

## The Association of MMP-8 Genotypes with Pterygium

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**Abstract.** *Background/Aim:* Pterygium is composed of proliferating fibrovascular tissue, and its formation and progression are closely related to the homeostasis of the extracellular microenvironment. However, few studies have examined the contribution of matrix metalloproteinases (MMP) to either diagnostic or prognostic potential in pterygium. In this study, we investigated the contribution of a polymorphism in the promoter region of MMP-8 (-799C/T) and two non-synonymous polymorphisms (Val436Ala and Lys460Thr) to pterygium. *Materials and Methods:* In this study, 134 patients with pterygium and 268 non-cancer controls patients were collected and the MMP-8 -799C/T, Val436Ala and Lys460Thr polymorphic genotypes of each subject were examined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *Results:* The results showed that the three polymorphisms investigated were not significantly associated with risk of pterygium. In addition, the stratified analysis showed that there was no interaction between MMP-8 genotype with age or gender on pterygium risk determination. *Conclusion:*

*Polymorphisms at MMP-8 -799C/T, Val436Ala and Lys460Thr may not mainly contribute to determining personal susceptibility to pterygium in the Taiwanese examined.*

Pterygium is a formation of fibrous tissue consisting of highly vascularized epithelial and subepithelial tissue that proliferates excessively and with an abnormal shape on the cornea. From the viewpoint of epidemiology, several physical and biological factors are reported to be associated with the pathogenesis of pterygium, including heat, dust, and other particles in the atmosphere, and immunological mechanisms, and regulations involving extracellular matrix reorganization, growth factors, cytokines, apoptosis, and angiogenesis. In recent years, mounting evidence has demonstrated that genetic factors play a significant role in the development of pterygium (1-4).

Matrix metalloproteinases (MMPs), also known as matrixins, are the major protein family in charge of regulating the homeostasis of extracellular matrix components (5). The MMPs perform a wide function in regulating the cell proliferation, differentiation, apoptosis, invasion, migration, metastasis, angiogenesis and immune surveillance during carcinogenesis (6). In literature, more and more evidence has shown that functional polymorphisms of MMPs may determine the inter-individual differences of susceptibility to several types of cancer (7-13). However, the difficulty in collecting enough samples from pterygium patients retarded the investigation of the role of MMPs in pterygium and only several reports with a small size of pterygium samples provided evidence for MMPs' playing a critical role in the development and progression of pterygium. For instance, a pilot study examined the

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Table I. Distribution of selected demographics of the 134 pterygium patients and the 268 non-terygium controls.

Characteristics	Controls (n=268)			Patients (n=134)			p-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age at onset (years old)			64.3 (6.0)			64.4 (7.0)	0.9660
Gender						1.0000	
Male	156	58.2%		78	58.2%		
Female	112	41.8%		56	41.8%		

<sup>a</sup>Based on Student's *t*-test; <sup>b</sup>based on the Chi-square test; SD: standard deviation.

expression of transcripts and proteins of MMPs in cultured human pterygium head, body, subconjunctival fibroblasts, and normal corneal and conjunctival fibroblasts. The results showed that MMP-1 and MMP-3, but not MMP-2, TIMP-1, TIMP-2 or uPA, were highly expressed in pterygium head, body and subconjunctival fibroblasts (14). Although Schellini and his colleagues demonstrated that the expression of MMP-9 was similar among the tissues from normal Tenon's capsule and primary or recurrent pterygia (15), Yang has oppositely proposed that the expression of MMP-9 by pterygium fibroblasts is significantly increased after the progression of pterygium (16). One of the reasons to explain the inconsistency is that the clinical sample sizes in the two researchers were only 6 and 15, respectively. Thus, the roles of MMPs in pterygium are still controversial and waiting to be revealed using not only a larger collection of samples, but also from studying the genetic in addition to the transcriptional or translational levels.

MMP-8 is a collagen regulator (17, 18), and the differential regulation patterns of MMP-8 may lead to different progression of cancers and pterygium among people of different genetic backgrounds of *MMP-8*. As early as 2007, *MMP-8* C-799T genotypes were found to be associated with breast cancer risk (19) and the electrophoretic mobility shift assays revealed differences in nuclear protein binding to oligonucleotides representing the -799C/T genotypes (20). In addition, the promoter constructs containing the CT and TT genotypes at the -799C/T had a 3-fold greater activity in chorion-like trophoblast cells compared to the constructs containing the C alleles (20). However, as mentioned above, the role of *MMP-8* genotypes has never been examined in pterygium. The current study aimed at investigating the association of *MMP-8* -799C/T, Val436Ala and Lys460Thr polymorphisms with the susceptibility of pterygium.

## Materials and Methods

**Patients and controls.** The protocol of the current study was approved by the Institutional Review Board of Changhua Christian Hospital and written informed consent was obtained from one or

both parents of all participants [Changhua Christian Hospital IRB numbers: 141208]. One hundred and thirty-four patients diagnosed with pterygium were recruited in this study from the Departments at Changhua Christian Hospital, Taichung, Taiwan, Republic of China. All of the clinical characteristics of these patients, including their histological details, were identified by expert surgeons. All pterygium patients voluntarily participated, completed a questionnaire, and provided peripheral blood samples. The inclusion criteria of pterygium patients were apex of pterygium invading the cornea for more than 1 mm. Healthy volunteers aged 45 years or more without pterygium or any type of cancer were enrolled as the control group for further selection. There were 78 males and 56 females in the pterygium group (age range of 48 to 89 years with an average age of 64.4). Finally, 268 non-terygium healthy participants were included in the study in order to match the population structure (double the number of cases and matched for their ages and genders) with our pterygium population. The overall agreement rate in the study was above 85%. Selected recorded characteristics of the subjects in pterygium and non-terygium control groups are summarized and compared in Table I.

**Methods for determining *MMP-8* genotypes.** Genomic DNA extracted from the peripheral blood leukocytes of each participant was extracted, aliquoted and stored as previously described (21). The primers for *MMP-8* -799C/T, Val436Ala and Lys460Thr polymorphisms were designed by our team as previously published (22, 23). Briefly, genotyping polymerase chain reaction (PCR) cycling conditions via My Cycler (Biorad, Hercules, CA, USA) for *MMP-8* were: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 sec, 57°C for 30 sec and 72°C for 30 sec and a final extension at 72°C for 10 min (22, 23).

**Statistical analysis.** Typical Pearson's Chi-square test without Yates' correction or Fisher's exact test was used to compare the distribution of the *MMP-8* genotypes between pterygium and non-terygium control groups. The associations between the *MMP-8* polymorphisms and pterygium risk were estimated by computing odds ratios (ORs) as well as their 95% confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounding factors when needed.

## Results

**Basic characteristics compared between pterygium patients and non-terygium control group.** The frequency distributions

Table II. Distribution of matrix metalloproteinase-8 (MMP-8) genotypic frequencies among the pterygium and the control subjects.

	Cases (%)	Controls (%)	Adjusted OR (95%CI) <sup>a</sup>	p-Value <sup>b</sup>
C-799T				
CC (wildtype)	74 (55.2)	138 (51.5)	1.00 (reference)	
CT	48 (35.8)	102 (38.1)	0.83 (0.61-1.27)	0.5645
TT	12 (9.0)	28 (10.4)	0.78 (0.39-1.54)	0.5484
CT+TT	60 (44.8)	130 (48.5)	0.83 (0.59-1.31)	0.4799
<i>P</i> <sub>trend</sub>				0.7574
Lys460Thr				
AA (wildtype)	130 (97.0)	265 (98.9)	1.00 (reference)	
AC	4 (3.0)	3 (1.1)	2.14 (0.45-9.36)	0.1776
CC	0 (0.0)	0 (0.0)	--	
Val436Ala				
TT (wildtype)	134 (100.0)	268 (100.0)	1.00 (reference)	
CT	0 (0.0)	0 (0.0)	--	
CC	0 (0.0)	0 (0.0)	--	

OR: Odds ratio; CI: confidence interval. <sup>a</sup>Data adjusted with confounding factors including age and gender. <sup>b</sup>Based on Chi-square test without Yates' correction or Fisher's exact test; \* $p < 0.05$ .

of the age and gender for 134 pterygium patients and 268 non-ptyerygium healthy controls are summarized and compared in Table I. The percentage of males was 58.2%. No difference was found between the two groups as for age and gender since we have matched them very well ( $p > 0.05$ ) (Table I).

*Association analysis of MMP-8 genotypes at -799C/T, Val436Ala and Lys460Thr with pterygium risk.* The distribution of genetic frequencies as for the MMP-8 polymorphisms for the pterygium patients and controls are presented and compared in Table II. First, there were noticeably no CT or CC genotypes at MMP-8 Val436Ala among either the cases or the controls (Table II, lowest part). That is to say, all the Taiwanese people investigated in this study had the MMP-8 Val436Ala TT genotype (Table II, lowest part). Second, the ORs with adjusting those possible confounding factors (age and gender) for the people carrying variant CT and TT genotypes at MMP-8 promoter C-799T were 0.83 (95%CI=0.61-1.27,  $p=0.5645$ ) and 0.78 (95%CI=0.39-1.54,  $p=0.5484$ ) respectively, compared to those carrying the CC wild-type genotype (Table II, upper part). The  $p$ -value for trend was not significant ( $p=0.7574$ ) (Table II). In the dominant model (CT plus TT versus CC), the association between MMP-8 promoter -799C/T polymorphism and the risk for pterygium was still not statistically significant (adjusted OR=0.83, 95%CI=0.59-1.31,  $p=0.4799$ ) (Table II, upper part). Last, a very small percentage of Taiwanese people carried the heterozygous variant AC genotype at MMP-8 Lys460Thr (3.0% and 1.1% in pterygium and control groups, respectively) and no association was found between MMP-8 Lys460Thr AC

genotypes and the risk for pterygium (adjusted OR=2.14, 95%CI=0.45-9.36,  $p=0.1776$ ) (Table II, medium part).

*Association of MMP-8 allelic frequencies at -799C/T, Val436Ala and Lys460Thr and pterygium risk.* Consistent with the findings in Table II, there is no differential distribution of allelic frequencies between the pterygium and control groups as for the MMP-8 promoter C-799T or Lys460Thr (Table III, upper part). In detail, the adjusted OR for the subjects carrying the T allele at MMP-8 promoter C-799T was 0.85 (95%CI=0.61-1.33,  $p=0.4398$ ), compared to those carrying the C wild-type allele (Table III, upper part). As for the allelic frequencies at MMP-8 Val436Ala and Lys460Thr polymorphic sites, there was no association between their genotypes and increased risk of pterygium (Table III, medium and lower parts).

## Discussion

MMPs play an important role in the homeostasis of extracellular matrix components, and any imbalance of the extracellular microenvironment is related to the initiation and progression of cancer. For instance, Yang and his colleagues detected the mRNA expression levels and protein activities in various stages of surgically excised pterygium specimens and cultured pterygium fibroblasts. In that report, they also investigated normal conjunctiva specimens and fibroblasts as controls for comparison. The results showed that the MMP-2 and MMP-9 expression levels in pterygium tissues and fibroblasts were higher than those of normal tissues and fibroblasts and closely relevant to the progression of pterygium. Also, in early-stage pterygium tissues and

Table III. Allelic frequencies for matrix metalloproteinase-8 (*MMP-8*) polymorphisms among the pterygium and control subjects.

Polymorphic Allele	Cases (%) N=268	Controls (%) N=536	Adjusted OR (95%CI) <sup>a</sup>	p-Value <sup>b</sup>
C-799T				
Allele C	196 (73.1)	378 (70.5)	1.00 (reference)	0.4398
Allele T	72 (26.9)	158 (29.5)	0.85 (0.61-1.33)	
Lys460Thr				
Allele A	264 (98.5)	533 (99.4)	1.00 (reference)	0.1795
Allele C	4 (1.5)	3 (0.6)	2.63 (0.44-8.71)	
Val436Ala				
Allele T	268 (100.0)	536 (100.0)	1.00 (reference)	--
Allele C	0 (0.0)	0 (0.0)	--	

OR: Odds ratio; CI: confidence interval. <sup>a</sup>Data adjusted with confounding factors including age and gender. <sup>b</sup>Based on Chi-square test without Yates' correction or Fisher's exact test; \**p*<0.05.

cultured fibroblasts, MMP-2 or MMP-9 was at low or undetectable levels, while in advanced-stage pterygium (pterygium head passed the papillary region), MMP-2 and MMP-9 could be obviously detected in pterygium tissues and fibroblasts (16). In addition, down-regulation of the expression levels of MMP-3 and MMP-13 was capable of suppression of the proliferation and migration of pterygium fibroblasts (24, 25). These findings support the idea that MMPs may play an important role in the progression of pterygium, and the genomic markers on *MMPs* can serve as valuable predictive biomarkers for the development or reoccurrence of pterygium. In the literature, the genotypes of SNPs at promoter region of many *MMP* genes were found to be associated with the risk of several types of cancer (7-13). However, no paper has investigated the role of polymorphisms of *MMPs* as risk factors for pterygium.

Mounting evidence supported the idea that *MMP-8* may play a critical role in the development of cancer. First, *MMP-8* was found to be highly expressed in Jurkat T leukemia cells, which was closely related to the suppression of invasiveness in T cells (26). Second, the mutant mice deficient in *MMP-8* were more susceptible to develop skin cancer (27, 28). From the viewpoint of a genomic study, some positive epidemiological findings reported that genotypes of promoter region of other *MMPs* such as *MMP-1* and *MMP-9*, may serve as promising markers for the prediction of lung cancer susceptibility and prognosis (29-31). From the molecular viewpoint, *MMP-8* might increase cell adhesion by rearrangement of cytoskeleton actin, thus decreasing cell invasion (32). However, there were few epidemiological studies investigating the role of *MMP-8* polymorphisms in the susceptibility of cancers (33). In 2008, the G variant allele at *MMP-8* +17 C/G was found to be

associated with a decreased risk of developing lung cancer (33). Thereafter in the present study, we firstly focused on the genotypes of *MMP-8* polymorphisms and assessed whether there was an association between the genotypes of the promoter region of *MMP-8* (-799C/T) and two nonsynonymous polymorphisms (Val436Ala and Lys460Thr) with pterygium risk. The results showed that no significant association was observed and our findings suggest that these three *MMP-8* polymorphisms may not play a critical role in mediating susceptibility to pterygium (Tables II and III). Furthermore, when the analyses were stratified by age and gender, no significant association between these genotypes and pterygium risk was observed (data not shown). The negative findings of *MMP-8* genotypes associated with pterygium, lung cancer (22) and oral cancer (23) hinted that *MMP-8* may influence personal susceptibility to these diseases *via* other mechanisms such as regulation of protein-protein interaction, but not simply from the level of genetic polymorphism.

There were several limitations of the study. First, the sample size is not as representative as those in common diseases such as cancers. The validation using larger sample sizes in multiple centers and populations is needed. Second, we did not perform the phenotypic measurements of *MMP-8* transcripts, protein level and activity among the patients and controls. Third, we did not stratify the pterygium population into subgroups according to their clinical characters, such as severity. There were several classification systems for pterygium. For instance, based on the relative translucency of the body, pterygium could be separated into atrophic, intermediate and fleshy types. It is expectable that the complete correlation of patient status, genotype and phenotype would be very helpful to understand the role of *MMP-8* in the development of pterygium.

In conclusion, this is the first study to examine the association of genetic polymorphisms of *MMP-8* with pterygium. Our results suggest that neither the genotypes of the promoter region C-799T nor nonsynonymous Val436Ala and Lys460Thr at *MMP-8* was significantly conferring susceptibility to pterygium. Further studies elucidating the contribution of the genotypes of other members of *MMPs* to pterygium development will be helpful and the negative findings in this study need to be validated in other populations.

## Conflicts of Interest

All the Authors declare no conflicts of interest.

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