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Research Article

EVIDENCE OF GENETIC HETEROGENEITY IN COMMUNITIES FROM UTTAR PRADESH USING IL-1RN GENE POLYMORPHISM

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ABSTRACT

In Uttar Pradesh (UP, India), there are people with distinct lifestyle, behavior, culture and socioeconomic status. However, genetic data of communities in UP are very limited. A 86bp variable number of tandem repeat (VNTR) in intron 2 of Interleukin 1 Receptor Antagonist (*IL-1RN*) gene has gained attention since cytokine genes are associated with several immune-inflammatory diseases such as type 2 diabetes and cancer. An attempt was made to study genetic difference amongst 5 different groups (Urban, Kanjar, Khatik, Kharwar and Saharia) using IL-1RN gene polymorphism. A total of 290 unrelated healthy subjects with individual consent were enrolled and 86bp *IL-1RN* VNTR polymorphism was carried out using PCR. Four alleles (I, II, III and IV) were observed (410, 240, 500, 325bp respectively). Allele IV was found in Saharia community like urban group. Genotype and allele frequencies of all four groups were compared with urban as reference (SPSS,v21.0) and that of Saharias was significantly different (P=0.037; P<0.001). In conclusion, the study demonstrated genetic heterogeneity with respect to *IL-1RN* 86bpVNTR. Allele IV may be used as a biomarker for Saharia community. Such studies shall provide a lead for future research in deciphering inheritance pattern of complex diseases on the basis of demographic differences.

INTRODUCTION

India comprises one of the largest ethnic populations with their unique cultural, linguistic and genetic diversity (Majumdar, 2001). India has diverse range of endogamous groups under broad categories of religions, castes and tribes. All Indian communities have their own/unique range of ethnic, religious, linguistic, socioculture and biological diversity. Long years ago, several community population of India were lived in virtually inaccessible forests/hilly areas, completely isolated from the global society. Individuals belonging to a particular community have evolved their own morphogenetic characteristics with respect to their ecological niches (Balgir, 2006). The endogamous behavior seems to have nurtured several hereditary diseases by limiting the flow of genes in the same group and therefore resulting into the formation of genetically isolated groups within the overall population of India. Since last two decades, a flood of investigation has been flourished undertaking the Indian population to explore the probable association between various diseases and genetic architecture. Uttar Pradesh (UP) is the largest populated state in India and is located in the northern region. The people of UP have distinct lifestyle, behavior, cultures and socioeconomic status and prefer to remain confined to their own communities. Several anthropological studies based on genetic markers have been reported earlier in order to understand whether genetic variations exist among the various communities/populations (Trajkov *et al.,* 2009).

It is well known that interleukins (ILs) are proinflammatory cytokines, which are secreted by monocytes, macrophages and dendritic cells in the body (Sampaio-Fernandes *et al.,* 2015). The IL-1 family comprises of the IL-1α, IL-1β and the IL-1 receptor antagonist (IL-1Ra) (Smith *et al.,* 2000; Banerjee and Saxena, 2014). Since last decade, polymorphisms associated with cytokine gene have earned noteworthy interest due to their probable role in the onset of immuno-inflammatory diseases (Bid *et al.,* 2004; Banerjee and Saxena, 2014).

VNTR, short DNA sequences are repeated in a "head-to-tail fashion". The number of tandem repeats at a locus varies between individuals. Such differences may alter the functional consequences of that particular gene as these repeats comprises

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of binding sites for transcription factors (Vamvakopoulos *et al.,* 2002). *IL-1RN* is a competitive inhibitor of pro-inflammatory activities induced by Interleukin 1 (*IL-1*). A 86 bp variable number of tandem repeats (86 bp VNTR) in Intron 2 of Interleukin 1 Receptor Antagonist (*IL-1RN*) gene has gained attention due to association with several diseases such as type 2 diabetes (Banerjee and Saxena, 2014), oral cancer (Gupta *et al.,* 2016), pulmonary tuberculosis (Hashemi *et al.,* 2015), coronary heart disease, periodontitis (Bashour, 2013). VNTR polymorphisms of *IL-1RN* gene, representing various copies of 86 bp of tandem repeats and each allele corresponds to a different number of repeats. The number of repeats in different individuals varies from I to VI. The allelic frequency of individual allele varies among ethnic populations. Genetic data of communities in the state of Uttar Pradesh are very limited. An attempt was therefore made to study the genetic difference, if any, amongst 04 different endogamous communities (Kanjar, Khatik, Kharwar and Saharia) using *IL-1RN* gene polymorphism.

MATERIALS AND METHODS

Subject selection and sample collection

A total of 290 unrelated healthy individuals were included in the present study, out of which 216 (54 each) belonged to 04 different endogamous communities (Kanjar, Khatik, Kharwar and Saharia) in different parts of Uttar Pradesh. The rest 74 individuals were randomly selected from the urban population of Lucknow. The study was conducted after obtaining proper informed written consent from all subjects as per inclusion/exclusion criteria.

Inclusion criteria for study subjects

- Age: 22-62 years.
- Gender: males and females.
- Endogamous subjects whose ancestors are confined to marriage within one's own community.
- Random urban group.

Exclusion criteria for study subjects

- Age: < 22 or >62 years.
- Gender: transgender.
- Diagnosed of psychotic disorder or hospitalized for depression.
- Pregnant or nursing mothers.

Sample collection and DNA extraction

About 2 ml of peripheral venous blood was collected using 0.5M ethylene diamine tetra acetic acid (EDTA) vials and kept at -80^{0}C. Sampling was done from all study and genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the salting out method (Miller *et al.,* 1988) along with slight modifications (Gautam *et al.,* 2011).

Genotyping

The polymorphic region of *IL-1RN* gene, 86 bp VNTR was amplified by standard Polymerase Chain Reaction (PCR) technique using specific primers 5'-CTCAGCAACACTCCTAT-3' (forward) and 5'-TCCTGGTCTGCAGGTAA-3' (reverse). PCR was performed in a final volume of 25 µl reaction mixture containing genomic DNA (100-150 ng), 10 pmol of each primer, 200 µM dNTPs, and 0.5U of Taq DNA polymerase (Takara, Japan) in a gradient Master Cycler (Eppendorf, Germany). The PCR conditions were: 94^{0}C for 5 min, 35 cycles at 94^{0}C for 30s, 60^{0}C for 30s and 72^{0}C for 30s and final extension at 72^{0}C for 10 min. The genotypes on individual basis were thereafter analyzed. The PCR products were visualized on 2.5% agarose gels and documented in a gel documentation system (Vilber-Lourmat, France). In order to ensure accuracy of genotyping, coded blind replicate samples (20%) were included in each assay.

Statistical analysis

Hardy-Weinberg equilibrium at individual locus for *IL-1RN* 86 bp VNTR polymorphism in all five groups were evaluated by Pearson's chi-square (χ^2) statistics using SPSS (version 21.0). Allele and genotype frequencies in all communities were compared by using Fisher's exact test. All *P*-values were two sided. Differences were deliberated by considering the random urban group as reference. Values showing $P<0.05$ were considered as statistically significant.

RESULTS

The *IL-1RN* 86 bp VNTR polymorphism was successfully genotyped in all study subjects. Four different alleles of 86 bp *IL-1RN* VNTR polymorphisms were observed *viz.* I (410 bp), II (240 bp), III (500 bp) and IV (325 bp) (Figure 1). All allele as well as genotypic frequencies were found to be in Hardy-Weinberg equilibrium.

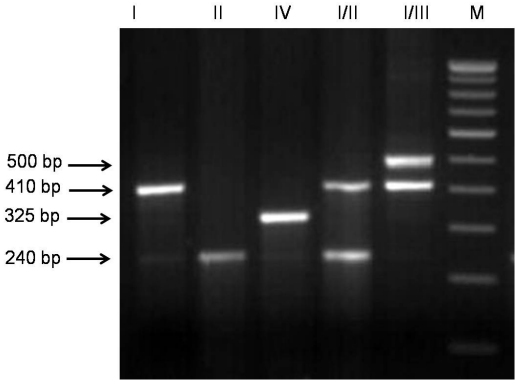

Figure 1 Agarose (2.5 %) gel showing allele variants of *IL-1RN*. M: 100 bp ladder.

Table 1 Genotypic and allelic frequencies of *IL-1RN* gene polymorphism in endogamous and control groups from Uttar Pradesh (India).

Subjects		Allelic frequency (%)				
		I	II	III	IV	*P*-value
Control group (n=148)		126 (85.135)	15 (10.135)	3 (2.027)	4 (2.702)	Ref.
Endogamous group (n=108 each)	Kanjar	89 (82.407)	13 (12.037)	6 (5.555)	0	0.908
	Khatik	72 (66.666)	34 (31.481)	2 (1.851)	0	0.084
	Kharwar	96 (88.888)	10 (9.259)	2 (1.851)	0	0.174
	Saharia	56 (51.851)	43 (39.814)	1 (0.925)	8 (7.407)	<0.001
Subjects		Genotype frequency (%)				
		I/I	II/II	I/II	I/III	IV/IV	
Control group (n=74)		58 (78.378)	4 (5.405)	7 (9.459)	3 (4.054)	2 (2.702)	Ref.
Endogamous group (n=54 each)	Kanjar	38 (70.370)	3 (5.555)	7 (12.962)	6 (11.111)	0	0.348
	Khatik	35 (64.814)	17 (31.481)	0	2 (3.703)	0	0.766
	Kharwar	47 (87.037)	5 (9.251)	0	2 (3.703)	0	0.098
	Saharia	25 (46.296)	19 (35.185)	5 (9.259)	1 (1.851)	4 (7.407)	0.037

In the randomly selected urban population, allele I (85.135%) was most common followed by alleles II (10.135%), III (2.027%) and IV (2.702%). The frequency distribution of allele I, II and III was similar in all the other 04 endogamous communities. However, allele IV was found in the urban (control) group and only one of the endogamous communities *i.e.* Saharia. Both The allele frequency in Saharia was significantly higher when compared to control group (P<0.001) (Table 1).

Five different genotypes (I/I, I/II, II/II, I/III and IV/IV) were identified (Figure 1). Most of the subjects were homozygous I/I in the study population *i.e.* 78.37% control urban individuals. In the endogamous communities, 70.37% Kanjar, 64.81% Khatik, 87.03% Kharwar and 46.29% Saharia had I/I genotype. Genotype I/II was found to be absent in Khatik and Kharwar. The frequency of genotype I/III was observed to be similar in Khatik and Kharwar *i.e.* 3.703%. The allele I appeared to be common either in homozygous or heterozygous condition. The genotype frequencies of Saharia were found to be significantly different from that of control group (P=0.037) (Table 1).

DISCUSSION

To the best of our knowledge, there are no previous reports on *IL-1RN* 86 bp VNTR gene polymorphism in different communities living in Uttar Pradesh (India). We defined allelic and genotypic frequencies in subjects from endogamous communities (Kanjar, Khatik, Kharwar and Saharia) that are residents of UP and compared them with the frequencies of subjects randomly selected from an urban population in Lucknow taken as control. It has been observed that frequency of tandem repeats varies among different ethnic/geographical populations (Bid *et al.,* 2004). Till date, six alleles are reported *i.e.* allele I: four repeats (410 bp); allele II: two repeats (240 bp); allele III: five repeats (500 bp); allele IV: three repeats (325 bp); allele V: six repeats (595 bp); allele VI: one repeat (155 bp) (Jaiswal *et al.,* 2012), suggesting the possible functional consequences of *IL-1RN* VNTR. In our study, we found only four alleles (I, II, III and IV). Most subjects were homozygous for genotype I as previously reported. The frequency distribution of alleles in our study population is similar to that of previous studies *i.e.* allele I is the most common allele followed by alleles II and III (Bid *et al.,* 2008). One important finding was that allele IV was present only in one of the endogamous communities *i.e.* Saharia just like in the urban population taken as control (Table 1). Allele IV may be used as a unique marker to distinguish Saharia from other communities in Uttar Pradesh. Both, alleles V and VI were not observed in the study populations.

Genetic research is focused on the study of variations in DNA sequences or single nucleotide polymorphisms (SNPs) in a variety of genes and their potential association with a particular trait or enhanced risk of developing a condition/disease (Dewberry *et al.,* 2000; Whyte *et al.,* 2000; Vijgen *et al.,* 2002; Olofsson *et al.,* 2009). The association of SNPs varies amongst various populations and even between communities (Xu *et al.,* 2010). As previously mentioned, polymorphism in *IL-1RN* VNTR has been found to be associated with various immunological diseases (Fischer *et al.,* 1992; Witkin *et al.,* 2002). Our preliminary objective of this study was to find out whether the genetic characters (genotype and allele frequencies) of 86 bp *IL-1RN* VNTR polymorphism can be used to differentiate between the endogamous communities living in UP. However, the sample sizes of our study groups are limited and the utility of 86 bp *IL-1RN* VNTR can be confirmed in a larger cohort before actually using it as a marker for Saharia.

Due to the high degree of polymorphic nature, *IL-1RN* VNTRs constitute useful tools in population genetics in order to understand ethnic differences at community level. Based on biological and pathological significance of *IL-1RN* 86 bp VNTR polymorphism, it is possible that variations/differences in number of repeats may contribute in determining disease susceptibility and help in clinical management at community levels in the Indian population. This may provide a lead in future research for studying the inheritance pattern of complex diseases on the basis of demographic differences.

Conflict of interest

The authors declare no conflict of interest.

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