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Expression of matrix metalloproteinase 2 and 9 and tissue inhibitor of metalloproteinase 1 in nonrecurrent vs recurrent nasal polyps

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ABSTRACT

Background: Matrix metalloproteinases (MMPs) 2 and 9 are a group of Zn²⁺-dependent endopeptidases to remodel the extracellular matrix. The association of these 2 endopeptidases with the development of morphologic changes in nasal polyps was previously described.

Objective: To determine whether MMP-2, MMP-9, and tissue inhibitor of MMP-1 (TIMP-1) play a role in the recurrence of nasal polyps.

Methods: To compare MMP-2, MMP-9, and TIMP-1 expression in recurrent vs nonrecurrent polyps, nasal polyps (NPs) and recurrent nasal polyps (RNPs) were obtained from 15 NP patients with chronic rhinosinusitis (CRS) undergoing endoscopic sinus surgery (ESS) and 15 RNP patients with CRS undergoing revision ESS. Fifteen specimens of inferior turbinate mucosa from patients undergoing nasal septal surgery were used as control. Through real-time polymerase chain reaction and immunohistochemistry, MMP-2, MMP-9, and TIMP-1 expressions were measured among controls, NP patients, and RNP patients.

Results: Expression of MMP-9 messenger RNA in the NP patients was significantly higher than in the controls. In addition, expression of MMP-9 messenger RNA in the RNP patients was significantly increased compared with NP patients. With immunohistochemistry, a more increased expression of MMP-9 was observed in NP patients than controls. Expression of MMP-9 in RNP patients was also significantly high compared with NP patients, particularly in stroma.

Conclusion: Expression of MMP-9 is increased in NP patients, and it is so more in the mucosa of RNP patients. Increased expression of MMP-9 is particularly found in the stroma of RNP patients, and it may contribute to the recurrence of NP.

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Introduction

Chronic rhinosinusitis (CRS) is one of the most common chronic diseases.¹ In addition to the economic burden associated with this disease, CRS has a considerable effect on patients' quality of life.² Nasal polyps (NPs) are common, abnormal lesions that originate from any portion of the nasal mucosa or paranasal sinuses, and they are considered a subgroup of CRS.^{3–5} CRS with NPs with recurrence potential is considered a more severe form of the disease and tends to be refractory to both medical and surgical interventions.⁴ Recurrence of polyposis is more common in patients with asthma, aspirin-exacerbated respiratory disease, and cystic

fibrosis.⁵ We have encountered recurrences in some patients without these diseases, and there is currently no measure to predict which cases are prone to recurrence after polypectomy.

The pathophysiology of NPs remains largely unclear. To date, no known pathway clearly explains the progression from insult to tissue change. The current understanding is limited to the interplay among different inflammatory promoters and pathways. Currently, the link between elevated cytokine and chemokine levels and the development of edema and extracellular matrix alterations are topic of discussion with regard to NP pathophysiology.^{5–7}

Previous research revealed that matrix metalloproteinases (MMPs) play a major role in normal physiologic tissue remodeling, inflammation, and tumor spread. MMPs are a group of Zn²⁺-dependent endopeptidases with at least 23 members that are able to degrade nearly every component of the extracellular matrix.⁸ Active forms of MMP-2 and MMP-9 share the ability to cleave type IV collagen. The extracellular activity of MMPs is regulated by tissue inhibitors of matrix metalloproteinases (TIMPs). Several studies noted increased MMP-2 or MMP-9 expression in NPs, and

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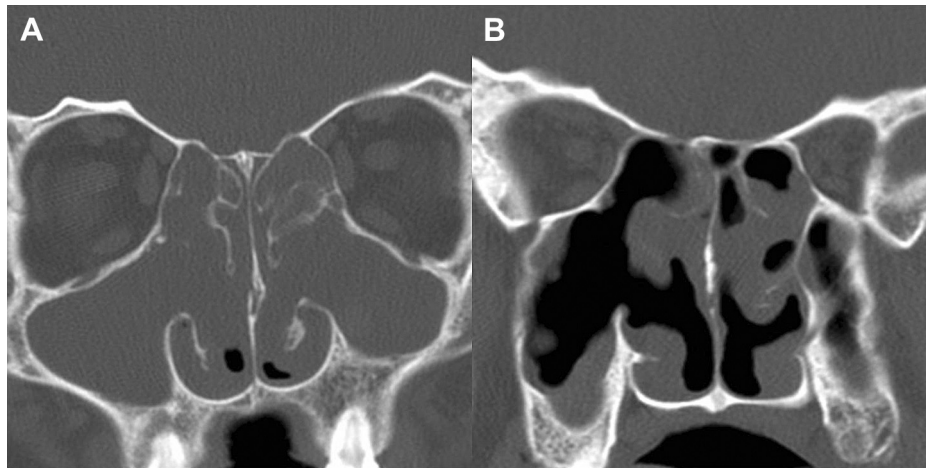


Figure 1. Paranasal sinus computed tomographic (CT) scans of patients in the recurrent nasal polyp group. (A) Coronal CT scans of the paranasal sinuses in a 24-year-old man reveal that his previous surgery had been completely performed but rhinosinusitis with nasal polyps recurred in his maxillary and ethmoid sinuses. (B) The other coronal CT scans of the paranasal sinuses in a 37-year-old woman also reveal recurrent nasal polyps in both sphenothymoidal recesses after previous endoscopic sinus surgery.

most of them indicated that there may also be significant differences in MMP and TIMP expression between patients with CRS without NPs and CRS with NPs.^{9–12} However, there is a paucity of research addressing differences in the expression of MMPs and TIMPs in patients with NPs and recurrent nasal polyps (RNPs). Therefore, we investigated differences in the expression of MMP-2, MMP-9, and TIMP-1 in the nasal tissues of patients with NPs and RNPs to determine whether these proteins may play a role in NP recurrence.

Methods

Patients and Study Design

The study group consisted of 45 participants (32 male and 13 female) aged 15 to 64 years who underwent functional endoscopic sinus surgery (ESS) or septoplasty by a single surgeon (N.K.Y.) at the Department of Oto-Rhino-Laryngology, Gangneung Asan Hospital. Fifteen patients had CRS with NPs, 15 patients had CRS with RNP, and 15 were controls. At screening, the diagnosis of sinus diseases was based on history, clinical examination, nasal endoscopy, and computed tomography (CT) of the sinuses according to the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 guidelines.¹³ None of the study participants had any history of malignancy, cystic fibrosis, or ciliary dyskinesia. We excluded patients with allergic symptoms, such as sneezing and nasal itching. Each patient had a negative response to the skin test, the *in vitro* test, or the multiple allergen simultaneous tests (AdvanSure Allergy Screen; LG Life Science, Seoul, Korea). The panel of allergens used in skin testing included molds (*Alternaria alternata* and *Penicillium notatum*), pollens (tree mix, grass mix, and ragweed), dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), cats, dogs, and cockroaches. A positive skin test response was defined by a wheal of 3 mm or greater than that produced by the 0.1% histamine positive control. Specific IgE antibodies in serum included a multiallergen panel, mold profile, weed profile, tree profile, grass profile, dust/mite profile, and dog and cat dander. Allergen specific IgE levels greater than 0.70 IU/mL were considered positive results. Patients with total IgE levels greater than 100 IU/mL were excluded. We also excluded patients with asthma or aspirin hypersensitivity based on their medical histories in addition to those patients with a forced expiratory volume in 1 second less than 80% by spirometry.

Patients with CRS with NPs (NP group) were diagnosed as having NPs in our clinic for the first time in their lives, and they had

no history of nasal surgery. Patients with CRS with RNP (RNP group) had undergone ESS more than once a minimum of 1 year in the past and had symptom-free periods of at least 6 months. They were checked as to whether their previous surgery had been completely performed by paranasal sinus CT scans (Fig 1). All patients were asked to stop taking oral corticosteroids for at least 2 months and topical medications for at least 4 weeks before surgery.

Before surgery, rhinosinusitis symptoms were evaluated using the Sinonasal Outcomes Test 20 (SNOT-20),¹⁴ and polyps were graded by size and extent in both the left and right nasal fossa on a scale of 0 to 3.¹³ CT scans were graded as described by Lund-Mackay.¹⁵ Samples of NPs were obtained from patients in both the NP and RNP groups. Inferior turbinate samples were obtained from patients without sinus disease undergoing septoplasty or rhinoseptoplasty. The study was approved by the Ethics Committee on Human Experimentation at Gangneung Asan Hospital (grant 2011-11), and written informed consent was obtained from all patients before collecting any samples for experimental purposes.

Tissue Preparation

NPs or inferior turbinate mucosae were divided into 2 parts. One part was immediately flash frozen in liquid nitrogen and stored at -70°C until RNA extraction or real-time polymerase chain reaction (PCR). The second part was fixed in buffered formalin and embedded in paraffin for conventional histopathologic examination and immunohistochemistry.

RNA Isolation and Complementary DNA Synthesis

All tissue samples were obtained from surgical specimens and immediately frozen at -70°C in a deep freezer. Total RNA was isolated with an RNeasy Plus Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The RNA concentration was determined by measuring absorbance at 260/280 nm using a BioSpec-nano spectrophotometer (Shimadzu, Kyoto, Japan). Complementary DNA (cDNA) was generated using a QuantiTect reverse transcription kit (Qiagen GmbH). The reactions were incubated at 42°C for 2 minutes, followed by 42°C for 15 minutes and 95°C for 3 minutes. The cDNA was stored at -70°C until further use.

Real-Time PCR Analysis

Real-time PCR was performed using 100 ng of cDNA with the SYBR Green fluorescent dye (Rotor-Gene SYBR Green PCR Kit;

Table 1

Sequences of real-time polymerase chain reaction primers used to amplify messenger RNA for MMP-2, MMP-9, TIMP-1, and β -actin

Gene	Forward primer	Reverse primer
MMP-2	5'-GCGACAAGAAGTATGGCTTC-3'	5'-TGCCAAGGTCAATGTCAGGA-3'
MMP-9	5'-CGCAGACATCGTCATCCAGT-3'	5'-GGATTGGCCTTGAAGATGA-3'
TIMP-1	5'-CAATTCCGACCTCGTCATCA-3'	5'-TCAGAGCCTTGGAGGAGCT-3'
β -actin	5'-CACCTTCTACAATGAGCTGC-3'	5'-AGGCAGCTCGTAGCTCTTCT-3'

Qiagen) to monitor DNA synthesis using specific primers. Primers for MMP-2, MMP-9, TIMP-1, and β -actin were designed using Beacon Designer 2.1 software (Bio-Rad, Hercules, California). Primer-set details are given in Table 1.^{16,17} To confirm the quality of individual primer pairs, we verified that detectable primer-dimers and nonspecific products were absent by performing conventional PCR before real-time PCR analyses.

The PCR was performed in a Rotor-gene Q system (Qiagen) using the following cycling protocol: a 95°C denaturation step for 5 minutes followed by 40 cycles of 95°C denaturation (5 seconds), 60°C annealing (10 seconds), and 72°C extension (10 seconds). Gene expression was normalized to the housekeeping gene β -actin. Subsequently, the samples were separated on 1% agarose gels and stained with ethidium bromide. A single narrow peak was obtained for each PCR product by melting curve analysis at the expected temperatures, and only a single band of the predicted size was observed after agarose gel electrophoresis, indicating specific amplification without significant byproducts. The amplified PCR product was analyzed with Rotor-gene Q software (Qiagen). The relative expression of MMP-2, MMP-9, and TIMP-1 messenger RNA (mRNA) was calculated: (cytokine concentration/ β -actin concentration) $\times$ 100.

Immunohistochemistry of MMP-2, MMP-9 and TIMP-1

All immunostaining of formalin-fixed, paraffin-embedded tissue sections was performed using the Bond-Max automatic immunostaining device (Leica Biosystems, Newcastle, United Kingdom) and a bond polymer intensity detection kit (Leica Biosystem). Five-micrometer-thick sections were obtained using a microtome, transferred onto adhesive slides, and dried at 62°C for 30 minutes. Antigen retrieval was performed in all cases (Table 2). Slides were counterstained with Harris hematoxylin. Breast cancer samples known to produce MMP-2, MMP-9, and TIMP-1 served as positive controls. Negative controls were obtained by omitting the primary antibody and replacing it with an irrelevant antibody of similar isotype.

The degree of immunostaining was reviewed and scored by a pathologist (D.W.E.) who was blinded to patient information. Scores were determined by combining the proportion of positively stained area and the staining intensity in the epithelium and stroma. Positive areas were scored based on the percentage of positive cells as follows: 0 (<5% positive cells), 1 (5%–25% positive cells), 2 (26%–50% positive cells), 3 (50%–75% positive cells), and 4 (>75% positive cells). Staining intensity was graded according to the following standard: 0 (no staining), 1 (weak staining, yellow

Table 2

Antibodies used for the study

Antibody	Source	Clone No.	Dilution	Antigen retrieval
TIMP-1	Leica Biosystem	6F6a	1:200	Heat (EDTA), 20 min
MMP-2	Leica Biosystem	17B11	1:150	Heat (EDTA), 20 min
MMP-9	Leica Biosystem	15W2	1:200	Heat (EDTA), 15 min

Abbreviations: MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase.

Table 3

Patient characteristics and symptom scores

Variable	Control group (n = 15)	NP group (n = 15)	RNP group (n = 15)	P value ^a
Age, mean (range), y	32.2 (19–67)	38.7 (19–64)	42.2 (18–63)	NS
Female/male, No.	5/11	4/11	4/11	NS ^b
Allergy, No. ^c	0/15	0/15	0/15	
Asthma, No. ^c	0/15	0/15	0/15	
Smoking, No.	5/15	3/15	5/15	NS ^b
Duration of symptoms, mean (range), y				
SNOT score, mean (range) ^d	22.4 (10–34)	27.7 (14–50)	29.3 (16–53)	0.044, ^e 0.032, ^f NS ^g
Bilateral polyp score, mean (range)		3.8 (2–6)	3.8 (2–6)	NS ^g
Bilateral CT score, mean (range)		14.6 (6–23)	17.5 (15–24)	NS ^g

Abbreviations: CT, computed tomography; MMP, matrix metalloproteinase; NP, nasal polyp; NS, nonsignificant; RNP, recurrent nasal polyp; SNOT-20, Sinusitis Outcomes Test 20; TIMP, tissue inhibitor of matrix metalloproteinase.

^aP values were obtained by analysis of variance.

^bFisher exact test. $P < .05$ was considered statistically significant.

^cWe deliberately excluded patients with allergy and asthma to eliminate the effect of them on the expression of MMP and TIMP.

^dSNOT score is the sum of the score composed 20 items to evaluate patients' physical and functional impairment related nasal symptoms. Each item is graded on a scale of 0 to 5. Final score of SNOT ranges from 1 to 100.

^eControl vs NP group.

^fControl vs RNP group.

^gNP vs RNP group.

brown), 2 (moderate staining, brown), and 3 (strong staining, dark brown). Final scores ranged from 0 to 12 and were calculated for the epithelium and stroma as the product of the staining intensity score and the area score. The sum of the epithelium and stroma scores yielded a total score ranging from 0 to 24.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software, version 19.0, for Windows (SPSS Inc, Chicago, Illinois). The nonparametric Kruskal-Wallis test or Mann-Whitney test was used for unpaired comparisons of relative MMP and TIMP expression. For comparisons between groups, the Kruskal-Wallis test was used to establish the significance of intergroup variability, and the Mann-Whitney test was then used for between-group comparisons. Baseline variables were analyzed by 1-way analysis of variance or the Fisher exact test. Statistical significance was set at $P < .05$.

Results

Patient Characteristics

Table 3 lists the demographic and clinical characteristics of the 3 groups of individuals enrolled in the study. The groups were similar with regard to demographic characteristics but had significant differences in SNOT-20 scores. Higher SNOT-20 scores reflect more severe nasal symptoms and worse quality of life for patients. SNOT-20 scores were significantly higher in the NP and RNP groups than in controls, but no difference was found between the NP and RNP groups. Bilateral endoscopic and CT scores did not differ significantly between the NP and RNP groups either.

Relative Expression of MMP-2, MMP-9, and TIMP-1 mRNA

MMP-2 mRNA was detected in all samples from the control, NP, and RNP groups. The mean (SD) relative expression values of MMP-2 mRNA in the control, NP, and RNP groups were 0.74 (0.08), 0.78 (0.53), and 0.99 (2.9), respectively. Statistical analysis revealed no significant difference between the relative expression of MMP-2 mRNA in samples from the control, NP, and RNP groups (Fig 2A).

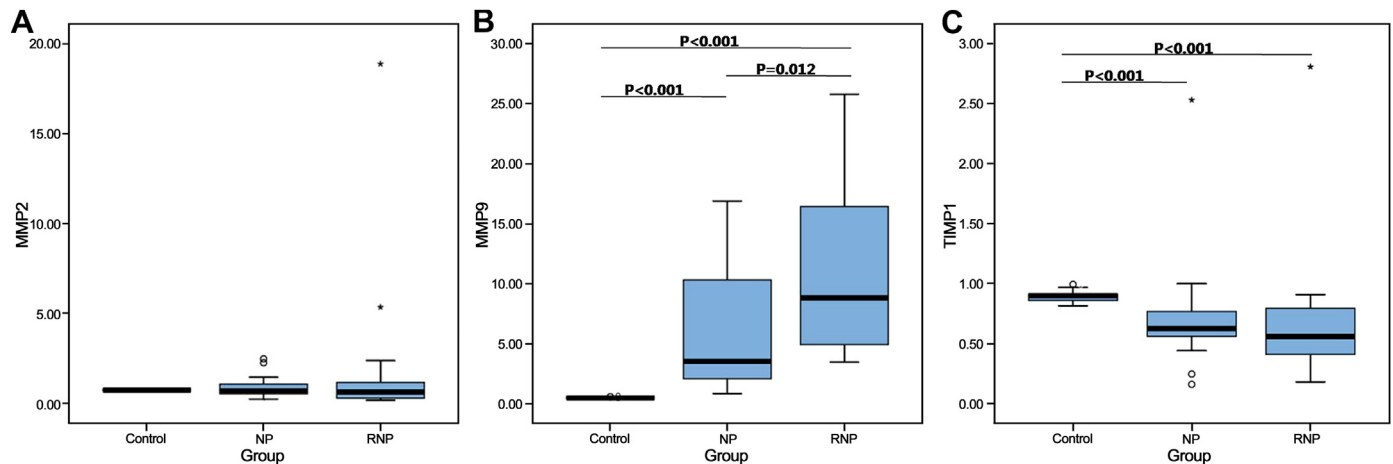


Figure 2. Comparison of relative expression of matrix metalloproteinase (MMP) 2 (A), MMP-9 (B), and tissue inhibitor of matrix metalloproteinase (TIMP) 1 (C) messenger RNA in the control, nasal polyp (NP), and recurrent nasal polyp (RNP) groups.

MMP-9 mRNA was also detected in all samples from the 3 study groups. The mean (SD) relative expression of MMP-9 mRNA in the control group was 0.51 (0.4). MMP-9 mRNA was more highly expressed in the NP (mean [SD] relative expression, 3.56 [4.71]) and RNP (9.00 [10.53]) groups compared with the control group ($P < .001$). Furthermore, MMP-9 mRNA expression was significantly higher in samples from patients with RNP than in samples from patients with NP ($P = .01$) (Fig 2B). TIMP-1 mRNA was also detectable in all examined tissue samples with mean (SD) relative expression values of 0.88 (0.12), 0.62 (0.51), and 0.55 (0.61) in the control, NP, and RNP groups, respectively. TIMP-1 mRNA expression was significantly lower in samples from patients with NP and RNP compared with controls ($P < .001$). However, no difference was found in the relative expression of TIMP-1 mRNA between the NP and RNP groups ($P = .44$) (Fig 2C).

Immunohistochemistry of MMP-2, MMP-9, and TIMP-1

Staining for MMP-9 was stronger in the surface epithelium than in the stroma, whereas MMP-2 and TIMP-1 staining was observed primarily in the stroma (Fig 3). In addition, MMP-2 and TIMP-1 stained more weakly than MMP-9, with TIMP-1 staining being the weakest and most heterogeneous. A comparison of immunostaining scores among the 3 groups revealed that the total MMP-9 score was much higher in the NP and RNP groups than in the controls (Table 4), and this was also true of the MMP-9 staining score for the epithelium. In comparing NP and RNP tissues, MMP-9 was found to be more highly expressed in the RNP group than in the NP group in the stroma. MMP-2 and TIMP-1 staining did not differ among the 3 groups (Table 4).

Discussion

In this study, we demonstrated that expression of MMP-9 was significantly higher in the tissue samples from patients with NP compared with controls and expression of MMP-9 in patients with RNP was significantly increased compared with NP patients. Interestingly, the higher expression noted in NP patients was associated with the epithelium, whereas that in the RNP group was observed in both the epithelium and stroma.

Because MMPs are key enzymes responsible for extracellular matrix degradation and contribute to progressive histologic changes, several researchers have studied the relationship between MMPs and NPs. Most studies agree that MMP-9 is likely to be associated with airway remodeling in nasal polyposis based on the increased expression of MMP-9 in NP patients compared with

controls.^{3,10–12,18,19} In contrast, the relevance of MMP-2 and TIMP-1 in NP patients is controversial in the literature. The presence of MMP-2 in NP patients was reported in several studies, but only Bhandari et al²⁰ detected higher MMP-2 mRNA expression in NPs from patients with mucopurulent discharge compared with inferior turbinate samples.²⁰ Other recent studies found no significant difference in MMP-2 mRNA or protein expression between NP and inferior turbinate samples.^{3,12,18} The pattern of TIMP-1 expression in NP patients is also unclear. Some studies noted that the TIMP-1 expression was significantly lower in NP patients compared with patients with maxillary sinus mucosa or concha bullosa mucosa.^{10,11} However, others reported that TIMP-1 expression did not differ significantly between patients with NPs and inferior turbinate mucosa.^{3,12}

As in previous studies, we found no difference in MMP-2 mRNA expression and elevated MMP-9 mRNA expression in NP compared with inferior turbinate samples via real-time PCR with immunohistochemical confirmation. TIMP-1 mRNA was significantly lower in NP compared with IT samples, but TIMP-1 expression on immunohistochemistry was not different between the 2 groups. This result was likely due to the very weak staining of TIMP-1 on immunohistochemistry.

Remarkably, we found that expression of MMP-9 in RNP patients was significantly higher than that in NP patients. To date, little is known about the expression of MMPs and TIMPs associated with tissue remodeling in RNPs. Few studies have investigated the relationship between growth factor expression and polyp recurrence. Cayé-Thomasen et al^{21,22} studied the expression of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) in NP vs RNP patients using a masked semi-quantification of tissue staining intensity method. They hypothesized that bFGF and VEGF might be associated with an increased risk of NP recurrence via their functions in angiogenesis and proliferation of stromal and epithelial tissue components; however, they concluded that the expression of bFGF and VEGF in NP and RNP patients was equivalent. In another study, Kösem et al⁴ found a significantly higher Ki-67 index in RNP patients compared with NP patients via immunohistochemistry, which is consistent with epithelial cell proliferation. Wang et al^{18,23} recently compared the expression of MMP-2 and MMP-9 between NP and RNP by immunohistochemistry. They found that the expression of MMP-2 and MMP-9 was not associated with the severity of CRS with NP and that there was no difference in the immunohistochemical labeling index of MMP-2 and MMP-9 between NP and RNP patients. In contrast, our study found that expression of MMP-9 was

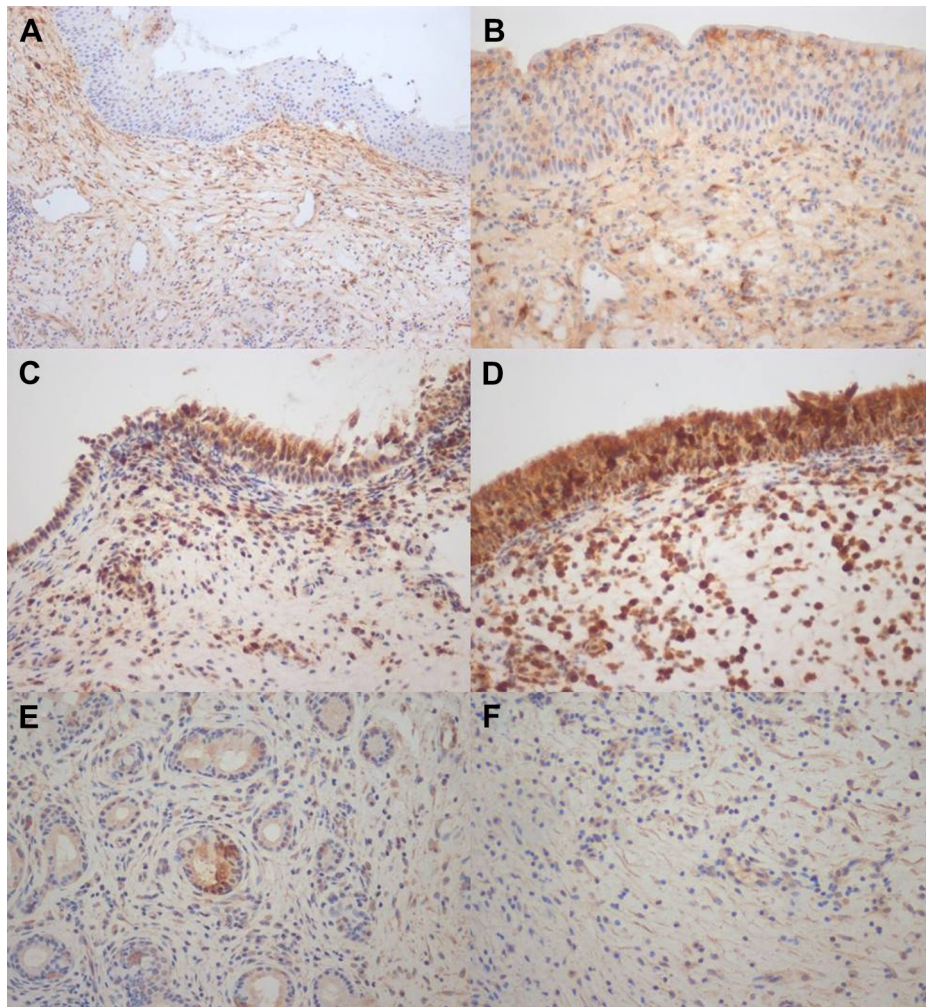


Figure 3. Representative matrix metalloproteinase (MMP) 2 (A and B), MMP-9 (C and D), and tissue inhibitor of matrix metalloproteinase (TIMP) 1 (E and F) immunohistochemistry staining in nasal polyp (NP) and recurrent nasal polyp (RNP) mucosa (original magnification $\times 200$).

significantly higher in RNP compared with NP patients. The difference in results may be due to methodologic differences between our study and previous work. We compared the expression of MMP-2, MMP-9, and TIMP-1 in NP and RNP patients by real-time PCR and immunohistochemistry. In addition, we excluded

patients with allergies and asthma to ensure we were evaluating the effect of MMP-2, MMP-9, and TIMP-1 only on the pathogenesis of NPs and recurrence. Lastly, we applied strict standards for patients' history and paranasal sinus CT analysis for selection of the RNP group. It is likely that the differences in expression of specific MMPs and TIMPs lead to specific differences in tissue remodeling in patients with NPs and RNPs.

Increased MMP-9 expression in patients with RNPs compared with those with NPs and controls was noted in the stroma, whereas increased MMP-9 expression in patients with NPs compared with controls was noted in the epithelium. Although the cellular mechanisms underlying the development of NPs remain uncertain, polyp formation results from the protrusion of connective tissue through an initial epithelial defect and remodeling.¹⁸ MMP-9 preferentially degrades gelatin, elastin, aggrecan, and collagens, and it has been suggested that this enzyme is likely to be associated with airway remodeling in nasal polyposis.^{9,12,24,25} MMP-9 could facilitate epithelial and endothelial cell migration observed during polyp development and growth, and it could also increase vascular permeability, leading to airway edema and inflammatory cell transmigration. Our results imply that increased expression of MMP-9 in the stroma induces the recurrence of these tissue remodeling processes and polyp formation. It is likely that the overall effect of MMP-9 in polyp formation is governed by the levels of TIMP-1, which binds pro-MMP-9 and thus limits the activity of

Table 4

Mean (SD) immunostaining scores for MMP-2, MMP-9, and TIMP-1

Variable	Control group	NP group	RNP group	P value ^a
MMP-2				
Epithelium	0.40 (0.91)	1.00 (2.11)	2.10 (2.11)	NS
Stroma	0.60 (0.95)	2.66 (2.32)	3.03 (3.17)	NS
Total	1.20 (1.11)	3.10 (3.06)	4.20 (4.18)	NS
MMP-9				
Epithelium	2.25 (3.33)	5.40 (3.42)	6.60 (3.45)	0.031, ^b 0.013, ^c NS ^d
Stroma	2.02 (1.82)	2.80 (1.69)	6.25 (3.01)	NS, ^b 0.001, ^c 0.032 ^d
Total	4.00 (4.03)	8.00 (3.58)	13.13 (5.12)	0.028, ^b 0.001, ^c 0.017 ^d
TIMP-1				
Epithelium	0.60 (0.50)	0.26 (0.45)	0.20 (0.34)	NS
Stroma	1.54 (3.15)	1.33 (1.29)	0.66 (1.04)	NS
Total	2.66 (3.03)	1.93 (1.48)	0.73 (1.03)	NS

Abbreviations: MMP, matrix metalloproteinase; NP, nasal polyp; NS, nonsignificant; RNP, recurrent nasal polyp; TIMP, tissue inhibitor of matrix metalloproteinase.

^aP values were obtained by the Kruskal-Wallis test and Mann-Whitney test.

^bControl vs NP group.

^cControl vs RNP group.

^dNP vs RNP group.

MMP-9. Although we observed decreased expression of TIMP-1 mRNA in NP patients compared with controls, we were not able to identify a pattern of TIMP-1 expression in RNP patients compared with NP patients. Our findings suggest a complex interaction among inflammatory cells, the respiratory epithelium, and endothelial cells to maintain a particular level extracellular matrix remodeling in NPs.

Our study had some limitations. First, because NP might recur even several years after surgery, an extended follow-up period in the NP group may have been appropriate. However, considering the similar ages of patients in the NP and RNP groups and the fact that patients in the RNP group had already undergone one or more ESSs due to RNPs, we are confident the patients were grouped appropriately. Second, this study might lack adequate power to detect meaningful differences because of the limited sample size. Third, we did not exclude allergic patients by nasal provocation tests or detection of local nasal IgE. Recently, increased levels of local IgE and positive reactivity on nasal allergen provocation tests have been demonstrated in nasal lavage fluid of patients with negative systemic allergy test results.^{26,27} To have completely controlled for the effect of allergies on the expression of MMP and TIMP, we would have had to exclude patients with positive tissue IgE and positive reactivity on nasal allergen provocation tests.

In conclusion, expression of MMP-9 was increased in NP and even more so in RNP mucosa. Elevated MMP-9 expression was detected in the stoma of RNP and may contribute to the recurrence of NP. Therefore, intensive manipulation of patients with elevated stromal expression of MMP-9 may serve as a suitable strategy to prevent additional unnecessary management of RNPs.

References

- [1] Anand VK. Epidemiology and economic impact of rhinosinusitis. *Ann Otol Rhinol Laryngol Suppl.* 2004;193:3–5.
- [2] Rudmik L, Smith TL. Quality of life in patients with chronic rhinosinusitis. *Curr Allergy Asthma Rep.* 2011;11:247–252.
- [3] Chen YS, Langhammer T, Westhofen M, Lorenzen J. Relationship between matrix metalloproteinases MMP-2, MMP-9, tissue inhibitor of matrix metalloproteinases-1 and IL-5, IL-8 in nasal polyps. *Allergy.* 2007;62:66–72.
- [4] Kosem M, Bulut G, Kaya Z. Analysis of Ki-67 immunoreactivity in recurring and nonrecurring nasal polyps. *J Otolaryngol Head Neck Surg.* 2010;39:464–467.
- [5] Georgy MS, Peters AT. Nasal polyps. *Allergy Asthma Proc.* 2012;33(suppl 1):S22–S23.
- [6] DeMarcantonio MA, Han JK. Nasal polyps: pathogenesis and treatment implications. *Otolaryngol Clin North Am.* 2011;44:685–695, ix.
- [7] Hsu J, Peters AT. Pathophysiology of chronic rhinosinusitis with nasal polyp. *Am J Rhinol Allergy.* 2011;25:285–290.
- [8] Shaida A, Kenyon G, Devalia J, Davies RJ, MacDonald TT, Pender SL. Matrix metalloproteinases and their inhibitors in the nasal mucosa of patients with perennial allergic rhinitis. *J Allergy Clin Immunol.* 2001;108:791–796.
- [9] Watelet JB, Bachert C, Claeys C, Van Cauwenberge P. Matrix metalloproteinases MMP-7, MMP-9 and their tissue inhibitor TIMP-1: expression in chronic sinusitis vs nasal polyposis. *Allergy.* 2004;59:54–60.
- [10] Can IH, Ceylan K, Caydere M, Samim EE, Ustun H, Karasoy DS. The expression of MMP-2, MMP-7, MMP-9, and TIMP-1 in chronic rhinosinusitis and nasal polyposis. *Otolaryngol Head Neck Surg.* 2008;139:211–215.
- [11] Shin HW, Han DH, Lim YS, et al. Nonasthmatic nasal polyposis patients with allergy exhibit greater epithelial MMP positivity. *Otolaryngol Head Neck Surg.* 2009;141:442–447.
- [12] Li X, Meng J, Qiao X, et al. Expression of TGF, matrix metalloproteinases, and tissue inhibitors in Chinese chronic rhinosinusitis. *J Allergy Clin Immunol.* 2010;125:1061–1068.
- [13] Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol Suppl.* 2012;3:1–298.
- [14] Wabnitz DA, Nair S, Wormald PJ. Correlation between preoperative symptom scores, quality-of-life questionnaires, and staging with computed tomography in patients with chronic rhinosinusitis. *Am J Rhinol.* 2005;19:91–96.
- [15] Lund VJ, Kennedy DW. Staging for rhinosinusitis. *Otolaryngol Head Neck Surg.* 1997;117:S35–S40.
- [16] Kontinen YT, Ainola M, Valleala H, et al. Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. *Ann Rheum Dis.* 1999;58:691–697.
- [17] Ivanoff A, Ivanoff J, Hultenby K, Sundqvist KG. Infiltrative capacity of T leukemia cell lines: a distinct functional property coupled to expression of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1). *Clin Exp Metastasis.* 1999;17:695–711.
- [18] Wang LF, Chien CY, Chiang FY, Chai CY, Tai CF. Correlation between matrix metalloproteinase 2 and 9 expression and severity of chronic rhinosinusitis with nasal polyposis. *Am J Rhinol Allergy.* 2012;26:e1–e4.
- [19] Mudd PA, Katial RK, Alam R, Hohensee S, Ramakrishnan V, Kingdom TT. Variations in expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in nasal mucosa of aspirin-sensitive versus aspirin-tolerant patients with nasal polyposis. *Ann Allergy Asthma Immunol.* 2011;107:353–359.
- [20] Bhandari A, Takeuchi K, Suzuki S, et al. Increased expression of matrix metalloproteinase-2 in nasal polyps. *Acta Otolaryngol.* 2004;124:1165–1170.
- [21] Caye-Thomasen P, Larsen K, Tinggaard P, Tos M. Basic fibroblast growth factor expression in recurrent versus non-recurrent nasal polyposis. *Eur Arch Otorhinolaryngol.* 2004;261:321–325.
- [22] Caye-Thomasen P, Larsen K, Tinggaard P, Tos M. Immunohistochemical demonstration and semi-quantitation of vascular endothelial growth factor in recurrent versus non-recurrent nasal polyps. *Acta Otolaryngol.* 2004;124:706–711.
- [23] Wang LF, Chien CY, Chiang FY, Chai CY, Tai CF. Expression of matrix metalloproteinase-2 and matrix metalloproteinase-9 in recurrent chronic rhinosinusitis with nasal polyposis. *Kaohsiung J Med Sci.* 2013;29:26–31.
- [24] Kostamo K, Tervahartiala T, Sorsa T, Richardson M, Toskala E. Metalloproteinase function in chronic rhinosinusitis with nasal polyposis. *Laryngoscope.* 2007;117:638–643.
- [25] Leonardi A, Brun P, Di Stefano A, Motterle L, Abatangelo G. Matrix metalloproteinases in vernal keratoconjunctivitis, nasal polyps and allergic asthma. *Clin Exp Allergy.* 2007;37:872–879.
- [26] Wise SK, Ahn CN, Schlosser RJ. Localized immunoglobulin E expression in allergic rhinitis and nasal polyposis. *Curr Opin Otolaryngol Head Neck Surg.* 2009;17:216–222.
- [27] Rondón C, Campo P, Galindo L, et al. Prevalence and clinical relevance of local allergic rhinitis. *Allergy.* 2012;67:1282–1288.