STUDY OF INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1Ra) GENE POLYMORPHISM IN HEALTHY INDIVIDUALS FROM NORTHERN INDIA

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ABSTRACT

Cytokines play a key role in immune responses and inflammation. IL-1Ra is a naturally occurring structural variant of IL-1 that competitively inhibits receptor binding of IL-1. We have investigated the polymorphism in intron-2 of the interleukin-1 receptor antagonist gene in North Indian population. This genetic variation has been of great interest due to its possible association with a variety of human diseases primarily of epithelial and endothelial cell origin such as urolithiasis etc. Allele frequencies of the IL-1Ra polymorphism vary among different populations but there is no data till date reported from India. The present study was carried out to determine the IL-1Ra gene Polymorphism in 165 normal unrelated individuals from North India. We obtained an allelic frequency of 63.94, 30.61, 4.55, 0.90 for A, B, C and D allele and percentage of genotypes AA, BB, CC, DD, A/B, A/C, A/D and B/C were 49.7, 18.2, 2.42, 0.60, 24.2, 3.63, 0.60, 0.60 respectively. Our results suggested that the frequency and distribution of this polymorphism in India is substantially different from other populations and ethnic groups.

KEY WORDS

IL-1 Ra Polymorphism, VNTR, Single nucleotide polymorphisms (SNPs).

INTRODUCTION

DNA sequences of the human genome reveal that many genes are polymorphic. In coding or noncoding regions of a specific gene, there may be either a single base pair substitution or a variable number of repeats of a short repetitive DNA sequence (VNTR). These variations may influence the rate of gene transcription, the stability of the messenger RNA, or the quantity and activity of the resulting protein. Thus, the susceptibility or severity of a number of disorders will be influenced by possession of specific alleles of polymorphic genes. Highly polymorphic segments (1, 2) are characterized by allelic variation, attributed to the difference in the copy number of the tandemly repeated DNA sequences (2, 3). VNTR,s are

informative markers for genetic characterization and are very useful for identifying parentage testing, disease susceptibility, linkage analysis and genome analysis. Cytokine gene polymorphisms have recently attracted considerable interest since it has been discovered that distinct alleles of cytokine genes are associated with different immunoinflammatory diseases (4-6). IL-1Ra is a naturally occurring competitive inhibitor of IL-1 induced proinflammatory activity. It has three intracellular isoforms but biological role of these isoforms remains unclear. In intron 2 of the IL-1Ra gene, a 86-bp of tandem repeat polymorphism leads to the existence of five different alleles (AA/410, BB/240, CC/500, DD/325 and EE/595). The genes encoding for IL-1Ra are located on the long arm of chromosome 2 in humans (2q13-14) (7). Polymorphism of the IL-1Ra gene, displaying different copies of 86-bp tandem repeat in intron 2, has been identified in 21% of a Caucasian population. (8) The number of times this sequence is repeated in different persons varies from 2 to 6 and the frequency of the individual alleles vary among different ethnic or geographic populations. IL1-Ra polymorphism is associated with many diseases e.g. rheumatoid arthritis, urolithiasis, inflammatory bowel disease (IBD) etc. The present

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study is an attempt to investigate IL-1Ra polymorphism in unrelated normal healthy individuals from northern India.

MATERIALS AND METHODS

Subjects

Blood samples were collected from 165 unrelated normal individuals (Male-120 & Female-45) (Mean age- 29.5 ± 6 Years) from the North Indian population in EDTA vials and kept frozen till DNA extraction. The genomic DNA was isolated from peripheral blood using standard phenol chloroform method (9).

Polymerase Chain Reaction

PCR analysis of IL-1 Ra gene polymorphism was carried out in a total volume of 50 μ l, containing genomic DNA (100-150ng); 2-6 pmol of each primer; 1X Taq polymerase buffer and 0.25 units of Taq DNA Polymerase (Bangalore Genei, India). The primers used were forward (5'-CTCAGCAACACTCCTAT-3') and backward (5'-TCCTGGTCTGCAGGTAA- 3') according to Cantagrel et al (10). PCR amplification was performed in thermal cycler (PTC-100, MJ Research) under the following conditions: 95°C for 5 min, 30 cycles at 95°C for 30s, 58°C for 30s and 72°C for 30 s and one cycle of extension at 72°C for 10 min. The PCR product were separated on 2% agarose gel and visualized by ethidium bromide staining and documented in a gel documentation system (Alphamager™ 1220, Alpha Innotech Corporation, USA).

Allele size determination

The size of PCR products was determined using a 100-bp DNA ladder (Roche, Germany). The molecular weight of each band was determined by using software in Alpha Imager 1220 v5.5 programme where the unknown samples were compared with the 100 bp DNA ladder. The alleles were designated according to their respective base pair size taking the 86 bp core repeat sequence of IL-1Ra VNTR marker from the literature. (8)

We performed the Fisher's Exact Test to compare

the allelic frequencies of different populations with the NCSS 6.0 software.

RESULTS

DNA extracted from the peripheral blood of 165 normal individuals from our population was screened and 8 different genotypes were identified. The distribution of IL-1Ra genotypes and allele frequencies in north India is shown in Table 1 and Fig 1. The most frequent allele observed was A (63.9%) followed by B (30.6%) and C (4.54%). D allele was rare and E allele was absent in our population like other world populations (Table 2). Genotype distribution was in agreement with Hardy-Weinberg equilibrium. Table 2 shows genotype distribution of the IL-1Ra alleles in our population with those reported in other populations and the p-values of different alleles. In the present study the genotype AA in our population was found to be significantly different from Taiwan and African-Americans ($p < 0.001$). The five populations were found to be significantly different when compared with Indians for genotype BB. Genotype CC of Indians was significantly different from Germany-England ($p < 0.05$) and African-Americans ($p < 0.05$). However for the other populations p-values were not significant. Genotype DD showed nonsignificant difference for all the comparable populations ($p > 0.05$). The genotype E being a rare allele has not been reported in other populations including ours. All the populations were found to be significantly different ($p < 0.05$) as compared to our population for genotype A/B. The rest of the genotypes A/C, A/D, B/C, B/D was found to be non-significant for all the five populations. ($p > 0.05$).

DISCUSSION

Identification of SNPs (Single nucleotide polymorphisms) in human genome has great implications in the study of disease susceptibility. The SNP in IL-1Ra gene (VNTR) has been found to be associated with different immunological diseases. Our basic objective of this study was to provide population genetic characterizations of VNTR polymorphism. Admittedly our sample sizes are rather limited, but this is the first attempt of reporting genotype and allele frequency distribution

Table 1 Frequency of alleles and genotypes of IL-1Ra VNTR in North Indian Population

Genotype (N = 165)									Allelic frequencies (%)			
AA	BB	CC	DD	EE	A/B	A/C	A/D	B/C	A	B	C	D
82	30	4	1	0	40	6	1	1	63.94	30.61	4.55	0.90

Fig. 1. PCR-based VNTR analysis of the IL-1 Ra gene Exon-2 polymorphism shown on 3% agarose gel to resolve the different alleles

Lane 1: B/C (240/500) heterozygous, Lane 2: A/B (410/240) heterozygous, Lane 3: A/D (410/325) heterozygous, Lane 4: A/A (410) homozygous, Lane 5: B/B (240) homozygous, Lane 6: D/D (325) homozygous, Lane 7: C/C (500) homozygous, Lane 8: A/C (410/500) heterozygous and M: 100 bp Marker

Table 2 Comparison of genotypes and their frequency distribution in various populations

Population (n)	Genotypes										Reference
	AA	BB	CC	DD	EE	A/B	A/C	A/D	B/C	B/D	
Taiwan (100)	92***	1***	NS	NS		10**	NS	NS	NS		Chen <i>et al.</i> (2001) (14)
Germany (141)			17***	*	NS	90*	9 NS	2 NS	3	NS	Mansfield <i>et al.</i> (1994) (15)
England (261)	NS										
American Caucasians (295)	150 NS	25***	2 NS	NS		110	6 NS	NS	NS	2 NS	Rider <i>et al.</i> (2000) (16)
African-American (182)	140***	6***	*	NS		23**	2 NS	5 NS	NS		Rider <i>et al.</i> (2000) (16)
Nether land (71)	40 NS	2***	NS	NS		29*	NS	NS	NS		Bioque <i>et al.</i> (1995) (17)
North India (165)	82	30	4	1		40	6	1	1		Present Study
* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, at 5% level of significance; NS = Not Significant ($p > 0.05$)											

data of VNTR loci in IL-1Ra gene in North Indian populations. The IL-1 gene family includes IL-1 α , IL-1 β and IL-1Ra. The genes encoding these proteins are located on the short arm of chromosome 2. IL-1Ra is a competitive inhibitor of IL-1 bioactivity. The concentration of IL-1Ra increases late during the course of an inflammatory event so that an induced acute inflammation can terminate and does not become chronic and damage healthy cells. (11) It has been reported that the frequency of the individual alleles varies among different ethnic or geographic populations. Five alleles were described, suggesting possible functional significance. (12) Allele (A) containing 4 repeats is more common than allele (B) containing 2 repeats. Our observations are in agreement to the studies of different populations reported (13-17), though the frequencies of genotypes differ. The remaining alleles, representing 3, 5 and 6 repeats, occurs in <1% in other populations. In every population studied to date, most persons are either IL1RN*A homozygous or IL1RN*A /IL1RN*B heterozygous. The prevalence of IL1RN*B homozygotes is typically <10% in black Africans and African Americans, the frequency of IL1RN*B homozygotes is considerably lower than in the white population. (16, 13) In the present analysis, variation at IL-1 Ra gene VNTR was measured solely on the basis of repeats of the basic core sequence. Due to their highly polymorphic content, VNTRs constitute useful tools in population genetic studies in understanding population and ethnic variations. Allelic association studies are in progress with several chronic inflammatory and degenerative diseases in which IL-1/IL1Ra may be involved. In the long run, these studies may help in determining disease susceptibility and clinical management of patients.

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