Impact of *CCL2* and Its Receptor *CCR2* Gene Polymorphism in North Indian Population: A Comparative Study in Different Ethnic Groups Worldwide

Vibha Singh · Neena Srivastava · Priyanka Srivastava
· Rama Devi Mittal

Received: 11 September 2012 / Accepted: 23 September 2012 / Published online: 5 October 2012
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Abstract Chemokine are small, inducible pro-inflammatory cytokines involved in many biological processes, such as migration of leukocytes, atherosclerosis, angiogenesis, tumor growth, and metastasis. Chemokine are also known to influence tumor cell's activity. Specifically, tumor cells express chemokine receptors in a non random manner suggesting a role of chemokine in metastatic destination of tumor cells. The present study was conducted to determine distribution of (Chemokine receptor 2) *CCR2* *V64I*, Chemokine ligand 2 *CCL2* *I/D*, and *CCL2* *2518A>G* gene polymorphisms in North Indian population and compare with different populations globally. Polymerase chain reaction (PCR)-based analysis was conducted in 200 normal healthy individuals of similar ethnicity. Allelic frequencies in wild type (GG) of *CCR2* *V64I* *G>A* were 63 % G; *CCL2* *I/D* 42 % *II*; *CCL2* *2518A>G* 40.5 % A. The minor variant allele frequency in our population was as follows: 19.5 % for *CCR2* *V64I*, 35.5 % for *CCL2* *I/D*, 35.3 % for *CCL2* *2518A>G*. We further compared frequency distribution for these genes with various published studies in different ethnicity. Our results suggested that frequency in chemokine genes exhibit distinctive pattern in India that could be attributed to ethnicity variation. This could assist in high-risk screening of human exposed to environmental carcinogens and cancer predisposition in different ethnic groups. Thus, they signify an impact of ethnicity and provide a basis for future epidemiological and clinical studies.

Keywords Chemokine gene · SNPs · Bladder cancer · PCR–RFLP · North India

Introduction

Genetic variation plays a critical but largely uncharacterized role in differentiation. Variations in the DNA sequences of humans can affect how they develop and respond to pathogens, chemicals, drugs, vaccines, and other agents. Genetic variation in human genome is an emerging resource for studying cancer, a complex disease characterized by both environmental and genetic contributions. Inflammation is a multistep process that includes injury, repair, and resolution. Chronic inflammation can incite carcinogenesis by inducing proliferative events and post-translational DNA modifications by enhancing the secretion of growth factors such as cytokines and chemokines, and inducing oxidative stress by the release of nitric oxide (NO) and reactive oxygen species (ROS). Chemokines are secreted, low molecular weight proteins (8–30 kDa) that bind to seven transmembrane protein-coupled receptors [1].

Chemokine/chemokine receptor interactions are important, which starts signaling (Fig. 1) through which wound healing, infection, and tissue maintenance occur. The expression of chemokine and chemokine receptors are often strongly up-regulated during tumorigenesis in case of different cancers like breast, lung, prostate, colon, ovary, and bladder [2]. C–C chemokine ligand 2 (*CCL2*), also known as monocyte chemo-attractant protein-1 (MCP-1), which is a member of the C–C beta chemokine family that is produced by macrophages, fibroblasts, and endothelial cells to stimulate chemotaxis of monocyte/macrophages and other inflammatory cells through its receptor, Chemokine receptor 2 (*CCR2*) [3]. Macrophage and lymphocytes infiltrating tumors are controlled by numerous cytokines, including macrophage chemoattractant protein 1

V. Singh · P. Srivastava · R. D. Mittal (✉)
Department of Urology and Renal Transplantation,
Sanjay Gandhi Post Graduate Institute of Medical Science,
Raebareli Road, Lucknow 226014, Uttar Pradesh, India
e-mail: ramamittal@gmail.com

N. Srivastava
Department of Physiology, CSM Medical University, Lucknow,
India

Fig. 1 Chemokine signaling
(self-drawn)

[4]. The polymorphism at A2518G in the regulatory region of the MCP-1 gene influences MCP-1 expression in response to inflammatory stimuli [5]. *CCR2* gene is localized on chromosome 3p21 within a cluster of chemokine receptor genes. It has two isoforms; *CCR2A* and *CCR2B* products of the *CCR2* gene as a result of alternative splicing. A single nucleotide polymorphism (SNP) of G to A at position 190 of *CCR2* gene changes amino acid valine (GTC) to isoleucine (ATC) at codon 64 [6]. C–C chemokine ligand 2 and its receptor *CCR2* play a key role in promoting tumorigenesis and metastasis via distinct mechanisms [7]. Inflammation involves a complex interaction of gene networks and is largely self-regulating, thus it is reasonable to assume that certain combinations of alleles may contribute to an imbalanced immune response and increased disease risk. This genetic polymorphism promises to help define pathophysiological mechanisms, to identify individuals at risk for disease, and to suggest novel targets for drug treatment. One of the main characteristics of cancer cells is their ability to proliferate, invade the surrounding tissues, and migrate to distant organs and form metastasis, thereby resulting in the emergence of disseminated metastases, which remains the primary cause of mortality in cancer patients [8–11].

The primary focus of this study is an attempt to investigate frequency distribution of *CCL2I/D*, *CCL2 A2518G*, and *CCR2 V64I* genes polymorphism by using a Polymerase chain reaction (PCR)-based restriction analysis in unrelated normal healthy individuals from North India thereby translating the observations in assisting high-risk screening of human exposed to environmental carcinogens and cancer

predisposition in different ethnic groups., thus providing basis for future epidemiological and clinical studies.

Materials and Methods

Subjects

Healthy and genetically unrelated individuals visiting the hospital for a routine checkup or health awareness camps and hospital employees were recruited as the controls ($n = 200$). All the controls were age and sex matched with similar ethnicity and had no evidence of malignancy or chronic disease. The mean age of the controls was 58.5 years, and M:F ratio as 175:25. The participation rate was 100 % and blood samples were available for all subjects. Ethnicity was based on self-report and categorized as North Indian. An epidemiologic questionnaire was designed for study participants to collect data on demographic characteristics, smoking history, occupation history, and other lifestyle factors. At the end of the interview, a 5-ml blood sample was drawn into coded tubes.

Informed consent was taken from all subjects when interviewing for the demographic details and blood sample collection. The Ethical Review Board of the Institute approved the study.

We evaluated three chemokine genes, *CCR2 V64I* (rs1799864), *CCL2 I/D* (rs3917887), and *CCL2 2518 A>G* (rs1024611) and identified a sufficient number of epidemiologic studies on chemokine to conduct a comparative analysis for genetic polymorphisms in inflammatory genes, focusing on *CCL2* and its receptor *CCR2*.

DNA Extraction

Five ml of blood was collected in EDTA vials and DNA was extracted from blood lymphocytes using a ‘salting out’ method [12].

Genotyping

Polymorphisms in chemokine *CCL2I/D*, *CCL2 2518 A>G* and *CCR2V64I* genes were analyzed using PCR–RFLP. Primer detail and PCR conditions of *CCL2I/D*, *CCL2 2518 A>G* [13] and *CCR2V64I* [14] respectively. Genotyping was done on 15 % PAGE using molecular weight markers and visualized after staining with ethidium bromide. Positive and negative controls were used in each genotyping assay, and 10 % of the samples were randomly selected and run in duplicates with 100 % concordance. The results were reproducible with no discrepancy in genotyping.

Prevalence of Gene Variants

We conducted a MEDLINE search using *CCR2 V64I*, *CCL2 I/D*, and *CCL2 2518 A>G* and ‘polymorphism’ for papers published up to 2012. The search was limited to human subjects without language restriction. For case–control studies, only genotype frequencies for the control population were considered. Studies that reported only allele frequencies and no genotype frequencies were not included. Studies based on fewer than 90 persons were excluded. When more than one article was identified for the same study population, we included the most recent publication. We identified ten publications reporting on the prevalence of *CCR2 V64I* polymorphism, three publications on *CCL2 I/D*, six studies on *CCL2 2518 A>G*, which were subsequently used for comparison with our study.

Statistical Analysis

Pearson’s χ^2 test was done to compare the genotype and allelic frequencies of different populations using the computer software SPSS for windows (version 11.5). Court-Lab (web-based software) was used to examine Hardy–Weinberg equilibrium (www.tufts.edu). $P < 0.05$ was considered statistically significant.

Results

The distribution of *CCR2 V64I*, *CCL2 I/D*, and *CCL2 2518 A>G* genotypes and allele frequencies in northern Indian population are shown in Table 1.

Genotype distributions were in agreement with Hardy–Weinberg equilibrium at all polymorphic sites in three

genes. The frequency distribution of different genotypes and alleles of these three genes with different populations with reference to ours were compared (Tables 2, 3, 4) using χ^2 tests. The minor variant allele frequency in our population was as follows: 19.5 % for *CCR2 V64I*, 35.5 % for *CCL2 I/D*, 35.3 % for *CCL2 2518 A>G*.

In case of *CCR2 V64I* significantly genotype and allele distribution were observed in two North Indian populations, South India, Northern Sweden, Czech, and Turkey population as compared to our population. Study of *CCL2 I/D* polymorphism is not common worldwide, only few studies have been reported. Based on reports, significantly different pattern of *CCL2 I/D* polymorphism and allele frequency were reported only in North India and South India. Genotype and allele distribution of *CCL2 2518 A>G* polymorphism was significantly different from North India, South India, Germany, Poland, and Turkey as compared to our population.

Discussion

Polymorphisms in cytokine/chemokine genes may result in inter individual variation in transcriptional regulation, and thus in differential inflammatory and pro-inflammatory molecules production.

Single nucleotide polymorphisms are scattered throughout the genome and high degree of variability make these informative genetic markers useful for disease susceptibility. Functional polymorphism of chemokine is thought to be of particular importance for implication in the pathogenesis of complex genetic disorders. Due to marked differences in the distribution of chemokine gene polymorphisms between various ethnicities, the data from ‘normal healthy’ populations are of special interest for the adequate evaluation of the relevance of the investigated genetic markers in susceptibility, manifestation, prognosis or treatment of diseases. However, it is noteworthy to conduct extensive investigations about the distribution of these genes in different ethnic groups.

Indian population is believed to be most diverse because of different socio-cultural traditions. The study of genetic variation can elucidate critical determinants in environmental exposure and cancer, which could have future implications for preventive and early intervention strategies. The differences in allele frequencies detected among these studies might be due to ethnic variation, heterogeneity of study populations, and different sample sizes. The variation in our Indian population from other world population signifies the impact of ethnicity. It is well recognized that ethnic background may influence the susceptibility to suffer from certain diseases [15].

In the *CCR2 V64I* polymorphism, the (A*) allele frequency in Indian population was 19.5 %, which was significantly lower in two North India population, South India, Czech, Turkey and higher in Northern Swedish and but no

Table 1 Genotypes and allele frequency distribution of *CCR2* V64I, *CCL2* I/D and *CCL2* 2518 A>G gene polymorphism in North India

Gene	Genotype	Observed, <i>n</i> (%)	Expected, <i>n</i> (%)	Minor allele frequency	<i>P</i> value (HWE)
<i>CCR2</i> V64I	GG	126 (63)	129.6 (64.8)	19.5	0.251
	G/A	70 (35)	62.8 (31.4)		
	(rs1799864) AA	4 (2)	7.6 (3.8)		
<i>CCL2</i> I/D	II	84 (42)	83.2 (41.6)	35.5	0.862
	DD	90 (45)	91.6 (45.8)		
	(rs3917887) DD	26 (13)	25.2 (12.6)		
<i>CCL2</i> 2518	AA	81 (40.5)	83.9 (41.9)	35.3	0.532
	A/G	97 (48.5)	91.3 (45.6)		
	(rs1024611) GG	22 (11.0)	24.9 (12.4)		

Table 2 Genotypes and allele frequency distribution of *CCR2* V64I gene polymorphism in various populations and *P* values in comparison to Northern Indian population

Gene	Country/ethnicity	<i>n</i>	Age (years), Mean age $\pm$ SD	Genotype			<i>P</i>	A ^a	Reference
				GG	GA	AA			
<i>CCR2</i> V64I (rs1799864)	North India	200	56.8 $\pm$ 10.8	126 (63.0)	70 (35.0)	4 (2.0)	Ref	19.5	Present study
	North India	277	60.24 $\pm$ 8.19	201 (72.6)	71 (25.6)	5 (1.8)	0.081	14.6	Singh et al. [19]
	North India	225	>10(17.07 $\pm$ 6.69)	189 (84.0)	34 (15.0)	2 (1.0)	<0.001	9	Prasad et al. [20]
	South India	206	31.3 $\pm$ 8.4	173 (84.0)	30 (14.6)	3 (1.4)	<0.001	8.7	Alagarasu et al. [17]
	Northern Swedish	148	64 (24 $\pm$ 89)	112 (75.7)	34 (23.0)	2 (1.0)	0.042	27.8	Zheng et al. [21]
	Czech	277	49.3 $\pm$ 7.9	218 (78.7)	58 (20.9)	1 (0.4)	<0.001	10.8	Petrkova et al. [22]
	Black South African	305	42.3 $\pm$ 9.0	189 (62.0)	112 (37.0)	4 (1.0)	0.786	19.5	Chatterjee et al. [16]
	Mixed-ancestry	1073	44.3 $\pm$ 8.4	704 (66)	356 (33)	13 (1.0)	0.569	17.5	Chatterjee et al. [16]
	Japanese	122	44.4 $\pm$ 14.1	66 (54.1)	48 (39.3)	8 (6.6)	0.064	26.2	Hizawa et al. [23]
	North India	180	36.3 $\pm$ 12.4	139 (77.1)	41 (22.8)	0 (0.0)	0.004	11.4	Manchanda et al. [24]
	Turkey	140	52.34 $\pm$ 11.91	112 (80.0)	24 (17.9)	4 (2.9)	0.001	11.4	Bektas-Kayhan et al. [25]

Bold values represents significant risk

^a Minor or variant allele frequency

Table 3 Genotypes and allele frequency distribution of *CCL2* I/D gene polymorphism in various populations and *P* values in comparison to Northern Indian population

Gene	Country/ethnicity	<i>n</i>	Age (years), Mean age $\pm$ SD	Genotype			<i>P</i>	D ^a	Reference
				II	ID	DD			
<i>CCL2</i> I/D (rs3917887)	North India	200	56.8 $\pm$ 10.8	84 (42.0)	90 (45)	26 (13)	Ref	35.5	Present study
	North India	255	58.1 $\pm$ 8.0	89 (35.0)	82 (32.0)	84 (33.0)	<0.001	49	Ahluwalia et al. [13]
	South India	92	53.5 $\pm$ 12.1	29 (32)	34 (36.0)	29 (32)	0.001	50	Ahluwalia et al. [13]
	Australia	160		82 (51.2)	63 (39.4)	15 (9.4)	0.189	29.1	Chinoy et al. [26]

Bold values represents significant risk

^a Minor or variant allele frequency

significant difference was observed from other North India population, Black South Africa, mixed-ancestry and Japan. The (D*) allele frequency in *CCL2* I/D polymorphism was

35.5 % in our population. This was significantly different and higher from North India and South India and no significant difference was observed from Australia. In *CCL2*

Table 4 Genotypes and allele frequency distribution of *CCL2* 2518 A>G gene polymorphism in various populations and *P* values in comparison to Northern Indian population

Gene	Country/ ethnicity	<i>n</i>	Age (years), Mean age $\pm$ SD	Genotype			<i>P</i>	<i>G</i> ^a	Reference
				AA	AG	GG			
<i>CCL2</i> 2518 A>G (rs1024611)	North India	200	56.8 $\pm$ 10.8	81(40.5)	97(48.5)	22(11.0)	Ref	35.3	Present study
	North India	255	58.1 $\pm$ 8.0	122 (48.0)	97 (38.0)	36 (14.0)	0.079	33.0	Ahluwalia et al. [13]
	South India	206	31.3 $\pm$ 8.4	93 (45.8)	81 (39.9)	29 (14.3)	0.201	37.4	Alagarasu et al. [17]
	Germany	140	38 $\pm$ 12.3	81 (57.9)	50 (35.7)	9 (6.4)	0.006	24.25	Steinmetz et al. [27]
	Poland	323	59.7 $\pm$ 11.2	154 (48.0)	145 (45.6)	24 (7.0)	0.169	30	Kruszyna et al. [18]
	Poland	325	60.7 $\pm$ 12.4	209 (64.0)	101 (31.0)	15 (5)	<0.001	20.0	Buraczynska et al. [28]
	Turkey	140	52.34 $\pm$ 11.91	94 (67.1)	45 (32.1)	1 (0.7)	<0.001	16.8	Bektas-Kayhan et al. [25]

Bold values represents significant risk

^a Minor or variant allele frequency

2518 A>G polymorphism, the (*G**) allele frequency in Indian population was 35.3 %, which was significantly different and lower in Germany, Poland, and Turkey population while no significant difference was observed from North India, South India and other Poland population.

In a study from Black South Africa and mixed-ancestry by Chatterjee et al. 2010, the minor variant allele frequencies were found to be almost similar with our northern population for *CCR2* V64I (19.5 vs. 19.5 % and 17.5 respectively) [16] whereas in case of *CCL2* 2518 A>G, a study from North India, South India and Poland by Ahluwalia et al. 2009, Alagarasu et al. 2009, and Kruszyna et al. 2010, the minor variant allele frequencies were found to be almost similar with our northern population i.e. (35.3 vs. 33 %, 37.4 %, and 30 % respectively) [13; 17; 18]. This suggested that there was no variability in two geographical zones from India. Large and combined analyses may be preferred to minimize the likelihood of both false-positive and false-negative results. When appropriate, confounding factors should be controlled for, with particular consideration of race and ethnicity. There are differences in the prevalence of chemokine polymorphisms across different populations. Hence, it is important to keep in mind that a susceptibility factor in one population may not hold true for another.

Such kind of study may form the basis for future establishment of epidemiological and clinical databases. This study suggest that *CCL2* and its receptor *CCR2* polymorphisms may be biomarkers of disease susceptibility and may be contributing factors in the risk of cancer development. A single larger study with thousands of subjects and tissue-specific biochemical and biological characterization is warranted to further evaluate potential gene-to-gene and gene-to-environment interactions on chemokine gene polymorphisms and cancer risk. The differences in these genes distribution between North Indian healthy population and

other ethnic groups may help in building a profile that would help in assessing the disease predisposition and prevalence.

Acknowledgments We are thankful to DBT (Department of Biotechnology, New Delhi) and ICMR (Indian Council of Medical Research, New Delhi) for providing financial support for the study. We are also thankful to the volunteers for providing the blood samples.

Conflict of interest There is no conflict of interest among the authors.

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