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ANATOMICAL PATHOLOGY

MMP-9-1562 C/T single nucleotide polymorphism associates with increased MMP-9 level and activity during papillary thyroid carcinoma progression

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Summary

Papillary thyroid carcinoma (PTC), a common form of thyroid malignancy, displays significant variations in clinical features and outcome. The malignant transformation of the thyroid is driven by altered expression of many matrix-modulating enzymes, including matrix metalloproteinase-9 (MMP-9). A single nucleotide polymorphism in its promotor (-1562 C/T) is suspected to cause over-expression of MMP-9, which in turn contributes to development of a tumour unfavourable phenotype. The aim of this study was to investigate the impact of *MMP-9* promotor genotype on MMP-9 expression in PTC samples, and to assess its value as a possible risk factor for developing PTC or its aggressive phenotype. A total of 105 PTC patients and 43 healthy controls were genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. In order to estimate MMP-9 expression, PTC tissue sections were stained immunohistochemically. Statistical analysis showed that PTC cases and controls did not differ significantly in genotype frequencies (OR = 2.27, CI = 0.854–6.022). In PTC samples, the presence of the T allele was accompanied by elevated MMP-9 expression ($p = 0.047$) as well as a higher risk of developing extrathyroid extensions ($p = 0.037$) and high TNM stages ($p = 0.009$). Moreover, we observed overexpression of MMP-9 in cases presenting with extrathyroid invasion ($p = 0.001$), lymph node metastasis ($p = 0.028$), large tumour size ($p = 0.031$) and advanced stage ($p = 0.005$) compared to indolent tumours, along with enhanced enzymatic activity demonstrated by *in situ* zymography. Data suggests that *MMP-9* (-1562 C/T) does not facilitate predisposition for PTC but affects the disease course by modulating MMP-9 expression. Genotyping *MMP-9* provides important information which may prove beneficial in risk stratification of PTC patients.

Key words: MMP-9; papillary thyroid carcinoma; single nucleotide polymorphism; carcinoma progression.

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INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of extracellular enzymes participating in extracellular matrix turnover. They are expressed at low levels under physiological conditions, and play important roles in tissue maintenance, wound healing, growth and development.[1] However, their inappropriate expression has been related to a number of pathological conditions, including the formation and progression of epithelially derived tumours.[2] Many steps of tumour progression require remodelling of the extracellular matrix, including cancer cell growth, epithelial to mesenchymal transition, dedifferentiation, migration, invasion and metastasis.[3] Next to sheer degraders of extracellular matrix, MMPs are currently regarded as general molecular switches in the microenvironment.[3] For instance, MMPs function as major regulators of tumour growth by catalysing the release or activation of growth factors, activation or shedding of membrane receptors, or cleavage of matrix/membrane-bound substrates involved in cell proliferation.[4,5]

A member of the matrix metalloproteinase family, MMP-9 degrades collagen IV, a main constituent of basement membranes, and thus plays an important role in the metastatic potential of cancer cells. Not surprisingly, it is reported to be overexpressed in a wide range of tumours, including oral, oesophageal, breast, renal, colorectal carcinomas and melanomas.[6–11] It is expressed not only by malignant cells but also by elements of the surrounding stroma, like endothelial cells, fibroblasts and lymphocytes.[12] A transgenic mouse study emphasised the role of MMP-9 in tumour development by showing that lack of MMP-9 decreases the incidence of invasive tumours, whilst transplanting MMP-9 expressing bone marrow cells to MMP-9 null mice restores the development of cancer.[13]

MMP-9 is synthesised as an inactive zymogen, and is extracellularly activated upon removal of the propeptide. Being an extremely potent enzyme, the action of MMP-9 is regulated at multiple points, most importantly at levels of transcription.[14] Therefore, functional polymorphisms in *MMPs* located in promoter regions may influence expression of these proteins and thus contribute to individual differences in cancer susceptibility and prognosis.[15]

One of these polymorphisms, a common substitution of cytosine with thymine at position -1562 (rs3918242) has been shown to affect gene transcription as well as MMP-9 protein levels. The study of Zhang *et al.* In 1999 showed that presence of the T allele at this position increased promotor activity by 1.5-fold compared to the C allele. They

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suggested that the T allele impairs binding of a putative transcription repressor resulting in increased gene transcription.[16] Another study from 2015 associated C-1562T with increased MMP-9 levels.[17] However, the association between *MMP-9* (-1562T) and cancer risk remains controversial due to conflicting results from various case-control studies.[10,18–20]

Papillary thyroid carcinoma (PTC) accounts for 80% of thyroid malignancies and is characterised by slow growth and an excellent prognosis. However, 10–15% of cases exhibit aggressive behaviour with hallmarks of local invasion, distant metastasis, treatment resistance and mortality.[21]

Regarding patient stratification and prediction, the clinician is still faced with considerable problems. Prognostic markers, competent for identifying high-risk patients in a timely manner are still largely lacking. The present study investigates the possibility that the polymorphism at position -1562 in the promotor of *MMP-9* could be one such factor. We hypothesised that the genetic C/T change could result in higher MMP-9 expression in PTC and indirectly lead to development of an invasive phenotype. In this case-control study, we aimed to link genetic alteration in the *MMP-9* promotor with modification of the MMP-9 expression profile, and consequently, with unfavourable behaviour of the carcinoma.

MATERIALS AND METHODS

Participant selection and clinical data

This study recruited a total of 148 subjects: 105 papillary thyroid carcinoma patients and 43 controls. Archival PTC samples were obtained from patients who had undergone thyroidectomy at the Centre for Endocrine Surgery, Clinical Centre of Serbia, Belgrade, between 2000 and 2015. The control group comprised systematically healthy individuals whose blood was sampled for purposes of clinical blood count and hormonal analysis at the Institute for the Application of Nuclear Energy, University of Belgrade. The inclusion criteria for the control group were normal ranges of thyroid hormones. The exclusion criterion was a history of thyroid disease.

For preparation of archival material, two different fixation methods were used prior to paraffin embedding. Formalin fixation was employed on tissue specimens later subjected to immunohistochemistry and DNA isolation, while alcohol fixation was utilised for the purpose of *in situ* zymography.

All specimens were verified by a pathologist to confirm the diagnosis of PTC according to the World Health Organization classification of thyroid malignancy.[22] The PTC cohort included conventional types of PTC (classical and classical with follicular areas). Carcinomas were staged in accordance with the American Joint Committee on Cancer tumour nodal metastasis system.[23]All subjects provided informed consent to participate in the study and to allow their biological samples to be analysed.

The study was approved by the Ethics Committee at the Centre for Endocrine Surgery, Clinical Centre of Serbia, Belgrade.

DNA isolation

Genomic DNA of control subjects was isolated from whole blood with TRIzol Reagent (Ambion, USA). Four mL of venous blood was placed in a 15 mL falcon tube together with 10 mL of erythrocyte lysis buffer (0.01 M Tris-HCL pH 7.4, 320 mM sucrose, 5 mM $MgCl_2$, 1% Triton X-100) and positioned on a rotating wheel for 10 min at room temperature (RT). The falcons were then centrifuged at 3000 *g* for 10 min at RT and the supernatant discarded. The pellets were washed three times in PBS and overlaid with 400 µL of TRIzol Reagent. Isolation DNA was carried out using the manufacturer's protocol.

DNA of PTC patients was isolated from formalin fixed, paraffin embedded sections using the Recover All Total Nucleic Acid Isolation kit (Ambion, USA) according to the manufacturer's instructions.

Polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) analysis of rs3918242

Subjects were genotyped by PCR-RFLP as previously described.[16] The following primers were used: 5′-GCC TGG CAC ATA GTA GGC CC-3′ (forward primer) and 5′-CTT CCT AGC CAG CCG GCA TC-3′ (reverse primer). PCR was carried out in a total volume of 25 µL with the following cycles: initial denaturation at 95°C for 3 min; 35 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s, with final elongation at 72°C for 7 min. The 435 bp PCR products were digested with Pael restriction enzyme (Thermo Fisher Scientific, USA) at 37°C overnight, producing fragments of 247 bp and 188 bp in the case of a T allele, or an undigested 435 bp band in the case of a C allele. Fragments were resolved on 2% agarose gel, stained with SYBR Safe (Thermo Fisher Scientific) and observed under ultra-violet light. The CC genotype showed one band of 435 bp, while TT gave two bands of 247 bp and 188 bp and CT exhibited three bands (435 bp, 247 bp and 188 bp).

Immunohistochemistry

The immunohistochemical study included 103 formalin fixed tissue slides. Briefly, the sections were deparaffinised in xylene, rehydrated in ethanol, and washed three times with PBS buffer. Endogenous peroxidases were blocked by hydrogen peroxidase treatment for 30 min. The samples were washed with PBS buffer and incubated in normal horse serum for 20 min to prevent non-specific antigen binding. The sections were incubated with diluted primary antibody against MMP-9 (mouse monoclonal MA5-14228; Invitrogen, USA), overnight at 4°C. The signal was enhanced with the avidin-biotin-peroxidase complex (Vectastain ABC kit; Vector Laboratories, USA), followed by visualisation of the reaction with 3,3′-diaminobenzidine-tetrahydrochloride (DAB) solution (Peroxidase Substrate Kit; Vector Laboratories). The slides were counterstained with haematoxylin, dehydrated through an ethanol series and xylene, and mounted. Negative controls were incubated with PBS buffer in place of the primary antibody and no positive staining was observed.

The tumour sections were simultaneously examined by two researchers for staining intensity and distribution of immunoreactivity within a single tissue section. The staining was graded as low if no reaction or only focal or diffuse weak reaction was observed; and high for moderate diffuse and intense diffuse reaction.

In situ zymography

In situ zymography was performed as described by Hadler-Olsen *et al.*[24] Twenty-eight alcohol-fixed, paraffin-embedded tissue sections were deparaffinised in xylene and rehydrated in graded alcohol baths. The slides were afterwards washed three times with PBS and then overlaid with a solution of 50 µg/mL dye-quenched gelatin (DQ gelatin; Molecular Probes, Thermo Fisher Scientific, USA) diluted in reaction buffer containing 13.7 nM ARP-100 (sc203522; Santa Cruz Biotechnologies, USA). At this molarity ARP-100 blocks the activity of MMP-2, leaving the activity of MMP-9 intact. The sections were then incubated in a dark humidity chamber at 37°C for 2 h allowing the reaction to occur. After digestion, the slides were washed twice with PBS and fixed with neutral buffered formalin. Finally, the tissue sections were overlaid with DAPI containing antifade solution (S7113; Merck Millipore, USA) to counterstain the nuclei and coverslipped.

Green fluorescence resulting from MMP-9 activity was observed under the 20× objective of the fluorescence microscope (Axio Imager 1.0 microscope with AxioCam HR monochrome camera, Carl Zeiss, Germany).

The level of autofluorescence was evaluated by incubation of control sections at −20°C for 2 h. To verify the contribution of metalloproteinases, control slides were preincubated with 20 mM EDTA for 1 h.

Statistical analysis

Obtained data were analysed statistically with the SPSS 12.0.1 software package (SPSS, USA) for Windows. χ^2 analysis was used to test for deviation of genotype distribution from Hardy Weinberg equilibrium. Odds ratios and corresponding confidence intervals were calculated in order to compare genotype and allele frequencies between patients and control subjects. The χ^2 or Fisher exact test, where appropriate, were used to check for association between genotypes and staining distribution, as well as their association with clinicopathological parameters. A probability (p) value smaller than 0.05 was taken to be statistically significant.

RESULTS

MMP-9 genotype distribution and allele frequencies in PTC patients and controls

Genotype and allelic frequencies of the functional -1562 C/T *MMP-9* polymorphism in cases and controls are summarised in Table 1. Genotype frequencies in this study were in Hardy-Weinberg equilibrium both in PTC patients and controls ($p > 0.05$). The frequencies of CC and CT genotypes were 73.1% and 25.8% in the patient group and 86% and 14% in the control group, respectively. Only one participant, belonging to the patient group, carried the rare homozygous TT genotype, while none was detected in the control group. Hence the dominant model was assumed, and the TT genotype was combined with the heterozygote for the purpose of further analysis. The combined CT+TT genotype was present in 26.9% of PTC patients and 14% of controls. No significant differences were found in genotype or allele frequencies between cases and controls. The calculated odds ratios and corresponding confidence intervals are given in Table 1.

Distribution of IHC staining of MMP-9 and gelatinolytic activity between -1562 C/T genotypes in PTC patients

Table 2 shows the distribution of MMP-9 expression and gelatinolytic profiles in PTC samples with altered -1562 C/T genotypes. The majority of common homozygotes displayed a low expression profile of MMP-9 (53/66) and poor gelatin breakdown (12/22), while a smaller proportion exhibited high levels of MMP-9 (13/56), with only one sample manifesting intense enzymatic activity. On the other hand, a comparable number of T allele carriers expressed low (15/25) and high (10/25) levels of MMP-9, a trend mirrored by gelatinolytic activity (4/9 low and 5/9 high). Statistical analysis demonstrated the differential staining and MMP-9 activity between genotypes to be significant ($p = 0.047$ and $p = 0.023$, respectively), implying an adverse effect of CC genotype on MMP-9 expression and, hence, activity in PTC. Representative micrographs of MMP-9 staining in PTC samples harbouring altered genotypes are presented in Fig. 1. A case of classical variant PTC genotyped as CC which did not express MMP-9 in malignant epithelium is demonstrated in Fig. 1A. A contrary example, a heterozygous sample overexpressing MMP-9, is shown in Fig. 1B. Typical *in situ* activity of MMP-9 in a PTC sample from a CC carrier is shown in Fig. 2A, displaying scarce lysis of gelatin represented by predominant blue fluorescence, while Fig. 2B illustrates high MMP-9 activity of an exemplary T allele carrier.

Association of *MMP-9* genotype, expression profile and gelatinolytic ability with clinicopathological factors of PTC

The effect of *MMP-9*-1562 C/T genotype, expression profile and activity on PTC severity was further assessed by establishing their relation with selected clinicopathological parameters (Table 3). Statistical analysis revealed that the genotypes were similarly distributed between age and gender groups. Correspondingly, immunoexpression and activity of MMP-9 also showed no association with these traits. Although the CT+TT genotype and the high expression group exhibited larger mean tumour size than their counterparts, the differences reached statistical significance only when expression profiles were compared ($p = 0.031$). In contrast, when comparing the results of zymography, the group with low MMP-9 activity portrayed larger average tumour size, yet this divergence was not significant. We were unable to demonstrate disparity between promotor genetic variants concerning tendencies to spread to lymph nodes. However, analysis showed that low immunoexpression and the low activity group gave rise to significantly fewer lymph node metastases ($p = 0.028$ and $p = 0.011$, respectively). The common homozygotes elicited fewer extrathyroid extensions ($p = 0.037$) and therefore were more often categorised to lower TNM stages ($p = 0.009$). The IHC and *in situ* zymography data in principal followed this trend, while the association of MMP-9 activity with TNM stage reached borderline statistical significance ($p = 0.051$).

DISCUSSION

Papillary thyroid carcinoma is regarded as a multifactorial disease caused by complex interplay between genetic and environmental factors. Previous studies provided evidence that risk factors such as age, gender, family history, iodine intake or radiation exposure contribute to clinical events leading to development and progression of PTC.[25–27] Moreover, known somatic mutations such as BRAF or RET/PTC repeatedly come up as genetic causes for the onset of malignancy.[28] Yet, it is also reasonable to assume that naturally occurring genetic variants may be the root of interpersonal differences in susceptibility to PTC or its invasive subtypes. Complex interactions between multiple risk alleles with relatively small effects on disease development may account for PTC cases otherwise not exposed to known risk factors.

Numerous studies have provided evidence that support the imperative role of MMP-9 activity in carcinoma progression.[29,30] This implies that polymorphisms affecting

Table 1 *MMP-9* genotype distribution and allele frequencies in PTC patients and the control group

Genotype	Patients *n* (%)	Controls *n* (%)	OR	95% CI	*p*
CC	68 (73.1)	37 (86)	1	Reference	
CT	24 (25.8)	6 (14)	2.176	0.82–5.80	0.12
TT	1 (1.1)	0	1.642	0.065–41.32	0.76
CT+TT	25 (26.9)	6 (14)	2.267	0.854–6.022	0.1
C	86	93	1	Reference	
T	14	7	2.163	0.833–5.612	0.113

CI, confidence interval; *n*, number of subjects; OR, odds ratio; Reference, genotype or allele indicator; $p < 0.05$ statistical significance.

Table 2 Distribution of immunohistochemical scores of MMP-9 expression and activity levels between CC and CT+TT genotypes

		Genotype		*p*
		CC	CT+TT	
MMP-9 expression	Low	53	15	**0.047**
	High	13	10	
MMP-9 activity	Low	12	4	**0.023**
	High	1	5	

p value indicates statistical significance of the χ^2 or Fisher exact test where appropriate.

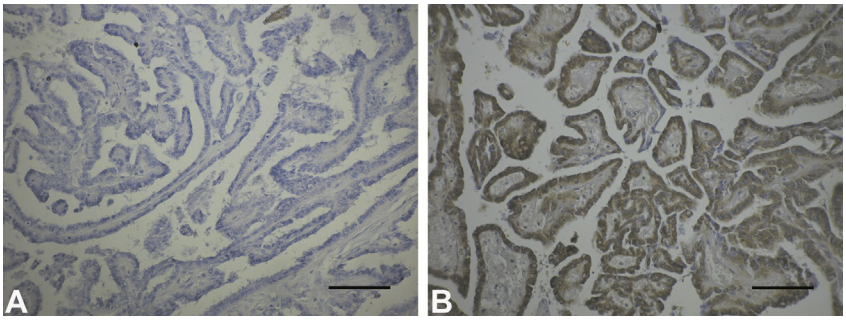

Fig. 1 Representative micrographs of immunostaining in thyroid tissue samples. (A). Absence of IHC staining for MMP-9 in a representative PTC sample genotyped as CC. (B) Moderate cytoplasmic staining for MMP-9 illustrating the High expression group in a PTC sample genotyped as CT. Scale bars = 50 µm.

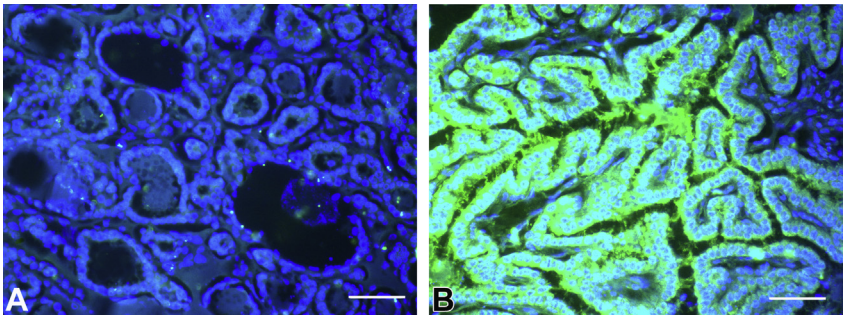

Fig. 2 *In situ* zymography of gelatinase activity in papillary thyroid carcinomas with and without extrathyroid invasion. (A) Low level of fluorescence originating from MMP-9 activity in a PTC sample genotyped as CC. (B) Intense fluorescence originating from MMP-9 activity in a PTC heterozygous sample detected by *in situ* zymography. Scale bars = 50 µm.

MMP-9 gene transcription may affect progression of the pathological process by altering protein expression. Transient transfection and DNA-protein binding experiments showed that the T allele on position -1562 of the *MMP-9* gene has an allele-specific down-regulating effect on promotor activity.[16] This finding motivated a handful of case-control studies to evaluate the possibility that the T allele plays an allele-specific role in the development of numerous types of cancer or increases metastatic potential.[31–35] These studies reported conflicting results. Apart from discrepancies in whether the T allele is a risk factor or not, there were also data indicating it as protective in comparison with the alternative C allele. Finally, the meta-analysis of Peng (2010) analysed a total of 51 studies with more than 40,000 subjects and reported lack of evidence that *MMP-9*-1562 C/T represents a major risk factor for most cancer types.[18] However, these pooled results did not cover many malignancies, including PTC. Moreover, to our knowledge, despite the well documented role of MMP-9 in PTC, so far there are no reports concerning -1562 C/T implications in PTC development or progression.

Therefore, our study was set to evaluate the significance of -1562 C/T polymorphism as a risk factor for developing PTC and/or an unfavourable clinical course of the disease. For this purpose, we analysed the frequency of -1562 C/T polymorphism in PTC samples and controls, investigated the relationship between the promotor genotype and the level of MMP-9 expression and finally estimated the significance of promotor genotype for MMP-9 enzymatic activity.

Our findings failed to provide evidence that genotyping the *MMP-9* promotor could be useful for screening individuals for risk of developing PTC. Genotype and allele frequencies did not differ significantly between PTC patients and control subjects. We then focused only on the PTC group, to examine whether the C/T alteration diverts malignant behaviour towards aggressive forms via changes in MMP-9 protein production. We found that the CC genotype carriers produced significantly less MMP-9 than T allele carriers. These results support previous findings that the C variant is more efficient in binding the putative transcriptional repressor.[16] Several studies covering other carcinomas also linked the promotor variant with expression levels of MMP-9.[36,37]

Table 3 Correlation between genotype frequencies of rs3918242, IHC scores of MMP-9 and extent of gelatinolytic activity with clinicopathological parameters in PTC

Clinicopathological parameters	Genotype			MMP-9 expression			MMP-9 activity		
	CC	CT+TT	*p*	Low	High	*p*	Low	High	*p*
Gender									
Male	10	1	0.156	11	2	0.342	1	1	0.916
Female	58	24		63	27		14	12	
Age, years									
<45	32	11	0.793	36	10	0.193	6	5	0.934
≥45	36	14		38	19		19	8	
Tumour size, mean	26.5	28.5	0.612	25.8	33.7	**0.031**	29.5	28.2	0.797
LNM									
0	51	15	0.158	57	16	**0.028**	14	6	**0.011**
1	17	10		17	13		1	7	
Ei									
0	53	14	**0.037**	58	13	**0.001**	13	4	**0.006**
1	15	11		16	16		2	9	
pT									
1	25	5	0.067	26	3	**0.025**	4	1	0.063
2	26	8		26	9		8	3	
3	10	4		12	10		1	5	
4	7	8		10	7		2	4	
TNM									
1+2	56	14	**0.009**	57	14	**0.005**	12	5	0.051
3+4	12	11		17	15		3	8	

Ei, extrathyroid extension; IHC score, immunohistochemical score; LNM, lymph node metastasis; pT, pT status; TNM, TNM stage (as described in the Materials and Methods section).
Bold numbers indicate statistical significance ($p < 0.05$).

It is important to underline that the antibody used for MMP-9 detection does not distinguish between the zymogen and the active form of the enzyme. It is also impossible to estimate the extent to which MMP-9 is blocked by endogenous inhibitors like TIMPs (tissue inhibitors of metalloproteinases). Therefore, in this case overexpression detected by immunohistochemistry does not immediately imply that the enzyme is operational.

To check for enzyme activity, we set up an experiment with zymography *in situ*, attempting to demonstrate a difference in capacity to degrade gelatin between carriers of disparate promotor genotypes. Although the number of analysed specimens is limited, we found a significant connection between promotor genotype and MMP-9 enzymatic activity. This link is probably indirect, a consequence of lower production of MMP-9 by the common homozygote rather than its ability to alleviate function, yet it demonstrates the influence of the aforementioned SNP on subsequent MMP-9 activity.

Although the genotypes were evenly distributed between PTC patients and healthy individuals, when we analysed the effect the T allele has on unfavourable clinicopathological features in the PTC cohort, we obtained more promising results. Statistical analysis showed that subjects carrying the T allele developed extrathyroid extensions more frequently than their counterparts and therefore were more often categorised in advanced TNM stages. Partially consistent with the genetic data, we also found that high levels of MMP-9 protein were associated with most of the unfavourable clinicopathological features of PTC: increased tumour size ($p = 0.03$), presence of lymph node metastasis ($p = 0.028$) and extrathyroidal invasion ($p = 0.001$) as well as advanced stage ($p = 0.025$). Finally, data obtained by *in situ* zymography revealed significant associations between MMP-9 activity and lymph node involvement ($p = 0.011$), extrathyroid extensions ($p = 0.006$), while correlation with high TNM stages was on the verge of statistical significance ($p = 0.051$). It is worth noting that pT and TNM staging in PTC are derived from more basic indications of carcinoma advancement: tumour size, the presence of extrathyroid invasion and lymphatic spreading, so the obtained low p values in these cases could be in part due to a close relation between these variables.

These data suggest that, although -1562 C/T genotyping failed to discriminate PTC patients from controls, it may still prove valuable in prognostic stratification. Several studies on other carcinomas provided similar evidence, discrediting -1562 C/T as a diagnostic tool but rather linking it with hallmarks of advanced malignancy. Schveigert *et al.* associated the *MMP-9* polymorphism variants with prostate cancer pathological stage and prognostic group.[37] Likewise, Li *et al.* related this polymorphism with breast cancer metastatic risk, while Glebauskiene *et al.* found a higher frequency of the CC allele in non-invasive pituitary adenomas.[8,38] On the other hand, expression profiles of MMP-9 in papillary thyroid carcinoma have been previously investigated in few studies, yielding conflicting results. While Maeta *et al.*[39] and Wang *et al.*[40] demonstrated a link only between MMP-9 expression and lymph node metastasis, other studies reported elevated MMP-9 levels in association with tumour stage[41] and the presence of distant metastasis.[42] A study by Marecko *et al.*[43] analysed separately the expression of total and active MMP-9 and found that only active MMP-9 correlates with parameters of tumour aggression. Reasons for differences in reported results may be also technically related, such as the choice of the primary antibody, detection or scoring system.

Our dataset demonstrated incomplete compliance between genetic findings, protein expression and enzymatic activity. This leads us to speculate that besides the -1562 C/T transition other elements contribute to regulation of MMP-9

expression. Also, there seems to be discrepancy between MMP-9 biosynthesis and activation. Although high levels of the enzyme can be found in samples displaying almost all forms of PTC threatening behaviour, only a portion undergoes downstream activation. High levels of this active form can be found solely in samples spreading to lymph nodes and invading surrounding tissue.

Therefore our and multiple other datasets strongly suggest that the T allele at -1562 of *MMP-9* does not bear a genetic risk for developing malignancy, but after the onset of malignancy due to other causes, deviates its behaviour towards severe phenotypes. It is also reasonable to assume that the effect of the mentioned single nucleotide polymorphism (SNP) depends on the genetic background of the individual, as well as environmental factors.

According to our findings, local aggressiveness of PTC is facilitated by overexpression of MMP-9 due to the presence of the T allele in the gene promotor, but requires downstream activation. As SNP markers are easy, inexpensive and non-invasive to measure, PTC patient management could benefit from genotyping *MMP-9* at -1562 in order to recognise patients in need of harsher treatment. Our findings should be treated as preliminary and wider studies covering multiple ethnicities should be undertaken in the future.

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