RESEARCH COMMUNICATION

Do IL-4 Intron 3 VNTR and IL-6 (-174) G/C Variants Reflect Ethnic Variation? A Comparative Study Between the Global and North Indian Populations

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Abstract

Variations in the production and activity of cytokines have been reported by several investigators which influence the susceptibility and/or resistance to various infectious agents and cancer. Differences in the cytokine production between individuals are often caused by single nucleotide polymorphism (SNP) in the promoter or coding regions of cytokine genes. Although the SNP cytokine gene variations are basically mutations, they are designated as polymorphisms, because these changes do not modify the alleles to rare or abnormal variants. The two important cytokine genes IL-4 and IL-6 of 343 unrelated healthy individuals from North India were compared with the published polymorphism of other populations. It was seen that our population differs from South Indian population as well as from other Caucasian populations except, Taiwanese population at IL-4 locus and Spanish and Polish population at the IL-6 gene locus. This study may be helpful for predicting clinical outcome of various infectious and immunoregulated disorders as well as explore for risk alleles for various cancers.

Key Words: Cytokines - polymorphisms - international comparison

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Introduction

Single nucleotide polymorphisms or SNPs and variable number of tandem repeats (VNTR) are common type of polymorphisms. The variants of DNA sequence can have a major impact on how humans respond to disease; environmental insults such as bacteria, viruses, toxins, and chemicals; and drugs and other therapies. SNPs do not cause disease, but they can help determine the likelihood that a particular individual may develop a particular disease (http://www.ornl.gov/sci/techresources/Human_Genome/faq/snps.shtml). In recent years there has been an enormous effort by numerous laboratories worldwide to identify the particular SNPs and VNTRs that play a major role in genetic predisposition to and disease progression in particular disease and cancers. Polymorphisms within the cytokine genes can play a role in the level of production of cytokines.

Cytokines are small molecules secreted by cells in response to specific stimuli and alter the behavior of the same or other cells. Cytokines act on target cells generally within the hematopoietic system by binding to specific receptors, initiating signal transduction and second messenger pathways within the target cell. Cytokines function as highly complex and coordinated network in which they modulate their own synthesis as well as that of other cytokines and cytokine receptors. Production of numerous cytokines by immune cells in response to both antigen specific and -nonspecific stimuli is critical to the outcome of inflammatory immune responses. IL-4 and IL-6 are considered important cytokines as they play an important role in B-cell activation and differentiation (Kishimoto, 1989). These may be of major help in cancer prevention.

IL-4 an anti-inflammatory cytokine plays a key role in activation and differentiation of B-cells, mast cells, erythroid progenitors and the development of the Th-2 subset of lymphocytes. Th2 cytokines such as IL-4, IL-2 and IL-10 primarily supports antibody production (Sosroseno et al., 1994). IL-4 is also known to inhibit macrophage activation and therefore may be involve in cancer.

A variable number of tandem repeat (VNTR) of 70 base pair repeat is situated in third intronic region of the IL-4 gene. Three repeat allele is most common and two repeat allele is rare. There is another much rare allele of four repeat, which is reported only in few populations (Mout et al., 1991). Three repeat allele is known to be high producer of IL-4 (Nakashima et al., 2002).

Interleukin-6 (IL-6) is a pleiotropic, proinflammatory cytokine involved in the regulation of the acute phase reaction, immune responses, and hematopoiesis. It has

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Table1. Genotypic and Allelic Frequency of IL-4 and IL-6 (n=343)

	Genotype Frequency			Allele Frequency	
	N(%)	N(%)	N(%)	N(%)	N(%)
IL-4 VNTR	3R/3R	2R/3R	2R/2R	3R	2R
	188 (54.8)	150 (43.7)	5(1.5)	526(76.67)	160(23.33)
IL-6	GG	GC	CC	G	C
	172(50.1)	120 (35.0)	51(14.9)	464(67.64)	222(32.36)

been reported to play a major role in B cell activation in to a antibody producing cells (Hirano, 1986).

IL-6 is particularly an important molecule since it is a pleiotropic cytokine that has a central role in host defense mechanism by regulating immune responses, hematopoiesis and induction of acute phase reaction (Kidd, 2003). It is localized on chromosome 7p21-24 with an upstream promoter containing 303 bp (Akira et al., 1993 and Bowcock et al., 1988). A common G>C polymorphism of the IL-6 promoter on position -174 has been investigated in a wide variety of diseases. The 174G>C promoter polymorphism in the IL-6 gene regulates the rate of IL-6 production and presence of "C" allele has been associated with decreased expression of IL-6 cytokine (Fishman et al., 1998).

The IL-6 system promotes inflammation through the activation and proliferation of lymphocytes, differentiation of B cells, leukocyte recruitment and induction of acute phase protein (Stenvinkel, 2005). It has been implicated in modulation of growth and differentiation in many malignant tumors and it is associated with poor progression in several solid and hematopoietic neoplasms such as renal cell carcinoma, ovarian cancer etc. (Matsumoto et al., 2000).

Keeping in view the importance of IL-4 and IL-6 cytokines in inflammatory diseases, we undertook the present study to evaluate the frequency of these cytokine genotypes and alleles in 343 unrelated healthy individuals of the North Indian population.

Materials and Methods

Subjects

The study was conducted on a cohort of 343 unrelated healthy individuals (males-251 & females-92)(age range-35-88 years) from Lucknow and its neighbouring area. Blood samples were collected with formal informed consent of each participant and approval for the study was obtained from the Ethical Committee of the Institute. Genomic DNA was isolated and purified from anti-coagulated blood (5 mL) by the salting out method (Miller et al., 1988).

Analysis of genotypes

The primer sequences and PCR conditions were standardized by referring to already published papers (Hegab et al., 2004; Tan et al., 2005 and Mittal et al., 2007).

Genotyping

IL-4 VNTR polymorphism

IL-4 variable number of tandem repeat (VNTR), which is a 70 base pair repeat, was genotyped using the thermocycler PTC–100 (MJ research). Alleles were named as follows: allele 3R= three repeats (254 bp), and allele 2R = two repeats (184 bp). The PCR products were resolved on 2% agarose gel. The alleles were named as 3R and 2R to minimize the confusion as other papers have named the same alleles as 1 and 2 or B1 and B2.

IL-6 (-174) G to C polymorphism

Genotyping was done by amplification refractory mutation system (ARMS–PCR) using two primer pairs for two alleles of SNP in a single PCR reaction. Two primer pairs were used to amplify the 2 alleles of IL-6 (i.e. G and C) in a single PCR reaction, (Tan et al., 2005). The genotype was indicated by the presence of following bands G/G homozygous (326 bp band and 205bp), G/C heterozygous (326bp, 205 bp band and 76bp) and C/C homozygous (326 bp and 176bp) (Tan et al., 2005)
All primers were synthesized commercially from (Gibco BRL Berlin Germany). PCR was performed in MJ Research PTC-100 thermocycler (USA) visualized under UV and photographed with an Alpha Imager-1220 v 5.5 Camera software. PCR product was sized using a 100-bp DNA ladder (Roche, Germany).

Statistical Analysis

Comparison between different populations was performed made using the χ^2 test to compare the genotype and allelic frequency distribution in healthy north Indian individuals with the SPSS software (version 11.5) using Fisher's exact test. P value <0.05 were considered statistically significant.

Results

The three repeat allele of IL-4 VNTR was present in 76.67% of our population and only 23.33 % of the population had two repeat allele. The percentage frequency of G and C allele, at -174 (promoter region) of IL-6 gene, was 67.64% and 32.36% respectively The genotyping results for IL-4 VNTR as well as IL-6 (-174)

Table 2. Comparison of IL-4 Genotypes and Alleles With Other Populations

Country/		Age range /	Genotype				Alleles			References
Ethnicity	No	Mean age±SD	3R/3R%	2R/3R%	2R/2R%	p	3R%	2R%	p	
Asian Population										
North India	343	35-78	54.8	43.7	1.5	Ref	76.7	23.3	Ref	Present Study
Taiwanese	100	--	64	33	3	0.115	80.9	19.1	0.208	Yu Su, 2007
Japanese	60	67.8±1.2	8.3	46.7	45	<0.0001	31.7	68.3	<0.0001	Hegab,2004
European Population										
French	104	--	74.0	25	1.0	0.002	86.4	13.6	0.03	Buchs, 2000
Egyptian	72	59.0±1.0	67.2	27.1	5.7	<0.0001	78.5	21.5	0.642	Hegab, 2004

Table 3. Comparison of IL-6 Genotypes and Alleles With Other Population

Country/ Ethnicity	No	Age range / Mean age±SD	Genotype GG%	GC%	CC%	p	Alleles G%	C%	p	References
Asian Population										
North India	343	35-78	50.1	35.0	14.9	Ref	67.6	32.4	Ref	Present Study
South Indian women	210	22-39	71.9	25.2	2.9	<0.0001	84.5	15.5	<0.0001	Bhanoori, 2005
Chinese	217	48.1±12.4	33.2	51.6	15.2	<0.0001	59	41	0.003	Jeng, 2005
Han Chinese	105	42.2±12.4	3.8	36.2	60	<0.0001	21.9	78.1	<0.0001	Hong Je, 2005
European Population										
Spanish	311	58-75	46.6	42.8	10.6	0.072	68.0	32.0	0.887	Landi, 2003
English	383	40-75	37.6	44.1	18.3	0.003	59.7	40.3	0.002	Fishman, 1998
Polish	50	42-88	32.0	56.0	12.0	0.015	60	40	0.13	Mazura, 2005
Czech	105	27-79	34.3	43.8	21.9	0.015	56.2	43.8	0.002	Vasku, 2004
American Population										
American Caucasian	65	50-75	30.8	46.2	23.1	0.014	53.8	46.2	0.002	Halverstadta, 2005
American	613	50-74	37.5	47.8	14.7	<0.0001	61.4	38.6	0.007	Michaud, 2006
Brazilian women	253	41-78	58.5	40.3	1.2	<0.0001	78.7	21.3	<0.0001	De Souza, 2006
Hawaiian women	218	--	4.1	21.1	74.8	<0.0001	14.7	85.3	<0.0001	Bushley, 2004

polymorphism is shown in (Table 1). Genotype distributions for both the genes were in agreement with Hardy-Weinberg equilibrium. In the literature search results we have found 3 papers in IL-4 VNTR and 11 in IL-6 polymorphisms. In comparison between our population and other population we have found non-significant difference in genotypic frequency (3R/3R=64%, 2R/3R=33%, 2R/2R=3%, p=0.115) and allelic frequency (3R= 80.9% and 2R=19.1%, p=0.208) of Taiwanese population and allelic frequency of Egyptian population in case of IL-4 (3R= 78.5% and 2R=21.5%, p=0.642) in comparison to genotypic (3R/3R=54.8%, 2R/3R=43.7%, 2R/2R=1.5%) and allelic frequency (3R= 76.7% and 2R=23.3%) of North in Indian population. In IL-6 non-significant difference in gene frequency (GG=46.6%, GC=42.8%, CC=10.6%, p=0.072) and allele frequency (G=68% and C=32%, p=0.887) of Spanish population and allelic frequency of Polish population (G=60% and C=41%, p=0.13) in comparison to North Indian population (Gene frequency-GG=50.1%, GC=35%, CC=14.9; Allele frequency- G=67.6%, C=32.4%). South Indian population was different from North Indian population (p<0.0001). Other populations showed significant difference in genotypic as well as allelic frequency (Tables 2 and 3).

Discussion

Several cytokine gene polymorphisms have been associated with different disease profiles as reported in the literature. Thus, they could become not only the potential markers for better understanding the etiopathogenesis of the disease but also as probable markers of disease susceptibility and severity.

B-cell plays an important role in immunity, as only B-cells can produce antibody. It is known that Interleukin-4 (IL-4) is required for early activation of resting B-cells, IL-5 for the growth of activated B-cells and IL-6 for B-cell differentiation in to antibody producing cell (Kishimoto, 1989). The effect of IL-6 is not restricted to B- cells but it can also act on T-cells. T cells express IL-6 receptors. In fact IL-6 was shown to induce IL-2 receptor and IL-2 production (Kishimoto, 1989).

Allelic distributions of cytokines vary in ethnically different populations (Bid et al., 2005; Meenagh et al., 2002). Few studies from our laboratory have been reported recently to emphasize the important role of various cytokine genes under normal as well as diseased state (Mittal, et al 2007). The results obtained in this study on IL-4 and IL-6 may be helpful in the future research probing association between cytokine variability and various immunological reactions in prostate and bladder cancers. A worldwide comparison of the distribution of IL-4 and IL-6 polymorphism in our population revealed certain key variations. Comparison between various population of world and North Indian population in IL-4 VNTR showed that gene frequency of French (p=0.002), Japanese (p<0.0001) and Egyptian population (p<0.0001) was significantly different. French and Japanese population were also significantly different in allelic distribution with p=0.03 and p<0.0001 respectively in comparison to our population. In a similar comparison of IL-6 polymorphism, we observed South Indian (p<0.0001), Chinese (p<0.0001), Han Chinese (p<0.0001), English (p=0.003), Polish (p=0.015), Czech (p=0.015), American Caucasian (p=0.014), American (p<0.0001), Brazilian (p<0.0001) and Hawaiian (p<0.0001), significant difference was seen in gene frequency in comparison to our population. We also found significant difference in allele frequency of South Indian (p<0.0001), Chinese (p<0.003), Han Chinese (p<0.0001), English (p=0.002), Czech (p=0.002), American Caucasian (p=0.002), American (p<0.007), Brazilian (p<0.0001) and Hawaiian populations (p<0.0001). Spanish study of Landi et al., 2003, with gene frequency (GG=46.6%, GC=42.8%, CC=10.6%) did not show any significant difference

(p=0.072).

In IL-4 a common trend was observed, with exception of Japanese population, were 3R allele was 31.7% in contrast to other population, which had 3R allele to be more common. In IL-6 we observed a common trend, with exception to Han Chinese and Hawaiian population were G allele, which is, most common in other populations is only 21.9% and 14.7% respectively. Indian population is one of the most diverse populations. The North Indian population is referred to as Aryans and South Indians as Dravidians. Both the population is entirely different due to different socio-cultural diversity. The different gene frequency between these groups is also suggestive of the same. This could be attributed to the fact that the South Indians are considered as the original inhabitants of Indian subcontinent and the North Indians are the migrant's population having a mixed gene pool (Coon, 1983). This facts support our results of IL-6 polymorphism. Allele frequency of G allele in North and South Indian population is 67.6% and 84.5% respectively and that of C allele is 32.4% and 15.5% respectively.Significant difference in Caucasian populations like French population in IL-4 and Spanish, Polish, Brazilian and American in IL-6 suggests ethnic variation at these particular gene loci. The oriental populations are also different in comparison to these loci. Three repeat allele of IL-4 VNTR frequency in Egyptians and Taiwanese is 78.5% and 80.9% respectively in comparison to 76.7% in North Indians. Frequency of Two repeat allele in Egyptians and Taiwanese is 21.5%and 19.1% respectively in comparison to 23.3 % of North Indians. In case of IL-6 polymorphism Polish (G= 60% and C=40%) and Spanish (G= 68% and C=32%) in comparison to North Indians (G= 67.6% and C=32.4%). This suggests similarity of Aryans and European Caucasoid. This may point toward the origin of Aryans race. Our earlier studies in which we have found non-significant difference in VDR gene locus in Greek and Swedish population (Bid et al, 2005) are also supportive of the same. However this cannot be confirmatory and suggests for further studies in large cohort of different population.

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