RESEARCH ARTICLE

# Association of the -2518 A/G Polymorphism of MCP-1 with Breast Cancer in Punjab, North-West India

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## Abstract

**Background: Monocyte chemoattractant protein-1 (MCP-1) is a major chemokine thought to be responsible for monocyte and T-lymphocyte recruitment in acute inflammatory conditions and recruitment of macrophages in tumors. It is also implicated in cardiovascular disease, rheumatoid arthritis and chronic obstructive pulmonary disease. The aim of the present study was to investigate the correlation between MCP-1 -2518 A/G polymorphism and breast cancer risk in patients from Amritsar city of Punjab state in North-West India. Materials and Methods: We screened DNA samples of 200 sporadic breast cancer patients and 200 age and gender matched unrelated healthy individuals for MCP-1 -2518 A/G polymorphism using the PCR-RFLP method. Results: A significantly increased frequency of the GG genotype was observed in patients as compared to controls. Individuals carrying the MCP1 -2518GG genotype had a two fold risk for breast cancer (OR=2.06, 95%CI, 1.06-3.98; p=0.03). Genetic models analysis revealed a significant association between MCP-1 -2518 A/G polymorphism and cancer risk in homozygous co-dominant (OR=2.06, 95%CI, 1.06-3.98; p=0.03) and recessive (OR=1.97, 95%CI, 1.05-3.70; p=0.03) models. Conclusions: We conclude that the GG genotype of the MCP-1-2518 A/G polymorphism is associated with increased risk to breast cancer in Punjab, North-West India.**

**Keywords:** Breast cancer - polymorphism - MCP-1 - chemokine - risk factor

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## Introduction

Chemokines or Chemotactic cytokines are small heparin-binding proteins that regulate the leukocytes trafficking during inflammation (Baggiolini, 1998; Yoshie et al., 2001) and also influence other cellular functions like proliferation, maturation, angiogenesis and malignant transformation (Hanahan and Weinberg, 2000). Several functional polymorphisms of chemokine and chemokine receptors have been reported to be associated with dysregulation of chemokine system, suggesting their critical role in inflammatory and other diseases (Navratilova, 2006; Attar et al., 2010; Narter et al., 2010). MCP-1 (OMIM 158105) is a member of the CC chemokine family, composed of 76 amino acids and is encoded by CCL2 localized on 17q12 (Coillie et al., 1999). MCP-1 has been described as a potent stimulator of macrophage recruitment in various cancer types like bladder (Amann et al., 1998), cervix (Riethdorf et al., 1996), ovary (Negus et al., 1995), lung (Arenberg et al., 2000) and breast (Ueno et al., 2000). Over expression of MCP-1 has been reported in many tumors such as glioma (Yoshimura et al., 1989), ovarian (Negus et al., 1995), breast (Valkovic et al., 1998) and esophageal cancer (Ohta et al., 2002). Correlation of MCP-1 expression with tumorigenesis and metastasis has also been reported in several solid tumors (Gu et al., 2011).

Worldwide, approximately 15-20% of all cancer related deaths are linked with infections and inflammation (Balkwill and Mantovani, 2001; Mantovani et al., 2008). MCP-1 is a major chemokine responsible for monocyte and T-lymphocytes recruitment in acute inflammatory conditions and is implicated in cancer, cardiovascular disease, rheumatoid arthritis and chronic obstructive pulmonary disease (Conti and Rollins, 2004).

Breast cancer is a heterogeneous disease comprising different subtypes with distinct molecular features and clinical behavior (Polyak, 2011). Genetic and non-genetic factors including menstrual and reproductive history, body mass index, alcohol intake and physical activity are involved in the etiology of breast cancer. Genetic polymorphisms in the promoter or exons might affect gene expression or protein functions and influence different characteristics among individuals. Functional genetic polymorphisms which alter the regulation of gene expression have a substantial influence on disease pathogenesis (Taylor et al., 2001). An *in vitro* study has shown that a single nucleotide polymorphism -2518 A/G

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(dbSNP: rs1024611) in the MCP-1 regulatory region affect the transcriptional activity of the distal regulatory region and monocyte MCP-1 production (Rovin et al., 1999).

It has been documented that breast cancer patients carrying GG genotype or G allele of MCP-1 -2518 A/G polymorphism are more susceptible to metastasis as compared with patients carrying AA genotype (Ghilardi et al., 2005). Carriers of AG and GG genotypes produce more MCP-1 as compared to AA genotype (Rovin et al., 1999). A meta-analysis of 11 case control studies including 1,422 cases and 2,237 controls revealed that Asian individuals mainly Chinese, with GG genotype had decrease risk of cancer as compared to individuals with AG and AA genotypes (Jia et al., 2013). Association of GG genotype or G allele with increased risk for various cancer types like endometrial (Attar et al., 2010) and oral (Bektas-Kayhan et al., 2011) in Turkish, ovarian (Li et al., 2015), gastric (Gu et al., 2011) and renal cell cancer (Liu et al., 2013) in Chinese population has been reported. Elevated MCP-1 levels has been correlated to vascular endothelial growth factor secretion, enhanced tumor proliferation, poor prognosis and early disease relapse (Moriya et al., 2014). India is a country having many population groups with varied ethnicities residing in different geographical regions. From India, only one study is available showing the association of GG genotype of MCP-1 -2518 A/G polymorphism with increased risk of breast cancer in Western Indians (Joshi et al., 2014). To date, there is no study on MCP-1 -2518 A/G polymorphism from North India in breast cancer. The aim of the present study was to investigate the possible correlation between -2518 A/G polymorphism of MCP-1 with breast cancer risk in patients from Amritsar city of Punjab state in North-West part of India where increased number of sporadic breast cancer patients have been observed.

## Materials and Methods

*Patients and controls*

This study was approved by the Ethics Committee of Guru Nanak Dev University, Amritsar, Punjab, India. For this case control study, patients were selected from Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar, Punjab. Two hundred clinically confirmed sporadic breast cancer patients and 200 age and gender matched normal healthy individuals were recruited as study subjects. Patients who had received chemotherapy, radiotherapy or blood transfusion before surgery or had history of any malignancy were excluded from the study. Controls were biologically unrelated to cancer patients and were from same geographical region as that of patients. Individuals with family history of any cancer or other chronic disease were not included in the control group. Epidemiological data was collected from each subject using pre-tested structured questionnaire which included demographic particulars, family history, disease history etc. After written informed consent, 5 ml peripheral venous blood sample was collected from each subject in 0.5M EDTA.

*DNA extraction and genotyping*

Genomic DNA was extracted from peripheral blood lymphocytes using standard phenol chloroform method (Adeli and Ogbonna, 1990). MCP-1 -2518 A/G polymorphism was screened by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using primers; Forward: 5' CCGCATTCAATTTCCCTTTAT 3', Reverse: 5' TTCCAAGCTGCCTCCTCA 3'. The total PCR reaction volume was 15$\mu$l containing 60ng DNA, 1.5$\mu$l 10X Taq buffer with 15mM $MgCl_2$, 0.4$\mu$l dNTPs mixture (Merck

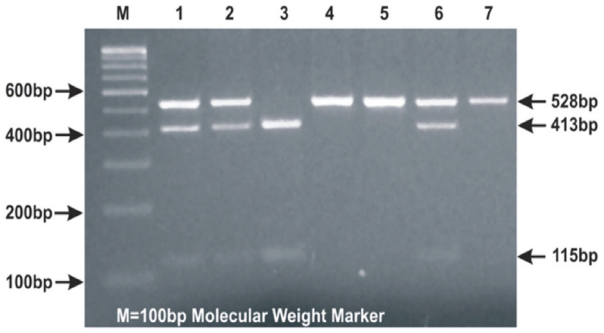


**Figure 1. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of MCP1 -2518A/G polymorphism.** Lanes 1, 2 and 6 = AG genotype; lane 3 = GG genotype; Lane 4 and 5 = AA genotype; lane 7= Undigested sample

**Table 1. Characteristics of Breast Cancer Patients and Controls**

| Characteristic | Patients | Controls | p value |
|---|---|---|---|
| No. of Subjects | 200 | 200 | |
| Gender | | | |
| Males | 6(3.0) | 6(3.0) | 1 |
| Females | 194(97.0) | 194(97.0) | |
| Age at Diagnosis (Years) | | | |
| <40 | 42(21.0) | 42(21.0) | |
| 40-49 | 61(30.5) | 61(30.5) | |
| 50-59 | 48(24.0) | 48(24.0) | |
| 60-69 | 34(17.0) | 34(17.0) | |
| 70-79 | 14(7.0) | 14(7.0) | |
| 80-89 | 1(0.5) | 1(0.5) | |
| Mean ± SD | 49.05±11.70 | 49.03±11.69 | 0.99 |
| Range | 25-85 | 25-85 | |
| Habitat | | | |
| Rural | 134(67.0) | 134(67.0) | 1 |
| Urban | 66(33.0) | 66(33.0) | |
| Diet | | | |
| Vegetarian | 127(63.5) | 112(56.0) | 0.13 |
| Non-Vegetarian | 73(36.5) | 88(44.0) | |
| Menstrual Status | | | |
| Premenopausal | 80(41.2) | 90(46.39) | 0.31 |
| Postmenopausal | 114(58.8) | 104(53.61) | |
| Histological Type | | | |
| Ductal | 189(94.5) | - | |
| Lobular | 4(2.0) | - | |
| Others | 7(3.5) | - | |
| Tumor stage | | | |
| I | 23(11.5) | | |
| II | 104(52) | - | |
| III | 54(27) | | |
| IV | 19(9.5) | | |

SD = Standard deviation; Figures in parentheses indicate percentages

Biosciences), 6 picomole of each primer (Sigma), 1 U Taq DNA Polymerase (Merck Biosciences). The PCR conditions were initial denaturation at 95°C for 5 min followed by 35 cycles with denaturation at 95°C for 45 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and final extension at 72˚C for 10 min in a Mastercycler gradient, (Eppendorf, Germany). A negative control without template DNA was included in each reaction to monitor the contamination. Amplified products were digested with PvuII restriction enzyme following the manufacturer recommendations (New England Biolabs, Beverly, MA). The resulting fragments were analyzed on 2.3 % ethidium bromide stained agarose gel and visualized under ultraviolet light. The genotype of each sample was assigned on the basis of fragments obtained after digestion. The presence of the G allele was indicated by bands of 413bp and 115bp, whereas undigested product of 528bp indicated the A allele (Figure 1). About 10% of the randomly selected samples were retested and reproducibility of results was 100%. Genotyping was performed without knowledge of case/control status.

*Statistical analyses*

Statistical analyses were performed using SPSS (Version 16, SPSS Inc, Chicago, IL). The association between cases and controls were examined by $\chi 2$ test. Odds ratio, their 95% confidence intervals (CI) ranges and corresponding p values were calculated using the Web-Assotest program (http://www.ekstroem.com). Deviations from Hardy Weinberg equilibrium (HWE) were tested using a $\chi 2$test. Analyses were also performed assuming dominant, co-dominant and recessive genetic models. Values for $p < 0.05$ were considered statistically significant.

## Results

The present case control study consisted of 200 patients with pathologically confirmed breast cancer patients and 200 healthy control subjects. The characteristics of breast cancer patients and controls are summarized in Table 1. Of 200 breast cancer patients, 194 (97%) were females whereas 6 (3%) were males. The mean age of breast cancer

**Table 2. Association of MCP1 -2518A/G Polymorphism with Breast Cancer Risk**

| | | Patients n(%) | Controls n(%) | OR(95% CI) | p value |
|---|---|---|---|---|---|
| Total No. of Subjects | | 200 | 200 | - | - |
| Total No. of Alleles | | 400 | 400 | | |
| Genotypes | AA | 86(43.0) | 97(48.5) | 1(Reference) | |
| | AG | 83(41.5) | 86(43.0) | 1.09(0.72-1.65) | 0.69 |
| | GG | 31(15.5) | 17(8.5) | 2.06(1.06-3.98) | **0.03** |
| Genetic Models | Dominant model AG+GG vs AA | | | 1.25(0.84-1.85) | 0.27 |
| | Over dominant model AG vs AA+GG | | | 0.94(0.63-1.40) | 0.76 |
| | Recessive model GG vs AA+AG | | | 1.97(1.05-3.70) | **0.03** |
| | Homozygous codominant GG vs AA | | | 2.06(1.06-3.98) | **0.03** |
| | Heterozygous codominant AG vs AA | | | 1.09(0.72-1.65) | 0.69 |
| Alleles | A | 255(63.8) | 280(70.0) | 1(Reference) | |
| | G | 145(36.2) | 120(30.0) | 1.33(0.99-1.78) | 0.06 |

**Table 3. Summary of Published Studies on MCP1 -2518A/G Polymorphism in Different Cancer Types**

| Type of Cancer | Study Location | No. of Cases/ Controls | Genotyping method | Key Findings | Reference |
|---|---|---|---|---|---|
| Breast | Italy | 83/141 | PCR-RFLP* | ↑ risk with G allele | Ghilardi et al., 2005 |
| | Poland | 160/323 | PCR-RFLP | No association | Kruszyna et al., 2011 |
| | Western India | 182/239 | PCR-SSP** | ↑ risk with GG genotype in younger patients | Joshi et al., 2014 |
| Endometrial | Turkey | 50/211 | PCR-RFLP | ↑ risk with GG genotype | Attar et al., 2010 |
| Ovarian | China | 275/293 | PCR-RFLP | ↑ risk with G allele and GG genotype | Li et al., 2015 |
| | China | 257 /273 | PCR-RFLP | ↑ risk with GG genotype | Wei et al., 2015 |
| Cervical | Taiwan | 86/253 | PCR-RFLP | ↑ risk with AA genotype | Wu et al., 2013 |
| Oral | Turkey | 129/140 | PCR-RFLP | ↑ risk with G allele and GG genotype | Bektas-Kayhan et al., 2012 |
| Gastric | China | 608/608 | PCR-RFLP | ↑ risk with GG genotype | Gu et al., 2011 |
| Colorectal | Spain | 377/326 | TaqMan assay | No association | Landi et al., 2006 |
| Renal cell | China | 416/458 | PCR-RFLP | ↑ risk with GG genotype | Liu et al., 2013 |
| Prostate | Turkey | 156/152 | PCR-RFLP | No association | Kucukgergin et al., 2012 |
| | India | 195/250 | PCR-RFLP | No association | Mandal et al., 2015 |
| | Spain | 296/311 | TaqMan assay | No association | Saenz-Lopez et al., 2008 |
| Bladder | Turkey | 142/197 | PCR-RFLP | No association | Kucukgergin et al., 2012 |
| | Turkey | 72/76 | PCR-RFLP | No association | Narter et al., 2010 |
| | India | 200/200 | PCR-RFLP | No association | Singh et al 2013 |
| | Mexico | 47/126 | PCR-RFLP | ↑ risk with AA genotype | Vazquezet al., 2009 |
| Lung | China | 112/82 | PCR-RFLP | ↑ risk with AA genotype | Yang et al., 2010 |
| | China | 102/344 | PCR-RFLP | No association | Yeh et al., 2010 |

* Polymerase chain reaction-restriction fragment length polymorphism; ** Polymerase chain reaction - Sequence specific primer

patients was 49.05±11.70 years (range 25-85 years) and controls was 49.03±11.69 years (range 25-85 years). Majority of the patients (94.5%) had infiltrating ductal carcinoma. Of 200 breast cancer patients, 23 had stage I, 104 had stage II, 54 had stage III and 19 had stage IV tumors. There was no significant differences between the breast cancer patients and controls for the gender, age, habitat, diet and menstrual status (p>0.05).

The frequencies of AA, AG and GG genotype of MCP1 -2518A/G polymorphism was 43 vs 48.5%, 41.5 vs 43% and 15.5 vs 8.5% in patients and controls respectively. The genotype distribution among cases ($\chi 2 = 2.085$; $p = 0.149$) and controls ($\chi 2 = 0.113$; $p = 0.736$) were in HWE. Individuals carrying MCP1 -2518GG genotype were associated with increased risk of breast cancer (OR=2.06, 95%CI, 1.06-3.98; p=0.03) (Table 2). There was marginally significant increase in frequency of G allele in patients and revealed 1.36 fold risk to breast cancer (OR=1.36; 95%CI, 1.01-1.82; p=0.06). We analyzed several genetic models and found significant association between polymorphism and cancer risk in homozygous codominant model (OR=2.06, 95%CI, 1.06-3.98; p=0.03) and recessive model (OR=1.97, 95%CI, 1.05-3.70; p=0.03) (Table 2).

The association between the MCP1 -2518A/G polymorphism and breast cancer risk was further examined by stratifying according to age, menopausal status, habitat, diet and tumor stage of breast cancer patients. No significant difference was observed in genotype distribution (data not shown).

## Discussion

Altered regulation of chemokines and their receptors has been shown to play a critical role in tumor development and progression through various mechanisms like proliferation, angiogenesis, invasiveness and recruitment of immune cells (Craig and Loberg, 2006). In the present case-control study, we investigated the potential association of MCP1 -2518A/G polymorphism with breast cancer in North-West Indians. We found that MCP1 -2518GG genotype was strongly associated with increased risk for breast cancer. The previous reports on association of MCP-1 -2518 A/G polymorphism in different cancer types have been inconsistent (Table 3). Association of GG genotype of MCP1 -2518A/G polymorphism has also been documented in Western Indian breast cancer patients aged ≤40 years (Joshi et al., 2014). MCP-1-2518 GG genotype has been reported to be associated with increased risk of endometrial cancer in Turkish (Attar et al., 2010) and ovarian cancer in Chinese patients (Li et al., 2015; Wei et al., 2015). A meta-analysis of 19 cases control studies including 4,162 cases and 5,173 controls suggested that MCP-1 -2518A/G polymorphism might have some relation to digestive system cancer susceptibility or cancer development in Caucasian (Da et al., 2013). In another meta-analysis on 1818 inflammatory disease (IBD) cases and 1319 controls revealed that MCP-1 -2518A/G polymorphism might be a protective factor for IBD in European populations (Li et al., 2015). On the other hand, no association of MCP-1 -2518 A/G polymorphism was observed in Spanish (Saenz-Lopez et al., 2008), Turkish (Kucukgergin et al., 2012) and Indian prostate cancer (Mandal et al., 2015) patients. Kruszyna et al., did not find significant association between MCP-1-2518 A/G polymorphism and breast cancer (Kruszyna et al., 2011). It has been reported that nasopharyngeal carcinoma patients with AA or AG were more prone to distant metastasis than those with the GG genotype (Tse et al., 2007).

Apart from cancer, G allele of MCP-1-2518 A/G polymorphism has also been associated with increased risk for diabetic retinopathy in Japanese (Katakami et al., 2010) and cardiovascular disease in Polish (Buraczynska et al., 2008) population. Carrier of A allele of MCP-1 -2518 A/G polymorphism has been reported to be associated with susceptibility to kidney failure in Korean type 2 diabetic patients (Moon et al., 2007). Down regulation of MCP-1 has been reported in advanced kidney cancer and low expression was described as a predictor of risk of relapse (Zirn et al., 2005).

Over expression of CCL2 chemokine might play a significant role in malignant transformation and cancer progression (Soria et al., 2008). It has been reported that MCP-1 plays an important roles in macrophage recruitment, expression of angiogenic factors and activation of matrix metelloproteinases in breast cancer patients (Saji et al., 2001). MCP-1 expression has been described as a regulator of tumor growth and metastasis and a poor prognostic factor in extra abdominal tumors like breast and prostate cancer (Li et al., 2013). In the present study, we did not observe a significant association between genotypes or allele of MCP-1-2518 A/G polymorphism and breast cancer clinicopathological characteristics similar to previous reports on nasopharyngeal carcinoma (Tse et al., 2007), hepatocellular carcinoma (Nahon et al., 2008), breast cancer (Kruszyna et al., 2011) and oral cancer (Bektas-Kayhan et al., 2012). The difference in cancer susceptibility in different populations could be attributed to various factors such as study design, sample size, ethnicity and genetic factors that predispose to various cancers.

In conclusion, we found an association between GG genotype of MCP-1-2518 A/G polymorphism and breast cancer risk in Punjab, North-West India. In future, studies in other ethnicities are required to better elucidate the involvement of MCP-1-2518 A/G polymorphism in breast cancer. It would be important to study the role of MCP-1-2518 A/G polymorphism on the expression of MCP-1 which may be helpful in the development of MCP-1 based therapeutic approach for breast cancer.

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