RESEARCH COMMUNICATION

Ethnicity Greatly Influences the Interleukin-1 Gene Cluster(IL-1β Promoter, Exon-5 and IL-1Ra) Polymorphisms: A Pilot Study of a North Indian Population

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Abstract

There is considerable evidence that polymorphisms in the regulatory regions of cytokine genes are highly influenced by ethnicity. Polymorphisms in interleukin 1-β (IL-1β) and IL-1 receptor antagonist (IL-1Ra) genes, respectively encoding a potent inflammatory agent and an antagonist, which combines with IL-1 receptors competitively, have been associated with a number of diseases like systemic lupus erythematosus, rheumatoid arthritis, sepsis, kidney diseases, and cancer. In this study, we therefore evaluated the distribution of interleukin-1 gene cluster (IL-1β promoter region, exon-5 and IL-1Ra) gene polymorphisms in 206 healthy north Indian subjects, using PCR-based restriction analysis. We also constructed various haplotypes and estimated the linkage disequilibrium (LD). We found that genotype and allelic frequencies for these cytokines were conspicuously different when compared among different ethnic populations. The haplotype 'T-E1-1' predominated (41.7%) while the least common was 'C-E2-2' (2%) in our population. Genetic linkage between three loci of IL-1 gene showed strong association among the variants in controls (D´=0.42, $p< 0.001$). Our results suggest that the frequency and distribution of the polymorphisms in India are substantially different from other populations and ethnic groups. Thus they signify an impact of ethnicity and provide a basis for future epidemiological and clinical studies.

Key Words: IL-1 gene cluster - SNPs - PCR-RFLP - ethnicity

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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 of one nucleotide for another (SNPs) or a variable number of repeats of a short repetitive DNA sequence (VNTR). Gene-environment interactions may be manifested in various ways, either by risk effects based on an individual's genotype, or differential gene risk effects based on exposure (Michael et al., 2002). The study of genetic polymorphisms promises to help define pathophysiological mechanisms, to identify individuals at risk for disease and to suggest novel targets for drug treatment.

Cytokines are signaling molecules contributing to the inflammatory response, and are key components in the pathogenesis of many diseases like cancer, metabolic disorders and inflammatory conditions (Anderson et al., 2001). Interleukin-1β, located at chromosome 2q12, is a potent proinflammatory agent that is central in immunoregulation, inflammation and cancer formation (Cantagrel et al., 1999). IL-1 receptor antagonist (IL-Ra), a structural variant of IL-1, binds to the same IL-1 receptor and acts as a competitive inhibitor of IL-1 bioactivity. In the second intron of the IL-1RN gene, there is a functional VNTR polymorphism, which is characterized as having an important role in regulating the serum IL-Ra levels, human immune response and cancer risk. (Hu et al., 2005). Interleukin (IL)-1 is known to act as a tumor growth factor by inducing angiogenic factors (Konishi et al., 2005).

Genetic variation in the human genome is an emerging resource for studying cancer, a complex set of diseases characterized by both environmental and genetic contributions. Risks of cancer are clearly influenced by polymorphisms in genes involved in carcinogen metabolism and the immune system. The primary focus of this article is the problem of identifying DNA sequence variation that is

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segregated in a population and has a causal connection with complex disease like cancer. One could perform tests of this association without consideration of the fact that the SNPs are not independent of one another, but the determination of critical values must be made in the context of linkage disequilibrium among SNPs. As there are no reports of allelic variations in IL-1 gene cluster from our subcontinent, the present study was performed to assess interleukin-1 (IL-1) genotype frequencies in our north Indian population, focusing on the IL-1β (promoter&exon-5) and IL-1Ra genes.

Materials and Methods

Subjects

The study involved 206 subjects (males 110 & females 96; age range 22-58 yrs) from the north Indian population. Unrelated healthy subjects for the study were drawn from the general population from the same geographical region. The hospital ethical committee approved the study and informed consent was obtained from the participating volunteers.

DNA Extraction

Five ml of blood was collected in EDTA vials and DNA was extracted from blood lymphocytes using a 'salting out' method (Miller et al., 1988).

Genotyping of IL-1 Gene Cluster

All subjects were genotyped for three polymorphisms in the interleukin-1 gene cluster: IL-1β promoter region –511, IL-1β exon 5 and IL-1Ra in intron 2, the details of which are presented in Table 1. The size of PCR product 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 version 5.5 programme where the unknown samples were compared with the 100 bp DNA ladder.

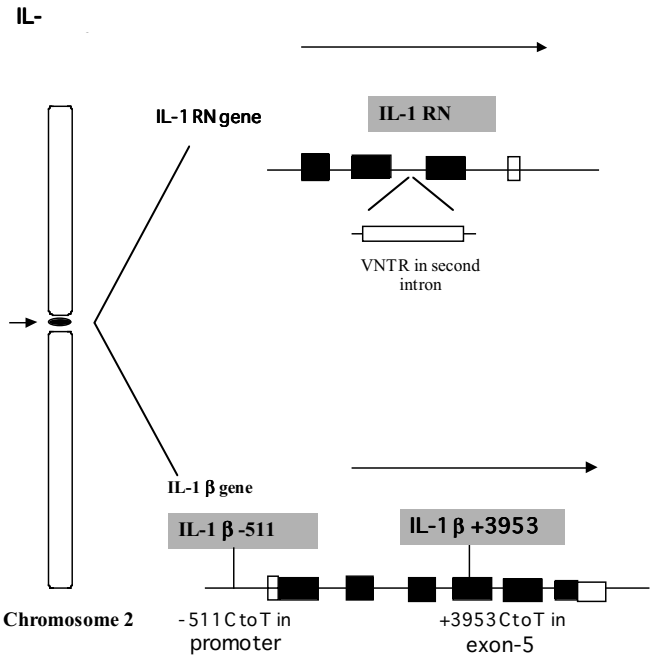

Figure 1. Schematic picture of the IL-1 gene map demonstrating the different restriction sites for IL-1β promoter region (-511), exon-5 (+3953) restriction enzymes and VNTR in IL-1Ra

Statistical Analysis

Two-tailed Fisher's exact test and chi square test was done to compare the allelic frequencies of different populations using the computer software SPSS for windows (version 11.5). P value <0.05 were considered statistically significant. SNPAnalyzer (web-based software) was used to examine Hardy-Weinberg equilibrium (HWE), haplotype estimation by maximum likelihood method, using expectation maximization algorithm and pair wise linkage disequilibrium between each pair of IL-1 loci. (Yoo et al., 2005).

Table 1. Main Characteristics of Cytokine Gene Polymorphisms and Techniques Used for Screening

	IL-1β (promoter region)	IL-1β (exon-5)	IL-1Ra
Type of polymorphism	Single base C/T	Single base C/T	86-bp VNTR
Site of polymorphism	–511	+3954	Intron-2
PCR primers			
Upstream	5'-TGGCATTGATC-TGGTTCATC-3'	5'-GTTGTCATCAG-ACTTTGACC-3'	5'-CTCAGCAAC-ACTCCTAT-3'
Downstream	5'-GTTTTAGGAAT-CTTCCCACTT-3'	5'-TTCAGTTCAT-ATGGACCAGA-3'	5'-TCCTGGTCTG-CAGGTAA-3'
PCR conditions			
Denaturation	95˚C, 0.5 min	95˚C, 0.5 min	95˚C, 0.5 min
Annealing	56˚C, 0.5 min	55˚C, 0.5 min	58˚C, 0.5 min
Extension	72˚C, 0.5 min	72˚C, 0.5 min	72˚C, 0.5 min
No. of cycles	35	35	30
Digestion	(Ava I)	(Taq I)	86-bp VNTR
Allele size, bp	*C: 190+114 *T: 304	*E1: 135+114 *E2: 249	I: 410, II: 240, III: 500, IV: 325, V: 595, VI: 154

Table 2. Genotypes and Allele Frequency Distribution of IL-1β (promoter &exon-5) and IL-1Ra Gene Polymorphism in North India

	Genotypes (%)			Allelic (%), N=412	
IL-1β promoter region					
	CC	CT	TT	C	T
N = 206	50 (24)	126 (61)	30 (15)	226 (55)	186 (45)
IL-1β exon-5					
	E1E1	E1E2	E2E2	E1	E2
N = 206	149(72)	46 (22)	11(6)	344 (84)	68 (16)

	Genotypes (%)							
IL-1Ra								
	1/1(410)	2/2(240)	1/2(410/240)	3/3(500/500)	4/4(325/325)	1/3(410/500)	1/4(410/325)	2/4 (240/325)
N=206	107(52)	36(18)	50(24)	4(2)	1(0.5)	6(3)	1(0.5)	1(0.5)

	Allele frequency (%)			
	Allele-1	Allele-2	Allele-3	Allele-4
N= 412	271(66)	123 (30)	14(3)	4 (1)

Results

A significant p-value ($p<0.001$) revealed that north Indian population is in HWE at all three polymorphic sites of the IL-1 gene i.e. the IL-1β (promoter & exon-5) and IL-1Ra. A schematic IL-1 gene map showing the different restriction sites for IL-1β promoter region (-511), exon-5 (+3953) restriction enzymes and VNTR in IL-1Ra is demonstrated in Fig. 1. Genotype and allele frequencies of IL-1β gene (promoter & exon-5) and IL-1Ra in the subjects are presented in Table 2 and 3 respectively. The observations made for allelic frequency were as follows: (55% vs 45%), (84% vs 16%) and (66%, 30%, 3% & 1%) for (C vs T) for IL-1β promoter, (E1 vs E2) for exon-5 and (1, 2, 3 &4) for IL-1Ra allele and the percentage of genotypes CC, CT and TT as 24%, 61% and 15%, E1E1, E1E2 and E2E2 as 72%, 22% and 6% and for 1/1, 1/2, 2/2 and 'others' (the rare genotypes like 4/4, 1/4, 1/3 & 2/4 were combined as 'others') as 52%, 18%, 24% and 6% respectively. The frequency distribution of different genotypes and alleles of IL-1 gene with different populations with reference to ours were compared (Table 4, 5 & 6) by using χ^2 tests. In case of IL-1β -511 (promoter region) significant difference was observed between Portugal, UK, Netherlands, Turkey, Caucasians, African-American, Austria and Egypt as compared to our population. Significant distribution of different genotypes frequency as reported earlier by several studies was observed in IL-1β +3953 (exon-5) polymorphism in Germany, China, Finland, Greece, Japan, Taiwan, Netherlands, Turkey and Egypt population. IL-1Ra, genotype distribution too, was

Table. 3 Genotypes and Allele Frequency Distribution of IL-1β -511 (promoter region) Gene Polymorphism in Various Populations and p-values in Comparison to North Indian Population

Country/ Ethnicity	No	Age (years)	Genotype (%) (CC)	(CT)	(TT)	P	Allele (%) (C)	(T)	P	Reference
North India	206	22-58	24	61	15	Ref	55	45	Ref	Present study
Portugal	218	19-61	46	40	14	**	66	34	NS	Machado et al, 2001
UK	54	23-85	41	48	11	*	65	35	NS	Carter et al, 2004
Germany	228	-	39	49	12	NS	64	36	NS	Grimm et al, 2004
Italy	272	59-76	36	44	20	NS	58	42	NS	Zienolddiny et al, 2004
China	361	21.2±1.4	34	48	18	NS	58	42	NS	Zeng et al, 2003
Finland	400	18-60	36	46	18	NS	59	41	NS	Makela et al, 2001
Taiwan	103	50-83	29	47	24	NS	53	47	NS	Tsai et al, 2004
United State (Whites)	124	-	35	50	15	NS	66	34	NS	Cantagrel et al, 1999
Korea	126	58.3±9.6	26	56	18	NS	54	46	NS	Lee et al, 2004
Netherlands	256	-	58	36	6	***	76	24	**	Stokkers et al, 1998
Spain	81	-	32	49	19	NS	57	43	NS	Pastor et al, 2005
Japan	160	40-82	27	49	24	NS	52	48	NS	Nishimura et al, 2002
Turkey	163	35.8±8.4	41	32	27	***	57	43	NS	Coskun et al, 2005
Caucasian	99	-	46	42	12	**	67	33	NS	Wetmore et al, 2005
African-American	98	-	11	58	31	**	40	60	*	Wetmore et al, 2005
Austria	310	37.3±14	42	47	11	*	66	34	NS	Westphal et al, 2003
Egypt	72	59±1.0	37	41	22	*	58	42	NS	Hegab et al, 2005

* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, at 5 % level of significance NS = Not Significant ($p>0.05$)

Table. 4 Genotypes and Allele Frequency Distribution of the IL-1β +3953 (exon-5) Polymorphism and p-values for Different Populations in Comparison to the North Indian Population

Country/ Ethnicity	No	Age (Years)	Genotype (%)				Allele (%)			Reference
			E1E1	E1E2	E2E2	P	E1	E2	P	
North India	206	22-58	72	22	6	Ref	84	16	Ref	Present study
UK	54	23-85	61	35	4	NS	79	21	NS	Carter et al, 2004
Germany	228	-	52	44	4	*	74	26	NS	Grimm et al, 2004
China	361	21.2±1.4	88	12	0	**	94	6	*	Zeng et al, 2003
Finland	400	-	52	41	7	*	72	28	NS	Maury et al, 2004
Caucasian	99	-	66	33	1	NS	83	17	NS	Wetmore et al, 2005
African-American	98	-	78	20	2	NS	88	12	NS	Wetmore et al, 2005
Greece	110	20-35	50	42	8	**	71	29	*	Sakellari et al, 2003
Austria	310	37.3±14	59	35	6	NS	77	23	NS	Westphal et al, 2003
Spain	81	-	61	37	2	NS	79	21	NS	Pastor et al, 2005
Japan	160	40-82	93	7	0	***	97	3	**	Nishimura et al, 2002
Taiwan	116	16-28	97	3	0	***	99	1	***	Hang et al, 2003
Netherlands	131	55-65	55	37	8	*	74	26	NS	Meulenbelt et al, 2004
Turkey	163	35.8±8	39	42	19	***	60	40	***	Coskun et al, 2005
Egypt	72	59±1.0	52	41	7	**	73	27	NS	Hegab et al, 2005

* = p< 0.05, ** = p<0.01, *** = p<0.001 , at 5% level of significance NS = Not Significant (p>0.05)

significantly different in Portugal, UK, Germany, Japan, Finland, China, Taiwan, Netherlands, Mexico, Korea, Austria and Egypt. We also examined the patterns of LD and haplotype distribution in our population, combining the three sites of IL-1 candidate genes using SNPAnalyzer. All the eight possible haplotypes were common in normals whose frequencies were higher than 1%. Haplotype 'T-E1-1' was 41.7% while the least common 2% was observed for 'C-E2-2'. Linkage disequilibrium was found to be significant between IL-1Ra and IL-1β (promoter region and exon-5) polymorphism in the controls (D´=0.42, $p< 0.001$).

Discussion

Inheritance of polymorphic cytokine gene alleles is dramatically influenced by ethinicity (Hoffmann et al., 2002). Polymorphisms in cytokine genes may result in inter-individual variation in transcriptional regulation, and thus in differential cytokine production. It has been widely hypothesized that genetic variants of cytokines could have phenotypic relevance and influence an individual's internal microenvironment (Loughrey et al., 1998, Saurez et al., 2003). In the present study, we investigated IL-1 gene cluster (IL-1β promoter, exon-5 and IL-1Ra) polymorphisms in the north Indian population and compared with the genotypes reported in different populations worldwide. The variation in our Indian population from the rest of the world population signifies the impact of ethnicity. In our population all the eight possible haplotypes were common and their frequency was > 1%, haplotype 'T-E1-1' being the most common (41%) The three polymorphic sites of IL-1 gene cluster were in strong linkage disequilibrium, which suggested that the three variants (IL-1β promoter, exon-5 and IL-1Ra) are strongly associated. .

Epidemiologic studies continue to suggest the significant contribution of genetic variation to cancer susceptibility.

Table 5. Genotypes and Allele Frequency Distribution of IL-1Ra Polymorphismand p-values for Different Populations in Comparison to the North Indian Population

Country/ Ethnicity	No	Age (Years)	Genotype (%)					Allele (%)				Reference
			1/1	1/2	2/2	others	P	1	2	O	P	
North India	206	22-58	52	18	24	6	Ref	66	30	4	Ref	Present study
Portugal	220	19-61	51	37	9	3	**	70	27	3	NS	Machado et al, 2001
UK	54	23-85	61	32	7	0	**	77	23	0	NS	Carter et al, 2004
Germany	234	-	46	41	6	7	***	69	27	4	NS	Hacker et al, 1997
Japan	160	40-82	91	6	0	3	***	94	3	3	***	Nishimura et al, 2002
Finland	400	18-60	50	39	9	2	**	70	28	2	NS	Makela et al, 2001
China	361	21.2±1.4	93	4	1	2	***	95	3	2	***	Zeng et al, 2003
Taiwan	103	50-83	86	10	1	3	***	91	6	3	***	Tsai et al, 2004
Netherland	130	55-65	55	37	8	0	**	74	26	0	NS	Meulenbelt et al, 2004
Mexico	215	18-92	41	44	12	3	**	63	34	3	NS	Garza et al, 2005
Korea	434	21-72	90	7	0	3	***	94	3	3	***	Chang et al, 2005
Austria	310	37.3±14	51	35	10	4	**	69	27	4	NS	Westphal et al, 2003
Egypt	72	59±1	69	21	10	0	**	80	20	0	*	Hegab et al, 2005

* = p< 0.05, ** = p<0.01, *** = p<0.001 , at 5% level of significance NS = Not Significant (p>0.05)

Table 6. Association Analysis of Haplotypes in Three LD Blocks (IL-1β promoter, exon-5 and IL-1Ra genes) between North Indian ESRD Patients and Controls

No.	Haplotype	Control (n=206)	
		N	frequency
1.	T-E1-1	86	0.4170
2.	C-E1-1	42	0.2058
3.	T-E1-2	31	0.1454
4.	C-E2-1	19	0.0945
5.	C-E1-2	11	0.0516
6.	T-E2-1	8	0.0414
7.	T-E2-2	5	0.0241
8.	C-E2-2	5	0.0203

(C, T, E1, E2 and 1,2 represent the alleles of IL-1β promoter, exon-5 and IL-1Ra genes)

Although involvement of numerous genetic factors in the pathogenesis of neoplasia is well proven, understanding of the complex molecular mechanisms underlying neoplastic growth is still disappointingly incomplete (Alexandre et al., 2004). Most human cancers are characterized by genomic instability, which is the result of the accumulation of multiple genetic alterations and allelic imbalance throughout the genome (Quek et al., 2004). A wide array of studies has further demonstrated differences in cytokine gene polymorphisms depending on ethinicity (Scarel-Caminaga et al., 2002 and Uboldi et al., 2003). Recently, association studies in humans have shown that polymorphisms of the IL-1 gene were associated with several cancers (Kato et al., 2001 and Ito et al., 2002). Our recently published study reported the association of IL-Ra gene polymorphism with prostrate cancer (Mittal et al., 2004).

Thus this kind of study may form the basis for future establishment of epidemiological and clinical databases. However, we are in the initial stages of characterising the tools (i.e., the single-nucleotide polymorphism, SNP) in rigorous analysis of the genetic contributions to complex diseases, such as cancer. Individual SNPs may serve as signposts for disease genes and haplotypes are believed to be superior for this purpose. Moreover, linkage analysis is comprehensive and locates genes that exert a major effect on disease susceptibility. This type of research has the initiative of developing a haplotype map of the human genome, the purpose of which is to relate human genetic variation with disease predisposition, specifically cancer. Thus, it is possible that differences in interleukin-1 distribution between north Indian healthy population and other ethnic groups may reflect a unique profile that may affect disease predisposition and prevalence.

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