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# Genetic Polymorphism of Five STR Markers among Four Groups of Punjabi Population in North-West Punjab, India

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**ABSTRACT** Historically, Punjab has served a major passage way for human migration to Indian subcontinent and has influenced the genetic structure of extant populations in Punjab. Therefore, Punjabi population possesses an exclusive genetic profile primarily due to the many migratory events in this region which caused an extensive range of genetic diversity. Hence, the present study is an attempt to understand the effect of these influences on genetic differentiation, diversity and population structure of Punjab. Genetic polymorphism at five highly polymorphic short tandem repeat loci (STR) is studied in four endogamous population groups of Punjab, India. The studied groups included Jat Sikh, Majbi Sikh, Brahmin and Ramdasia to evaluate their significance in human identification and genetic study. All selected groups practice endogamy and a total of 358 individuals belonging to these four endogamous groups were studied for five highly polymorphic with greater power of exclusion STR loci: THO1, TPOX, D7S820, CSF1PO and vWA. The highest observed heterozygosity was found in Ramdasia population for almost all the markers except vWA which had highest observed heterozygosity among Majbi Sikh population group. In this study, the average sub-ethnic differentiation (Fst) among the four populations of north-west Punjab was 0.0821. The marker with the highest contribution to interpopulation genetic difference was observed to be vWA.

## INTRODUCTION

The multiallelic and hyper variable nature of STRs make them highly informative markers to study the population genetic structure and evolutionary relationship between human populations, forensic sciences and human gene mapping (Nakamura et al. 1987; Deka et al. 1995; Shazia et al. 2009; Ferdous et al. 2010;He and Guo 2013; Liu et al. 2013; Vieira et al. 2013; Soltyszewski et al. 2014). Therefore, in the present study, the researchers report allele frequency data of five autosomal polymorphic microsatellite loci (THO1, TPOX, D7S820, CSF1PO and vWA) from four distinct endogamous groups of Punjabi population in India to obtain a reference population genetic database.

### Population Information

Ethnic Punjabi population shows enormous cultural, linguistic, and genetic diversity due to its positioning on the crossroads of many historic and pre-historic human migrations (Sekhon 2000). The hierarchical caste system dominates the social structure of the Punjabi populations. The origin of the caste system in India is a matter of debate with many linguists and anthropologists suggesting that it began with the arrival of Indo-European speakers from Central Asia about 3500 years ago. Also, Indian castes have been found to be more closely related to the Central Asians than to the Indian tribal groups (Cordaux et al. 2004; Nair et al. 2011). Punjabi population possesses an exclusive genetic profile primarily due to the many migratory events in this region which caused an extensive range of genetic diversity. Hence, the present study is an attempt to understand the effect of these influences on genetic differentiation, diversity and population structure of Punjab. Till date no study has reported the microsatellite diversity among these population groups in Punjab. Of all the states in India, Punjab has the highest number of Scheduled Caste group. They constitute an impressive 28.9% of the total population of Punjab. The caste groups selected for the present study were Jat Sikh (higher caste of Sikh religion), Majbi Sikh (lower caste of Sikh religion), Brahmins (higher caste of Hindu religion) and Ramdasia/Valmiki (lower caste of Hindu religion) with their informed consents. All selected groups practice a high degree of endogamy (Sekhon 2000).

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***Jat Sikh***: Jats are the biggest group in terms of numbers (66%) among Sikh religion and recognized as higher caste group. They own more than 80%, of available agricultural land in Punjab. Besides agriculture, which is their signature trade, Jat Sikhs are now very well educated and have taken up various professions. They often reside in the rural areas, and are economically influential in the state. Jat Sikhs are known for their lively spirit and easy-going nature. The Jats have probably originated as one of the late immigrants to the subcontinent.

***Majbi Sikh*:** They are mainly found in the Punjab, Kashmir and Rajastan regions (Sekhon 2000) and are considered lower caste group in Sikh religion. In the glorious Sikh Regiment in Indian army, Majbi Sikhs were recruited in good numbers due to their bravery, physical strength and self-sacrifice. Their houses are generally located on the outskirts of the town and are economically very poor. The urban Mazbhis have made social and economic progress over the years, however, poverty and illiteracy is still rampant among them.

***Brahmin:*** They are recognized as the highest class group among Hindu religion. In Punjab most of the Brahmins are Saraswat Brahmins and they were recognized as the intellectual and priestly class from ancient civilization to till date. They are highly respected and honored for creating the world's oldest literary and religious traditions. They were the original propagators of the revered texts such as the Vedas and the Upanishads. They have also excelled as educators, law makers, scholars, doctors, warriors, writers, poets, land owners and politicians.

***Ramdasia***: They generally belong to socially low class society among the Hindu religion. The Ramadasia community has traditionally been relegated to the most menial labour with negligible possibility of upward mobility and also subject to social disadvantages and exclusion in comparison to the wider community. More than 80% of Ramadasias in Punjab are living in villages.

A very few number of genetic studies (Badaruddoza et al. 2007, 2008; Badaruddoza and Sudhir 2012; Saini et al. 2012) have been carried out on Punjabi population. However, no STR marker based study on present study populations have been reported in the literature till date. Therefore, the present data would be used in forensic and individual identification for these selected population groups and these genetic data would enrich Indian genetic informational resources. The study was approved by Ethical Research Committee of Guru Nanak Dev University, Amritsar, Punjab.

## MATERIAL AND METHODS

### Sample Collection

3ml venous blood samples were obtained from a total of 358 (112 Jat Sikh, 105 Majbi Sikh, 71 Brahmin and 70 Ramdasia) unrelated healthy subjects through simple random sampling from four endogamous groups of north-west border districts of Punjab such as Amritsar, Fazilka, Ferozepur, Gurdaspur, Pathankot and Tarn Taran. Blood was immediately transferred to pre-labelled blood collecting vial containing 0.5M EDTA (as anticoagulant). All samples were transported on ice from the place of sample collection to laboratory and were stored at -20°C till further analysis.

### DNA Extraction, PCR Amplification and STR Genotyping

Genomic DNA was extracted using phenol-chloroform method (Sambrook et al. 1989) and quantified using NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific™, Pittsburgh, US). In the present study five STR markers were selected: THO1, TPOX, CSF1PO, vWA and D7S820 on the basis of their heterozygosity and their feasibility for rapid analysis (through PCR) in lab. Selection of the present STR marker has also been based on the global survey carried out by Perez-Lezaun et al. (1997).

The PCR amplification was done on Mastercycler ® Personal (Eppendorf, Germany). using locus specific primer pairs (Table 1). PCR program used was initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 45 sec, 58°C for 45 sec, 72°C for 45 sec, followed by final extension at 72°C for 10 min for THO1, TPOX and CSF1PO loci done in a multiplex. Same protocol was used for other two loci also except the annealing step which was 56°C for vWA and 53°C for D7S820. The PCR reaction mixture of 15 µl consists of 50 ng of DNA, Taq polymerase buffer, 200 µmol/l dNTPs, and 1.0 U Taq polymerase and 5 pmol of each primer. PCR products were checked on 2.5% agarose gel and then ana-

lyzed on 10% native polyacrylamide gel electrophoresis (PAGE) and various alleles were detected after proper silver staining.

### Statistical Analysis

Various statistical analyses were performed on the STR data to accomplish the objectives of the study. The allele frequencies, observed and expected heterozygosity, genotype distribution to the Hardy-Weinberg equilibrium was performed using GENEPOP software pakage (version 3.3). Locus informativeness parameters, such as matching probability (MP), power of discrimination (PD), polymorphism information content (PIC), power of exclusion (PE) and paternity index (PI) were analyzed using PowerStat v12 software (Promega Corporation, USA). The phylogenetic tree was drawn by unweighted group-method with arithmetic mean (UPGMA). Pair wise genetic distances were calculated based on the allele frequencies of STR loci using Nei's method.

## RESULTS

The distributions of the observed allele frequencies for five STR loci in four Punjabi population groups are compared in Table 2. Seven for THO1, eight for TPOX and D7S820 each, 10 for CSFIPO and 12 for vWA different alleles were observed. The maximum allele repeats for THO1, TPOX, D7S820, CSF1PO and vWA have been observed 9, 8, 10, 12 and 16 respectively in Jat Sikh; 9, 8, 8, 13 and 17 respectively in Majbi Sikh; 6, 8, 11, 14-15 and 17 respectively in Brahmin; 9, 7, 10, 13 and 17 respectively in Ramdasia groups. It is also noticed that 8, 9 and 17 allele repeats are most common in all four groups. Different populations contain alleles with repeated number varying from 5 to 23. In all four populations, the distributions of allele frequencies are bimodal with major peak in 8 and secondary peak may be found at alleles 9 and 17. The highest frequency (44.64%) observed for allele 12 for CSFIPO locus among Jat Sikh, whereas, the lowest frequency (0.45%) observed for allele 8 for the same locus among Jat Sikh.

For all the markers, the researchers determined the observed and expected heterozygosity values (Table 3). The highest observed heterozygosities were found in Ramdasia group for four loci (0.8462, 0.7692, 0.9231 and 0.7692 for THO1, TPOX, D7S820 and CSF1PO loci respectively). However, the observed heterozygosity varied from 0.5893 in Jat Sikh for CSFIPO locus to 0.9231 in Ramdasia for D7S820 locus. In general, the average observed heterozygosity is lower than expected heterozygosity in five STR markers in four population groups. In this study the average sub-ethnic differentiation (Fst) among four population groups of northwest Punjab was 0.0821. The D7S820 marker has showed small inter-population differences ($F_{st}$=0.0145), whereas, vWA showed the highest contribution to inter-population genetic differences ($F_{st}$=0.1577).

With respect to PIC value, THO1, TPOX, D7S820, CSF1PO and vWA loci were classified as highly informative markers (PIC >0.70) (Table 4). All five studied loci have considerable discriminating power, PD values lying in the range from 0.834 for THO1 in Ramdasia to 0.949 for vWA in Jat Sikh. Therefore, in total sample there were no two individuals with the same genotype of the five marker system.

Based on the genetic distance, dendrogram was constructed to depict the genetic affinities (Fig. 1) among four population groups. The dendrogram showed the low genetic distance bem-

**Table 1: STR markers with primer sequences and product sizes**

| *STR marker* | *Primers* | *Product size (bp)* |
|---|---|---|
| HUMTHO1 | For: ATTCAAAGGGTATCTGGGCTCTGG | |
| | Rev: GTGGGCTGAAAAGCTCCCGATTAT | 171-215 |
| HUMTPOX | For: ACTGGCACAGAACAGGCACTTAGG | |
| | Rev: GGAGGAACTGGGAACCACACAGGTTA | 216-264 |
| HUMCSF1PO | For: AACCTGAGTCTGCCAAGGACTAGC | |
| | Rev: TTCCACACACCACTGGCCATCTTC | 287-331 |
| HUMVWA | For: CCCTAGTGGATGATAAGAATAATC | |
| | Rev: GGACAGATGATAAATACATAGGATGGATGG | 122-182 |
| D7S820 | For: TGTCATAGTTTAGAACGAACTAACG | |
| | Rev: CTGAGGTATCAAAAACTCAGAGG | 198-234 |

**Table 2: Allele frequency data of THO1, TPOX, CSF1PO, vWA and D7S820 microsatellite markers among four Punjabi population groups (n=358)**

| *Locus* | *Allele* | *Populations* | | | |
|---|---|---|---|---|---|
| | | *Jat Sikh (n=112)* | *Majbi Sikh (n=105)* | *Brahmin (n=71)* | *Ramdasia (n=70)* |
| *THO1* | 5 | 0.0089 | 0.0147 | 0.0373 | 0.0962 |
| | 6 | 0.1964 | 0.2843 | 0.2910 | 0.1154 |
| | 7 | 0.1518 | 0.1765 | 0.2537 | 0.2115 |
| | 8 | 0.2500 | 0.1912 | 0.1866 | 0.1923 |
| | 9 | 0.3170 | 0.2892 | 0.1567 | 0.3269 |
| | 9.3 | 0.0759 | 0.0441 | 0.0672 | 0.0577 |
| | 10 | 0.0000 | 0.0000 | 0.0075 | 0.0000 |
| *TPOX* | 5 | 0.0000 | 0.0000 | 0.0217 | 0.0000 |
| | 6 | 0.0893 | 0.0882 | 0.1087 | 0.0577 |
| | 7 | 0.1607 | 0.1667 | 0.2609 | 0.3654 |
| | 8 | 0.2991 | 0.2794 | 0.2826 | 0.3269 |
| | 9 | 0.1920 | 0.2206 | 0.2101 | 0.1731 |
| | 10 | 0.1295 | 0.1520 | 0.0870 | 0.0769 |
| | 11 | 0.1116 | 0.0784 | 0.0217 | 0.0000 |
| | 12 | 0.0179 | 0.0147 | 0.0072 | 0.0000 |
| *D7S820* | 7 | 0.0357 | 0.1188 | 0.0652 | 0.0000 |
| | 8 | 0.1250 | 0.2327 | 0.1884 | 0.1154 |
| | 9 | 0.0848 | 0.0693 | 0.1087 | 0.2308 |
| | 10 | 0.3571 | 0.1931 | 0.1377 | 0.3269 |
| | 11 | 0.1920 | 0.2129 | 0.2464 | 0.2115 |
| | 12 | 0.1339 | 0.1584 | 0.1957 | 0.0577 |
| | 13 | 0.0491 | 0.0149 | 0.0580 | 0.0577 |
| | 14 | 0.0223 | 0.0000 | 0.0000 | 0.0000 |
| *CSF1PO* | 7 | 0.0000 | 0.0000 | 0.0000 | 0.0577 |
| | 8 | 0.0045 | 0.0000 | 0.0072 | 0.0385 |
| | 9 | 0.0045 | 0.0147 | 0.0217 | 0.1346 |
| | 10 | 0.2054 | 0.0980 | 0.0725 | 0.1731 |
| | 11 | 0.2723 | 0.1863 | 0.1377 | 0.1346 |
| | 12 | 0.4464 | 0.2745 | 0.1304 | 0.1538 |
| | 13 | 0.0670 | 0.2794 | 0.1377 | 0.1923 |
| | 14 | 0.0000 | 0.1078 | 0.2391 | 0.0962 |
| | 15 | 0.0000 | 0.0392 | 0.2391 | 0.0192 |
| | 16 | 0.0000 | 0.0000 | 0.0145 | 0.0000 |
| *vWA* | 11 | 0.0367 | 0.0300 | 0.0000 | 0.0000 |
| | 12 | 0.0550 | 0.0650 | 0.0145 | 0.0000 |
| | 13 | 0.0229 | 0.0400 | 0.0000 | 0.0192 |
| | 14 | 0.1514 | 0.1350 | 0.1232 | 0.0192 |
| | 15 | 0.1239 | 0.0800 | 0.0290 | 0.0000 |
| | 16 | 0.1560 | 0.2350 | 0.2391 | 0.2308 |
| | 17 | 0.0780 | 0.3000 | 0.3768 | 0.3077 |
| | 18 | 0.1055 | 0.0500 | 0.1014 | 0.2885 |
| | 19 | 0.1055 | 0.0650 | 0.0870 | 0.0962 |
| | 20 | 0.0826 | 0.0000 | 0.0290 | 0.0385 |
| | 21 | 0.0780 | 0.0000 | 0.0000 | 0.0000 |
| | 23 | 0.0046 | 0.0000 | 0.0000 | 0.0000 |

tween Majbi (lower caste group of Hindu religion) and Brahmin (higher caste group of Hindu religion). The tree also reflected the ethnic background of the four populations. Considering the linguistics and genetic affinities, the four groups have same origin with recent separation from common stock. However, the Majbi and Brahmin groups showed extensive gene flow between each others.

## DISCUSSION

The present study was undertaken to produce population genetic database based on STR

**Table 3: Observed and expected heterozygosity values and genetic differentiation coefficients in four Punjab population groups (n=358)**

| *Locus* | *Parameter* | *Jat Sikh (n=112)* | *Majbi Sikh (n=105)* | *Brahmin (n=71)* | *Ramdasia (n=70)* | *Mean* |
|---|---|---|---|---|---|---|
| *THO1* | $H_{obs}$ | 0.6518 | 0.7941 | 0.7463 | 0.8462 | 0.7596 |
| | $H_{exp}$ | 0.7730 | 0.7694 | 0.7915 | 0.8009 | 0.7837 |
| | $F_{st}$ | | | | | 0.0307 |
| *TPOX* | $H_{obs}$ | 0.6429 | 0.7451 | 0.7246 | 0.7692 | 0.7205 |
| | $H_{exp}$ | 0.8140 | 0.8122 | 0.7933 | 0.7345 | 0.7885 |
| | $F_{st}$ | | | | | 0.0862 |
| *D7S820* | $H_{obs}$ | 0.7679 | 0.7129 | 0.8841 | 0.9231 | 0.8220 |
| | $H_{exp}$ | 0.7942 | 0.8231 | 0.8332 | 0.7903 | 0.8102 |
| | $F_{st}$ | | | | | 0.0145 |
| *CSF1PO* | $H_{obs}$ | 0.5893 | 0.6961 | 0.6522 | 0.7692 | 0.6767 |
| | $H_{exp}$ | 0.6829 | 0.7928 | 0.8307 | 0.8756 | 0.7955 |
| | $F_{st}$ | | | | | 0.1493 |
| *vWA* | $H_{obs}$ | 0.6789 | 0.7700 | 0.6812 | 0.6154 | 0.6864 |
| | $H_{exp}$ | 0.8954 | 0.8208 | 0.7715 | 0.7722 | 0.8150 |
| | $F_{st}$ | | | | | 0.1577 |
| General $F_{st}$ | | | | | | 0.0821 |

**Table 4: Informativeness of the five microsatellite markers studied**

| *Locus* | *Parameter* | *Jat Sikh* | *Majbi Sikh* | *Brahmin* | *Ramdasia* |
|---|---|---|---|---|---|
| *THO1* | MP | 0.086 | 0.114 | 0.089 | 0.166 |
| | PD | 0.914 | 0.886 | 0.911 | 0.834 |
| | PIC | 0.74 | 0.73 | 0.75 | 0.75 |
| | PE | 0.367 | 0.599 | 0.523 | 0.687 |
| | PI | 1.46 | 2.50 | 2.06 | 3.25 |
| *TPOX* | MP | 0.070 | 0.069 | 0.101 | 0.154 |
| | PD | 0.930 | 0.931 | 0.899 | 0.846 |
| | PIC | 0.78 | 0.78 | 0.76 | 0.67 |
| | PE | 0.345 | 0.501 | 0.467 | 0.543 |
| | PI | 1.40 | 1.96 | 1.82 | 2.17 |
| *D7S820* | MP | 0.082 | 0.066 | 0.070 | 0.115 |
| | PD | 0.918 | 0.934 | 0.930 | 0.885 |
| | PIC | 0.77 | 0.79 | 0.80 | 0.74 |
| | PE | 0.541 | 0.448 | 0.763 | 0.843 |
| | PI | 2.15 | 1.74 | 4.31 | 6.50 |
| *CSF1PO* | MP | 0.158 | 0.081 | 0.070 | 0.077 |
| | PD | 0.842 | 0.919 | 0.930 | 0.923 |
| | PIC | 0.62 | 0.76 | 0.80 | 0.84 |
| | PE | 0.278 | 0.422 | 0.358 | 0.543 |
| | PI | 1.22 | 1.65 | 1.44 | 2.17 |
| *vWA* | MP | 0.051 | 0.066 | 0.105 | 0.118 |
| | PD | 0.949 | 0.934 | 0.895 | 0.882 |
| | PIC | 0.88 | 0.79 | 0.73 | 0.72 |
| | PE | 0.396 | 0.545 | 0.393 | 0.310 |
| | PI | 1.56 | 2.17 | 1.55 | 1.30 |

MP-matching probability; PD-power of discrimination; PIC-polymorphism information content; PE-exclusion potential; PI-paternity index

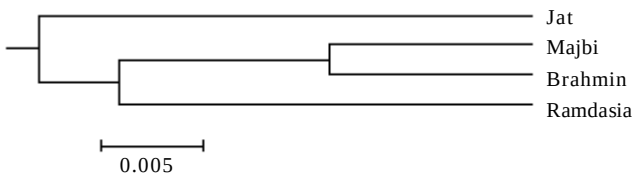


**Fig. 1. Nei's genetic distances was used to generate the dendogram illustrating the phylogenetic relationship of four studied population groups**

loci among four Punjabi caste groups to compare allele frequencies. Presently, there has been increasing use of microsatellite loci to understand the genetic relationship between closely related populations (Chu et al. 1998; Reddy et al. 2001, 2005; Krithika et al. 2008). In the present study the researchers used five autosomal STR microsatellite markers to understand the population structure and variation of four caste populations distributed in north-west Punjab. These STR loci are highly polymorphic within each of the population. The distributions of allele frequencies are moderately uniform across the population, suggesting relative homogeneity among the four castes of Punjabi population. No deviation was observed of the studied loci from Hardy Weinberg Equilibrium (HWE). The least average heterozygosity value among Jat Sikh for CSF1PO loci (~59%), THO1 (~65%) and TPOX (~64%) might be explained by their preferential marriage practices among clans prohibiting external gene flow. In the present study, D7S820 was the only marker showing very small differences among the population studied, whereas, the marker with highest contribution to inter-population genetic differences was vWA. Furthermore, not many studies have been done in these population groups to compare the present results (Reddy et al. 2005; Khan et al. 2007; Noor et al. 2009). The results suggested that the present marker system actually meets all the existing requirements and can be used for DNA typing and population studies.

The phylogentic analyses of four caste populations showed closer proximity between Majbi Sikh which is lower caste of Sikh religion and Brahmin which is higher caste of Hindu religion. This probably suggested their common genetic affinity and possible admixture between these two populations. Therefore, the clustering between Brahmin and Majbi Sikh also points towards the possibility of the central Asian immigrants appointed themselves to predominantly belonged to caste of higher rank and subsequently, the lower caste was admixed with them. The similar type of clustering between higher and lower caste has been observed in many north Indian population (Bamshad et al. 2001; Khan et al. 2007). However, the results should be confirmed in further study. Overall, Jat Sikh population (higher caste) are well differentiated with other neighboring populations. Bamshad et al. (2003) expressed the view that a large number of microsatellite loci are required to differentiate populations. However, the present five autosomal STR markers that are highly polymorphic and widely used for forensic investigations, for investigating local population structure and to construct the evolutionary relationship between different groups of population (Langsteieh et al. 2004; Reddy et al. 2005).

## CONCLUSION

The phylogenetic analysis of four endogamous caste populations of the north-west Punjab based on five STR loci have revealed the information about the genetic affinities between Jat Sikh (higher caste of Sikh religion), Majbi Sikh (lower caste of Sikh religion), Brahmin (higher caste of Hindu religion) and Ramdasia (lower caste of Hindu religion). The present results have demonstrated that intra-population differences were marginal, however, there was a definite pattern of genetic variation found in four different caste populations.

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