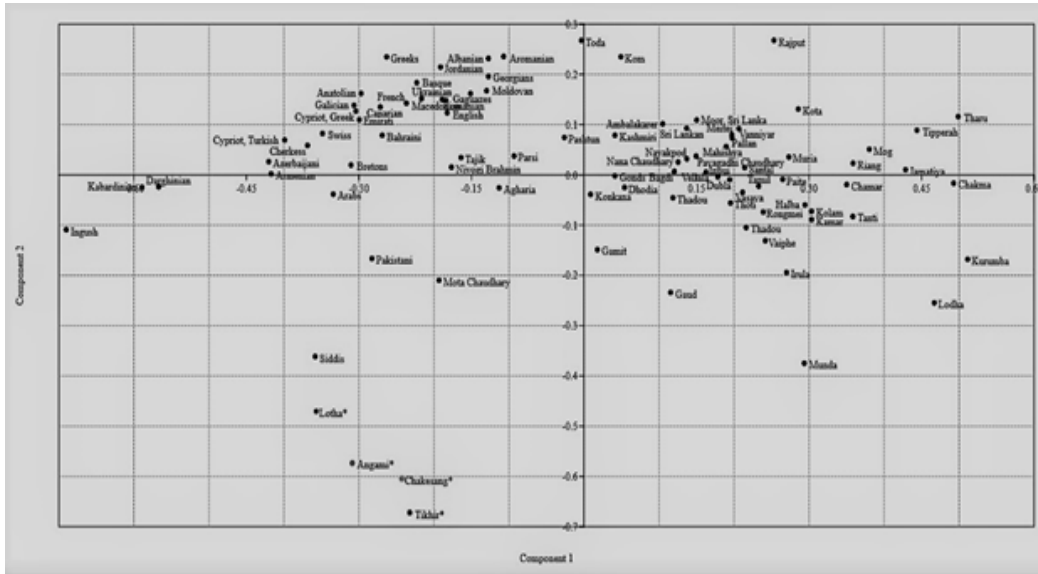


Fig. 3. Principal Coordinates (PCoA) Analysis of study populations with other Indian populations and global populations (ALU)

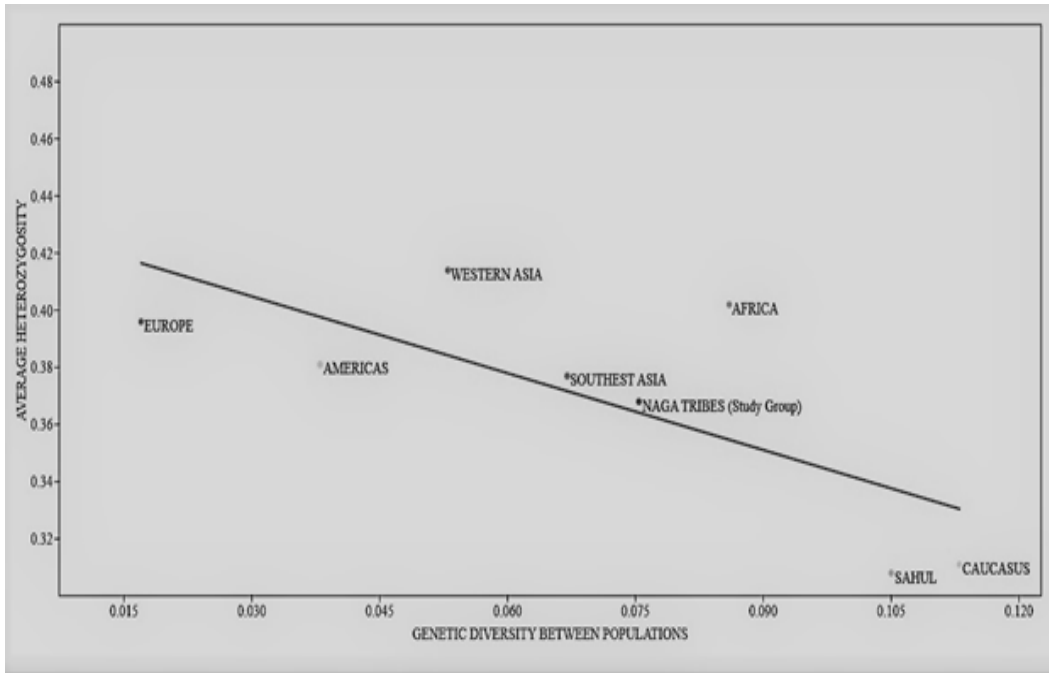


Fig. 4. Plot of heterozygosity vs. distance from centroid of four study populations in addition, world populations based on allele frequency data of eight loci

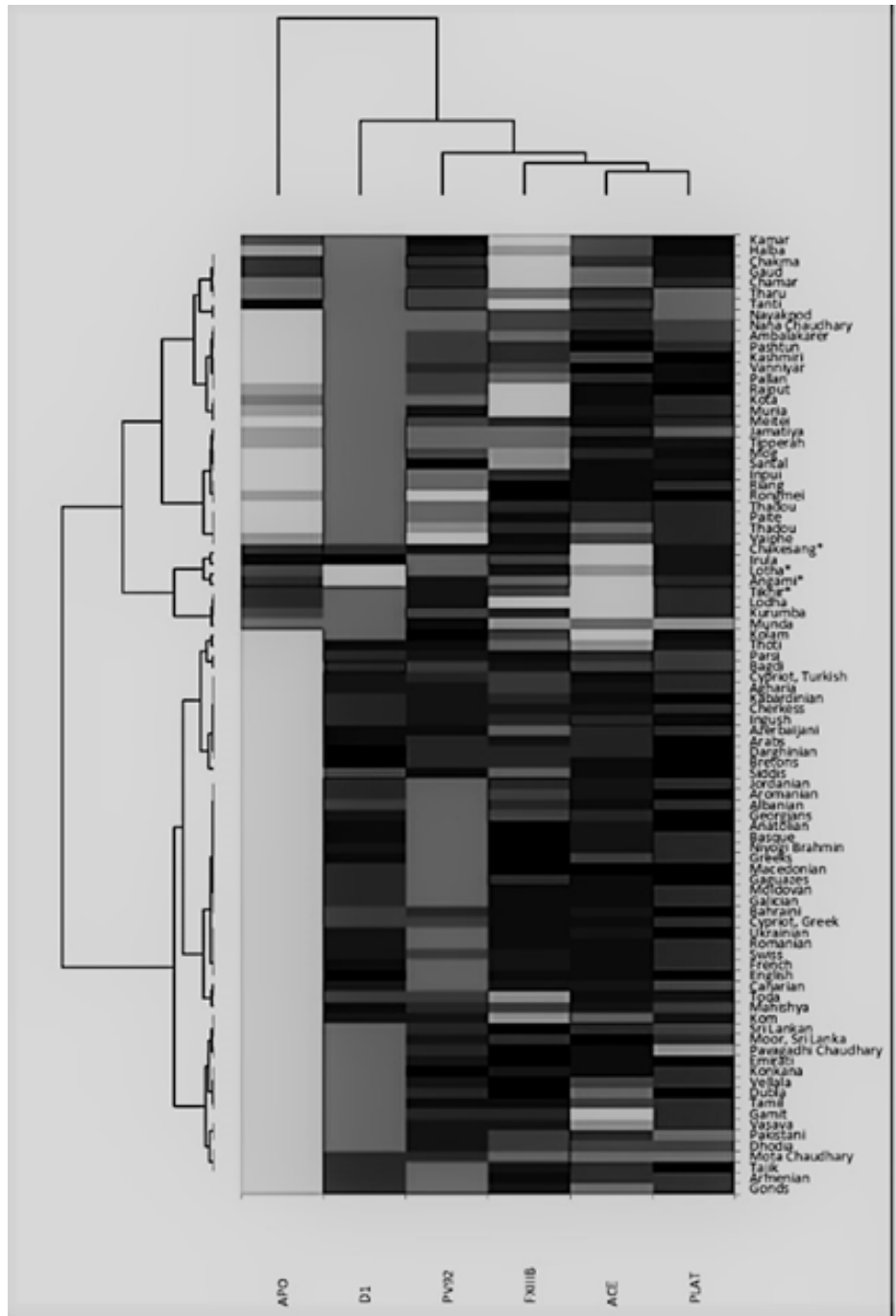


Fig. 5. Heat Map of the four tribal population with other population of India

pared to Manipur population where a study conducted using 20 markers having average heterozygosity that differs from 0.372 in Vaiphei reaching to 0.394 in Rongmei, it shows that the average heterozygosity in both the groups of population are very similar. Depending on the type of markers employed, different average heterozygosity values exist. The average heterozygosity levels of the four study populations are therefore lesser than the described heterozygosity values of different Indian populations, going up to high theoretical value of 0.5 when matched with studies done on a similar collection of 18 markers.

The gene differentiation value (G_{ST} value) in the current study was 7.5 percent, which is much higher than the G_{ST} value of 5.2 percent carried out on the Manipur population by Gangaina et al. (2021), through the usage of 20 autosomal loci. However the G_{ST} value of both the studies were detected in which they are higher than that detected in some other studies in Indian population (Kshatriya et al. 2011; Saraswathy et al. 2008; Veerajulu et al. 2001; Mukherjee et al. 2000) and greater than the records from various studies (Saraswathy et al. 2009; Vishwanathan et al. 2004) such as study by Vishwanathan et al. (2004) where they conducted a study on ethnic population of Tamil Nadu of South India during which they found the G_{ST} value to be 6.70 percent through the usage of 24 autosomal DNA markers. On the other hand, Meitei et al. (2010) carried out a research between the various inhabitant groups of Manipur through 7 aluInDel tools and detected that GST level between tribal people is higher, which is 7.4 percent when compared to non-tribal populations, which accounts only for 2.2 percent. As a result, it becomes clear that tribal groupings are more diverse than non-tribal groups. The strong endogamous nature of these populations, which suggests a separate origin for these populations, may be the cause of the high level of genetic differentiation (5.2%).

The four tribal communities of Nagaland's current study therefore emphasises the genetic similarities and variations amongst them. Genetic differentiation (GST) and molecular variance analyses have shown that there are genetic differences in all four tribal populations' allele distribution patterns. Thus it indicates that the tribal population of Nagaland undoubtedly has their differences. Finally, despite some genetic, cultural, and linguistic diversity among Naga tribes, yet all the four tribal groups seem genetically closer to the

Siddis tribe of Andhra Pradesh and Mota Chaudhary populations nationally and southeast Asian and west Asians populations globally rather than the Europeans.

The results of the heat map on the other hand show the affinities of the four tribal populations with that of the Irula tribe of Tamil Nadu and vaiphe whereby the Chakhesang tribe have a closer affinity to both the groups. The Lotha tribe on the other hand shows closer affinity with that of the Irula tribe.

A population study (Silva et al. 2017; Basu et al. 2016) revealed different genetic representations of Indian populations, thus making the populations history further difficult. In order to strengthen the ongoing discussion of Indian populations' genetics, the current work also aimed to study the genomic diversity of four Naga tribal populations, namely Angami, Lotha, Tikhir and Chakhesang. Several methods have been put forth to discuss the genomic relationship of the following populations with other population groups. The results specify that allele frequencies of In all four of the populations analysed, alu indel loci exhibit high polymorphism. The allele occurrence distribution design of the studied tribal populations shows great affinities with other Indian populations (Gangaina et al. 2021; Yadav and Arora 2010; Panjaliya et al. 2012; Saraswathy et al. 2008; Dada et al. 2011; Kshatriya et al. 2011; Krishnaveni and Prabhakaran 2015).

Due to the fact that there is much higher inter-individual variation within each research population than between populations, the level of population variance and incidence of the average GST value for all markers are fairly high in the four tribal groups (7.5%). As low as 2.0 percent (Kshatriya et al. 2011), to high level of 8.3 percent (Kshatriya et al. 2011), other studies have observed GST values that are comparatively lower (Vishwanathan et al. 2003).

Owing to their topographical position and environmental condition, the phylogenetic analysis reveals that the study groups are closer with each other and have high affinities genetically than to various Indian and global populations.

The current work shows that the studied Naga tribal groups from North East India reveal high polymorphism and high heterozygosity, however shows genomic differentiation and are genetically far from other population of Indian tribes as well as global populations.

CONCLUSION

The present study proposes that the tribal groups of Nagaland, regardless of phenotypic traits are genetically more similar to some Indian ethnic groups and South East Asia than it is to European nations. Based on eight autosomal loci, they seem to display significant levels of genetic diversity and genetic variation.

RECOMMENDATIONS

Nagaland, a north eastern state of India is situated in the eastern most part of India sharing its international borders with countries like Myanmar. It is therefore very less explored particularly in the field of genetics. The Naga tribal tribes, which are distinguished by their distinctive cultural customs and endogamy, indicate a primitive, Mongoloid demographic ancestry. The current study suggests a broader study on the field of genetics in the Naga tribes. Expansion of such studies is required in the Naga population so as to expand in the field of research and genetic knowledge among the Naga people.

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