Expression of *mammaglobins A* and *B* in nasal polyps is similar in patients with and without allergic rhinitis

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ABSTRACT

Background: *The causes of nasal polyposis remain unclear. Mammaglobins have been implicated in its pathogenesis. However, their association with the occurrence of nasal polyps in the presence of allergic rhinitis (AR) has not been explored. The aim of this study was to compare the expression levels of mammaglobins A and B with the nasal polyps of patients with and without AR.*

Methods: *Thirty-one patients with bilateral nasal polyposis underwent skin-prick tests to specific aeroallergens. Nasal polyp tissues were obtained from all patients and divided into two groups as nasal polyps with and without AR depending on clinical history and the skin-prick test results. All polyp tissues were analyzed for the levels of mammaglobin A and mammaglobin B by using real-time quantitative polymerase chain reaction technique.*

Results: *Of the 16 samples from patients having nasal polyps with AR, only 1 sample expressed a detectable level of mammaglobin A (1/16). There was no detectable expression of mammaglobin A in tissues from the group of nasal polyps without AR (0/15). Expression of mammaglobin B was detected in all nasal polyp tissues from both groups. The expression of mammaglobin B was not significantly different between nasal polyps with AR (median, 25th–75th percentiles; 0.023, 0.013–0.046) and nasal polyps without AR (0.032, 0.007–0.16).*

Conclusion: *Expression levels of mammaglobins A and B in nasal polyps are not different between patients with and without AR. Our findings suggest that mammaglobins' implication in the pathogenesis of nasal polyps is independent of an underlying AR.*

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Key words: Allergic rhinitis, etiology, gene expression, mammaglobin A, mammaglobin B, nasal polyps, pathogenesis, real-time quantitative polymerase chain reaction

Nasal polyposis is a chronic inflammatory disease of the nasal cavities. Treatment with intranasal corticosteroids remains the first line.[1] However, some patients do not respond to medical treatment requiring endoscopic sinus surgery and polypectomy. In addition, recurrence of nasal polyps after surgical removal is frequently seen.[2] Understanding of its molecular pathogenesis, therefore, may lead to a better preventive measure and a more effective treatment.

Although its definite causes have not been clearly identified, most studies support the role of inflammation and local immunologic imbalance in the development of nasal polyps.[3] With the recent advance in genetic techniques, *mammaglobins A* and *B* are the two that have been implicated.[4–6] The first study implicating *mammaglobin* in nasal polyposis was performed by Fritz *et al.* in 2003. They found that the most up-regulated gene in nasal polyps of patients with allergic rhinitis (AR) compared with nasal mucosa of patients with AR but without polyps was *mammaglobin 1* (or *mammaglobin A*).[5] Another study observed a similar trend showing that mammaglobin (type not specified) was modestly up-regulated in nasal polyps when comparing with normal sinus tissues.[6] Subsequently, Benson *et al.* showed that the expression of *mammaglobin B* in nasal polyps increased after glucocorticoid treatment.[4] However, they found that expression of *mammaglobin B* did not differ between untreated nasal polyps and healthy nasal mucosa.[4] Because the involvement of *mammaglobins* in the pathogenesis of nasal polyps still is inconclusive, additional studies of their association with the disease are justified.

Several studies have reported a lower incidence of nasal polyposis in patients with AR than in the general population.[2,7–10] However, other studies found the prevalence of atopy was similar to[11] or more in patients with nasal polyps.[12] Therefore, we hypothesize that the involvement of *mammaglobins* in the pathogenesis of nasal polyps may relate to the underlying AR. Specifically, the aim of this study was to compare the expression levels of *mammaglobins A* and *B* between nasal polyps with and without AR.

MATERIALS AND METHODS

Subjects

We included all patients presenting with bilateral nasal polyposis who entered the Department of Otolaryngology, King Chulalongkorn Memorial Hospital, and were willing to participate in the study. Written informed content was obtained from patients before their recruitment. The Ethic Committee of the Faculty of Medicine, Chulalongkorn University, approved the study. Treatment with antihistamines, intranasal corticosteroids, oral corticosteroids, and leukotriene receptor antagonists was discontinued for at least 2 weeks before undergoing skin-prick tests to specific aeroallergens and nasal polyp biopsy. The aeroallergen extracts included house dust,

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Table 1 **Primers and Taqman probe sequences**

Targets	Primers (P) or Taqman probes (T)	Sequences (5′-3′)
Mammaglobin A	P-SCGB2A2-F79	CTCATGCTGGCGGCCCTCTC
	P-SCGB2A2-R236	ATGGCATTTGTAGTGGCATTGTC
	T-SCGB2A2-P106	(FAM)-TGCTACGCAGGCTCTGGCTGCCC-(BHQ1)
Mammaglobin B	P-SCGB2A1-F261	TCCTCAACCAGTCACATAGAAC
	P-SCGB2A1-R380	CCCTCTGAGCCAAACGCCTT
GAPDH	P-GAPDH-F85	GTGAAGGTCGGAGTCAACGG
	P-GAPDH-R191	TCAATGAAGGGGTCATTGATGG
	T-GAPDH-P121	(HEX)-CGCCTGGTCACCAGGGCTGC-(BHQ1)
GAPDH	P-GAPDH-F6	GAAGGTGAAGGTCGGAGTC
	P-GAPDH-R231	GAAGATGGTGATGGGATTTC

FAM = 6-carboxyfluorescein; BHQ1 = black hole quencher; HEX = hexachloro-6-carboxy-fluorescein.

dust mites, cockroaches, cats, dogs, feathers, Bermuda grasses, Johnson grasses, and molds. A reaction was considered positive if the wheal was ≥3 mm in mean diameter with surrounding erythema. Patients who had clinical history of chronic persistent rhinitis and positive skin test responses were classified as nasal polyps with AR, and those patients who tested negative were classified as nasal polyps without AR. All patients underwent nasal polyp biopsies under local anesthetic and topical decongestant using standard nasal endoscopy with 4-mm rigid endoscope. All biopsied polyp tissues were analyzed for the level of *mammaglobin* expressions by using real-time quantitative polymerase chain reaction (RTQ-PCR) technique.

Analysis of Mammaglobin RNA Level

Total RNA was isolated from the biopsied tissues using the QIAamp RNA Blood Mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Total RNA was reverse transcribed into complementary DNA (cDNA) using ImProm-II reverse transcriptase (Promega, Madison, WI), according to the company recommendations. All RNA samples were stored at −70°C before use.

RTQ was performed using Roter-Gene 6000 (Corbett Robotics, Inc., Mortlake, NSW, Australia) according to the manufacturer's instructions. The TaqMan primer/hybridization probe real-time PCR approach was used to assay the expression level of *mammaglobin A*. The primers for all mRNA assays were intron spanning. The sequences of the amplification primers and TaqMan binding probes for *mammaglobin A* and *GAPDH* (P-GAPDH-F85, P-GAPDH-R191, and T-GAPDH-P121) are listed in Table 1. *GAPDH* was used as a control for normalization. *Mammaglobin A* RNA level was determined by Biotools QuantiMix Easy Probes Kit (Biotools, Madrid, Spain). The PCR reactions were set up according to the manufacturer's instructions in a reaction volume of 10 μL.

For *mammaglobin B* expression, SYBR Green I (10,000× concentration in DMSO; Molecular Probes, Eugene, OR) was used as the detection format. The sequences of the amplification primers for *mammaglobin B* and *GAPDH* (P-GAPDH-F6 and P-GAPDH-R231) are listed in Table 1. Amplification was performed in a total volume of 10 μL. A nontemplate control was run with every assay and each sample was assayed in triplicate.

A standard curve from the amplification data for each primer was generated using a dilution series of cDNA as templates. The expression levels of *mammaglobins* were normalized to the expression of GAPDH. All data were analyzed

Table 2 **Characteristics and expressions of *mammaglobins A* and *B* in polyps with and without AR**

	Polyps with AR	Polyps without AR	p Value
Sex (n)			
F	6	6	0.589
M	10	9	
Age (mean [SD], range [yr])	45.9 (13.7, 18–67)	40.9 (18.2, 21–71)	0.389
Number of subjects expressing *mammaglobin A*	1/16	0/15	NS
Number of subjects expressing *mammaglobin B*	16/16	15/15	NS
Level of *mammaglobin B* expression* (Median, 25th–75th percentiles)	0.023 (0.013–0.046)	0.032 (0.007–0.16)	0.984

**The level of mammaglobin B expression has no units because it is a relative amount to that of GAPDH*[13]

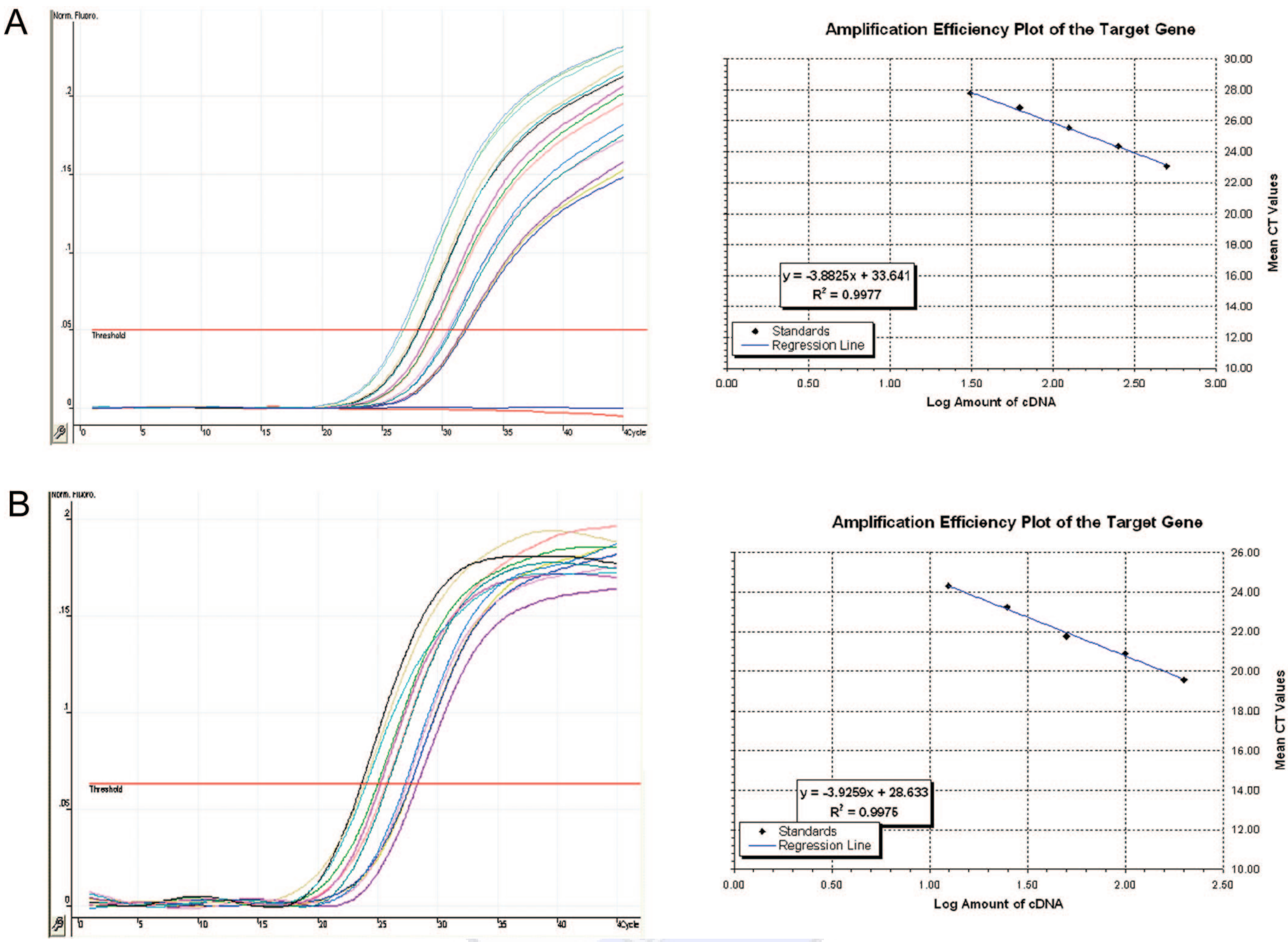

Figure 1. Quantitation of mammaglobins A and B mRNA-positive cells by real-time PCR in the Rotor Gene System. The left panel shows logarithmic plots of fluorescence signals during amplification. The right panel shows standard curves of RTQ-PCR of serial cDNA dilutions extracted from four samples. (A) Mammaglobin A; (B) mammaglobin B.

using Q-Gene software,[14] a widely used program for relative quantitation.

Statistical Analysis

The variables were the levels of *mammaglobin* expressions in nasal polyp tissues, sex, age, and number of subjects expressing *mammaglobins*. The differences of the expression levels of *mammaglobin B* between nasal polyps with and without AR were compared using the Mann-Whitney test. The levels of expressions were shown as median and interquartile range. Data for age, sex, and number of subjects expressing *mammaglobins* were analyzed using chi-square tests to compare the differences between two groups.

RESULTS

Thirty-one patients with bilateral nasal polyps were recruited. The skin-prick test results were positive in 16 patients (6 women, aged 18–67 years; average, 45.9 years) and negative in 15 patients (6 women, aged 21–71 years; average, 40.9 years; Table 2). Polyp tissues from the patients were analyzed for the expression levels of *mammaglobins A* and *B* by using the real-time PCR technique. *GAPDH* was used as a control for normalization. Of the 16 samples from patients having nasal polyps with AR, only one expressed a detectable level of *mammaglobin A* (expression level 0.023). None of the 15 samples from patients having nasal polyps without AR had a detectable level of *mammaglobin A*. Expression of *mammaglobin B* was detected in all nasal polyp tissues from both groups. The median normalized expression of *mammaglobin B* in tissues from nasal polyps with AR was 0.023 (0.013–0.046), and that from nasal polyps without AR was 0.032 (0.007–0.16). The expression levels of *mammaglobin B* between both groups were not statistically different ($p = 0.984$; Table 2).

DISCUSSION

Mammaglobins have been implicated in the pathogenesis of nasal polyps but their roles still are inconclusive. Some studies found that *mammaglobin A* was the most up-regulated in polyp mucosa compared with normal nasal mucosa,[5] and another showed that *mammaglobin* (type not specified) was only modestly up-regulated.[6] Interestingly, *mammaglobin B* was found in another study to be up-regulated only after

glucocorticoid treatment but its expression did not differ between untreated nasal polyps and healthy nasal mucosa.[4] We hypothesize that the discrepancy may relate to the presence of AR. Therefore, we compared the expressions of *mammaglobins A* and *B* in nasal polyps with and without AR.

We found that only one of 16 samples in the group of nasal polyps with AR expressed *mammaglobin A*, and none of the 15 samples in the group of polyps without AR expressed it. The finding that only one of 31 polyps expressed *mammaglobin A* was different from the study by Fritz *et al*.[5] They detected *mammaglobin A* protein in three of the five samples from nasal mucosa of patients with polyps but none in controls without nasal polyps.[5] The difference is unlikely to be caused by a technical problem of our study in quantitative assay by RTQ-PCR because we were able to detect its expression in one subject (Fig. 1 *A*). Instead, this could be partly due to differences in pathogenesis among different ethnics and geographic distributions. In addition, it could be from the difference in tissue samplings between the two studies. In our study, we performed biopsies at the polyps whereas in Fritz's study, the biopsies were done at the origin of the polyps. Nonetheless, our study supported that of Liu *et al.* and Benson *et al.*, which found no significant differences in expression of *mammaglobins* between untreated polyps and healthy nasal mucosa.[4,6]

With regard to *mammaglobin B*, we found that it was expressed in all 31 samples we studied. However, the expression levels were not significantly different between nasal polyps with and without AR. A previous study found that the mean expression levels of *mammaglobin B* in nasal polyps were lower but did not significantly differ when comparing with healthy nasal mucosa.[4] These results suggest that *mammaglobin B*, if involved in nasal polyps' pathogenesis, is not related to an underlying AR. The 2-week period of discontinuation of medications used in our study was used by some other groups[5] but may be considered short by others. Whether the similarity of the expression of *mammaglobins* in these two groups of patients found in this study relates to the period of medication washout needs additional investigation.

In conclusion, expressions of *mammaglobins A* and *B* are not different between nasal polyps with and without AR. Our findings suggest that *mammaglobin* implication in the pathogenesis of nasal polyps is independent of an underlying AR.

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