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Study of DYS 390 Polymorphism among Khatri Population of Punjab in Comparison to Other Indian and World Population

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ABSTRACT The Y chromosome specific polymorphism (Y-STR and Y-SNPs) can be used to track paternal lineages as well as male specific movements and admixture among humans. The objective of the present work is to study the genetic polymorphism at Y chromosome specific STR locus, DYS390 among Khatri population of Punjab and generate a comparative data with respect to other Indian and world populations. The DNA was isolated from blood samples of Khatri male individuals through organic method and were amplified by PCR using specific primers for DYS390 locus. The PCR products were electrophoresed on polyacrylamide gels and silver staining was done to resolve and observe different alleles. Gel-Pro 3.1 software was used to confirm the allele size. Chi-Square test was applied to test whether differences between the allele frequencies among Khatri, other Indian and world populations were statistically significant. Network Joining (NJ) tree was constructed using Network analysis software. The results showed 7 different alleles ranging from 22 to 28 in Khatri population and revealed a significantly high degree of genetic heterogeneity at this locus. The gene diversity was found to be 0.8363. The Network Joining tree showed Khatri population as an independent branch alongside Chinese. Therefore, it can be inferred that Khatri population is probably a result of conglomeration of different lineages, like most other North-West Indian population.

INTRODUCTION

Khatri caste is one of the major business communities of Punjab (India). In pre independence era, they were mostly settled in the Western part of undivided Punjab state but subsequently after independence, they migrated to Indian part of Punjab and other North Western states of India like Haryana, Himachal Pradesh, Delhi and Uttar Pradesh. The aim of the present study is to bring the most mobile Khatri population of the state of Punjab on the molecular genetic map of the world through the study of genetic polymorphism at Y-chromosome specific STR locus DYS 390.

The male-specific essentially haploid Y-chromosome is one of the smallest human chromosomes with an average size of 60 million base pairs. The non-recombining portion of Y-chromosome contains different kinds of polymorphisms with known mutation rates. It is inherited as a single unit or haploid through paternal line of transmission (Vandenberge et al. 1999; Ploski et al. 2002). Recently, there has been a rapid growth in the discovery of new Y-STR markers (Kayser et al. 1997; Jobling et al. 1998; Ayub et al. 2000; Bosch et al. 2000; Butler 2003; Jobling and Tyler-Smith 2003; Deng et al. 2004; Shephered et al. 2004) to investigate the population affinities, their origin and subsequent distribution in various parts of globe. The Y-STR loci became also useful markers to identify male lineage in forensic practice.

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MATERIALS AND METHODS

Blood samples of individuals belonging to Khatri population were collected randomly after obtaining informed written consent from the donor and for the DYS390 polymorphism survey, a total of 146 samples were analysed. DNA was extracted from blood samples by organic method (Gill et al. 1987) through phenol/chloroform extraction. The quality and quantity of DNA was checked by agarose gel electrophoresis. The PCR conditions for DYS390 consisted of an initial denaturation at 95°C for 2 minutes followed by 30 cycles of denaturation at 94°C for 1 minute, primer annealing at 50°C for 1 minute and primer extension at 72°C for 1 minute. The final extension step was extended to 10 minutes followed by incubation at 4°C. The primers used for PCR to amplify DYS 390 were as follows:

Forward: 5'-TTATTTTACACATTTTTGGG CC-3'

Reverse: 5'-TGACAGTAAAATGAACACA TTGC-3'

After PCR amplification, the samples were electrophoresed on non-denaturing 10% polyacrylamide gel (Maniatis et al. 1989) for 18 hr at constant voltage of 100 V. The gels were then silver stained to visualize various alleles at this locus and photographed.

Gene diversity D was used to measure the informativeness of each locus. Values for each locus were calculated as per Nei (1987):

$D=1-\sum x_i^2$, where the x_i is the allele frequency in a population. The measure of genetic similarity (I_N) and distance (D_N) were also calculated as per Nei and Kumar (2000), I_N

$$=\sum_{i=1}^{m}(p_{ix}\,p_{iy}) / \sqrt{(\sum_{i=1}^{m} p^2_{ix})\,(\sum_{i=1}^{m} p^2_{iy})}$$

and then transformed this to obtain Nei's Genetic Distance: $D_N = - \ln (I_N)$.

Where p_{ix} is the frequency of allele i in the population p_{iy} is the frequency of allele i in the population Y, m is the number of alleles at the locus and ln is logarithm to the base e. The Network Joining (NJ) trees were calculated through PHYLIP3.6 (Phylogeny inference package) and TREVIEW 1.6.6 software.

RESULTS AND DISCUSSION

Y-chromosome polymorphisms especially Y-STRs and Y-SNPs can be used to track paternal lineages as well as male specific movements and admixtures (Butler 2003). From the mini-sequenencing 7 regional population groups in India and 10 continental population groups from the data published by Gresham et al. (2001), Kayser et al. (2001), Qamar et al. (2002) and Saha et al. (2005) were used for comparison with the present data (Table 1 and 2). A total of seven different alleles from 22 to 28 were observed with a size range of 207-231 bp in Khatri population. The highest allele frequency was found for allele 24 and lowest for allele 22. This shows a significant high degree of genetic polymorphism at this locus. Most of the allele combinations were found to be balanced between the different Indian and world populations. However, the present Khatri population is characterized by a

Table 1: Comparison of Y-STR allele distributions and frequencies for microsatellites DYS390 among Khatri and other reported Indian population (Saha and Bamezai 2000; Saha *et al.* 2005)

Populations	*Allele frequencies*									
	20	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*Σ*
Khatri	0	0	0.06	0.12	0.22	0.18	0.10	0.20	0.12	1.00
Kashmiri Pandits	0	0.093	0.148	0.278	0.148	0.203	0.13	0	0	1.00
Kashmiri Muslims	0.026	0.051	0.103	0.205	0.308	0.307	0	0	0	1.00
Uttar Pradesh (UP)	0	0.026	0.053	0.237	0.158	0.158	0.263	0.105	0	1.00
West Bengal (WB)	0	0	0.111	0.333	0.222	0.223	0	0.111	0	1.00
Punjab	0	0	0.421	0.158	0	0.263	0.105	0.053	0	1.00
Bihar	0	0.026	0.154	0.205	0.179	0.179	0.205	0.052	0	1.00
South India	0	0	0.316	0.105	0.263	0.158	0.158	0	0	1.00

Table 2: Comparison of Y-STR allele distributions and frequencies for microsatellite DYS-390 among Khatri and other reported world population (Gresham *et al.* 2001; Kayser *et al.* 2001; Qamar *et al.* 2002).

Populations	*Allele frequencies*									
	20	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*Σ*
Khatri	0	0	0.06	0.12	0.22	0.18	0.10	0.20	0.12	1.00
Pygmies	0	0.323	0.065	0.29	0.065	0.257	0	0	0	1.00
Chinese	0	0.029	0.057	0.543	0.229	0.142	0	0	0	1.00
German	0	0	0.080	0.322	0.287	0.253	0.058	0	0	1.00
Indonesian	0	0.116	0.058	0.246	0.319	0.247	0.014	0	0	1.00
Inuit Greenland	0	0	0.048	0.065	0.855	0.032	0	0	0	1.00
Mongolian	0	0	0.075	0.250	0.375	0.275	0	0.025	0	1.00
Romani Europe	0	0	0.753	0.118	0.112	0.017	0	0	0	1.00
Pakistani Burusho	0.011	0	0.309	0.255	0.233	0.181	0.011	0	0	1.00
Pakistani Balti	0	0	0.231	0.154	0.154	0.461	0	0	0	1.00
Italian	0	0.01	0.152	0.394	0.383	0.051	0.01	0	0	1.00

more uniform distribution of genotypes and also showed the presence of longest allele 28 which is not found in any other population from India and the world. The lesser common alleles 26 and 27 are present in some world population with low frequency, and are absent in others. It can be seen that four alleles as 22, 23, 24 and 25 are forming a cluster in most of the world populations. Thus, the genetic affinity of Khatris with neighbouring sub-continental, European and East Asian population is evident.

To compare the degree of gene diversity between Khatri and other populations, Nei's (1987) method was used. The gene diversity ranges for different categories of populations are given in Table 3. The relatively higher value of gene diversity (0.8363) for DYS 390 STR locus has been found in Khatri population and it is lowest for Chinese group (0.628171). This indicates that Khatri is a large population and has high genetic admixture with external gene flow. This is a characteristic feature of mainland population. Low gene diversity indicates that it has little genetic admixture and is a characteristic feature of tribal population (Kumar and Reddy 2003; Reddy et al. 2005).

It has also been found that the present Khatri population exhibits more similarity to some populations of UP (India) and Burusho (Pakistan). The allele 28 for DYS 390 locus is not found in any other reported ethnic groups, thus the Khatri population of Punjab represents a distinct ethnic group in the region.

Based on the allele frequency data, Nei and Kumar's (2000) standard genetic distances were computed and processed in PHYLIP 3.6 software to get an 'outfile'. The given outfile was opened in TREEVIEW to get the Neighbour Joining Tree (Fig. 1). It shows Khatris in an independent branch alongside Chinese. This is followed by a group of pygmies and south Indian population.

A perusal of above given facts makes it possible to conclude, that Khatri population is most probably a result of conglomeration of different lineages like most other North-West

Table 3: Comparison of gene diversity values among Khatri and other reported Indian and world populations

Population	*Gene Diversity Value*
Khatri	0.836800
Kashmiri Muslim	0.754361
Kashmiri Pandits	0.811783
Uttar Pradesh (UP)	0.780224
West Bengal (WB)	0.765901
Punjab	0.714792
Bihar	0.824875
South India	0.770022
Pygmies	0.736557
Chinese	0.628171
German	0.740289
Indonesian	0.760191
Mongolian	0.715000
Italian	0.671403
Pakistani Balti	0.685763
Pakistani Burusho	0.751735
Inuit Greeland	0.261422
Romani Europe	0.406234

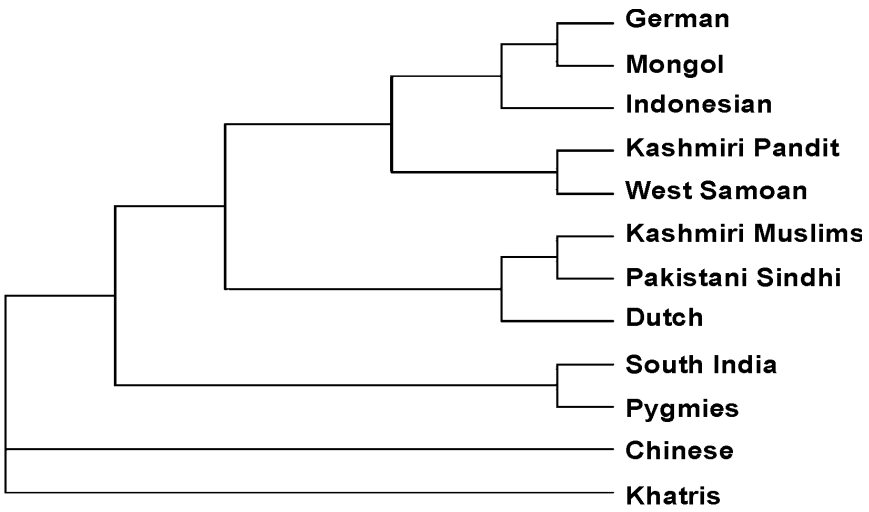

Fig. 1. Unrooted NJ tree showing relation between Khatris and different world population based on DYS390-STR marker

Indian populations (Majumder 2001; Saha et al. 2003; Saha and Bamezai 2000). The results of this study can only be reinforced by further study with larger sample size as well as more DNA markers.

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