

ORIGINAL ARTICLE

Chronic rhinosinusitis with and without nasal polyps is associated with decreased expression of glucocorticoid-induced leucine zipper

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Summary

Background Chronic rhinosinusitis without nasal polyps (CRSsNP) and with nasal polyps (CRSwNP) is characterized by persistent inflammation of sinonasal mucosa. Glucocorticoid-induced leucine zipper (GILZ) is a recently described anti-inflammatory mediator.

Objective Here we analysed the expression of GILZ in CRSsNP and CRSwNP, its association with response to surgery, and its cytokine-driven expression regulation in the upper airways.

Methods The messenger RNA (mRNA) and protein expression of GILZ in 33 CRSsNP, 32 CRSwNP, and 11 control samples was assessed by means of a quantitative RT-PCR and immunohistochemistry, respectively. Nasal explant culture was used to investigate the effect of IFN- γ , IL-4, IL-13, IL-1 β , and TNF- α on GILZ mRNA expression in normal sinonasal mucosa.

Results The GILZ mRNA and protein expression was significantly suppressed in both CRSsNP and CRSwNP patients compared with controls. No significant difference in GILZ expression was found between CRSsNP and CRSwNP patients. Comparing patients responsive and patients recalcitrant to surgery, a significant further decrease of GILZ expression was found in recalcitrant patients both in the CRSsNP and in the CRSwNP group. IL-1 β , TNF- α , IL-4, and IL-13 reduced, whereas IFN- γ enhanced GILZ mRNA levels in the sinonasal mucosa.

Conclusion Down-regulated expression of GILZ may contribute to the pathogenesis of CRSsNP and CRSwNP and associate with response to surgery. GILZ expression in the upper airways can be regulated differentially by different cytokines.

Keywords chronic rhinosinusitis, cytokine, glucocorticoid-induced leucine zipper, nasal polyp, regulation

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Introduction

Chronic rhinosinusitis (CRS) is a major public health problem. Based on the clinical manifestation and pathological features, CRS is frequently divided into two types: CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP). CRS is often associated with poorer outcomes after standard medical therapy and endoscopic sinus surgery (ESS) [1, 2]. Although multiple predisposing factors have been suggested to contribute to CRS development, no one causative factor fully explains or adequately accounts for the pathologic and clinical manifestations of CRS. To date, one of the most important characteristics of CRS is the prolonged and exaggerated inflammatory reaction in the paranasal mucous mem-

brane [1, 2]. Our previous studies showed that the expression of the Clara cell 10 kDa protein, a steroid-inducible protein with anti-inflammatory and immunomodulatory effects, was significantly inhibited in CRSsNP and CRSwNP and negatively correlated with total infiltrating inflammatory cells and goblet cells [3, 4], suggesting that the dysfunction of anti-inflammatory network may lead to the imbalance of pro-inflammatory and anti-inflammatory responses and may therefore initiate and/or sustain inflammation exacerbation.

Glucocorticoid-induced leucine zipper (GILZ), initially isolated as a dexamethasone-responsive gene from a thymus subtraction library [5], is a newly described anti-inflammatory and immunosuppressive molecule. It has been found to block the key inflammatory signalling mediators including NF- κ B and AP-1 [6, 7]. Overexpression of this gene in dexamethasone-treated or genetically

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modified immune cells may prevent the activation of these cells driven by antigens or pathogens [7–9]. GILZ can diminish the expression of CD80 and CD86 on macrophages and dendritic cells and inflammatory chemokine production by these cells [8, 9], and inhibit inflammatory cytokine-induced cyclooxygenase-2 expression in mouse bone marrow-derived mesenchymal stem cells [10]. Recently, GILZ has also been demonstrated to be expressed by human airway epithelial cells and suppress IL-1 β -induced IL-8 expression through interfering with NF- κ B-mediated gene transcription in human airway epithelial cells [11]. All these previous studies indicate a vital modulatory role for GILZ in the inflammatory reaction. However, currently, very few studies have investigated the role of GILZ in human diseases and the relationship between GILZ and inflammatory airway diseases is unknown.

Several regulators of GILZ expression have been identified, such as INF- γ , IL-4, IL-10, TNF- α , and IL-1 β . However, these cytokines may exert different effects on GILZ production in different kinds of cells [8, 9, 11, 12]. During CRS development, the sinonasal mucosa is exposed to dramatically altered levels of a variety of cytokines. But whether GILZ expression in the sinonasal mucosa could be regulated by these cytokines is unclear.

This study, was designed to investigate whether GILZ plays a role in CRS, and its amenability to surgical therapy, and how the expression of GILZ is regulated by pro-inflammatory cytokines in the sinonasal mucosa.

Methods

Subjects

Seventy-six patients who underwent ESS or septal surgery were recruited for quantitative RT-PCR and immunohistochemistry experiments to investigate the GILZ expression in CRS. Patients were divided into three groups: controls, CRSsNP patients, and CRSwNP patients. The details of subjects' characteristics are included in Table 1. Patients undergoing septoplasty because of anatomic variations and not having any sinus disease were considered control subjects, and inferior turbinate mucosal samples were taken during surgery. The diagnosis of CRS was based on medical history, nasal endoscopy, and computed tomography of the paranasal cavities according to the current European EAACI Position Paper on Rhinosinusitis and Nasal Polyps [1] and American guidelines [2]. Diseased sinus mucosal tissues from most hypertrophied and hyperaemic regions or NP tissues from the apex part of the polyps were collected during surgery.

Additional inferior turbinate mucosal samples of 24 patients who underwent septal surgery and having no evidence of any sinus disease were obtained for nasal

explant culture experiments to investigate the GILZ expression regulation via cytokines (Table 1).

In this study, subjects were excluded if they had received any oral steroid or antihistamine 3 months before the surgery. Topical medications were withheld for a minimum of 1 month before the study. None had received anti-leukotrienes or immunotherapy. Patients who underwent a previous sinus surgery were excluded. Pre- and post-operation medical management strategies were identical in both the CRS groups [1]. Before surgery, all CRS patients received antibiotics for 3–5 days. All CRS patients were followed for a minimum of 12 months after ESS and assessed for recurrence or persistence of CRS. Six CRSsNP patients and 10 CRSwNP patients developed recurrence or persistence of CRS within 12 months after surgery (Table 1). These patients were classified as 'recalcitrant' for the purposes of this study.

All the patients provided informed consent before their participation, and this study was approved by the ethical committee of Tongji Medical College of Huazhong University of Science and Technology.

Routine staining, GILZ immunohistochemistry, and quantification

Samples obtained during surgery were fixed in formalin and embedded in paraffin. Paraffin sections (4 μ m) were prepared from each block and stained with haematoxylin and eosin and periodic acid-Schiff. Sections stained with haematoxylin and eosin were used to count the number of eosinophils, mononuclear cells, and total infiltrating cells, and sections stained with periodic acid-Schiff were used to count the number of goblet cells as described previously [3]. The number of inflammatory cells per high-power field (HP) was determined by counting 10 randomly selected fields in a blinded fashion at $\times 400$ magnification. Goblet cells were observed in the epithelium using the same methods, but the results were expressed as cells per millimetre of epithelium [3].

Protein expression of GILZ was examined by means of immunohistochemical staining. Sections were rehydrated and then subjected to heat-induced antigen retrieval using Antigen Retrieval Solution (Dako, Carpinteria, CA, USA). All washings between steps were performed using Tris-buffered saline. For endogenous peroxidase inhibition, 3% hydrogen peroxide was used. Afterwards, tissue sections were blocked for non-specific binding with normal goat serum. Sections were then stained with rabbit anti-human GILZ antibody (1 : 100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and detected using the streptavidin-peroxidase complex method using a histostain-plus kit (Zhongshan Golden Bridge Biotechnology, Beijing, China) according to the manufacturer's instructions. Immunostaining was visualized by addition of 3'-diaminobenzidine, which rendered positive cells brown. Tissue

Table 1. Patients' clinical data

	Quantitative RT-PCR and immunohistochemistry			Nasal explant culture
	Control group	CRSsNP	CRSwNP	
No. of subjects	11	33	32	24
Sex (male/female)	6/5	20/13	18/14	15/9
Age (years)	18–46	19–53	16–62	17–55
No. of patients with asthma	0	5	8	0
No. of patients with positive skin prick test results	0	12	13	0
No. of patients responsive to ESS	–	27	22	–
No. of patients recalcitrant to ESS	–	6	10	–

CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; ESS, endoscopic sinus surgery.

sections were counterstained with haematoxylin. Negative control experiments were performed using normal rabbit IgG. Quantitative measurement of GILZ protein expression was analysed using the HPIAS-1000 automated image analysis system as described elsewhere (Olympus, Tokyo, Japan) [13, 14]. Ten microscopic fields were randomly selected from each slide under $\times 400$ magnification. Results were presented as 1/grey scores. The grey score reflects the optical density of the selected field of analysing. Thus, 1/grey scores positively correlated with the intensity of immunoreactivity [13, 14].

Nasal explant culture

Normal inferior turbinate mucosal tissues were obtained during surgery and immediately cut into multiple fragments of approximately 6 mm³. One was processed for histologic evaluation and the others were used for tissue culture as described previously [13, 14]. Briefly, sections of tissue were placed on 0.4 μ m-well inserts (Millipore Corp., Billerica, MA, USA) in 2 mL of Dulbecco's modified Eagle's medium/F-12 supplemented with 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. The tissue was oriented with the epithelium being exposed to the air, forming an air–liquid interface to mimic the *in vivo* situation. Tissue was incubated in the absence or presence of various concentrations of dexamethasone (10^{-6} and 10^{-7} M, Sigma, St Louis, MO, USA), INF- γ (0.1, 1, and 10 ng/mL), IL-4 (1, 10, 20, and 100 ng/mL), IL-13 (1, 10, 20, and 100 ng/mL), IL-1 β (1, 10, 20, and 100 ng/mL), and TNF- α (1, 10, 20, and 100 ng/mL) for various durations of time between 4 and 24 h. All these cytokines were purchased from R&D Systems (Minneapolis, MN, USA). The tissues were cultured at 37 °C with 5% CO₂ in humidified air for 4–24 h.

Quantitative reverse transcriptase polymerase chain reaction

RNA was extracted from homogenized mucosa using an RNeasy Mini kit (Qiagen, Valencia, CA, USA) and treated using a DNA-free kit (Ambion, Austin, TX, USA) to remove contaminating DNA. cDNA was reverse transcribed from 0.5 μ g of total RNA with random hexamer primers. Quantitative PCR was performed on the Light-Cycler system (Roche Diagnostics, Mannheim, Germany) using the SYBR Premix Ex Taq kit [TaKaRa Biotechnology (Dalian), Dalian, China] with the appropriate primers and samples according to the manufacturer's protocol. Specific GILZ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were as follows: GILZ, 5'-TCTGCTTGG AGGGGATGTGG-3' (sense) and 5'-ACTTGTGGGGATTTCG GGAGC-3' (antisense); GAPDH, 5'-GAAGGTGAAGGTTCG GAGTC-3' (sense) and 5'-GGAAGATGGTGATGGGATT-3' (antisense). The PCR mixture contained 1 μ L cDNA, 10 μ L 2 $\times$ SYBR Premix Ex Taq master mix, 8 μ L RNase-free water, and 1 μ L of each primer (5 μ M), forming a final volume of 20 μ L. The PCR conditions consisted of an initial denaturation at 95 °C for 30 s, followed by amplification for 45 cycles of amplification of 5 s at 95 °C and an annealing step of 20 s at 66 °C. After PCR, a melting curve was constructed by increasing the temperature from 65 to 95 °C with a temperature transition rate of 0.1 °C/s. Relative gene expression was calculated using the comparative CT method [13, 14]. A sinus mucosa sample from one CRSsNP patient was used as a calibrator in the GILZ mRNA expression study, whereas tissues without any treatment were used as calibrators in the *ex vivo* culture study. GAPDH served as a housekeeping gene for standardization and a 'no template' sample was used as a negative control.

Statistical analysis

Data are presented as mean $\pm$ SD. Kruskal–Wallis *H*- and Mann–Whitney *U*-tests were performed to analyse paired sets of RT-PCR and immunohistochemistry data. The paired *t*-test was used in tissue culture data analysis. Spearman's test was applied to determine correlations. The study power estimation of the sample size was above 0.8 for each set of experiments, except for the comparison between asthmatics and non-asthmatics, providing sufficient power in data analysis. Differences were considered statistically significant at a *P*-value of <0.05.

Results

Glucocorticoid-induced leucine zipper expression in chronic rhinosinusitis patients with and without nasal polyps

Using the RT-PCR assay, we found that the GILZ mRNA expression in sinonasal mucosa and immunohistochemical

staining further revealed that GILZ was mainly expressed by epithelial cells and some infiltrating cells in the sinonasal mucosa (Figs 1a–c). Compared with controls, we found that the mRNA and protein expression of GILZ in sinonasal mucosa was significantly decreased in both CRSsNP and CRSwNP patients (Figs 1e and f). However, no significant difference in GILZ expression levels was found between CRSsNP and CRSwNP patients (Figs 1e and f). Although a trend towards decreased GILZ expression was found in asthmatic patients compared with non-asthmatic patients both in the CRSsNP and in the CRSwNP group, the difference in GILZ expression between asthmatic and non-asthmatic patients failed to reach statistical significance (Figs 1g and h). Comparing responsive and recalcitrant patients, a significant decrease of GILZ expression was found in patients recalcitrant to ESS both in the CRSsNP and in the CRSwNP group (Figs 1i and j). No difference in GILZ levels was found between atopic and non-atopic patients, and between male and female patients (data not shown). The numbers of goblet cells, eosinophils, mononuclear cells, and total infiltrating cells in the CRSsNP and CRSwNP groups were listed as follows: 88.90 ± 7.84 vs. 89.06 ± 8.35 cells/mm ($P > 0.05$), 3.74 ± 0.49 vs. 12.90 ± 1.68 cells/HP ($P < 0.01$), 31.82 ± 3.58 vs. 44.75 ± 5.50 cells/HP ($P < 0.01$), and 62.69 ± 4.60 vs. 66.32 ± 5.01 cells/HP ($P < 0.01$), respectively. Because of the potential effect of antibiotics on neutrophil infiltration, neutrophil counting was not performed. Analysing the relationship between GILZ staining intensity and the number of eosinophils, mononuclear cells, goblet cells, and total infiltrating cells, we found that GILZ staining intensity inversely correlated with the numbers of goblet cells ($r = -0.42$ and -0.38 in the CRSsNP and the CRSwNP group, respectively; $P < 0.05$ for both) and the total infiltrating cells ($r = -0.56$ and -0.45 in the CRSsNP and the CRSwNP group, respectively; $P < 0.05$ for both) in both the CRSsNP and the CRSwNP group, but correlations between GILZ staining intensity and numbers of eosinophils ($r = -0.31$ and -0.33 in the CRSsNP and the CRSwNP group, respectively) or mononuclear cells ($r = -0.24$ and -0.24 in CRSsNP and CRSwNP group, respectively) did not reach statistical significance.

Effect of cytokines on glucocorticoid-induced leucine zipper messenger RNA production in the sinonasal mucosa

To determine the potential factors contributing to the regulation of GILZ gene expression in the sinonasal mucosa, the modulation of GILZ mRNA expression by dexamethasone, selected T-helper type 1 (Th1) (INF- γ), Th2 (IL-4 and IL-13), and proinflammatory cytokines (IL-1 β and TNF- α) was examined. We found that IL-1 β , TNF- α , IL-4, and IL-13 significantly inhibited GILZ mRNA levels in a dose-dependent manner (Figs 2a–d). The time-course study showed that IL-1 β , TNF- α , IL-4, and IL-13

significantly decreased GILZ mRNA expression as early as 4 h following addition of these cytokines and this suppression effect peaked at 8–12 h (Figs 2h and i). In contrast, IFN- γ and dexamethasone were found to enhance GILZ mRNA expression levels in a dose-dependent manner (Figs 2e and f). The increase in GILZ expression was detected 4 h after stimulation with IFN- γ and dexamethasone, and close to the maximum at 12 h (Fig. 2g).

Discussion

GILZ is a newly found glucocorticoid-inducible protein with anti-inflammatory and immunosuppressive effects. Although the evidence showing the involvement of GILZ in various immunological events is increasing, little has been explored about the relationship between GILZ and human diseases. The present study provides the first demonstration that GILZ was expressed in the sinonasal mucosa and its expression was dramatically decreased in both CRSsNP and CRSwNP, which may result from, at least in part, the regulation of cytokines.

Previous studies showed that the GILZ gene is constitutively expressed in different haematopoietic cell lineages [8, 15]. Recently, the presence of GILZ was also observed in normal human bronchial epithelial cells [11]. Consistent with these previous reports, we found that GILZ was locally expressed by epithelial cells and some infiltrating cells in the sinonasal mucosa, indicating that the epithelial and inflammatory cells are the main sources of GILZ production in the upper airway. Our previous study showed that the expression of CC10, another glucocorticoid-inducible protein, was further decreased in CRSwNP compared with CRSsNP [4]. However, in terms of GILZ, no significant difference was found in the expression levels between CRSsNP and CRSwNP, indicating that GILZ may have a similar impact on the pathogenesis of CRSsNP and CRSwNP. Although a trend towards decreased GILZ expression was found in asthmatic patients compared with non-asthmatic patients both in the CRSsNP and in the CRSwNP group, the difference in GILZ expression between asthmatic and non-asthmatic patients did not achieve statistical significance, which might be because of the limited study population of asthmatic subjects in our present study. Further studies are warranted to explore the impact of asthma on GILZ expression in CRS patients. When we explored the correlations between GILZ expression and different kinds of inflammatory cells in CRSsNP and CRSwNP, we found significant negative correlations between GILZ staining intensity and numbers of total inflammatory cells and goblet cells. This suggests that the loss of GILZ expression may lead to the dysfunction of the anti-inflammation network in the upper airway and the hyper-inflammation states associated with CRS.

ESS is necessary in certain patients with CRS when medical therapy fails. Prediction of ESS failure and

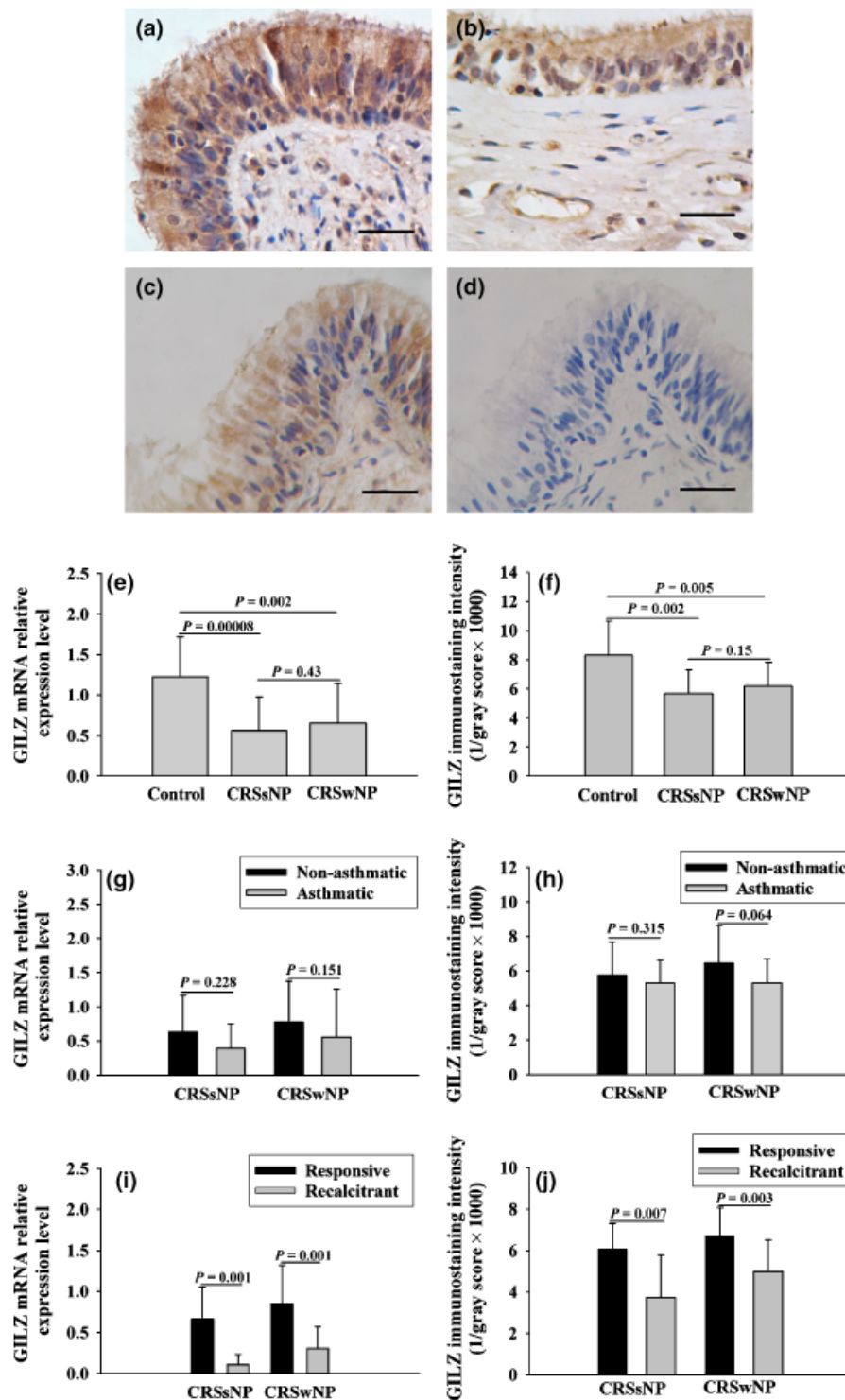


Fig. 1. Expression of glucocorticoid-induced leucine zipper (GILZ) in the sinonasal mucosa. (a–c) Representative photomicrographs of sinonasal tissue sections showing immunohistochemical staining of GILZ from controls (a), CRSsNP patients (b), and CRSwNP patients (c). (d) A photomicrograph of a serial section adjacent to the section used in (c) stained with normal rabbit IgG. Scale bars, 50 μ m. (e–j) Quantification of GILZ messenger RNA (mRNA) levels in tissues with a real-time RT-PCR (e, g, and i) and protein expression levels by means of immunohistochemistry (f, h, and j). In the control group, $n = 11$; in the CRSsNP group, $n = 33$ (five asthmatic/28 non-asthmatic, 27 responsive/six recalcitrant); and in the CRSwNP group, $n = 32$ (eight asthmatic/24 non-asthmatic, 22 responsive/10 recalcitrant). CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps.

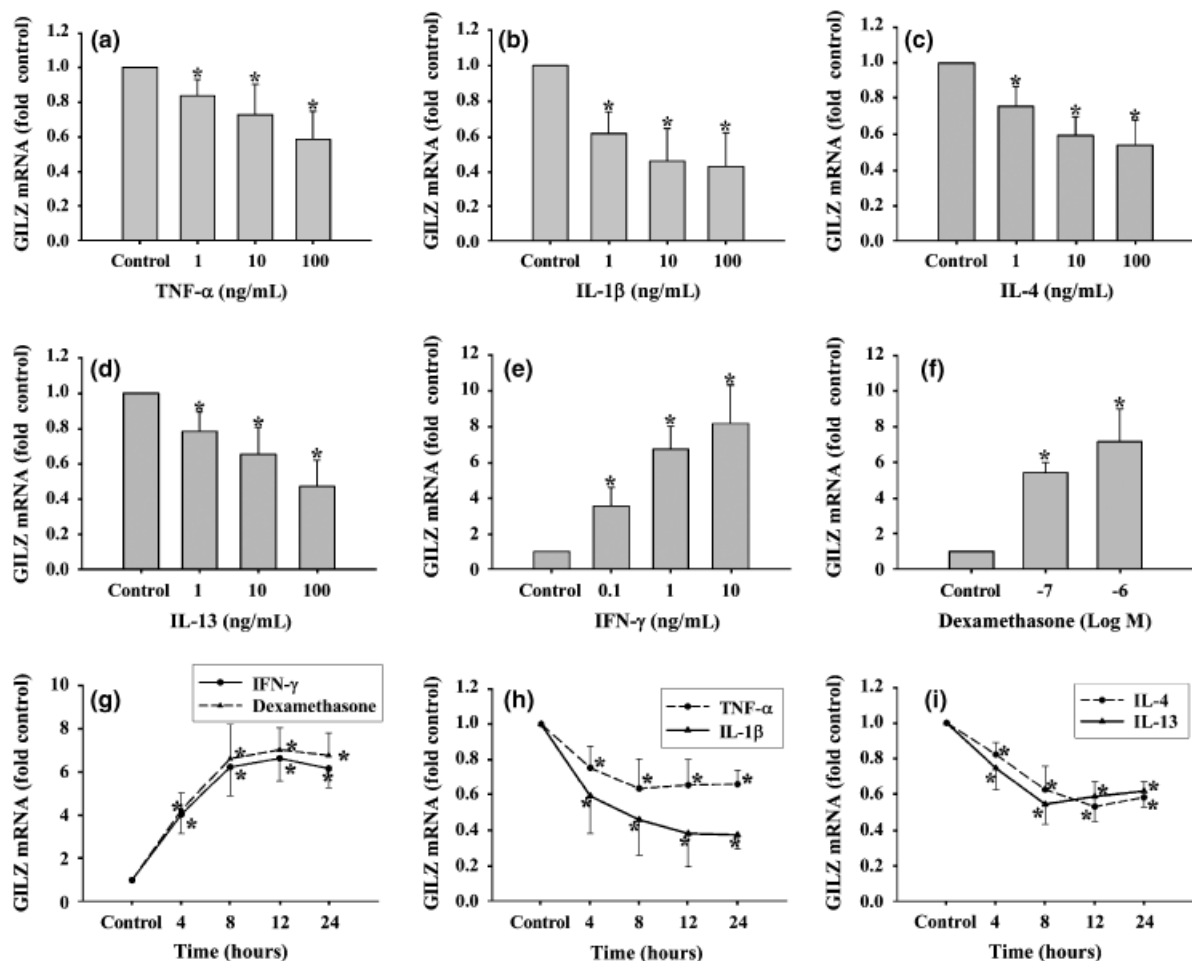


Fig. 2. Effect of TNF- α , IL-1 β , IL-4, IL-13, IFN- γ , and dexamethasone on glucocorticoid-induced leucine zipper (GILZ) messenger RNA (mRNA) expression in *ex vivo*-cultured normal inferior turbinate mucosa. (a–f) Tissues were incubated with cytokines and dexamethasone at various concentrations for 12 h ($n = 6$). (g–i) Tissues were incubated with dexamethasone (10^{-6} M), IFN- γ (1 ng/mL), IL-1 β (20 ng/mL), TNF- α (20 ng/mL), IL-4 (20 ng/mL), and IL-13 (20 ng/mL) for various durations of time ($n = 6$). * $P < 0.01$ compared with untreated mucosa.

detection of potential responders among candidates for surgical treatment would be of considerable clinical value. Our findings showed that GILZ expression levels negatively correlated with responsiveness to surgery. Patients who were recalcitrant to surgical therapy tended to have significantly lower GILZ levels compared with patients responsive to surgical interventions. Recent studies showed that the CRS patients recalcitrant to ESS show more severe inflammation in the sinonasal mucosa compared with patients responsive to ESS, which may contribute to the resistance to surgery in CRS patients. For example, higher levels of acidic mammalian chitinase and macrophage-inflammatory protein α were found in recalcitrant CRSwNP [16, 17]. The current study showed that the lower GILZ expression may result in greater inflammatory cell infiltration and goblet cell hyperplasia in CRS. Therefore, the greater extent of GILZ dysfunction in recalcitrant CRS patients may lead to a more severe inflammatory reaction in the local mucosa and contribute

to the therapy resistance. This result not only further proves the involvement of GILZ in the pathogenesis of CRS but also suggests that GILZ may be a predictor of surgical response. However, further randomized-controlled studies are needed to determine the prognostic value of GILZ.

GILZ belongs to the TGF- β 1-stimulated clone 22 domain (TSC22D) family, termed TSC22D3. It has to be stated that three GILZ isoforms produced by alternative splicing have been recognized recently: TSC22D3-1, -2, and -3. Different isoforms may have distinct tissue expression profiles and functions [18]. In rat and mouse tissues, TSC22D3-1 was the highest-expressed isoform in general; however, TSC22D3-2 displayed the highest levels in the lungs [18]. TSC22D3-2 was the most potent one in the repression of NF- κ B activity in kidney epithelial cells, whereas TSC22D3-3 appeared to be the strongest suppressor of proliferation [18]. In most published researches, the GILZ studied is TSC22D3-2. In the present study, the

primers for GILZ were constructed from published sequences [8]. They can only amplify the fragment of TSC22D3-2, which has the highest expression levels in the airways and can inhibit the activation of NF- κ B. On the basis of the availability of commercial antibodies, a polyclonal antibody was used in an immunohistochemistry study, which was raised against amino acids 1–134 representing full-length TSC22D3-2 of human origin. Because the other isoforms share sequences with TSC22D3-2 partially, they could be identified by this antibody too. Therefore, further studies are required to determine the mRNA and protein expression patterns of individual GILZ isoforms in CRS.

In our previous study, we detected the expression of TNF- α , IL-1 β , IL-4, and INF- γ in CRS and found that the levels of TNF- α and IL-1 β were significantly increased in both CRSsNP and CRSwNP [4]. On comparing CRSsNP and CRSwNP, significantly higher levels of INF- γ were found in CRSsNP, which is in line with Van Zele's study [19], in which CRSsNP revealed a Th1 polarization, whereas CRSwNP displayed a Th2-skewed inflammation. Previous studies demonstrated that GILZ expression in haematopoietic cells could be modulated by several cytokines. Given the decreased GILZ expression in CRS and the well-documented abnormal expression levels of a variety of cytokines in CRS, we investigated the regulation of GILZ expression in normal sinonasal mucosa by selected Th1 (INF- γ), Th2 (IL-4 and IL-13), and proinflammatory cytokines (IL-1 β and TNF- α). We generated sinonasal mucosa explants and performed an *ex vivo* culture, which simulated the *in vivo* nasal environment. Because previous studies showed that glucocorticoids could induce GILZ expression [11], we used dexamethasone as a positive control for our *ex vivo* culture experiment. We found that dexamethasone did induce GILZ mRNA expression in a time- and dose-dependent manner. In agreement with the report of airway epithelial cells by Eddleston et al. [11], we found that GILZ expression was suppressed by IL-1 β and TNF- α . Our previous study showed that IL-1 β and TNF- α concentrations were significantly elevated in both CRSsNP and CRSwNP [4]; therefore, the GILZ gene down-regulation in CRS may result from, at least in part, enhanced expression of IL-1 β and TNF- α in diseased sinonasal mucosa. In terms of INF- γ , in contrast to Eddleston's finding that GILZ expression in airway epithelial cells was inhibited by INF- γ [11], we found that GILZ expression was enhanced by INF- γ in the sinonasal mucosa. The reason for this discrepancy is not clear, but may be linked to the technical differences concerning the types of tissues and culture systems used. In our study, whole sinonasal tissue explants and air-liquid interface cultures were used to mimic the *in vivo* situation, while in Eddleston's report only airway epithelial cells were cultured in suspension, lacking normal interactions with other resident cell populations and the air-liquid inter-

face, which may modulate their functions and gene activities. Moreover, in our study, we found that Th2 cytokines could inhibit the GILZ expression in the sinonasal mucosa. However, Th2 cytokines displayed no influence on the GILZ mRNA level in mast cells [12], but induced GILZ expression in macrophages [8]. The discordance between these studies might have resulted from the differences between the different cells and tissues investigated. Van Zele's study and our previous study demonstrated that CRSsNP and CRSwNP have distinct cytokine expression profiles. CRSsNP manifests a Th1 polarization with a high INF- γ concentration, whereas CRSwNP presents a Th2 polarization with eosinophilic inflammation [4, 19]. Our finding that Th2 cytokines reduced, whereas Th1 cytokines increased GILZ expression in the sinonasal mucosa, prompted us to hypothesize that there should be a lower GILZ expression in CRSwNP compared with CRSsNP. However, we did not find a significant difference in the GILZ expression levels between CRSsNP and CRSwNP, suggesting that some other factors may have contributed towards the regulation of GILZ expression in CRS, counteracting the impact of Th2 and Th1 cytokines. Obviously, this requires further studies. Moreover, it should be pointed out that although we discovered the effect of these cytokines on GILZ expression *in vitro*, their exact roles in GILZ gene regulation in CRS *in vivo* still need to be defined.

To the best of our knowledge, this is the first report exploring the association between GILZ and CRS. Our results indicate that down-regulated GILZ may be involved in the hyper-inflammatory process in CRS and could be related to the response to surgery, and GILZ expression in the upper airways can be modulated differentially by different cytokines.

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