

The 5A/6A Polymorphism of the Matrix Metalloproteinase 3 Gene Promoter and Breast Cancer

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ABSTRACT

Purpose: The matrix metalloproteinase 3 (MMP3), also known as stromelysin-I, is a key-player for carcinogenesis and tumor growth. A 5A/6A promoter polymorphism is associated with differences in MMP3 activity and has been linked to cancer susceptibility in some studies. In the present study we evaluated the role of this polymorphism for breast cancer risk.

Experimental Design: A case-control study was performed including 500 patients with histologically confirmed breast cancer and 500 female, age-matched, healthy control subjects from population-based screening studies. The MMP3 5A/6A polymorphism was determined by a 5'-nuclease (TaqMan) assay.

Results: Prevalences of 5A/5A, 5A/6A, and 6A/6A genotypes were similar among patients (20.6, 51.8, and 27.6%, respectively) and controls (23.3, 47.3, and 29.4%, $P = 0.34$). The odds ratio of carriers of a MMP3 5A allele for breast cancer was 1.09 (95% confidence interval, 0.83–1.44). Patients with the 5A/5A genotype had a higher proportion of lymph-node metastases than those with a 5A/6A or 6A/6A genotype ($P = 0.010$).

Conclusions: The MMP3 5A/6A promoter polymorphism does not appear to influence breast cancer susceptibility but may be linked to a higher risk for metastasizing among breast cancer patients.

INTRODUCTION

Matrix metalloproteinases (MMPs) comprise a family of enzymes that are able to degrade components of the extracellular matrix. Degradation of the basement membrane and extracellular matrix is important for tumor progression and metastasis and MMPs are known to be key-players for carcinogenesis and tumor growth (1). MMP3, also known as stromelysin-I, is up-regulated in a variety of tumors and has been shown to influence tumor initiation and neoplastic risk (2).

A common adenine insertion/deletion polymorphism (5A/6A) at position –1171 of the MMP3 gene promoter (National Center for Biotechnology Information SNP identification no. rs3025039) influences transcription factor binding and MMP3 promoter activity. *In vitro* promoter activity as well as *in vivo* gene expression of the 5A variant is about 2–4-fold higher than that of the 6A allele (3, 4). An increased risk for breast cancer in carriers of a 5A allele has been reported (5, 6), but was not confirmed by a subsequent study (7).

Here we present data on the role of MMP3 5A/6A for breast cancer from a large Austrian case-control study.

MATERIALS AND METHODS

The study included 500 consecutive female patients with histologically confirmed breast cancers without synchronous and/or metachronous secondary malignancy and a population-based and age-matched control group of 500 healthy women. Characteristics of the study population have been described previously (8–11).

The study was performed according to the Austrian Gene Technology Act and the guidelines of the Ethical Committee of the Universitätsklinik Graz. Written informed consent was obtained from all of the participating subjects.

Genotyping was done by a 5'-nuclease assay (TaqMan). Primer and probe sets were designed and manufactured using Applied Biosystems "Assay-by-Design" custom service (Applied Biosystems, Austria). The PCR reaction was performed in a Primus 96 plus thermal cycler (MWG Biotech AG, Ebersberg, Germany) using a total volume of 5 μ l containing 2.5 μ l of SuperHot-Master-Mix (Bioron GmbH, Ludwigshafen, Germany), 0.125 μ l of Assay-by-design Mix (Applied Biosystems, Austria), 0.375 μ l of H₂O, and 2 μ l of DNA. Reactions were overlaid with 15 μ l of mineral oil. Cycling parameters were: 1 min at 94° for primary denaturation, followed by 45 cycles of 15 s at 92° and 1 min at 60°. Fluorescence was measured in a Lambda Fluoro 320 Plus plate reader (MWG Biotech AG) using excitation/emission filters of 485 nm/530 nm for FAM-labeled probes (5A-allele) and 530 nm/572 nm for VIC-labeled probes (6A-allele). The data were exported into Excel format and analyzed as scatter plot. As a quality control, 95 samples were reanalyzed, and results were identical for all samples.

Statistical analysis was performed using SPSS 11.0 for Windows. Numeric values (*e.g.*, age at diagnosis) were analyzed

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Table 1 MMP3 5A/6A genotype and allele frequencies of breast cancer patients and healthy controls

	Patients	Controls	P
5A/5A, n (%)	103 (20.6)	115 (23.3)	0.34
5A/6A, n (%)	259 (51.8)	233 (47.3)	
6A/6A, n (%)	138 (27.6)	145 (29.41)	
6A allele frequency, %	53.5	53.0	0.84

P was calculated by χ^2 test.

by ANOVA, proportions (e.g., genotype frequencies) of groups were compared by a χ^2 test. Odds ratio (OR) and 95% confidence interval (95% CI) was calculated to estimate the risk of the MMP3 genotype for breast cancer. OR of the MMP3 genotype for lymph node positivity was calculated including tumor size, tumor grade, and hormone receptor positivity as potential confounders. Threshold for significance was $P < 0.05$.

RESULTS

At the time of breast cancer diagnoses, patients were between 28 and 84 years of age, with a mean age of 57 ± 11 years. Controls were age-matched to the case subjects (± 1 year), the mean age was 57 ± 11 years with a range of 28–84 years. Mean time between diagnosis of breast cancer and study entrance was 73 ± 50 months.

The MMP3 genotype was successfully determined in all of the patients and 493 (98.6%) of the controls. Genotype distribution did not deviate from the Hardy Weinberg equilibrium in patients or controls. MMP3 genotype and allele frequencies were not significantly different between patients and controls (Table 1). The OR for breast cancer was 1.09 (95% CI, 0.83–1.44) for carriers of the high-activity MMP3 5A variant. This OR overlapped with previously reported ORs for this allele (Fig. 1).

The MMP3 genotype was furthermore not associated with tumor size, histological grading, estrogen or progesterone receptor status, or age at diagnosis (Table 2). Patients with the homozygous 5A/5A genotype had a higher proportion of lymph node metastases in the primary axillary dissection than those with a 5A/6A or 6A/6A genotype ($P = 0.010$). The OR of the homozygous 5A/5A genotype for breast cancer was 1.78 (95% CI, 1.14–2.76). This did not substantially change after adjust-

ment for tumor size, tumor grade, or receptor positivity (estrogen and/or progesterone receptor; OR, 1.78; 95% CI, 1.12–2.82).

DISCUSSION

In the present study, the functional MMP3 5A/6A promoter polymorphism was not associated with breast cancer. The MMP3 has been previously analyzed in breast cancer association studies in populations from Italy (6), Czech (7) and Sweden (7). ORs from these studies and the present one are presented in Fig. 1. The OR of pooled data were 1.1 (95% CI, 0.9–1.4), suggesting that the MMP3 polymorphism is at most a modest risk factor for breast cancer. Currently, determination of the MMP3 genotype is not a useful tool to estimate individual breast cancer risk.

This result seems to be contrary to the observation that MMP3 activity plays a pivotal role for tumor growth and development (2). Although the MMP3 promoter polymorphism has been shown to be functional, its effect on breast cancer risk may be too subtle to be detected in common case-control studies. It is furthermore possible that regulatory effects other than the 5A/6A promoter polymorphism may be more relevant during tumor growth. Although our data suggest that this polymorphism is not a major risk factor for breast cancer, our results do not question the role of MMP3 activity itself for carcinogenesis.

In a case-control study from Poland, the MMP3 5A/6A polymorphism was not associated with the presence or histological stage of ovarian cancer (12). Interestingly, in a study by Hinoda *et al.* (13), the low-activity MMP3 6A/6A genotype was found more frequently in patients with colorectal cancer than in controls. This unexpected result may have been due to an observed linkage disequilibrium between MMP3 and the adjacent MMP1 locus. Haplotype analysis of the MMP gene cluster on chromosome 11q22, which includes the MMP10, MMP1, MMP3, and MMP13 genes, will probably bring more insight into the complex relation between the MMP3 polymorphism and cancer risk (13, 14).

In the present study, homozygotes for the high-activity MMP3 5A/5A genotype had a significantly higher proportion of lymph node metastases than carriers of other genotypes. This is

Fig. 1 Results of previous studies and the present one on the MMP3 5A/6A gene polymorphism and breast cancer risk. ■ [diao2f], odds ratios for carriers of a MMP3 5A allele; horizontal lines, the 95% confidence interval. Odds ratios < 1 , decreased risk for breast cancer; odds ratios > 1 , increased risk for breast cancer.

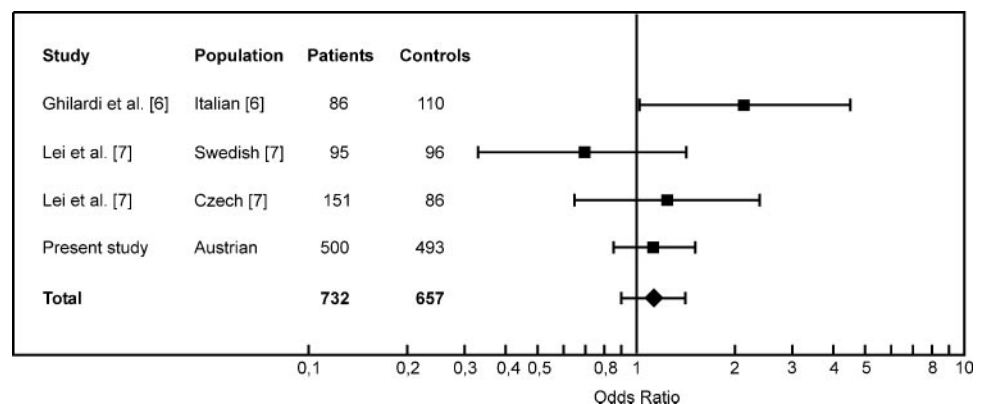


Table 2 MMP3 genotypes and tumor characteristics
Numbers are n (%) or mean \pm SD.

	5A/5A	5A/6A	6A/6A	P
Tumor size				
≤ 2 cm	55 (53.9)	132 (52.4)	66 (49.3)	0.75
> 2 cm	47 (46.1)	120 (47.6)	68 (50.7)	
Histological grade				
1 and 2	59 (57.3)	114 (46.4)	64 (48.8)	0.61
3 and 4	44 (42.7)	132 (53.7)	67 (51.1)	
Lymph node metastases				
Negative	43 (42.2)	146 (58.6)	70 (52.2)	0.018 ^a
Positive	59 (57.8)	103 (41.4)	54 (47.8)	
Estrogen receptor				
Negative	26 (25.7)	57 (22.5)	37 (28.0)	0.48
Positive	75 (74.3)	196 (77.5)	95 (72.0)	
Progesterone receptor				
Negative	38 (38.0)	77 (30.7)	54 (40.9)	0.11
Positive	62 (62.0)	174 (69.3)	78 (59.1)	
Age at diagnosis, years	57.6 \pm 10.4	56.9 \pm 11.4	55.3 \pm 10.5	0.23

^a P = 0.010 comparing 5A/5A homozygotes with carriers of a 6A allele (5A/6A+6A/6A).

in line with results of Ghilardi *et al.* (6), who reported an overrepresentation of the 5A/5A genotype in metastasized breast cancer patients compared with controls or patient without metastasis. Nevertheless, longitudinal studies are needed to confirm the role of the MMP3 polymorphism for metastasizing.

Limitations of our study are its retrospective case-control design, which could have led to a survival bias, and the fact that some classical breast cancer risk factors such as menarche or number of pregnancies were not retrieved from study probands.

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