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Distribution of CC-chemokine receptor-5- Δ 32 allele among the tribal and caste population of Vidarbha region of Maharashtra state

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BACKGROUND: Genetic relationships among the ethnic groups are not uniform across the geographical region. Considering this assumption, we analyzed the frequency of the CC-chemokine receptor-5 (CCR5)- Δ 32 allele of the CCR5 chemokine receptor, which is considered a Caucasian marker, in *Bhil* tribal and *Brahmin* caste sample sets from the population.

MATERIALS AND METHODS: 108 blood samples were collected from 6 tribe's populations and a caste population from the district of Vidarbha region.

RESULTS AND DISCUSSION: The presence of low frequencies of CCR5- Δ 32 in an individual of *Bhil* tribe (0.034, χ^2 value 0.017) in the present study implies that these communities may have a better resistance toward human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) than the other studied tribe sample, as non-show such mutation.

CONCLUSION: The marginal presence of the allele seen in the studied tribal population could be due to gene flow from the people of European descent. However, lack of the homozygous CCR5- Δ 32 mutation and the low prevalence of heterozygous CCR5- Δ 32 mutations suggest that the Indians are highly susceptible to HIV/AIDS, and this correlates with the highest number of HIV/AIDS infected individuals in India.

Key words: Allele frequency, CC-chemokine receptor-5- Δ 32, India, genetic polymorphism, tribes, Vidarbha

Introduction

Human immunodeficiency virus (HIV)-1 infection has spread to all population groups in India and has reached epidemic proportions.^[1] The rate of progression of HIV-1 disease exhibits a remarkable variation among different individuals. Many host genetic factors are now known to affect the disease progression rates, especially polymorphisms in genes encoding chemokine receptors.^[2-5] Although, no studies on chemokine receptor polymorphisms have been reported in the endogamous population of State of Maharashtra state so far, only one study has been carried out in healthy individuals of tribes and Muslim ethnic groups of Andhra Pradesh, south India.^[6]

Certain members of the chemokine family of receptors serve as critical portals for the entry of HIV-1 into target cells. A mutant allele (CC-chemokine receptor-5 [CCR5]- Δ 32) of the β -chemokine receptor gene CCR5 carrying a 32 base-pair deletion prevents cell invasion by the primary transmitting strain of HIV-1. Individuals who are homozygous for the CCR5- Δ 32/ Δ 32 allele are highly resistant to HIV-1 infection; the heterozygote state does not protect against HIV-1 infection, although, heterozygotes have been found to have significantly lower viral loads.^[7] Early reports indicated that the CCR5- Δ 32 allele maybe absent in indigenous non-European populations. The CCR5_32 allele appears to have originated quite recently (approximately 7,000 years ago) in northeastern

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Europe.^[8] Although its frequency has now reached a relatively high level in Europeans, e.g., 16.3% in Finns and 15.8% in Moravians, it is not present among African populations, and is only so at low levels in the Asian.^[9] Hence, by application it is possible to evaluate the influence of the European population on the genetic constitution of others.

In India population one study has been carried out in healthy individuals of the tribes and Muslim ethnic groups of Andhra Pradesh, South India.^[6] Majumder and Dey,^[10] reported absence of CCR5-Δ32 in various ethnic populations of India, both tribal and non-tribal, except for some populations of the northern and western regions where this allele may have been introduced by Caucasian gene flow. Although a few studies on chemokine, chemokine receptor, and DCSIGN exon 4 repeat number polymorphisms have been reported in north Indian (Aryan descent) HIV patients and healthy controls,^[11-13] there is a dearth of reports on the HIV patients with and without tuberculosis (TB) of South Indian (Dravidian descent) origin.

CCR5-Δ32 exhibit variable frequencies in distinct populations^[14-17] and possibly, their phenotypes depend on the ethnicity analyzed.^[15-20] The Brazilian population presents a complex genetic background, characterized by a high degree of miscegenation.^[21,22] In major Brazilian cities, CCR5-Δ32 was found at frequencies of between 2% and 7%.^[23-26]

Genetic relationships among caste groups are not uniform across the geographical region of India.^[27,28] India is known for the enormous cultural and genetic diversity of its people.^[27] Such diversity is some time attributed to the positioning of the Indian peninsula at the tri-junction of the three continents, viz. Africa, Europe, and Asia. The contemporary Indian population is stratified as tribal and non-tribal, i.e., caste population. The origin of the caste in India is an enigma,^[29] though many are known to have a tribal origin.^[30,31]

The Maharashtra state of India forms a huge irregular triangle with its base on the west coast of India, overlooking the Arabian Sea. Historically, the state is comprised of three sub-regions, Western Maharashtra, the Vidarbha, and the Marathwada. Vidarbha lies on the eastern side and thus mainly contributes to the region

broadly referred to as central India. Apart from the tribal population, many other Ethnic Communities mainly Hindus, Muslim, Buddhist and Sikhs, inhabit the region. The Vidarbha has a hoary past and has been under the domination of many Hindus, Muslims, and tribal-Gond Kingdoms. The Vidarbhan strip served as a bridge between Northern and Southern India. It is assumed that the relationship between these various populations may define the present genetic landscape of India.

Taking this assumption and geographical and ethnic diversity into account in the present study, we investigated the distribution of CCR5-Δ32 alleles in tribes from the Vidarbha region (Maharashtra) Central India.

Material and Methods

Population

The Kolam (tribes)

Besides inhabiting the adjoining state, a substantial number of these people inhabit in few district of Vidarbha. They speak the Gondi dialect which belongs to the Dravidian linguistic group. We sampled 15 samples from this group Village of Yavatmal district.

The Bhil (tribes)

Bhils are listed as Adivasi residents of the states of Gujarat, Madhya Pradesh, Chhattisgarh, Maharashtra, and Rajasthan in Western and Central India as well as in Tripura in far-eastern India on the border with Bangladesh. *Bhils* are divided into a number of endogamous territorial divisions, which in turn have a number of clans and lineages. Most *Bhils* now speak the language of the region they reside in, such as Marathi and Gujarati. We sampled 15 samples from this group Village of Yavatmal district.

The Korkus (tribes)

The *Korkus* are a typical tribal population from Amravati district and found only in the *Satpuda* mountain ranges spanning Maharashtra and Madhya Pradesh. They are mainly concentrated in *Melghat* a scheduled area of *Korku* comprising 89% of the tribal population. The *Korkus* speak *Korku* dialect belonging to Austro-Asiatic linguistic group. The Austro-Asiatic speakers are considered as the first settler of Indian subcontinent.^[32] We sampled 15 samples from this group.

The *Paradhi* (tribes)

Phase *Paradhi* or Phasse *Paradhi* is a tribe in India. The tribe often faces harassment by Indian law enforcement agencies. The tribe is found mostly in Maharashtra and parts of Madhya Pradesh. The Phasse are a sub tribe of the *Paradhi* caste, which includes sub-castes like *Gav Paradhi*, *Berad-Paradhi*, *Gay-Paradhi*, *Chita Paradhi*. *Paradhi* is the term for “hunter.” There are only three surnames among them, Chauhan, Pawar, and Solanke. We sampled 15 samples from this group from Akola district.

The *Andh* (tribes)

A low cultivating caste of Berar, who numbered 52,000 persons in 1911, and belongs to the Yeotmal, Akola, and Buldana Districts. The *Andhs* appear to be a non-Aryan tribe of the Andhra or Tamil country, from which they derive their name. There were 8228 *Andh* in Andhra Pradesh in 1991. According to Singh *et al.*,^[33] in their 2004 book people of India there are over 74,000 *Andhs* in Maharashtra. We sampled 16 samples from this group.

The *Gonds* (tribes)

This tribe falls under the primitive tribes category and spread much over the central India. *Gond* generally speaks “*Gondi*” dialect, which belongs to the Dravidian linguistic family, after Indo-European, in India. We sampled 17 blood samples from this tribe from the Village Gadchiroli district.

Brahmin caste

Brahmin is a class of priests and preachers of ‘*Dharma*’ and considered as the torch bearer of Hinduism. Majority of *Brahmin* in Maharashtra speak Marathi, one of the major languages of Indo-Aryan linguistic group. The population of *Brahmins* in Amravati district is 21,500 or 3% of the population. We collected 15 samples of *Brahmins* living in Amravati district.

Blood sample collection and DNA extraction

We have collected 108 blood samples on Whatman FTA mini cards (GE Health-Care, UK, Ltd) from 6 tribe’s populations and a caste population from the district of

Vidarbha region. Every card was labeled with appropriate code as per tribes/caste and district with informed consent obtained from each volunteer. Approximately, (200 µl [2-5 drop]) of blood by veni-puncture was directly spotted on the FTA mini card within a printed circle area and dried at room temperature. Genomic DNA was isolated from each dried blood sample by following protocol.^[34]

Amplification and restriction digestion of DNA

Polymerase chain reaction was performed following previously prescribed protocol by.^[35] Briefly, 100 ng of genomic DNA was denatured at 94°C for 10 min, following, which it was subjected to 30 cycles of denaturation, annealing, and extension. The last cycle was followed by an incubation at 72°C for 10 min. The reaction mixture of 50 µl contained, 50 mmol KCl, 10 mmol Tris-HCl, pH 8.3, 800 µmol dNTPs, 100 µg/ml gelatin, 10 pmoles of each of the CCR5-specific primer forward: CCR5 – F: (5'-CCTGGCTGTCGTCCATGCTG-3') and reverse: CCR5-R: (5'-CTGATCTAGAGCCATGTGCAC AACTCT-3')., and 1.5 units of Taq polymerase enzyme (Xcelris Genomics, Ahmedabad, India).

Genotyping for CCR5 Δ-32 polymorphism

The genotypes were visualized by running digested product on 2% agarose gel at 100 V for about 2 h and the results were recorded in gel documentation system. The *EcoRI* Restriction enzymes digest the amplified polymerization Chain Reaction (PCR) product of 735 base pairs (bps). The amplified product was digested with 10 U of *EcoRI* at 37°C for 2 h. After digestion, the products were analyzed on a 2% agarose gel and bands were visualized on a Ultraviolet (UV)-transilluminator. PCR amplified a 735 bp region of genomic DNA that spanned a 32-bp deletion differentiating the CCR5-₃₂ allele from its wild-type counterparts at the CCR5 locus. After restriction digestion with *EcoRI*, with wild gene yielded band at 332 bp and for mutated gene, the bands were at 332 and 403 Figure 1.

Statistical analysis

Statistical analysis of allele frequencies was performed using Chi-square statistics. Genotype distribution for polymorphism was first compared to predictable values

from Hardy–Weinberg equilibrium. In all cases, *P* values less than 0.05 were considered to be statistically significant.

Observation and Results

Our data on distribution of CCR5-Δ32 mutation among the selected tribe and a caste is depicted Table 1. Genotype and phenotype for the heterozygous mutation among the tribes sample suggested that it is either absent or present at low frequency 1.08% (1 in 93 tribe’s samples and 1 in 15 samples of a caste). None of the tribes and control caste was found to be homozygous for the CCR5-Δ32 mutation, while the *Bhil* tribe and control caste show the heterozygous for the CCR5-Δ32 mutation in negligible frequency (0.034). The analysis of χ^2 suggested that the prevalence of the CCR5-Δ32 is significantly low ($\chi^2 = 0.02$, $P > 0.05$). The aggregate frequencies of the entire sample for the wild-type allele CCR5 and the CCR-5-Δ32 variant were found to be 0.991 and 0.009, respectively. Among the tribes *Kolam*, *Korku*,

Paradhi, *Andh* and *Gonds* showed the highest (wild type) homozygous genotype frequency (100%). while only the *Bhil* tribe shows heterozygous genotype frequency (06.97%) and allelic frequency (0.034) for CCR5/Δ32. Only one bearer of this mutation was found in the blood sample of *Bhil* tribe collected from Yavatmal district of Vidarbha region.

No significant deviations from the HWE were observed ($P > 0.05$, Chi-squared goodness of fit). Table 1 shows the frequency of the CCR5-Δ32 allele in the six tribes and one control caste *Brahmin* population.

Discussion on Conclusion

This study describes the genotype and allele frequencies of the polymorphisms CCR5-Δ32 in selected six tribal and one caste populations from Vidarbha region. Most importantly, however, this study is the first to be conducted in Vidarbha that investigates the genetic polymorphisms CCR5-Δ32 among different ethnic tribal population settlement in the districts of Vidarbha region.

CCR5, a coreceptor for HIV-I virus, has been shown to be the most important for the HIV transmission.^[36,37] A 32-nucleotide deletion of CCR5 homozygous (CCR5-Δ32/Δ32) display a high degree of the natural resistance to HIV transmission whereas CCR5-Δ32 heterozygosity (CCR5+/Δ32) demonstrate a slower progression to acquired immunodeficiency syndrome (AIDS) than CCR5 wild type (CCR5+/+).^[3,38] However, this genetic mutation is found in Caucasian rather than non-Caucasian population including India.^[10] The CCR5-Δ32 genotype frequency among our study tribes sample was absent or negligible, the average genotype frequency were common homozygous

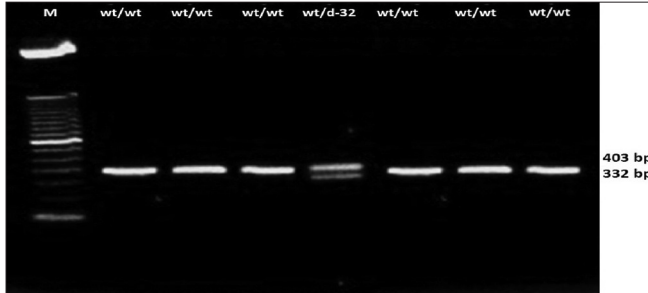


Figure 1: CC-chemokine receptor-5 genotyping among the tribes Lane 1, 2, 3, 5, 6 and 7 represent the PCR product from samples with homozygous wild type genotypes (fragments of 332 bp wt/wt). Lane 4 represents the Δ-32 genotype (with the presence of both fragment of 332 and 403 wt/Δ32)

Table 1: Genotypic distribution and gene frequencies of the CCR5 allele in different population samples of Vidarbha region of Maharashtra state

Population	Genotype of CCR5 Δ32			Allelic frequency (λ)		Δ32	χ^2	<i>P</i> value (1 df)
	wt/wt	wt/Δ32	Δ32/Δ32	<i>P</i> ²	<i>q</i> ²			
<i>Kolam</i>	15 (100)	0	0	1.00	0.00	0	NA	NA
<i>Bhil</i>	14 (93.3)	1 (06.9)	0	0.966	0.034	0.034	0.017	0.893
<i>Korku</i>	15 (100)	0	0	1.00	0.00	0	NA	NA
<i>Paradhi</i>	15 (100)	0	0	1.00	0.00	0	NA	NA
<i>Andh</i>	16 (100)	0	0	1.00	0.00	0	NA	NA
<i>Gond</i>	17 (100)	0	0	1.00	0.00	0	NA	NA
<i>Brahmin</i>	14 (93.3)	1 (06.9)	0	0.966	0.034	0.034	0.02	0.893
Total	106 (98.1)	2 (01.8)	0	0.991	0.009	0.01	0.009	0.922

NA: Not applicable, CCR5: CC-chemokine receptor-5

wt/wt (98.3%), heterozygous wt/mt (1.87%) and rare homozygous mt/mt (0%), from the control group, revealing CCR5-Δ32 allele frequency of 6.97%. The frequency of the CCR5-Δ32 allele among our study population seems to be remarkably similar to previously reported frequencies in other Asian populations.

The CCR5-Δ32 allele frequency among Asians is very low in Rajasthan Indians (0.05%), Andhra Pradesh Indians (0-0.03%),^[39] North Indians (1.5%),^[12] South Indians (1-3%),^[6] and ethnic population of Kashmir (3-4%).^[40] A similar study conducted from the Island of Crete, Greece showed allele frequency of 3.25%, with a 95% confidence interval (CI) for conformity with Hardy-Weinberg equilibrium of 0.74-5.7%.^[41] The CCR5-Δ32 polymorphism is found all across Europe at different allele frequencies, with a North to South decreasing gradient and lower distribution in the regions of Southeast Mediterranean.^[42] The frequency of the CCR5-Δ32 allele in the studied tribal population is consistently similar with data reported from other populations with non-European ancestors.^[9,16] CCR5-Δ32 allelic frequencies were not different when the self-reported racial characteristics of the individuals evaluated were considered.^[25] This allele has not been found among South American native Indians, corroborating the hypothesis of a European origin of this allele and its introduction to the continent through migration.^[43]

Within the Middle-Eastern populations the frequency of the mutant CCR5-Δ32 allele reached it's the highest among Iranians, 2.4%; Saudi, 2.1%; and it's the lowest among Kuwaitis, 1%; and the Egyptians, 0.5%; and is completely absent in individuals from the United Arab Emirates.^[44] Our results suggest that the CCR5-Δ32 allele is detected at very low frequency in studied tribal populations from Vidarbha. The presence of low frequencies of CCR5-Δ32 in an individual of *Bhil* tribe (0.034, χ^2 value 0.017) in the present study implies that these communities may have a better resistance to HIV/AIDS than other studied tribe sample, as non show such mutation. However, the CCR5-Δ32 allele is observed mostly in European populations.

The marginal presence of the allele seen in the studied tribal population could be due to gene flow from the people of European descent. However, CCR5-Δ32

is completely absent in the populations from Africa, Oceania, and the Americas. However, lack of the homozygous CCR5-Δ32 mutation and the low prevalence of heterozygous CCR5-Δ32 mutations suggest that the Indians are highly susceptible to HIV/AIDS, and this correlates with the highest number of HIV/AIDS infected individuals in India.

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