

## Original Article

# Polymorphisms in the promoters of MMP-2 and TIMP-2 genes in patients with acne vulgaris

Kursat Oguz Yaykasli<sup>1</sup>, Hakan Turan<sup>2</sup>, Ertugrul Kaya<sup>3</sup>, Omer Faruk Hatipoglu<sup>4</sup>

<sup>1</sup>Department of Medical Genetics, Faculty of Medicine, Duzce University, Duzce, Turkey; <sup>2</sup>Department of Dermatology, Faculty of Medicine, Duzce University, Duzce, Turkey; <sup>3</sup>Department of Pharmacology, Faculty of Medicine, Duzce University, Duzce, Turkey; <sup>4</sup>Department of Pathology, Faculty of Medicine, Pittsburg University, Pittsburg, USA

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**Abstract:** Acne, a chronic inflammatory skin disease, can be seen at any age but it most often occurs in adolescents and young people. Several factors, including increased sebum production, abnormal cornification of the pilosebaceous units, proliferation of *Propionibacterium acne*, and extracellular matrix (ECM) remodeling, are thought to be associated with the pathogenesis of the acne. The remodeling of the ECM is regulated by a balance between matrix metalloproteinases (MMPs) and their inhibitors called tissue inhibitors of metalloproteinases (TIMPs). The current study investigated the potential association between MMP-2 (-1306 C/T) and TIMP-2 (-418 G/C) polymorphisms and the risk for acne in a Turkish population. The study was conducted with 85 subjects who presented to the Dermatology Department of Duzce University Hospital. DNA was isolated from 2 ml of peripheral blood taken from each subject, and their genotypes were analyzed with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The CC, CT, and TT genotypes for MMP-2 (-1306 C/T) polymorphism were similar between the patient and control group (24 [55.8%], 17 [39.5%], and 2 [4.7%], respectively, vs. 21 [50%], 18 [42.9%], and 3 [7.1%], respectively). However, the distribution of the GG, GC, and CC genotypes for TIMP-2 (-418 G/C) polymorphism were different between the patient and control group (30 [69.8%], 9 [14.8%] and 4 [9.3%], respectively, vs. 26 [61.9%], 14 [33.3%], and 2 [4.8%], respectively). The results demonstrated that the TIMP-2 (-418 CC) genotype was nearly two times more common in the patient group compared to the control group ( $p=0.686$ , OR=1.45). It may be possible that the TIMP-2 (-418 CC) genotype increases the tendency to develop acne vulgaris by disrupting the balance between MMPs and TIMPs. Further investigations are needed to clarify more precisely the relationship between acne and MMP-TIMP genes.

**Keywords:** Acne vulgaris, MMPs, TIMPs, polymorphism

## Introduction

Acne vulgaris is a chronic inflammatory skin disease involving pilosebaceous follicles. It is typically classified into two groups: noninflammatory (open and closed comedones) and inflammatory (papules and pustules) [1, 2]. Acne commonly begins in adolescence and is perceived as a teenage disease; however, it often persists into adulthood [3, 4]. It is very difficult to estimate the demographic prevalence of acne due to the variety of methods used to assess its severity. Acne vulgaris affects 40-50 million people in the U.S. [5, 6]. It is also estimated that 50.9% of women and

42.5% of men are affected by acne throughout their 20 s [7].

The pathogenesis of acne is known to be multifactorial. Several pathogenetic factors have been identified, including sebum overproduction by the sebaceous gland, *Propionibacterium acnes* follicular colonization, alteration in the keratinization process, and release of inflammatory mediators into the skin [8]. A number of studies have investigated the relationship between diet and acne, but a link has not been proven yet [9, 10]. Although triggering mechanism of acne remains unclear, recent studies demonstrated the potential role of inflammato-

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ry mediators, including metalloproteinases (MMPs). MMPs are zinc-dependent extracellular proteinases. They are involved in a wide range of normal biological functions, including morphogenesis, wound healing, and angiogenesis. The activities of MMPs are regulated by tissue inhibitors of metalloproteinases (TIMPs) [11, 12]. MMPs and TIMPs are essential for the remodeling of the extracellular matrix (ECM), and inflammatory diseases, such as acne, can emerge in the case of an imbalance between MMPs and TIMPs [13].

It is well established that inflammation in acne is caused by *P. acnes*, which induces monocytes to secrete proinflammatory cytokines [14]. Studies demonstrated that increased levels of inflammatory cytokines stimulate MMPs in acne vulgaris [15, 16]. To date, several polymorphism studies have related gene polymorphisms of inflammatory mediators to the pathogenesis of acne vulgaris. Although the relationship between MMPs and acne vulgaris has been demonstrated, the polymorphisms of MMP and TIMP genes have not been investigated in acne vulgaris yet. The polymorphisms of MMP-2 (-1306 C/T) and TIMP-2 (-418 G/C) located at the SP1 binding site of the promoter region and the substitution of C to T and G to C may result in down-regulation of MMP-2 and TIMP-2 genes, respectively, by eliminating the SP1 binding site. Therefore, studies have investigated the role of these polymorphisms as risk factors for several diseases [17, 18]. This study investigated the potential association between MMP-2 (-1306 C/T) and TIMP-2 (-418 G/C) polymorphisms and the risk for acne in a Turkish population.

### Materials and methods

#### Study subjects

The study population comprised 43 patients with acne who presented to the Dermatology Department of Duzce University Hospital and 42 healthy controls without any systematic or dermatological disease. The study was approved by the local ethics committee and conformed to the Declaration of Helsinki. Each participant was informed about the aim of the study, and their written informed consent forms were obtained from each participant. The demographic and biochemical characteristics of the participants, including their age, body

mass index (BMI), glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and testosterone, were recorded.

#### DNA extraction and genotyping

DNA extraction and genotyping were done as explained previously [19]. Briefly, blood samples were obtained in 2 mL EDTA tubes from the patient and control groups. Genomic DNA was obtained from peripheral blood leukocytes by a standard method using a PureLink DNA isolation kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and the samples were stored at -20°C until analysis by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The oligonucleotide primers, forward 5'-CTT-CCTAGGCTGGTCTTACTGA-3', reverse 5'-CTGAGACCTGAAGAGCTAAAGAGCT-3', forward 5'-CGTCTCTGTGGCTGGTCA-3', and reverse 5'-CCTCTCAGCTCGACTCTGGAG-3' were used to amplify the MMP-2 -1306 C/T and TIMP-2 -418 G/C polymorphisms, respectively. PCR was performed in total volume of 25 µL with 0.5 µL Taq DNA polymerase (Fermantas, Lithuania). The amplification conditions were 5 min at 94°C, followed by 35 cycles of 30 s at 94°C and 45 s at 58°C for MMP2 -1306 C/T and at 59°C for TIMP2 -418 G/C and 45 s at 72°C, with a final step at 72°C for 10 s [20].

The PCR products were digested by Bfal (XspI) (NEB, UK) and Aval (BsoBI) (NEB, UK) restriction enzymes for MMP2 -1306 C/T and TIMP2 -418 G/C, respectively. The incubation was performed at 37°C in a 25 µL reaction containing 10 U restriction enzymes. The digested products were separated on a 4% agarose gel stained with ethidium bromide.

The primers for MMP-2 -1306 C/T generated a 193 base-pair (bp) PCR product. After digestion, the C allele resulted in 188 + 5 bp bands, and the T allele resulted in 162 + 26 + 5 bp bands on agarose gel. The primers for TIMP-2 -418 G/C generated a 304 bp PCR product. After digestion, the G allele resulted in 230 + 51 + 23 bp bands, and the C allele resulted in 253 + 51 bp bands on agarose gel.

#### Statistical analysis

Statistical analyses were performed using PASW 18 statistical software (ver. 18.0 for Windows; SPSS Inc., Chicago, IL, USA). Categorical

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**Table 1.** The characteristics in the patient and control groups

	Total (85)	Acne (43)	Control (42)	p
Age (Year)	19.9 ± 3.5	19.3 ± 3.6	20.57 ± 3.2	0.095
BMI (kg/m <sup>2</sup> )	22.4 ± 3.2	22.2 ± 3.1	22.5 ± 3.4	0.736
Glucose (mg/dL)	90.2 ± 11.4	90.8 ± 10	89.5 ± 12.6	0.61
HDL (mg/dL)	50.5 ± 13.5	52.7 ± 13.2	48.3 ± 13.7	0.14
LDL (mg/dL)	92.2 ± 30.6	90.7 ± 27.4	93.6 ± 33.5	0.682
Triglycerides (mg/dL)	102 (74-141.7)	94 (63-124.5)	110 (82-161)	0.008
Testosterone (mg/dL)	51.9 (23.2-411.5)	52.9 (32.7-460)	50 (17.9-328.5)	0.182

**Table 2.** MMP2 -1306 C/T SNP genotypes distributions in the patient and control groups

Genotype	Total (85)	Control (42)	Acne (43)	p (χ <sup>2</sup> )	OR* (95% CI)	P*
CC n (%)	45 (52.9)	21 (50)	24 (55.8)		Ref	Ref
CT n (%)	35 (41.2)	18 (42.9)	17 (39.5)	0.812	0.75 (0.3-1.86)	0.532
TT n (%)	5 (5.9)	3 (7.1)	2 (4.7)		0.42 (0.06-2.91)	0.381
CT + TT n (%)	40 (47.1)	21 (50)	19 (44.2)	0.591	0.7 (0.29-1.69)	0.427

OR: odds ratio, CI: confidence interval. \*For logistic regression.

variables were compared in two groups by using Pearson's and Fisher's Chi-square test, and the results were expressed as frequency and percentage. Binary logistic regression analyses were performed to identify independent predictors for acne disease. An independent sample *t*-test was performed to compare normal distributed continuous variables in the two groups, and these variables were expressed as mean ± standard deviation (SD). If the continuous variables were not normally distributed, the Mann-Whitney *U* test was used, and the data were expressed as median values (25-75th percentiles). A *p* value of less than 0.05 was considered as statistically significant.

### Results

The characteristics of the patient and control groups are summarized in **Table 1**. The patient group included 43 subjects with a mean age of 19.3 ± 3.6 years, and the control group included 42 subjects with a mean age of 20.57 ± 3.2 years. No statistical significance was determined (*p*=0.095). Similarly, there was no statistically significant difference between the two groups in terms of BMI, glucose, HDL, LDL and testosterone. The serum triglyceride level in the acne patients (94 [63-124.5]) was significantly lower compared to that in the control group (110 [82-161]) (*p*=0.008).

As for the MMP-2 -1306 C/T SNP, the CC genotype was found in 24 (55.8%), CT genotype in

17 (39.5%), and TT genotype in 2 (4.7%) subjects in the patient group. In the control group, the CC genotype was found in 21 (50%), CT in 18 (42.9%), and TT in 3 (7.1%) subjects. The CT + TT genotypes were detected in 21 (50%) and 19 (44.2%) subjects in the control and patient group, respectively. No statistical significance was determined (*p*>0.05) (**Table 2**).

Regarding the TIMP-2 (-418 G/C) SNP, the GG genotype was found in 30 (69.8%), GC in 9 (20.9%), and CC in 4 (9.3%) subjects in the patient group. In the control group, the GG genotype was found in 26 (61.9%), GC in 14 (33.3%), and CC in (4.8%) subjects. The GC + CC genotypes were found in 16 (38.1%) and 13 (30.2%) subjects in the control group and patient group, respectively. Although no statistical significance was determined, the TIMP-2 CC genotype was nearly two times more common in the patient group compared to the control group (*p*=0.686, OR=1.45) (**Table 3**).

### Discussion

In the present study, the possible relationship between MMP2 (-1306 C/T) and TIMP2 (-418 G/C) polymorphisms and acne vulgaris was investigated. The results showed that the frequency of the (-418 CC) genotype in the patient group was higher than in the control group (*p*=0.686, OR=1.45). It can be concluded that the TIMP- (-418 CC) genotype is a risk factor for acne vulgaris by disrupting the balance between

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**Table 3.** TIMP2 -418 G/C SNP genotypes distributions in the patient and control groups

Genotype	Total (85)	Control (42)	Acne (43)	p ( $\chi^2$ )	OR*	P*
GG n (%)	56 (65.88)	26 (61.9)	30 (69.8)		Ref	Ref
GC n (%)	23 (43.1)	14 (33.33)	9 (20.9)	0.363	0.581 (0.21-1.58)	0.29
CC n (%)	6 (7.06)	2 (4.8)	4 (9.3)		1.45 (0.23-8.87)	0.686
GC + CC n (%)	29 (34.1)	16 (38.1)	13 (30.2)	0.445	0.7 (0.28-1.76)	0.451

OR: odds ratio, CI: confidence interval. \*For logistic regression.

MMPs and TIMPs. Inflammation has a pivotal role in the pathogenesis of acne. It is well established that the secretion of proinflammatory cytokines, such as interleukin-1 $\alpha$ , interleukin-8, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), contributes to the inflammatory nature of acne vulgaris [21, 22]. Therefore, several studies have investigated the relationship between acne vulgaris and these proinflammatory gene polymorphisms. Most of these studies have found an association between them [23, 24]. The release of the proinflammatory mediators in acne inflammation also affects the activity of MMP in matrix remodeling. MMPs, a group of zinc-dependent endopeptidases, are capable of degradation of components of the ECM, such as collagen, and the activity of the MMPs is controlled by TIMPs. MMPs and TIMPs have a crucial role in the remodeling of the ECM, and any imbalance in the ratio of MMPs to TIMPs causes atrophic or hypertrophic scars in acne vulgaris patients [25, 26].

One of the most comprehensive studies of gene expression patterns in acne was conducted by Trivedi et al. [16]. They compared gene expression profiles between lesional and non-lesional skin and found that 211 genes, including MMP-1 and MMP-3, are upregulated in lesional skin compared to nonlesional skin. Similar results were obtained by several other groups. Papakonstantinou et al. [15] reported the upregulation of the expression of MMP-1 mRNA in skin from acne patients. Kang et al. [27] reported that MMP-1, MMP-3, and MMP-9 mRNA levels were significantly elevated compared to controls. Another study done by Choi et al. [28] reported that *P. acne* stimulates pro-MMP-2 expression via TNF- $\alpha$  in human dermal fibroblasts. Although proinflammatory cytokines have the potential to upregulate the expression of MMPs, the elevated expression of MMPs in acne might be Tol-like receptor2 (TLR2)-dependent. Jalian et al. [29] found that

the expression of MMP9 was TLR2-dependent in acne, but the upregulation of MMP1 was TLR2 independent.

After clarification of the upregulation of MMPs in patients with acne, investigators focused on conventional therapeutic inhibitors to decrease the activity of MMPs. In the latest study conducted by Sato et al. [30], the authors demonstrated that clindamycin decreased the production of pro-MMP-2 in hamster sebocytes. Emanuele et al. [31] reported use of a topical preparation containing nicotinamide, retinol, and 7-dehydrocholesterol for the same purpose because of its anti-inflammatory effects. Their gene expression study revealed not only significant down-regulation of MMP-1, MMP-2, MMP-9, and MMP-14, but also upregulation of TIMP-1, TIMP-2, and TIMP-3 after use of the topical preparation.

These experimental data point to the prominent role of MMPs and TIMPs in the pathogenesis of acne. It is well known that gene polymorphisms may affect gene expression levels. However, no previous study investigated the relationship between acne and MMPs-TIMPs polymorphisms. The current study is the first clinical investigation of the relationship between them. The experimental results demonstrated no statistically significant differences between the groups. However, the TIMP-2 (-418 CC) genotype was nearly two times more common in the patient group compared to the control group. Therefore, it might be speculated that the TIMP-2 (-418 CC) genotype increases the tendency to develop acne vulgaris. The main limitation of the present study was the small number of subjects, and this study should be supported by further studies with a larger population. In addition, other MMP and TIMP gene polymorphisms should be investigated to clarify the relationship between acne and MMPs-TIMPs.

**Disclosure of conflict of interest**

None.

**Address correspondence to:** Dr. Kursat Oguz Yaykasli, Department of Medical Genetics, Faculty of Medicine, Duzce University, Konuralp Campus, 81620, Duzce, Turkey. Tel: +90 380 542 11 00 / 4160; Fax: +90 380 542 13 02; E-mail: kursatyay@yahoo.com

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