

## MATRIX METALLOPROTEINASES IN ORAL DISEASES (WITH ACCENT ON MATRIX METALLOPROTEINASE-1 IN CHRONIC PERIAPICAL LESIONS)

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### Abstract

Matrix metalloproteinases (MMPs) are zinc and calcium dependent enzymes capable of degrading almost all extracellular matrix and basement membrane components. This group of proteolytic enzymes believed to be implicated in the breakdown of extracellular matrix in normal physiological processes, as well as, plays an important role in many destructive pathological oral processes, such as periodontal tissue destruction, root caries, tumor invasion, and chronic periapical inflammation (CPL). The aim of this study was to analyze polymorphism in the gene MMP-1 and their association and influence on clinical manifestation of chronic periapical lesions in order to provide new advances regarding the involvement of MMPs in various oral diseases associated with the inflammatory process.

A total of 240 unrelated Macedonian subjects were included in the present study. Polymorphism -1607 1G/2G in the gene MMP-1 detected with restriction enzymes AluI, XmnI and polymorphism -519 A/G in the gene MMP-1 detected with restriction enzyme KpnI was study in 120 patients with CPL and 120 controls without any signs of chronic or acute inflammatory process in the jaw. The amplification of the region of selected gene was made with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Our results showed that there was a differences in the allele and genotype frequencies of the MMP-1 polymorphism between patients with CPL and controls ( $p < 0.05$ ). Also this study suggests that MMP-1 polymorphism -1607 1G/2G detected with restriction enzymes AluI, XmnI was a risk for expression of CPL (OR = 18.38 < 4.06 < OR < 115.46; OR = 7.73 < 3.1 < OR < 19.55) and MMP-1 polymorphism -519 A/G detected with restriction enzyme KpnI was a risk for expression of CPL (OR = 12.11 < 4.64 < OR < 32.30).

Detection of this genetic polymorphism is relevant for obtaining providential treatment of patients who are at high risk of chronic periapical inflammation.

**Key words:** Matrix metalloproteinases, Oral diseases, Polymerase chain reaction, Gene mutation.

### 1. Introduction

Matrix metalloproteinases (MMPs) are zinc and calcium dependent enzymes capable of degrading almost all extracellular matrix and basement membrane components [1].

This group of proteolytic enzymes is believed to be implicated in the breakdown of extracellular matrix in normal physiological processes, such as: embryonic development, blastocyst implantation, organ morphogenesis, nerve growth, ovulation, cervical dilatation, postpartum uterine involution, endometrial cycling, hair follicle cycling, bone remodeling, wound healing, angiogenesis, apoptosis, etc. [2].

Matrixins play a critical role in pathological processes (e.g.: arthritis, cancer, cardiovascular disease, nephritis, neurological disease, breakdown of blood brain barrier, periodontal disease, skin ulceration, gastric ulcer, corneal ulceration, liver fibrosis, emphysema, fibrotic lung disease, etc.) [3].

According to the important role of MMPs in inflammation [4], it has been shown that genetic variation affecting the expression of MMPs influences the susceptibility and progression of various oral diseases [5].

Matrix metalloproteinases are strongly associated with levels of inflammation and play a major role in bone remodeling and bone resorption [6].

Bone matrix is comprised mostly of mineralized fibrillar type I collagen. MMPs are capable of cleaving native, non-denatured collagens with long uninterrupted triple helices and can function as collagenases *in vivo* [7].

Expression of MMPs is low in normal cells, and these low levels allow for healthy connective tissue remodeling. In pathologic conditions, however, the level of MMPs expression increases considerably, resulting in aberrant connective tissue destruction. Expression of MMPs is regulated primarily at the transcriptional level where the promoter of the genes responds to different regulators [8]. Some functional polymorphisms have been described in the regulatory region of MMPs genes [5]. Because destructive enzymes of the MMPs family are involved in this process, allele polymorphisms of MMPs genes are interesting candidates for analyses of the influence, susceptibility and severity of chronic periapical lesions (CPLs).

Subfamily of human collagenases has three members, from which MMP-1 (fibroblast type of collagenase, collagenase-1) is one of our interest [9].

Gen of the MMP-1 is localized on chromosome 11q22 and its expression was shown in various types of healthy cells (stromal fibroblasts, macrophage, endothelial, and epithelial cells), as well as in different inflammatory and carcinoma cells [10].

Level of expression of MMP-1 can be under the influence of different single nucleotide polymorphism in promontory region. Insertion or deletion of the guanine from the position -1607 can be identified in humans in promoter of the gen of MMP-1 developing two different alleles: one have single guanine (1G), and the other two guanine (2G) [11].

Patients who carried 2G allele have predisposition to develop several types of carcinoma or their rapid progression [12], to develop arthritis [5], arteriosclerosis,

periodontitis [13], unsuccessful osteointegration of the implant [14], and coronary heart disease in patients with diabetes mellitus [15].

The aim of this study was to analyze polymorphism in the gene MMP-1 and their association and influence on clinical manifestation of chronic periapical lesions in order to provide new advances regarding the involvement of MMPs in various oral diseases associated with the inflammatory process.

## 2. Materials and Methods

### 2.1 Materials (patients' samples)

Selection of the patients was made according to internal criteria, on the basis of sated clinical diagnosis, after detail analysis and clinical examination with analysis of X-ray changes. In this study patients who have antibiotic or immuno-suppressive therapy and those patients who have clinical signs of parodontopathia were excluded. Two groups were formed. First group was formed from 40 patients after clinically and radiographic verified chronic periapical lesion. Second (control) group formed 120 patients without endodontic treated teeth and absents of chronic periapical process or acute odontogenic infection.

In the aseptic conditions, from each patient with vene-punction, sample of vein blood (10mL) was taken in sterile epruvete (Vacutainer<sup>o</sup>) in which was anticoagulant (EDTANa<sup>2</sup>). Ich sample was frozen at -80 °C as soon as possible and stored until the analysis.

### 2.2 Methods

Standard isolation of genomic DNA from the nuclear cells was made with natrium chlorid extraction and afterwards precipitation with ethanol [16].

**Table 1. Sequence of PCR primers with their restriction enzyme**

Gene	Polymorphism	Primer	PCR conditions	Length of PCR amplificat.	Restriction enzyme	Restriction digestion
MMP-1	-1607 1G/2G	(F) 5'-TGA CTT TTA AAA CAT AGT CTA TGT TCA-3' (R) 5'- TCT TGG ATT GAT TTG TTG AGA TAA GTC ATA GC-3'	35 cycles: 94 °C 30 s, 54 °C 30 s, 72 °C 30 s	269 bp	<i>AluI</i>	(241 + 28) bp
MMP-1	-1607 1G/2G	(F) 5'-TCG TGA GAA TGT CTT CCC ATT-3' (R) 5'-TCT TGG ATT GAT TTG AGA TAA GTC ATA-3'	35 cycles: 93 °C 30 s, 5 °C 30 s, 72 °C 30 s, 72°C 5 min.	118 bp	<i>XmnI</i>	(29 + 89) bp
MMP-1	-519 A/G	(F) 5'-CAT GGT GCT ATC GCA ATA GGG T-3' (R) 5'-TGC TAC AGG TTT CTC CAC ACA C-3'	30 cycles: 94 °C 30 s, 49 °C 30 s, 72 °C 20 s, 72 °C 4 min.	200 bp	<i>KpnI</i>	(176 + 24) bp

Samples of DNA isolates were aliquoted in few epruvettes, from which, one was kept from +4 °C till +8 °C, and that one was used for analysis. The other EDTA tubes were kept as a reserve in the bank of samples at temperature from -18 till -20 °C. Amplification of the regions of elected genes was made with polymerase chain reaction (PCR) and performed in PCR machine (Perkin-Elmer Gene Amp System 2400).

Presence or absence of some normal or mutated sequence in PCR product followed by restriction digestion results with different size of fragments (Table 1).

Successful amplification was verified with presence of electrophoresis marker (PCR Marker, Bio-Rad) with agarose electrophoresis (Bio-Rad, USA). Production of digestion were visualized with fluorescence painting of the gel with etidium bromid.

### 3. Results and Discussion

#### 3.1 Results

Representation of the polymorphism -1607 1G/2G of the gene of MMP-1 detected with restricted enzyme *AluI* in the patients from examined groups is shown in Table 2.

Statistical analysis confirm that there is a statistical significance between gene polymorphism of MMP-1 detected with restriction enzyme *AluI* and CPL (Pearson Chi-square: 40.9120, df = 2, p = 0.000000). Polymorphism -1607 1G/2G for the gene of MMP-1 detected with restrictive enzyme *AluI* was risk factor for expression of chronic periapical lesion (OR = 18.38 < 4.06 < OR < 115.46).

Representation of the polymorphism -1607 1G/2G of the gene of MMP-1 detected with restricted enzyme *XmnI* in the patients from examined groups is shown in Table 3.

Statistical analysis confirm that there is a statistical significance between gene polymorphism of MMP-1 detected with restriction enzyme *XmnI* and CPL (Pearson Chi-square: 57.5222, df = 2, p = 0.000000). Polymorphism -1607 1G/2G for the gene of MMP-1 detected with restrictive enzyme *AluI* was risk factor for expression of chronic periapical lesion (OR = 7.73 < 3.1 < OR < 19.55).

Representation of the polymorphism -519 A/G of the gene of MMP-1 detected with restricted enzyme *KpnI* in the patients from examined groups is shown in Table 4.

Statistical analysis confirm that there is a statistical significance between gene polymorphism of MMP-1 detected with restriction enzyme *KpnI* and CPL (Pearson Chi-square: 45.8285, df = 2, p = 0.000000). Polymorphism -519 A/G for the gene of MMP-1 detected

**Table 2. Representation of the polymorphism -1607 1G/2G for the gene of MMP-1 detected with restricted enzyme *AluI* in patients from examined groups**

Polymorphism of MMP-1 detected with restricted enzyme <i>AluI</i>	N° and %	Examined groups		Total
		Chronic periapical lesions	Control group	
-/- (homozygotes for absence)	N°	2	59	61
	%	5%	49.2%	43.6%
+/+ (homozygotes for presence)	N°	19	10	29
	%	47.5%	8.3%	20.7%
-/+ (heterozygotes for presence)	N°	19	51	70
	%	47.5%	42.5%	50%

**Table 3. Representation of the polymorphism -1607 1G/2G for the gene of MMP-1 detected with restricted enzyme *XmnI* in patients from examined groups**

Polymorphism of MMP-1 detected with restricted enzyme <i>XmnI</i>	N° and %	Examined groups		Total
		Chronic periapical lesions	Control group	
-/- (homozygotes for absence)	N°	9	83	92
	%	22.5%	69.2%	68.3%
+/+ (homozygotes for presence)	N°	24	7	31
	%	60%	5.8%	22.1%
-/+ (heterozygotes for presence)	N°	7	30	37
	%	17.5%	25%	26.4%

**Table 4. Representation of the polymorphism -519 A/G for the gene of MMP-1 detected with restricted enzyme *KpnI* in patients from examined groups**

Polymorphism of MMP-1 detected with restricted enzyme <i>KpnI</i>	Number	Examined groups		Total
		Chronic periapical lesions	Control group	
-/- (homozygotes for absence)	N	18	109	127
	%	45%	90.8%	90.7%
+/+ (homozygotes for presence)	N	9	/	9
	%	22.5%	/	6.4%
-/+ (heterozygotes for presence)	N	13	11	24
	%	32.5%	9.2%	17.1%

with restrictive enzyme *KpnI* was risk factor for expression of chronic periapical lesion (OR = 12,11 < 4.64 < OR < 32.30).

Detected frequency of 2G allele in polymorphs -1607 1G/2G of the gene for MMP-1 detected with restricted enzyme *XmnI* was significant higher in patients with CPL than those from the control group ( $f(2G) = 0.18$ ). With that we can confirm protective role of 1G allele for developing an inflammatory process. Frequency of the genotypes was  $f(1G/1G) = 0,2$ ;  $f(1G/2G) = 0,2$ ;  $f(2G/2G) = 0,6$  and  $p < 0.05$  ( $p = 0,0002$ ) witch shows that there is an equilibrium (Table 5).

Frequency of the genotypes was  $f(1G/1G) = 0,7$ ;  $f(1G/2G) = 0,2$ ;  $f(2G/2G) = 0,1$  and  $p > 0.05$  ( $p = 0,0002$ ) witch shows that there is no equilibrium (Table 6).

Detected frequency of 2G allele in polymorphs -1607 1G/2G of the gene for MMP-1 detected with restricted enzyme *AluI* was significant higher in patients with CPL than those from the control group ( $f(2G) = 0.30$ ). Frequency of the genotypes was  $f(1G/1G) = 0,0$ ;  $f(1G/2G) = 0,5$   $f(2G/2G) = 0,5$  and  $p > 0.05$  ( $p = 0,3133$ ) witch shows that there is no equilibrium (Table 7).

Frequency of the genotypes was  $f(1G/1G) = 0,5$ ;  $f(1G/2G) = 0,4$ ;  $f(2G/2G) = 0,1$  and  $p > 0.05$  ( $p = 0,8259$ ) witch shows that there is no equilibrium (Table 8).

Detected frequency of 2G allele in polymorphs -519 A/G of the gene for MMP-1 detected with restricted enzyme *KpnI* was significant higher in patients with CPL than those from the control group ( $f(G) = 0.05$ ). With that we can confirm protective role of A allele in this polymorphism for developing an inflammatory process. Frequency of the genotypes was  $f(A/A) = 0,5$ ;  $f(A/G) = 0$ ;  $f(G/G) = 0,2$  and  $p < 0.05$  ( $p = 0,0461$ ) witch shows that there is an equilibrium (Table 9).

Frequency of the genotypes was  $f(A/A) = 0,9$ ;  $f(A/G) = 0,1$ ;  $f(G/G) = 0,0$  and  $p > 0.05$  ( $p = 0,5988$ ) witch shows that there is no equilibrium (Table 10).

### 3.2 Discussion

MMPs play an important role during periapical pathology development [17], and it has been suggested that the modality of root canal treatment could interfere with MMPs expression [18].

Due to their various roles in bone remodeling, immune responses, caries, and dental development, it is hypothesize that variation in MMPs and TIMP genes may alter the level of bone destruction and remodeling and contribute to the formation of more extensive periapical lesions in teeth affected by deep carious lesions. [19, 20].

Results in our study indicate that individual genetic predisposition have influence on periapical lesion formation. We can confirm that polymorphism of the

**Table 5. Frequency of the alleles in the polymorphism -1607 1G/2G for the gene of MMP-1 detected with restricted enzyme *XmnI* in patients with CPL**

Genotype	1G/1G	1G/2G	2G/2G	Total	Frequency of the alleles
Number n	9	7	24	40	80
Allel 1G	18	7	0	25	0,31
Allel 2G	0	7	48	55	0,69

**Table 6. Frequency of the alleles in the polymorphism -1607 1G/2G for the gene of MMP-1 detected with restricted enzyme *XmnI* in patients from controle group**

Genotype	1G/1G	1G/2G	2G/2G	Total	Frequency of the alleles
Number n	83	30	7	120	240
Allel 1G	166	30	0	196	0,82
Allel 2G	0	30	14	44	0,18

**Table 7. Frequency of the alleles in the polymorphism -1607 1G/2G for the gene of MMP-1 detected with restricted enzyme *AluI* in patients with CPL**

Genotype	1G/1G	1G/2G	2G/2G	Total	Frequency of the alleles
Number n	2	19	19	40	80
Allel 1G	4	19	0	23	0,29
Allel 2G	0	19	38	57	0,71

**Table 8. Frequency of the alleles in the polymorphism -1607 1G/2G for the gene of MMP-1 detected with restricted enzyme *AluI* in patients from controle group**

Genotype	1G/1G	1G/2G	2G/2G	Total	Frequency of the alleles
Number n	59	51	10	120	240
Allel 1G	118	51	0	169	0,70
Allel 2G	0	51	20	71	0,30

**Table 9. Frequency of the alleles in the polymorphism -519 A/G for the gene of MMP-1 detected with restricted enzyme *KpnI* in patients with CPL**

Genotype	A/A	A/G	G/G	Total	Frequency of the alleles
Number n	10	22	8	40	80
Allel A	20	22	0	42	0,52
Allel G	0	2	16	38	0,48

**Table 10. Frequency of the alleles in the polymorphism -519 A/G for the gene of MMP-1 detected with restricted enzyme *KpnI* in patients from controle group**

Genotype	A/A	A/G	G/G	Total	Frequency of the alleles
Number n	59	51	10	120	240
Allel A	118	51	0	169	0,70
Allel G	0	51	20	71	0,30



gene for MMP-1 contributes for increasing individual frequency of apical tissue destruction as a reaction to deep caries lesion.

Our results are in correlation with findings of Rutter *et al.*, [11] in which is show that this polymorphism can have functional consequences because can affect the function of MMP-1, as well as, is polymorphism who is candidate with strong biological significance for developing inflammatory process.

Also our result are in correlation with finding of Cao *et al.*, [21] in which genetic variations in promontory region of the gene of MMP-1 have influence on the transcription level and as a results of this, the gen of MMP-1 have crucial role in degradation of connected tissue in periodontitis.

Beside this, Itagaki *et al.*, [9], have not detected differences between allele of the gene of MMP-1 and genotypic distribution in healthy Japanese and those with chronic periodontitis.

Findings about the detected frequency of G allele in polymorphs -519 A/G of the gene for MMP-1 in our study are in relationship with findings of Oh *et al.*, [22], where is observed similar distribution of the genotypes and frequencies of the alleles.

Polymorphism -1607 1G/2G for the gene MMP-1 detected with restricted enzymes *XmnI* and *AluI* is an risk factor for developing chronic periapical lesion. Individuals who are carriers of 2G/2G genotype and individuals with 1G/2G genotype (2G carriers) in this polymorphism shows affection for developing chronic periapical lesion.

Polymorphism -519 A/G for the gene MMP-1 detected with restricted enzymes *KpnI* is a risk for developing chronic periapical lesion and individuals who are carriers of G/G genotype and individuals with A/G genotype (G carriers) in this polymorphism shows affection for developing chronic periapical lesion.

#### 4. Conclusions

- With this study we can identify the genetic factors that are of enormously meaning for establishing the risk profile of the patients who have affection for developing chronic periapical lesions in Macedonian population, and variations in gene alleles of the MMP-1 are markers for monitoring the risk and progression of this inflammatory process.

- Our findings are fundamental for identification of gene polymorphism of MMP-1 in Macedonian population, as a starting point in further investigations of MMPs gene expression, enable to predict the course and development of clinical sign of inflammatory process, indirectly pointed the adequate therapeutic method.

#### 5. References

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