RESEARCH COMMUNICATION

Influence of Matrix Metalloproteinase Gene Polymorphisms in Healthy North Indians Compared to Variations in other Ethnic Groups Worldwide

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Abstract

Matrix metalloproteinases have a range of biological functions, including the liberation of cytokines and membrane-bound receptors, with roles in promotion of tumor invasion and angiogenesis. Several polymorphisms in MMPs have been implicated in the development of cancer as well as other diseases. Since their frequency distributions in the general North Indian population is not known the present study was conducted with the focus on MMP-1(-519) A>G, MMP-1(-1607) 1G>2G, and MMP-7(-181) A>G gene polymorphisms. PCR-based analysis was conducted for 200 normal healthy individuals of similar ethnicity. Allelic frequencies in wild type of MMP-1(-519) A>G were 71.2% A; MMP-1(-1607) 1G>2G 48.2% 1G; MMP-7(-181) A>G 60.7% A. The variant allele frequencies were 29% A in MMP-1(-519) A>G; 52% 2G in MMP-1(-1607) 1G>2G; and 39.3% G in MMP-7(-181) A>G respectively. We further compared frequency distribution for these genes with various published studies in different ethnicity globally. Our results suggest that frequency in these MMP genes exhibit distinctive patterns in India that could perhaps be attributed to ethnic variation. This study is important as it can form a baseline for screening individuals who are at high risk when exposed to environmental carcinogens. More emphasis is needed on evaluating polymorphisms, alone or in combination, as modifiers of risk from relevant environmental/ lifestyle exposures.

Key Words: Ethnicity - haplotype - metalloproteinase - polymorphism - RFLP

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Introduction

Inherited genetic variation plays a critical but largely uncharacterized role in human differentiation. Variations in the DNA sequences of humans can affect diseases 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. The extracellular microenvironment is a dynamic entity and provides regulatory signals on an intricate network of pathways that include cell adhesion, differentiation, division, and apoptosis. Therefore, cells with disruption of these pathways may acquire tumorigenic properties, such as loss of contact inhibition, aberrant cell division, and evasion of apoptosis (Hojilla et al., 2003).

Matrix metalloproteinases (MMPs), a family of structurally and functionally related zinc endopeptidases, are essential regulators of the microenvironment of the cell, through their control of extracellular proteolysis, and have been implicated in invasion and metastasis of tumor cells (Chambers et al., 1997; Werb et al., 1997; Curran et al., 1999; Stamenkovic et al., 2000). They have been well documented to have important functions in cancer invasion and metastasis mediated principally by their activities in degrading extracellular matrix and basement membrane (Deryugina et al., 2006). Recent studies also suggested that MMPs have other functions related to cancer development and progression, such as activating growth factors (Fridman et al., 2003; Yu et al., 2000). In the process of tumour dissemination and metastasis, matrix metalloproteinases (MMPs) play an important role in the invasion of tissue, vascular and lymphatic basal membranes and the subsequent coordinated proteolytic breakdown and reconstitution of extracellular matrix (Kohn and Liotta, 1995). Matrix metalloproteinases also modulate cell proliferation, apoptosis and host immune surveillance (Egeblad and Werb, 2002). Several lines of evidence indicate a significant association between variations in MMP genes and susceptibility to cancer.

Functional SNPs in MMP genes promoter regions may modify the production of proteolytic enzymes, and in turn modify the risk for melanoma progression (Cotignola et al., 2007). These proteolytic enzymes are present in normal healthy individuals and up-regulated in almost every type of human cancers. The study of genetic polymorphisms promises to help define

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pathophysiological mechanisms, to identify individuals at risk for disease and to suggest novel targets for drug treatment.

Polymorphisms have recently been demonstrated in the promoter region of some MMPs, and there is growing evidence to indicate that naturally occurring sequence variations in the promoters of MMP genes may result in differential expression of MMPs in different individuals (Ye, 2000). Hence this study was undertaken to evaluate MMP-1(-519) A>G, MMP-1 (-1607) 1G>2G, and MMP-7(-181) A>G gene polymorphisms in North Indian population which was then compared with different populations globally

Materials and Methods

Subjects

Healthy and genetically unrelated individuals either visiting the hospital for a routine checkup or health awareness camps or healthy 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. An epidemiologic questionnaire was designed for study participants to collect data on demographic characteristics, smoking history, occupation history, and other lifestyle factors were employed. At the end of the interview, a 5-ml blood sample was drawn into coded tubes.

Informed and written 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 Matrix metalloproteinase genes, MMP-1(-519) A>G, MMP-1(-1607) 1G>2G, and MMP-7(-181) A>G and reviewed adequate number of epidemiologic studies on MMPs to conduct a comparative analysis for genetic polymorphisms in proteinases genes, focusing on MMP-1 and 7.

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

All study samples were genotyped for three SNPs in three MMP genes that included MMP-1(-519) A>G, MMP-1(-1607) 1G>2G, and MMP-7(-181) A>G using PCR-RFLP (shown in Fig.1a, 1b and 2).

Prevalence of gene variants

We conducted a MEDLINE search using MMP-1(-519) A>G, MMP-1(-1607) 1G>2G, and MMP-7(-181) A>G and ''polymorphism'' for papers published before 2004. 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 3 publications reporting on the prevalence of MMP-1(-519) polymorphism, 6 publications on MMP-1(-1607), 6 studies on MMP-1(-181) which were subsequently used for comparing our study.

Statistical analysis

Pearson's $\chi 2$ 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 value <0.05 was considered statistically significant.

Results

The distribution of MMP-1(-519) A>G, MMP-1(-1607) 1G>2G, and MMP-7(-181) A>G genotypes and allele frequencies in northern Indian population are shown in Table 1.

The genotype distributions in DNA of healthy individuals were in agreement with Hardy–Weinberg equilibrium at all the selected 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) using _2 tests. The minor variant allele frequency in our population was as follows: 28.7% for MMP-1 (-519) A>G, 51.8% for MMP-1 (-1607) 1G>2G, 47% for MMP-7 (-181) A>G. In case of MMP-1 (-1607) 1G>2G significant frequency distribution were observed in Japan and China as compared to our population. Study of MMP-1 (-519) A>G polymorphism is not common worldwide, only few studies have been reported. Based on reports, significantly different pattern of MMP-1 (-519) A>G polymorphism and allele frequency was reported only in Caucasian. Genotype and allele distribution of MMP-7 (-181) A>G polymorphism was significantly different from China, Shanghai, Europe as compared to our population. Ethnicity taken in any case for comparison [Japan/China in MMP-1 (-1609), Europe in MMP-1 (-519), China (Shanghai), Europe in MMP-7 (-181)] showed significant differences.

Table 1. Genotypes and Allele Frequency Distribution of MMP-1 (-519), MMP-1 (-1607) and MMP-7 (-181) Gene Polymorphisms in North India

Gene	Genotype	Observed n (%)	Expected n (%)	Minor allele %	P-value (HWE)
MMP-1(-519)	AA	105 (52.5)	102 (50.8)	28.7	0.231
A/G	AG	75 (37.5)	82 (39.2)		
	GG	20 (10.0)	16 (10.0)		
MMP-1(-1607)	1G/1G	53 (26.5)	46 (23.3)	51.8	0.068
1G/2G	1G/2G	87 (43.5)	100 (49.9)		
rs1799750	2G/2G	60 (30.0)	54 (26.8)		
MMP-7(-181)	AA	73 (36.5)	74 (36.9)	39.3	0.809
A/G	AG	97 (48.5)	95 (47.7)		
rs11568818	GG	30 (15.0)	31 (15.4)		

Table 2. Genotypes and Allele Frequency Distribution of MMP-1 (-519), MMP-1 (-1607) and MMP-7 (-181) Gene Polymorphisms in Various Populations and P-values in Comparison to the North Indian Population

Country/ethnicity	n	Mean age ± SD	Genotype			p value	Variant	Reference
MMP-1 (-519)			AA	AG	GG		G	
North India	200	56.8±10.8	105 (52.5)	75 (37.5)	20 (10.0)	Ref	29	Present Study
Europe	100	48.7±11·3	37 (37.0)	38 (38.0)	25 (25.0)	0.001	44	Jurajda et al, 2002
Belgium	164	---------	78 (47.6)	70 (42.7)	16 (9.8)	0.591	31.1	Hlavaty et al, 2005
MMP-1 (-1607)			1G/1G	1G/2G	2G/2G		2G	
North India	200	56.8±10.8	53 (26.5)	87 (43.5)	60 (30.0)	Ref	52	Present Study
Japan	177	64.9±22.6	13 (7.3)	91 (51.5)	73 (41.2)	<0.001	67	Hirata et al, 2004
Brazil	118	59.9±12.5	32 (27.1)	59 (50.0)	27 (22.9)	0.356	48	Piccoli et al, 2007
US	555	64 (24–89)	110 (19.8)	288 (51.9)	157 (28.3)	0.072	54.2	Kader et al, 2006
China	151	49.3±7.9	25 (16.6)	50 (33.1)	76 (50.3)	<0.001	67	Yan, 2006
Turkey	94	58.7±9.9	29 (30.9)	43 (45.7)	22 (23.4)	0.471	46.3	Tasci, 2007
MMP-7 (-181)			AA	AG	GG		G	
North India	200	56.8±10.8	73 (36.5)	97 (48.5)	30 (15.0)	Ref	39.3	Present Study
North India	162		49 (30.0)	82 (50.6)	31 (19.4)	0.363	44	Hari Om et al, 2008
China	350	49.3 ±7.9	316 (90.3)	33 (9.4)	1 (0.3)	<0.001	5.0	Zhang et al, 2005
Shanghai	1,082	47.0 ±8.7	900 (83.2)	177 (16.4)	5 (0.5)	<0.001	8.6	Fadiel et al, 2008
Europe	169	35–91	46 (27.2)	106 (62.7)	17 (10.1)	0.023	41	Kubben et al, 2006
France	565	60.7±12.4	187 (33.1)	259 (45.8)	119 (21.1)	0.173	44	Lièvre et al, 2006

Discussion

SNPs scattered throughout the genome with high degree of variability make these informative genetic markers useful for disease susceptibility. Functional polymorphism of MMPs is thought to be of particular importance for implication in the pathogenesis of complex genetic disorders. Due to marked differences in the distribution of MMP 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.

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 (Kittles and Weiss, 2003). 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.

In the MMP-1 (-519) polymorphism, the (G*) allele frequency in Indian population was 29%, which was significantly higher in Europe whereas no significant difference was observed in Belgium subjects. The (2G*) allele frequency in MMP-1 (-1607) polymorphism was 52% in our population. This was significantly different and lower from Japan and China and no significant difference was observed from Brazil, US and Turky. In MMP-7 (-181) polymorphism, the (G*) allele frequency in Indian population was 39.3% which was significantly different and lower in China while significantly higher in Europe. No significant difference was observed in France. In a study from Europe and France by Kubben et al, 2006 and Lièvre1, 2006, the minor variant allele frequencies were found to be almost similar with our northern population for MMP-7 (-181) (39.3 vs. 41% and 44 respectively). This suggested that there was no variability in above two geographical zones compared to India.

Epidemiological investigations of MMPs polymorphisms are therefore important (Wacholder et al., 2004). Large numbers 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 MMP 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.

The advantage of such kind of study may form the basis for future establishment of epidemiological and clinical databases. The analyses suggest that MMP-1 and MMP-7 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 now warranted to further evaluate potential gene-to-gene and gene-to-environment interactions on MMP polymorphisms and cancer risk. The differences in the distribution of these genes between North Indian healthy population and other ethnic groups may help in building a profile that would facilitate assessing the disease predispositions in relation to prevalence.

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References

Beeghly-Fadiel A, Long JR, et al (2008). Common MMP-7 polymorphisms and breast cancer susceptibility: a multistage study of association and functionality. *Cancer Res*, **68**, 6453-9.

Chambers AF, Matrisian LM (1997). Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst*, **89**, 1260-70.

Cotignola J, Roy P, Patel A, et al (2007). Functional polymorphisms in the promoter regions of MMP2 and MMP3 are not associated with melanoma progression. *J Negat Results Biomed*, **6**, 9.

Curran S, Murray GI (1999). Matrix metalloproteinases in tumour invasion and metastasis. *J Pathol*, **189**, 300-8.

Deryugina EI, Quigley JP (2006). Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev*, **25**, 9-34.

Egeblad M, Werb Z (2002). New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer*, **2**, 161-74.

Fridman R, Toth M, Chvyrkova I, Meroueh SO, Mobashery S (2003). Cell surface association of matrix metalloproteinase-9 (gelatinase B). *Cancer Metastasis Rev*, **22**, 153-66.

Hirata H, Okayama N, Naito K, et al (2004). Association of a haplotype of matrix metalloproteinase(MMP)-1 and MMP-3 polymorphisms with renal cell carcinoma. *Carcinogenesis*, **25**, 2379-84.

HlavatiT, Pierik M, Henckaerts L, et al (2005). Polymorphisms in apoptosis genes predict response to infliximab therapy in luminal and fistulizing Crohn's disease. *Aliment Pharmacol Ther*, **22**, 613-26.

Hojilla CV, Mohammed FF, Khokha R (2003). Matrix metalloproteinases and their tissue inhibitors direct cell fate during cancer development. *Br J Cancer*, **89**, 1817-21.

Jurajda M, Muzík J, Izakovicová Hollá L, Vácha J (2002). A newly identified single nucleotide polymorphism in the promoter of the matrix metalloproteinase-1 gene. *Mol Cell Probes*, **16**, 63-6.

Kader AK, Shao L, Dinney CP, et al (2006). Matrix metalloproteinase polymorphisms and bladder cancer risk. *Cancer Res*, **66**, 11644-8.

Kittles RA, Weiss KM (2003). Race, ancestry, and genes: implications for defining disease risk. *Annu Rev Genomics Hum Genet*, **4**, 33-67.

Kohn EC, Liotta LA (1995). Molecular insights into cancer invasion: strategies for prevention and intervention. *Cancer Res*, **55**, 1856-62.

Kubben FJ, Sier CF, Meijer MJ, et al (2006). Clinical impact of MMP and TIMP gene polymorphisms in gastric cancer. *Br J Cancer*, **95**, 744-51.

Lièvre A, Milet J, Carayol J, Le Corre D, Milan C, et al (2006). Genetic polymorphisms of MMP1, MMP3 and MMP7 gene promoter and risk of colorectal adenoma. *BMC Cancer*, **6**, 270.

Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*, **16**, 1215.

Piccoli MF, Figueira M, Andreoni C, et al (2007). Lack of association between matrix metalloproteinase-1 (MMP-1) promoter polymorphism and risk of renal cell carcinoma. *Int Braz J Urol*, **33**, 622-9.

Singh H, Jain M, Mittal B (2008). MMP-7 (-181A>G) promoter polymorphisms and risk for cervical cancer. *Gynecol Oncol*, **110**, 71-5.

Stamenkovic I (2000). Matrix metalloproteinases in tumor invasion and metastasis. *Semin Cancer Biol*, **10**, 415-33.

Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N (2004). Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst*, **96**, 434-42.

Werb Z (1997). ECM and cell surface proteolysis: regulating cellular ecology. *Cell*, **91**, 439-42.

Ye S (2000). Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol*, **19**, 623-9.

Yu Q, Stamenkovic I (2000). Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev*, **14**, 163-76.

Zhang J, Jin X, Fang S, et al (2005). The functional polymorphism in the matrix metalloproteinase-7 promoter increases susceptibility to esophageal squamous cell carcinoma, gastric cardiac adenocarcinoma and non-small cell lung carcinoma. *Carcinogenesis*, **26**, 1748-53.