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Common matrix metalloproteinase-2 gene variants and altered susceptibility to breast cancer and associated features in Tunisian women

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Abstract

A role for matrix metalloproteinase polymorphisms in breast cancer development and progression was proposed, but with inconclusive results. We assessed the relation of matrix metalloproteinase-2 variants with breast cancer and related phenotypes in Tunisians. This case-control retrospective study involved 430 women with breast cancer and 498 healthy controls. Genotyping of matrix metalloproteinase-2 rs243866, rs243865, rs243864, and rs2285053 was analyzed by allelic exclusion. The minor allele frequency of rs2285053 was significantly lower in women with breast cancer cases as compared to control women; minor allele frequencies of the remaining single-nucleotide polymorphisms were similar between cases and control women. The distribution of rs243865 and rs2285053 genotypes was significantly different between breast cancer patients and control subjects. This persisted when key covariates were controlled for. None of the matrix metalloproteinase-2 variants were associated with estrogen receptor positivity, progesterone receptor positivity, or with double estrogen receptor–progesterone receptor positivity in breast cancer patients. Matrix metalloproteinase-2 rs243866, rs243865, and rs243864 were positively associated with menstrual irregularity and histological type, while rs243866 and rs2285053 were negatively associated with menarche and nodal status. In addition, rs2285053 was negatively associated with triple negativity, tumor size, distance metastasis, molecular type, and chemotherapy. Haploview analysis revealed high linkage disequilibrium between matrix metalloproteinase-2 variants. Four-locus Haploview analysis identified haplotypes GCTT and GTTC to be negatively associated with breast cancer, which remained statistically after controlling for key covariates. Matrix metalloproteinase-2 alleles and genotypes, along with four-locus haplotypes, are related to reduced susceptibility to breast cancer in Tunisian women, suggesting a protective effect.

Keywords

Breast cancer, genotypes, haplotypes, matrix metalloproteinase-2

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Introduction

Breast cancer is a highly prevalent malignancy in females and a leading cause of cancer-related mortality in women worldwide (https://gco.iarc.fr/).[1] In Tunisia, the prevalence of breast cancer is estimated at 15.6% and is responsible for 8.4% of all cancer-related deaths among females (https://gco.iarc.fr/). Despite its national significance as a major health problem,[2] available screening programs for breast cancer in Tunisia remain inadequate.[2,3] Early screening of breast cancer is an important determinant of disease prognosis and in reducing breast cancer-related mortality.[4,5] Current screening of breast cancer relies on mammography, ultrasonography, and genetic screening.[6,7] However, these screening tests suffer from limited sensitivity,[8] risk of false-positive/negative results,[6] and limited or no availability in remote areas.[9,10]

Several studies evaluated new biomarkers of breast cancer.[7,11,12] Blood-based biomarkers[13,14] and genome-wide association[15,16] were explored for possible diagnostic and prognostic roles, and several loci linked to the pathogenesis of breast cancer were identified.[15,17] These included matrix metalloproteinase (*MMP*) gene variants, an enzyme family which controls tumor invasion and metastasis.[18] Particular attention was given to the gelatinase family, in particular, matrix metalloproteinase-2 (*MMP2*), which act by degrading collagen type IV. As it is an extracellular matrix (ECM) component,[19] this results in the loss of cellular structural support and thus destabilization of the basal membrane, an essential step for the spread of cancer.[19,20]

Altered *MMP2* activity resulting from the presence of specific *MMP2* variants was implicated in the destruction of ECM[21] and disruption of basement membrane barriers.[18,22] Insofar as *MMP2* is overexpressed in breast cancer,[20–23] genetic variation in *MMP2* gene affecting its expression was shown to contribute to cancer susceptibility.[24–26] Few studies evaluated the (likely) link between *MMP2* promoter gene variants with altered breast cancer susceptibility and were reported for different populations, but with mixed outcome.[25–29] A recent Tunisian study involving 251 breast cancer cases documented a protective effect of rs243865 (promoter) variant in breast cancer development.[30] Future studies involving larger number of subjects, and testing additional variants, are needed to confirm a role for *MMP2* as at-risk breast cancer locus.

Several studies proposed that genetic polymorphism in *MMP2* promoter region control *MMP2* expression[23,31] and that sustained increased *MMP2* levels might render the carriers more susceptible and aggressive to tumorigenesis. In this context, we examined the relationship between breast cancer susceptibility and the presence of *MMP2* (promoter) variants: rs243864 (−790 G/T), rs243865 (−1306 C/T), rs243866 (−1575 G/A), and rs2285053 (−735 C/T) as potential risk biomarkers of breast cancer.

Subjects and methods

Study subjects. A case-control retrospective study involved 430 women with breast cancer (age: 45.6 ± 9.3 years) and 498 cancer-free women serving as control (age: 46.8 ± 11.1 years). Cases and controls were recruited from the outpatient surgery and oncology services of Salah Azaiez Institute (Tunis, Tunisia) between June 2018 and October 2018. Control women, who comprised hospital staff or volunteer women, were free of personal or family history of breast cancer and were similar in self-declared ethnic origin to patients. Diagnosis of breast cancer was consistent with the guidelines of the American Cancer Society (www.cancer.org). This included mammography and testing of breast biopsies for confirmation of breast cancer; these were for done all patients.

None of the subjects (cases and controls) have unrelated comorbidities. Demographic profile and clinical biodata were collected for patients from medical records and interviews using a structured questionnaire by physicians or senior resident. These comprised age at entry into the study, age at first diagnosis of breast cancer, status of menopause, and disease stage at presentation. Histological assessment included stage of disease and nuclear grade, estrogen receptor (ER) and progesterone receptor (PR) status, along with treatment regimen (chemotherapy, surgery, and radiation). The study was done per Helsinki II declaration, and approval was obtained from the Research and Ethics Committee of Salah Azaiez Institute (IRB number: ISA/2018/19); all patients and control subjects provided written informed consent.

MMP2 genotyping. Four single-nucleotide polymorphisms (SNPs) in *MMP2* gene, having minor allele frequency (MAF) >10% in Tunisians and clinical relevance, were identified using National Center for Biotechnology Information (NCBI) Gene SNP Geneview (www.ncbi.nlm.nih.gov/projects/SNP/). These comprised rs243866 (context sequence [VIC/FAM]: TAG CTG TGA TGA TCA AGA CAT AAT C[A/G] TGA CCT CCA ATG CCC CCC ACA AGT A), rs243865 (context sequence [VIC/FAM] TCC CCA TAT TCC CCA CCC AGC ACT C[C/T] ACC TCT TTA GCT CTT CAG GTC TCA G), rs243864 (context sequence [VIC/FAM] CAG TGG GGT CTT TGT

Table 1. Characteristics of study subjects.

	Cases (430)	Controls (498)	p[a]
Age (yr)[b]	45.6 ± 9.3	46.8 ± 11.1	0.066
BMI (kg/m)[b]	28.5 ± 4.8	27.1 ± 5.0	<0.001
Obesity (BMI > 30 kg/m^2)[c]	133 (30.9)	157 (31.9)	0.776
Menarche (yr)[b]	12.5 ± 1.4	12.2 ± 1.1	0.021
Smokers[c]	26 (6.0)	14 (2.8)	0.022
Breastfeeding[c]	310 (72.1)	449 (90.2)	1.1×10^{-11}
Menstrual history[c]			
Regular	266 (61.9)	364 (73.1)	3.2×10^{-4}
Irregular	164 (38.1)	134 (26.9)	
Menopausal status[c]			
Pre-menopausal	220 (51.2)	264 (53.0)	0.598
Post-menopausal	210 (48.8)	234 (47.0)	
Oral contraception users[c]	128 (29.8)	74 (14.9)	4.8×10^{-7}
Triple negative[c]	102 (23.7)	N/A	N/A
ER positive[c]	291 (67.7)	N/A	N/A
PR positive[c]	224 (52.1)	N/A	N/A
HER-2 positive[c]	117 (27.2)	N/A	N/A
ER positive/HER-2 negative[c]	211 (49.1)	N/A	N/A

BMI: body mass index; ER: *estrogen receptor*; PR: progesterone *receptor; HER-2*: human epidermal growth factor receptor 2.
[a]Student's *t*-test (continuous variables) and Pearson's χ^2 (categorical variables).
[b]Mean ± SD.
[c]Number of subjects (percent total).

GAC CTC TAT C[G/T]T ATT AAA CCA GTC TTG CCC AAT TTC), and rs2285053 (context sequence [VIC/FAM] TCA TCC TGT GAC CGA GAA TGC GGA C[C/T]C TCC TGG GAG TGC AGC CCA GCA GGT). *MMP2* genotyping was done by the allelic discrimination method, using (VIC- and FAM-labeled) assay-on-demand TaqMan assays, which were supplied by Applied Biosystems (Dubai, UAE). Polymerase chain reaction (PCR) was performed with a volume of 6 μL in StepOnePlus system (Applied Biosystems). Genotyping reproducibility was verified by inclusion of replicate blinded samples; concordance was >99%. The average successful genotyping rate for each sample and SNP exceeded 98.9%.

Statistical analysis. Statistical analysis was done on SPSS 24 (IBM, Armonk, NY, USA). Mean (± SD) and percent total were used in presenting continuous and categorical data, respectively. Means differences and inter-group significances were evaluated using Student's *t*-test and Pearson χ^2 test, respectively. Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/cgi-bin/cc2k.cgi) was employed for calculating the study power, considering the number of study subjects (430 patients and 498 controls), MAF of the included variants, breast cancer prevalence in Tunisia (estimated), and relative risk for heterozygous (1/2) and minor allele homozygous (2/2) genotypes. The overall power (87.3%) was calculated as the average of included SNPs. Haploview 4.2 (www.broad.mit.edu/mpg/haploview) was used for Hardy–Weinberg equilibrium (HWE) calculation. Allele frequencies established in this study were compared frequencies reported for Caucasians (CEU), African Americans of the American Southwest (ASW), and Yoruban in Ibadan, Nigeria (YRI) in HapMap release #28.

Analyses were done assuming additive genetic effect. Linkage disequilibrium (LD) between any pair of SNPs and haplotype patterns (done by the expectation maximization method) were checked by Haploview 4.2. Among the 16 theoretical *MMP2* haplotypes, 4 were found to be common with frequency exceeding 2%, capturing 97.3% of all haplotypes. Calculation of odds ratios (ORs) and 95% confidence intervals (95% CIs) associated with the risk of breast cancer was determined using logistic regression analysis; statistical significance was set at $p < 0.05$.

Results

Study subjects

Demographic and clinical characteristics of study participants are listed in Table 1. Significant differences between breast cancer patients and control women were noted in body mass index (BMI; $p < 0.001$), menarche ($p = 0.021$), and number of smokers ($p = 0.022$). Higher frequency of women with irregular menses ($p < 0.001$), past users of oral contraceptives ($p < 0.001$), and lower frequency of breastfeeding women ($p < 0.001$) were noted in patients than in control women. As such, these covariates were selected as the main covariates that were controlled for later analysis.

Table 2. Distribution of *MMP2* alleles in breast cancer cases and control women.

SNP[a]	Position[b]	Alleles[c]	HWE	Cases[d]	Controls[d]	χ^2	*p*	OR (95% CI)[e]
rs243866	55477625	G > T	0.95	123 (0.17)	136 (0.14)	2.45	0.12	1.24 (0.95–1.61)
rs243865	55477894	C > T	0.23	150 (0.18)	188 (0.19)	0.43	0.51	0.92 (0.73–1.17)
rs243864	55478410	T > G	0.11	148 (0.17)	144 (0.15)	2.85	0.09	1.24 (0.97–1.59)
rs2285053	55478465	C > T	0.43	111 (0.14)	213 (0.21)	17.51	2.9×10^{-5}	0.59 (0.46–0.75)

MMP2: matrix metalloproteinase-2; SNP: single-nucleotide polymorphism; HWE: Hardy–Weinberg equilibrium; OR: odds ratio.
[a]SNP genotyping was done by allelic exclusion method, using VIC- and FAM-labeled primers.
[b]Location on chromosome based on dbSNP build 125.
[c]Major allele > minor allele.
[d]Minor allele number (frequency).
[e]Crude (unadjusted) OR.

Table 3. Association of *MMP2* genotypes with breast cancer.

SNP	1/1[a]		1/2[a]		2/2[a]		χ^2	*ap*[c]
	Cases[b]	Controls[b]	Cases	Controls	Cases	Controls		
rs243865	291 (68.1)	350 (70.3)	122 (28.6)	108 (21.7)	14 (3.3)	40 (8.0)	13.43	0.001
rs243864	297 (69.6)	366 (73.5)	112 (26.2)	120 (24.1)	18 (4.2)	12 (2.4)	3.23	0.199
rs243866	251 (69.1)	352 (73.3)	101 (27.8)	120 (25.0)	11 (3.0)	8 (1.7)	2.84	0.242
rs2285053	303 (75.2)	307 (61.6)	89 (22.1)	169 (33.9)	11 (2.7)	22 (4.4)	18.69	8.7×10^{-5}

MMP2: matrix metalloproteinase-2; SNP: single-nucleotide polymorphism; BMI: body mass index.
[a]Genotypes were coded as per "1" = major allele and "2" = minor allele.
[b]Number of subjects (percent total).
[c]*p* values adjusted for age, BMI, menses pattern and menarche, breastfeeding, smoking, and use of oral contraceptives.

Association studies

The genotype distribution of the investigated *MMP2* SNP was in HWE in study subjects (Table 2). MAFs of the tested *MMP2* SNPs in breast cancer patients and control women are presented in Table 2. Compared to control women, MAF of rs2285053 was lower in breast cancer patients ($p < 0.001$); MAF of the other tested *MMP2* SNPs was comparable between breast cancer cases and controls.

The distribution of *MMP2* genotypes in breast cancer cases and control women is shown in Table 3. The distribution of rs243865 ($p = 0.001$) and rs2285053 ($p < 0.001$) genotypes was significantly different between breast cancer patients when compared to control women. The association of *MMP2* rs243865 ($p < 0.001$) and rs2285053 ($p < 0.001$) with breast cancer remained significant after controlling for the covariates age, BMI, menses pattern and menarche, breastfeeding, smoking, and use of oral contraceptives.

Association of MMP2 genotypes with breast cancer features

We tested the possible association of *MMP2* genotypes with breast cancer according to ER and PR status. Of the included breast cancer patients, 291 (67.7%) were ER positive and 224 (52.1%) were PR positive. Data from Table 4 demonstrate that none of the tested *MMP2* variants were associated with ER positivity or PR positivity in breast cancer patients. Furthermore, none of the tested *MMP2* variants were associated with double ER–PR positivity in breast cancer patients.

We investigated the possible association of the tested *MMP2* variants with breast cancer features. Results from Table 5 show that rs243866, rs243865, and rs243864 were positively associated with menstrual irregularity and histological type, while rs243866 and rs2285053 were negatively associated with menarche and nodal status. In addition, rs2285053 was negatively associated with triple negativity ($p = 0.014$), tumor size ($p = 0.002$), distance metastasis ($p = 0.001$), molecular type ($p = 0.024$), and outcome of chemotherapy ($p < 0.001$).

Haploview analysis

High LD was noted between *MMP2* variants (Figure 1), and four-locus haplotypes were determined based on the MAF of individual variants and LD pattern between them. Of the potential 16 haplotypes, 4 were assigned as common (>2% of total), capturing 98.6% (patients) and 96.3% (controls) of all haplotypes.

Table 4. Association of *MMP2* variants with risk of breast cancer according to ER and PR status.

	ER positive[a]			PR positive[a]		
	p^b	1/2[c]	2/2[c]	p^b	1/2[c]	2/2[c]
rs243866	0.41	0.44 (0.09–2.14)	0.39 (0.08–1.85)	0.52	0.50 (0.14–1.81)	0.58 (0.17–2.02)
rs243865	0.96	1.15 (0.37–3.51)	1.18 (0.37–3.76)	0.83	0.97 (0.32–2.92)	1.12 (0.39–3.29)
rs243864	0.83	1.29 (0.46–3.60)	1.35 (0.51–3.60)	0.79	1.12 (0.43–2.90)	0.97 (0.36–2.61)
rs2285053	0.15	0.61 (0.16–2.35)	1.71 (0.41–7.17)	0.76	1.13 (0.34–3.78)	1.94 (0.55–6.85)

MMP2: matrix metalloproteinase-2; ER: *estrogen receptor*; PR: progesterone *receptor;* ANOVA: *analysis of variance.*
[a]ER and PR positivity were determined by immunocytochemistry of formalin-fixed and paraffin-embedded breast tissue sections; specimens were considered positive for ER and PR if $\geqslant$ 1% of tumor cells showed a positive nuclear staining.
[b]Two-way ANOVA.
[c]Alleles were designated as "1" (major allele) and "2" (minor allele).

Table 5. Matrix of correlation between *MMP2* variants and breast cancer features and outcome.

	rs243866		rs243865		rs243864		rs2285053	
Parameter	ρ^a	p	ρ	p	ρ	p	ρ	p
Menstrual irregularity	**0.139**	**0.008**	**0.136**	**0.005**	**0.117**	**0.015**	0.020	0.682
Menarche (yr)	**−0.113**	**0.031**	−0.048	0.320	−0.036	0.461	**−0.200**	**5.4×10^{-5}**
Triple negative	0.005	0.927	0.050	0.304	0.070	0.149	**−0.123**	**0.014**
Histological type (ductal/lobular/mixed)	**0.169**	**0.001**	**0.148**	**0.002**	**0.153**	**0.001**	0.083	0.097
Tumor size	0.036	0.496	0.084	0.082	0.068	0.161	**−0.155**	**0.002**
Distant metastasis	−0.001	0.991	0.012	0.798	0.003	0.946	**−0.166**	**0.001**
Nodal status (N0, N1, N2)	**−0.114**	**0.030**	−0.039	0.422	−0.051	0.292	**−0.161**	**0.001**
Molecular type (HR (+ /–)/HER-2 (+ /–))	−0.018	0.729	−0.009	0.860	0.026	0.588	**−0.112**	**0.024**
Chemotherapy	0.013	0.800	0.058	0.237	0.056	0.253	**−0.194**	**9.1×10^{-5}**

MMP2: matrix metalloproteinase-2; *HER-2*: human epidermal growth factor receptor 2.
Bold values indicate statistically significant differences.
[a]Spearman's correlation coefficient.

Haploview analysis identified haplotypes GCTT ($p = 2.4 \times 10^{-3}$) and GTTC ($p = 4.9 \times 10^{-6}$) to be negatively associated with breast cancer. This association of GCTT ($ap = 1.4 \times 10^{-3}$) and GTTC ($ap = 1.1 \times 10^{-4}$) with breast cancer remained statistically after controlling for key covariates (Table 6).

Discussion

Breast cancer is the leading cause of cancer-related deaths worldwide. A number of studies have demonstrated increased expression of *MMP2* in breast cancer,[21,32,33] and genetic functional variants in *MMP2* gene were associated with altered breast cancer susceptibility.[26,27,29,34] In this regard, a link between *MMP2* polymorphisms and the risk of breast cancer was reported for several populations.[26,27,30,35] Our study confirmed the association of rs243865 and rs2285053 *MMP2* SNP with the presence and the aggressiveness of breast cancer.

Located on chromosome 16, *MMP2* gene contains several polymorphisms, of which (the promoter) rs243865 variant is linked with lower promoter activity, owing to disruption of Sp1-type promoter site (CCACC box). Results shown here revealed a negative association of rs243865 (–1306 C/T) polymorphism with breast cancer, in agreement with a recent Tunisian study involving 210 breast cancer patients and 250 healthy control women, which also documented negative association of the minor (T) allele of rs243865 with breast cancer.[30] Our results were in agreement with Mexican[36] and Chinese[34] studies, which also documented negative association of rs243865 with the risk of breast cancer. Our findings were in apparent disagreement with Swedish[35] and Brazilian[25] studies, which suggested no association between rs243865 and the risk of breast cancer. Functionally, the rs243865 minor (T) allele was associated with disruption of the binding of SP1 binding elements, resulting in decreased promoter activity.[26,27,37]

Similarly, *MMP2* rs2285053 (−735 C/T) was negatively associated with breast cancer, as its minor [T] allele was enriched in breast cancer patients. This was in sharp contrast to Chinese[27] and Iranian[29] studies,

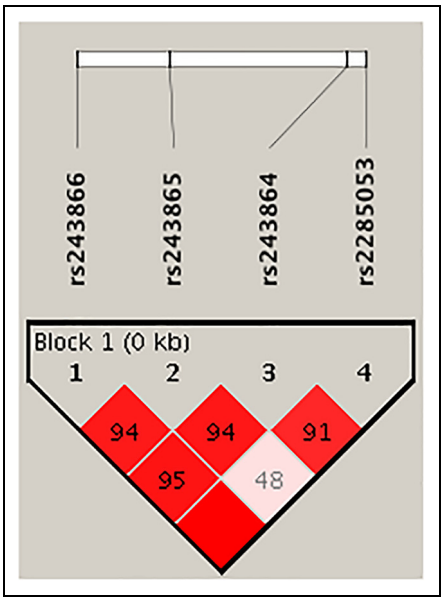

Figure 1. Haploview analysis of the typed *MMP2* variants.
The nucleotide positions of the included *SHBG* variants are shown above Haploview output; the color scheme denotes LD between pairs of *MMP2* variants; LD calculation was based on D values (D divided by the theoretical maximum for the observed allele frequencies) multiplied by 100; absence of LD is expressed as values near zero, while values close to 100 suggest complete LD; the colored red square represents varying degrees of LD (<1) and logarithm of odds (LOD; >2) scores; darker shades represent stronger LD.

which demonstrated that the major [C] allele was positively associated with increased risk of breast cancer. These discrepancies are explained by ethnic differences, selection of cases, and the statistical methods in assessing the association. By disrupting Sp1 binding site,[37] the C-to-T transition at position −735 and −1306 in *MMP2* promoter region induces low *MMP2* enzyme expression in stromal and neoplastic cells.[31] This explains, in part, the beneficial effect of lower expression of T allele in reducing excessive degradation of fibrillation collagen[29] and other ECM components and thus development of breast cancer.[34,38]

Neither rs243864 (–790 G/A) nor rs243866 (–1575 G/A) *MMP2* variants was associated with breast cancer in Tunisians, as MAF distribution of both variants was comparable between breast cancer cases and control women. A lone Chinese study which investigated the association of 9 promoter (including the 4 tested variants in this study), 19 intron, 1 exon (exon 9), and 7 3' flanking region (FR) *MMP2* variants with breast cancer in 6066 Chinese women also showed lack of association of rs243864 (–790 G/A) and rs243866 (–1575 G/A) *MMP2* with altered susceptibility to breast cancer.[27] *MMP2* is an estrogenic responsive gene,[39] and many genetic variants of *MMP2* (rs243865 and rs243866) are located within an operational ER binding site and reduce the transcriptional response to estrogen. Accordingly, ER-negative tumors, by expressing low *MMP2* levels compared to ER-positive tumors, are associated with reduced transcriptional responsiveness to estrogen.[33] Future studies involving additional ethnic groups and larger sample size are needed to confirm, or alternatively rule out, any association of rs243864 or rs243866 with altered breast cancer susceptibility.

Overexpression of *MMP2* was shown to be related to invasiveness and metastasis of certain cancers,[18,19] including breast cancer.[20,23,24,39] In this study, rs2285053 was negatively correlated with triple negative tumors, tumor size, distant metastasis, and lymph nodes. While not tested here, we speculate that *MMP2* rs2285053 (–735 C/T) pro-cancer capacity resides in its ability to modulate the degradation of the ECM, as was suggested elsewhere.[31] Furthermore, carriage of *MMP2* rs243865 and rs2208553 minor alleles in ER-negative tumors is expected to express low *MMP2* activity. Our results are in disagreement with earlier reports, which demonstrated that both *MMP2* variants are correlated with ER negativity and/or PR negativity.[30,33] To the best of our knowledge, this is the study to analyze the association of rs243864 (–790 G/T) and rs243866 (–1575 G/A) with altered risk of breast cancer. As such, this does not provide for comparison of our results with others.

This study has several strengths, in particular, the relatively large sample size, and thus was sufficiently powered. Additional strengths are in the ethnicity of

Table 6. *MMP2* four-locus haplotypes in breast cancer cases and control women.

Haplotype[a]	Total[b]	Cases[b]	Controls[b]	χ^2	p	aOR (95% CI)[c]
G C T C	0.636	0.673	0.605	9.11	2.5×10^{-3}	1.00 (reference)
G C T T	0.169	0.141	0.194	9.12	2.4×10^{-3}	0.49 (0.25–0.94)
T T G C	0.148	0.168	0.130	5.06	0.024	1.35 (0.71–2.56)
G T T C	0.020	0.004	0.034	20.87	4.9×10^{-6}	0.39 (0.19–0.81)

MMP2: matrix metalloproteinase-2; aOR: adjusted odds ratio; BMI: body mass index.
[a]four-locus *MMP2* haplotype consists of 243866, rs243865, rs243864, and rs2285053 alleles; minor alleles are designated as underlined.
[b]Haplotype frequencies.
[c]Covariates that adjusted for were age, BMI, menses pattern and menarche, breastfeeding, smoking, and use of oral contraceptives.

the studied population (only Tunisian Arabic-speaking cases and controls) and that it investigated the correlation of *MMP2* variants with phenotypic aspects of breast cancer. This study had some limitations, which limited the interpretation of the results. It involved a limited number of *MMP2* variants, thereby prompting the speculation of additional variants acting as at-risk loci for breast cancer. The retrospective case-control design was another shortcoming, as it failed to address the cause–effect relationship between carriage of the *MMP2* variants and the phenotypic presentation of breast cancer and that *MMP2* levels as determined by the presence of *MMP2* variants were not performed. Additional larger designed studies are needed to better evaluate the effect of these and other likely *MMP2* variants on breast cancer risk.

Conclusion

At the genetic level, *MMP2* constitutes an at-risk locus of risk of breast cancer, and *MMP2* gene variants rs2285053 (–735 C/T) and rs243865 (–1306 C/T) are linked with altered risk of breast cancer development and progression. Of these, rs2285053 (–735 C/T) appears to influence some breast cancer phenotypic features, including metastasis, lymph node involvement, and tumor size. This suggests that analysis of *MMP2* variants may constitute potential biomarkers for breast cancer susceptibility and for early identification of individuals at high risk to develop breast cancer.

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Author contributions

A.F.H. and R.M.G. contributed to the data entry and drafting of the manuscript. H.B. and A.D. contributed to patient screening, selection, and referral. M.H.-A. contributed to literature search and data analysis. A.M., S.Z., M.H., and K.R. contributed to patient screening, selection, and referral. B.Y.-L. contributed to data analysis. W.Y.A. was the project leader and contributed to the statistical analysis.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The study was done according to Helsinki II declaration and approved by the Research and Ethics Committee of Salah Azaiez Institute (IRB number: ISA/2018/19); all participants provided written informed consent.

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