Increased airway surface liquid and decreased mucin expression in pendrin-deficient human airway epithelia

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Increased airway surface liquid and decreased mucin expression in pendrin-deficient human airway epithelia

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학위과정 동안 많은 가르침과 도움을 주신 모든 분들께 감사의 말씀을 전합니다. 먼저, 지도교수님으로서 많은 조언과 가르침을 주신 최재영 교수님께 감사 드립니다. 연구자로서 가야 할 길과 목표를 제시해 주신 윤주헌 교수님, 이비인후과 발전을 위해 힘쓰시는 김창훈 교수님, 가까이서 격려와 조언을 해주신 이상남 선생님, 유지환 선생님 모두 감사 드립니다.

바쁘신 와중에도 학위논문에 대한 검토와 실험적 조언을 해주신 이민구 교수님, 김철훈 교수님, 복진웅 교수님, 김성헌 교수님께도 감사 드립니다.

지난 학위과정 동안 많은 시간을 함께하며 고생했던 실험실 동료들에게도 감사의 인사를 전합니다.

힘이 들 때 옆에서 힘이 되어 주고, 기쁠 때 같이 기쁨을 나눠 주었던 친구들에게도 고맙다는 말을 전합니다.

마지막으로 사랑하는 가족들. 긴 시간 동안 공부할 수 있도록 무한한 사랑과 격려를 아끼지 않으셨던 아버지, 어머니. 많은 관심 주시고 함께 걱정해 주신 장인어른, 장모님. 바쁘다는 핑계로 소홀했던 저의 역할을 대신해준 동생. 짧지 않은 시간 동안 참고 기다려준 아내와 눈에 넣어도 아프지 않을 딸 소윤이에게 감사하다는 말을 전합니다.

이제 또 다른 시작이라는 마음으로 겸손한 태도를 가지고 좀 더 발전해 나아가는 연구자가 되도록 노력하겠습니다.

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TABLE OF CONTENTS

ABSTRACT·····1

I. INTRODUCTION·····3

II. MATERIALS AND METHODS·····6

1. Subjects and tissue harvesting·····6

2. Cell culture and IL-13 treatment·····7

3. Quantitative Real-time PCR·····7

4. Measurement of ASL thickness·····8

5. Short circuit current·····9

6. Measurement of anion channel activity·····9

7. Histology and periodic acid-Schiff (PAS) staining·····10

8. Statistics·····11

III. RESULTS·····12

1. ASL thickness; DFNB4 patients vs. controls·····12

2. Expression pattern of mRNAs encoding Na^+ and Cl^- channels·····13

3. Anion exchanger expression and activity·····16

4. *MUC5AC* expression and goblet cell differentiation·····19

IV. DISCUSSION·····24

V. CONCLUSION·····28

REFERENCES·····29

ABSTRACT (IN KOREAN)·····34

PUBLICATION LIST·····36

LIST OF FIGURES

Figure 1. ASL thickness in human nasal epithelia from patients harboring *SLC26A4* mutations (DFNB4) and controls·····12

Figure 2. Expression of epithelial sodium channels (ENaC) and Cl^- channels (CFTR and ANO-1) in human nasal epithelial cells from patients harboring *SLC26A4* mutations (DFNB4) and control·····14

Figure 3. Changes in ion channel expression in response to IL-13 treatment·····15

Figure 4. Changes in ion channel activity in response to IL-13 treatment ·····16

Figure 5. Anion exchanger expression in cultured human nasal epithelia from controls·····18

Figure 6. Anion exchange activity in human nasal epithelia cultured from patients with *SLC26A4* mutations (DFNB4) and controls·····19

Figure 7. *MUC5AC* expression and goblet cell differentiation in cultured human nasal epithelial cells·····20

Figure 8. PAS staining of inferior turbinates·····21

Figure 9. The hypothetical role of pendrin in the regulation of airway surface liquid (ASL)·····22

LIST OF TABLES

Table 1. Subject Characteristics·····6

ABSTRACT

Increased airway surface liquid and decreased mucin expression in pendrin-deficient human airway epithelia

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Pendrin is an anion exchanger whose mutations are known to cause hearing loss. However, recent data support the linkage between pendrin expression and airway diseases, such as asthma. To evaluate the role of pendrin in the regulation of the airway surface liquid (ASL) volume and mucin expression, we investigated the function and expression of pendrin and ion channels and anion exchangers.

Human nasal epithelial cells were cultured from 16 deaf patients carrying pendrin mutations (DFNB4) and 17 controls. The cells were treated with IL-13 to induce mucus hypersecretion. ASL thickness was measured and real-time polymerase chain reaction was performed targeting various transporters

and *MUC5AC*. Anion exchanger activity was measured using a pH-sensitive fluorescent probe. Periodic acid-Schiff staining was performed on the cultured cells and inferior turbinate tissues.

The ASL layer of the nasal epithelia from DFNB4 subjects was thicker than the controls, and the difference became more prominent following IL-13 stimulation. There was no difference in anion exchange activity after IL-13 treatment in the cells from DFNB4 patients, while it increased in the controls. Goblet cell metaplasia induced by IL-13 treatment seen in the controls was not observed in the DFNB4 cells. Furthermore, the periodic acid-Schiff staining-positive area was lesser in the inferior turbinate tissues from DFNB4 patients that those from controls.

Pendrin plays a critical role in ASL volume regulation and mucin expression as pendrin-deficient airway epithelial cells are refractory to stimulation with IL-13. Specific blockers targeting pendrin in the airways may therefore have therapeutic potential in the treatment of allergic airway diseases.

Key words: *SLC26A4*, asthma, anion channel

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I. INTRODUCTION

Pendrin, encoded by *SLC26A4*, is a membrane protein that exchanges anions such as HCO_3^- and I^- for Cl^-.[1] The protein is predominantly expressed in the inner ear, thyroid and kidney.[2-5] Pendrin presumably acts as an HCO_3^-/Cl^- exchanger in the inner ear, and mutations cause prelingual hearing loss with enlarged vestibular aqueducts (DFNB4).[6-8] In the thyroid, pendrin is essential for I^- uptake, and defects in this protein may cause goiter and possibly hypothyroidism. Goiter combined with hearing loss is known as Pendred syndrome.[9,10]

Pendrin is also expressed in the airway epithelia[11-16] and recent data suggest that pendrin expression is associated with airway diseases. Pendrin expression

is up-regulated in airway tissues of patients with asthma and allergic rhinitis.[11-14,17,18] Moreover, recent study showed that patients harboring bi-allelic *SLC26A4* mutations do not experience asthma,[14] although the prevalence of asthma among these patients is not significantly lower compared to controls due to the low numbers of subjects. Animal studies also showed the role of pendrin in the pathogenesis of asthma. Ovalbumin-induced airway hyperresponsiveness and inflammation are attenuated in pendrin-knockout mice[13,19] and the overexpression of pendrin in airway epithelia of mice resulted in increased airway hyperresponsiveness.[13] These findings suggest that pendrin may play an important role in the development of asthma.

Homeostasis of the volume and composition of the airway surface liquid (ASL) is essential for maintaining the mucociliary clearance system.[20,21] ASL volume is regulated by the coordinated interactions of various ion transporters, such as epithelial sodium channels (ENaC) and cystic fibrosis transmembrane conductance regulators (CFTR). A recent report[13] indicates that pendrin is involved in ASL volume regulation. ASL thickness was shown to increase following allergic cytokine, interleukin (IL)-13, stimulation in the airway epithelia of pendrin-knockout mice. Another interesting discovery is that pendrin may strongly participate in the secretion of airway mucus. Overexpression of pendrin induces mucus hypersecretion in mice, and pendrin has been shown to be a key modulator of IL-13-induced mucus secretion.[14]

Based on these recent findings, pendrin may be a potential new drug target for asthma and other inflammatory airway diseases. However, before deciding on the value of pendrin as a new drug target, further elucidation of the physiological role of pendrin in human airways is necessary because the inner ear and the thyroid phenotypes of pendrin knockout mice have shown to be different from those of patients harboring *SLC26A4* mutations and the physiology underlying ASL homeostasis and mucus secretion in human airways is quite different from that in animal models.

In this study, we compare the ASL thickness, mucin expression and the response to stimulation with IL-13 in nasal epithelial cells cultured from patients with *SLC26A4* mutations (DFNB4) to those in controls.

II. MATERIALS AND METHODS

1. Subjects and tissue harvesting

This study was approved by the Institutional Review Board of the Yonsei University Health System. Human nasal mucosa was obtained from the inferior turbinates of 16 deaf patients harboring bi-allelic *SLC26A4* mutations (DFNB4) during cochlear implantation. Nasal mucosa was also harvested in 17 controls whom underwent septoplasty for correction of deviated nasal septum. These controls had no history of allergic rhinitis or chronic sinusitis, and a genetic study confirmed they did not carry any *SLC26A4* mutations. A genetic analysis confirmed the presence of the bi-allelic *SLC26A4* mutation in all 16 deaf patients. The genotypes of the DFNB4 patients were as follows: H723R homozygote (n=6), H723R/IVS7-2A>G (n=6), IVS7-2A>G homozygote (n=2), IVS7-2A>G/M147V (n=1) and IVS7-2A>G/I302K (n=1). (Table 1) shows the characteristics of the subjects.

Table 1. Subject Characteristics

	N	Age	Male (no. [%]
DFNB4*	16	18 ± 12	8 (50)
Control	17	25 ± 7	10 (59)

* patients with bi-allelic mutation in *SLC26A4*.

2. Cell culture and IL-13 treatment

Primary cultures of human nasal epithelial (HNE) cells were obtained as previously described.[22] Passage-2 cells were seeded into a Transwell-Clear culture insert with a 0.45-μm pore size (Costar Co., Cambridge, MA, USA) at a density of 2 x 10^5 cells/cm^2. The cells were maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium (Lonza, Walkersville, MD, USA) and bronchial epithelial growth medium (Lonza), supplemented with the following growth factors according to the manufacturer's instructions: hydrocortisone (0.5 μg/ml), insulin (5 μg/ml), transferrin (10 μg/ml), epinephrine (0.5 μg/ml), triiodothyronine (6.5 μg/ml), gentamicin (50 μg/ml), amphotericin B (50 μg/ml), retinoic acid (15 ng/ml), bovine pituitary extract (50 μg/ml), bovine serum albumin (1.5 μg/ml) and epidermal growth factor (0.5 ng/ml). The cells were maintained submerged for the first 7 days, after which they were exposed to the apical air interface for the remainder of the culture period. The cells were used between 14 and 21 days after the establishment of the air-liquid interface. At all stages of culture, the cells were maintained at 37°C under 5% CO_2 in an air incubator. To stimulate the cells, they were treated basolaterally with 10 ng/ml of recombinant human IL-13 (R&D Systems, Minneapolis, MN, USA) for 7 days.

3. Quantitative Real-time PCR

Total RNA was isolated from primary cultures of HNE cells from five patients harboring bi-allelic *SCL26A4* mutations and four controls. RNA was isolated using the TRIzol reagent (Invitrogen, San Diego, CA, USA) according to the manufacturer's protocol. Purified RNA samples were reverse transcribed with a cDNA Synthesis Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Quantitative Real-time PCR for pendrin, epithelial sodium channel (ENaC) α, β, γ, CFTR, ANO-1, Anion exchanger (AE) 2, AE3, AE4 and SLC26A3, SLC26A6, SLC26A7, SLC26A8, SLC26A9, SLC26A11, MUC5AC was performed using the 7300 Real-Time PCR System (Applied Biosystems) and the DyNAmoHSSYBR Green qPCR Kit (Finnzymes, Espoo, Finland). The primers employed for real time PCR are listed in Table S1.

4. Measurement of ASL thickness

The thickness of the ASL was measured in HNE cells using a modified version of the method described by Terran et al.[21] Briefly, the cells were washed, and 20 μl of PBS containing 0.2% (v/v) Texas Red-dextran (Invitrogen) was added onto the apical cell surface to label the ASL layer. A 100-μl aliquot of perfluorocarbon (Fluorinert FC-770; 3M, St. Paul, MN, USA) was then added to the apical surface to prevent evaporation of the ASL. After 12 hr, the cultures were transferred to the stage of an inverted confocal

microscope (LSM 700; Carl Zeiss MicroImaging Inc.), and the height of the ASL was measured at five predetermined points in the cultures (one central, four circumferential) via XZ scans. Images were reconstructed 3-dimensionally and analyzed using Imaris 7.1 software (Bitplane Co, Zurich, Switzerland).

5. Short circuit current

Human nasal epithelial cells were mounted in Ussing chambers (Physiologic Instruments, San Diego, CA). Amiloride, CFTRinh-172, ATP were added to the apical solution, and an equal volume of vehicle was added at the same time to the basolateral solution. Symmetrical $HCO3^-$-buffered solutions (120 mM NaCl, 5 mM KCl, 1 mM MgCl2,1mM CaCl2, 10 mM D-glucose, 5 mM HEPES, and 25 mM NaHCO3, pH 7.4)were used for human nasal epithelial cells. Cells were bathed for a 10 min stabilization period and aerated with 95% O2/5% CO2 at 37 °C or room temperature. Short circuit current was measured using an EVC4000 MultiChannel V/I Clamp (World Precision Instruments, Sarasota, FL) and recorded using PowerLab/8sp (AD Instruments, Castle Hill, Australia).

6. Measurement of anion exchange activity

Measurements of the pH_i in the HNE cells were performed with a pH-sensitive fluorescent probe, bis-carboxyethyl-carboxyfluorescein (BCECF) (Invitrogen, San Diego, CA, USA), as described previously.[23] Briefly, the measurements were performed in PBS- or IL-13-treated cells, in which a cluster of cells showing green fluorescent protein (GFP) fluorescence was exposed to BCECF, and the pH_i was monitored. After adding BCECF, the cells were perfused with HCO_3^-buffered solution (120 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 D-glucose, 5 HEPES and 25 $NaHCO_3$ in mmol/l, pH 7.4), and BCECF fluorescence was recorded at excitation wavelengths of 490 nm and 440 nm at a resolution of 2 sec on a recording setup (Delta Ram; PTI Inc., Birmingham, NJ, USA). The Cl^-/HCO_3^- exchange activities were estimated from the initial rate of the pH_i increase as a result of Cl^- removal in the HCO_3^--containing buffer (25 mM HCO_3^- with 5% CO_2). The intrinsic buffer capacity (β_i) was measured as described previously.[23]

7. Histology and periodic acid-Schiff (PAS) staining

Periodic acid-Schiff (PAS) staining was performed with a PAS staining kit (Sigma, St. Louis, MO, USA) according to the manufacturer's protocol. The harvested nasal mucosa and HNE cells on the transwell inserts were gently washed with PBS and fixed with 4% paraformaldehyde. After deparaffinization and hydration, the slides were immersed in periodic acid

solution for 5 min at room temperature. After being rinsed in distilled water, the slides were immersed in Schiff's reagent for 15 min at room temperature, and then washed in running tap water for 5 min. Slides were counterstained in Hematoxylin Solution for 90 sec and then rinsed in running tap water, dehydrated, cleared and permanently mounted. For morphometric analysis, images of the PAS-stained slides (five different slides of tissue from each patient) were acquired with a Leica DM750 LED microscope and PAS-positive cells were counted.

8. Statistics

Statistical analysis was performed with SPSS software for PC, version 19 (SPSS Inc., Chicago, IL). Differences between groups regarding anion exchange activities and the rates of pendrin positivity were evaluated via one-way analysis of variance. A p-value of less than 0.05 was considered statistically significant.

III. RESULTS

1. ASL thickness; DFNB4 patients vs. controls

First, we measured the ASL thickness in HNE cells from patients harboring *SLC26A4* mutations (DFNB4) and controls. Twelve hours after PBS/Texas Red-dextran loading, the ASL thickness in the DFNB4 patients (14.1 ± 2.9 μm) was thicker than that in the controls (8.0 ± 2.1 μm, $p<0.05$). The increase in ASL thickness following IL-13 stimulation was more prominent in DFNB4 cells (31.3 ± 7.6 μm) than in controls (13.5 ± 2.8 μm) **(Figure 1)**.

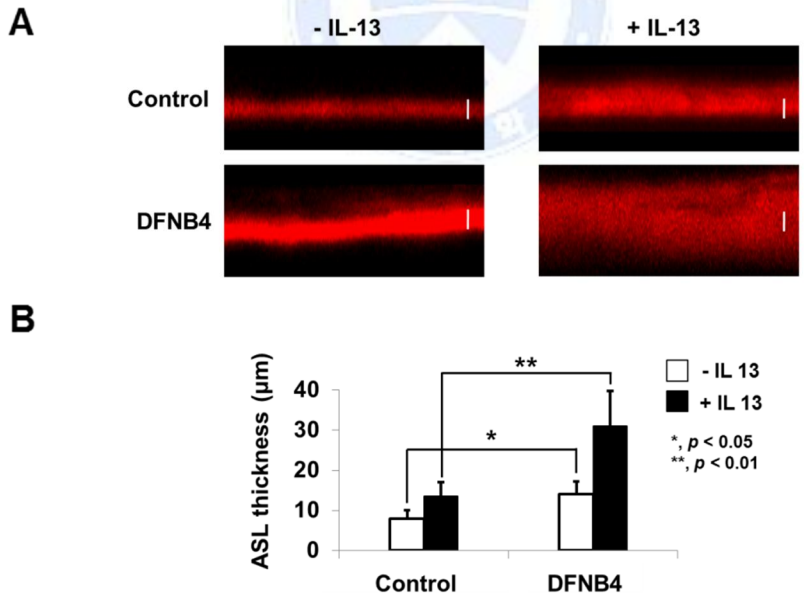

Figure 1. ASL thickness in human nasal epithelia from patients harboring *SLC26A4* mutations (DFNB4) and controls. Confocal images (A) and summary of ASL thickness measurements (B) at 12 hr after the apical addition of 20 μl of PBS containing Texas Red-dextran to primary nasal epithelial cultures from DFNB4 patients (n=5) and controls (n=4) in the absence or presence of IL-13 (10 ng/ml). Scale bars, 10 μm. * $p<0.05$, ** $p<0.01$,compared with the controls. Error bars, S.E.

2. Expression pattern of mRNAs encoding Na^+ and Cl^- channels

We compared the mRNA expression patterns of ion transporters involved in ASL volume regulation to rule out the possibility that the difference in ASL thickness between the pendrin mutants and controls stemmed from the differential expression of these transporters. The expression patterns of ENaC subtypes α, β, and γ and ANO-1 were similar between DFNB4 patients and controls. However, CFTR showed lower expression in the DFNB4 patients (0.36 ± 0.25 fold of that in the controls) **(Figure 2)**.

When the HNE cells from controls were treated with IL-13, ENaC subtypes β and γ were down-regulated, whereas ANO-1 (49 ± 18 fold of PBS) was up-regulated. As previously reported, pendrin expression was also greatly increased by 104 ± 27 fold following treatment with IL-13. The response of the pendrin-deficient epithelial cells to IL-13 treatment was similar to the

response in the controls: ENaC subtypes β and γ were down-regulated, and ANO-1 was up-regulated (61 ± 26 fold) **(Figure 3)**.

Short circuit current measurements in HNE cells showed these expression patterns. IL-13-treated HNE cells from control showed decreased amiloride sensitive ENaC current and increased CaCC current. In DFNB4 cells, IL-13 treatment had no effect on ENaC and CFTR current, but had increased CaCC current following ATP stimulation **(Figure 4)**.

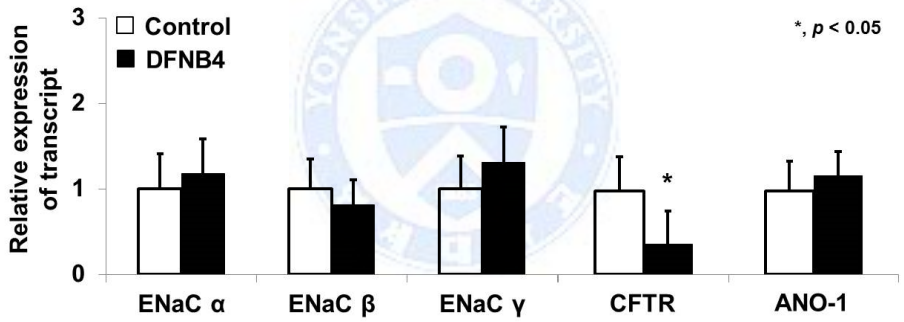

Figure 2. Expression of epithelial sodium channels (ENaC) and Cl^- channels (CFTR and ANO-1) in human nasal epithelial cells from patients harboring *SLC26A4* mutations (DFNB4) and controls. The expression patterns in the cells from patients with DFNB4 (n=3) are similar to those in controls (n=3), except for CFTR, which shows low levels of

expression in the epithelia from DFNB4 patients. * indicates p<0.05, compared to controls.

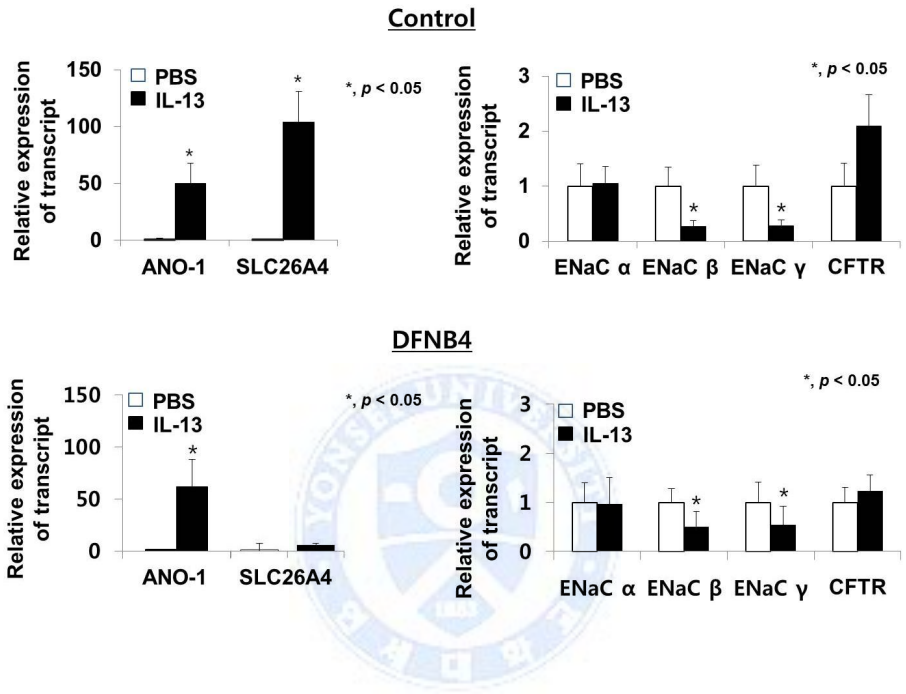

Figure 3. Changes in ion channel expression in response to IL-13 treatment (10 ng/ml). In both the control (n=3) and *SLC26A4* mutant (DFNB4) (n=3) nasal epithelia, ENaC β and γ expression are suppressed, and ANO-1 expression is greatly increased (by more than 50 fold) following IL-13 treatment. In the control epithelia, pendrin is also upregulated (approximately 100 fold) following IL-13 treatment. * indicates p<0.05.

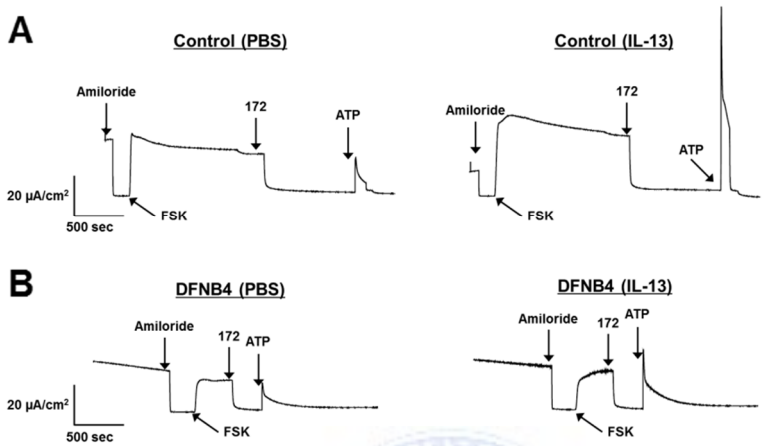

Figure 4. Changes in ion channel activity in response to IL-13 treatment (10 ng/ml). (A) Short circuit current measured in HNE cells from control. Amiloride (10 μM)-sensitive Na^+ current was decreased by IL-13 treatment. CFTR was activated by 10 μM forskolin and inhibited by $CFTR_{inh}$-172 (10 μM). ATP-induced CaCC current was increased by IL-13. (B) Short circuit current measured in HNE cells from DFNB4. ENaC and CFTR currents were not different between PBS and IL-13 treatment. But, ATP-induced CaCC current was increased by IL-13 treatment.

3. Anion exchanger expression and activity

In addition to *SLC26A4*, various anion exchangers, including *AE2, AE3, AE4, SLC26A3, SLC26A6, SLC26A7, SLC26A8, SLC26A9* and *SLC26A11*,

were found to be expressed in the HNE cells of the controls. When the cells were treated with IL-13, *SLC26A3* was up-regulated (by 8.2 ± 5.4 fold compared to PBS treatment) along with *SLC26A4*. The other anion exchangers did not respond to IL-13 stimulation **(Figure 5)**. We also examined anion channel activity by measuring the Cl^-/HCO_3^- exchange ratio. The anion channel activity in epithelia from the patients harboring *SLC26A4* mutations (DFNB4) (0.12 ± 0.07 ΔpH/min) was not different from that in the controls (0.15 ± 0.06 ΔpH/min) under basal conditions. When the HNE cells from the controls were treated with IL-13 for 7 days, the anion channel activity was significantly increased (0.23 ± 0.17 ΔpH/min, $P<0.05$), but not in the epithelia from patients with *SLC26A4* mutations (0.18 ± 0.06 ΔpH/min) **(Figure 6)**. The molecular and functional data indicate that pendrin is a major anion exchanger that responds to IL-13 and that the intracellular and ASL pH may be regulated by various anion exchangers other than pendrin under basal conditions.

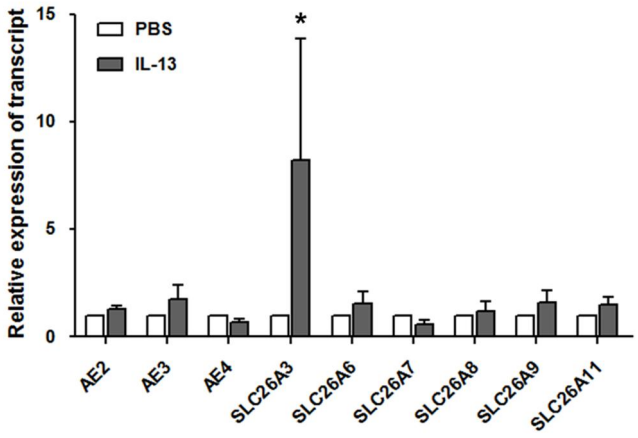

Figure 5. Anion exchager expression in cultured human nasal epithelia from controls. Along with pendrin, various anion exchangers (*AE2, AE3 and AE4*) and *SLC26A* family transporters (*SLC26A3, SLC26A6, SLC26A7, SLC26A8, SLC26A9* and *SLC26A11*) are expressed in nasal epithelia from controls (n=5). Only *SLC26A3* is increased by IL-13 (10 ng/ml). * indicates $p<0.05$ compared with the PBS-treated cells.

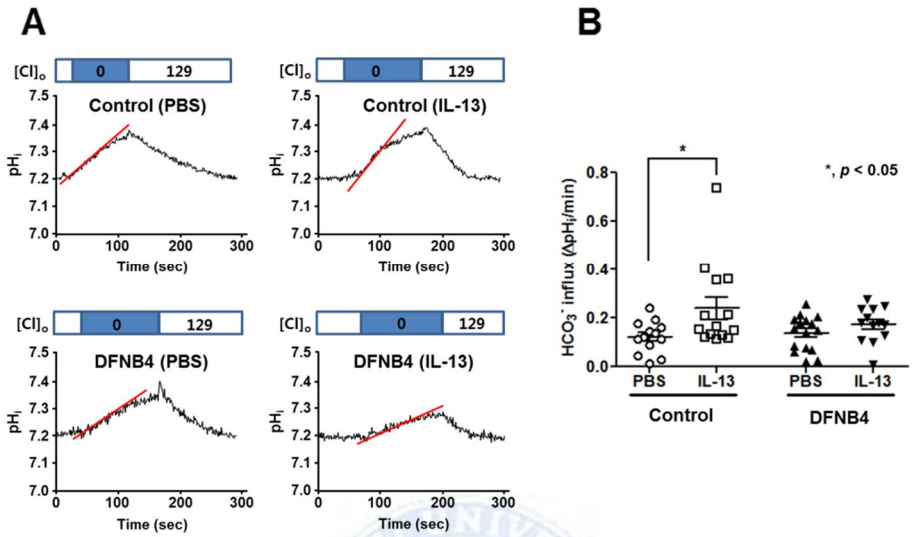

Figure 6. Anion exchange activity in human nasal epithelia cultured from patients with *SLC26A4* mutations (DFNB4) and controls. (A) Representative tracings of anion channel activity. (B) Measurements of anion exchanger activity showing significantly increased activity following IL-13 treatment (10 ng/ml) in the controls (n=4) but not in epithelia from patients harboring *SLC26A4* mutations (DFNB4, n=5). * indicates p<0.05 compared with the PBS-treated cells.

4. *MUC5AC* expression and goblet cell differentiation

We also examined the role of pendrin in mucus secretion in the airway epithelia. Quantitative real-time PCR data showed that the expression of *MUC5AC,* a goblet cell marker, was greatly increased by IL-13 treatment in

cultured cells from controls. In contrast, *MUC5AC* expression was comparatively low in cultured cells from DFNB4 patients, and it was not up-regulated by IL-13 treatment **(Figure 7A)**. PAS staining revealed goblet cell hyperplasia in the control HNE cells following IL-13 treatment, but this was not observed in the epithelia from DFNB4 subjects **(Figure 7B)**. Histologic examination of the inferior turbinates also revealed that there was a much lower number of PAS-positive cells (3.7 ± 2.1/mm) in the nasal mucosa from DFNB4 patients compared with that from controls (10.3 ± 3.7/mm) **(Figure 8)**.

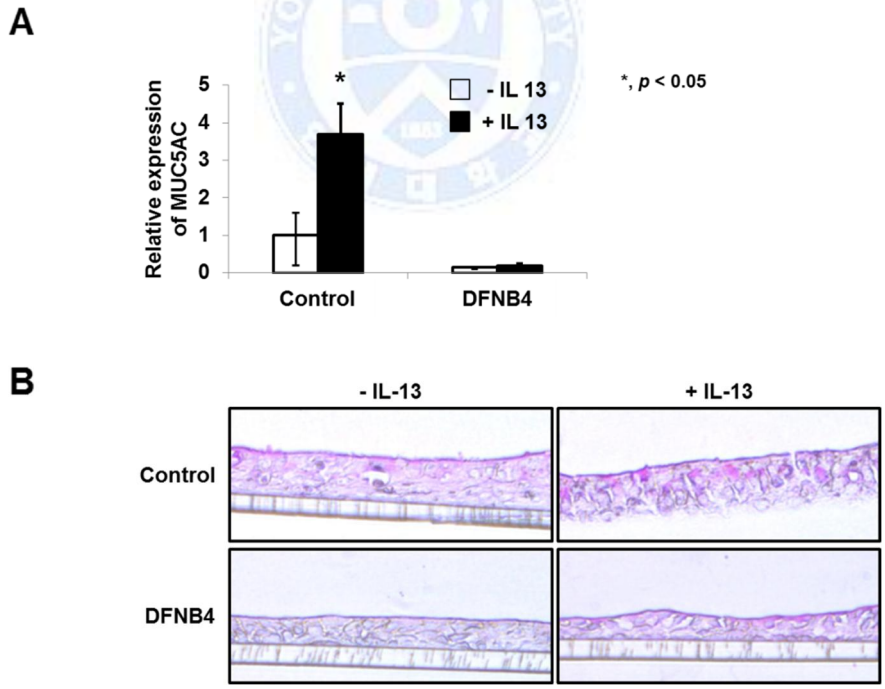

Figure 7. *MUC5AC* expression and goblet cell differentiation in cultured human nasal epithelial cells. (A) Real-time PCR analysis showing up-regulation of *MUC5AC* by IL-13 (10 ng/ml) in controls (n=3). In contrast, *MUC5AC* is weakly expressed and does not respond to IL-13 in epithelia from patients carrying *SLC26A4* mutations (DFNB4, n=3). * indicates $p<0.05$. (B) PAS staining showing goblet cell hyperplasia in nasal epithelial cells from controls following IL-13 treatment (10 ng/ml) for 7 days, but not in epithelia from DFNB4 patients. Scale bar represents 10 μm.

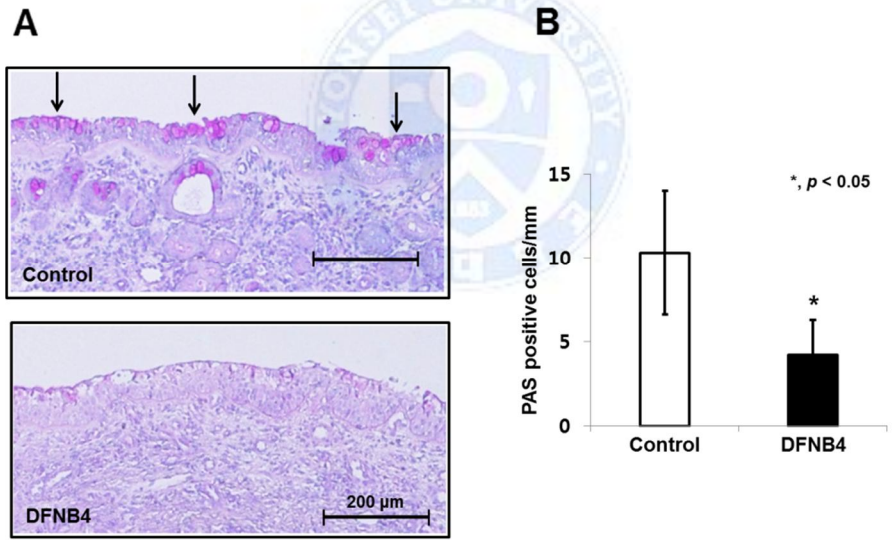

Figure 8. PAS staining of inferior turbinates. (A) Representative images shows a much lower amount of PAS staining in turbinate tissue from patients harboring *SLC26A4* mutations (DFNB4) compared with the tissues from

controls (bars indicate 200 μm). (B) Quantitative analysis of the abundance of PAS-positive cells in 6 DFNB4 and 6 controls. * indicates $p<0.05$.

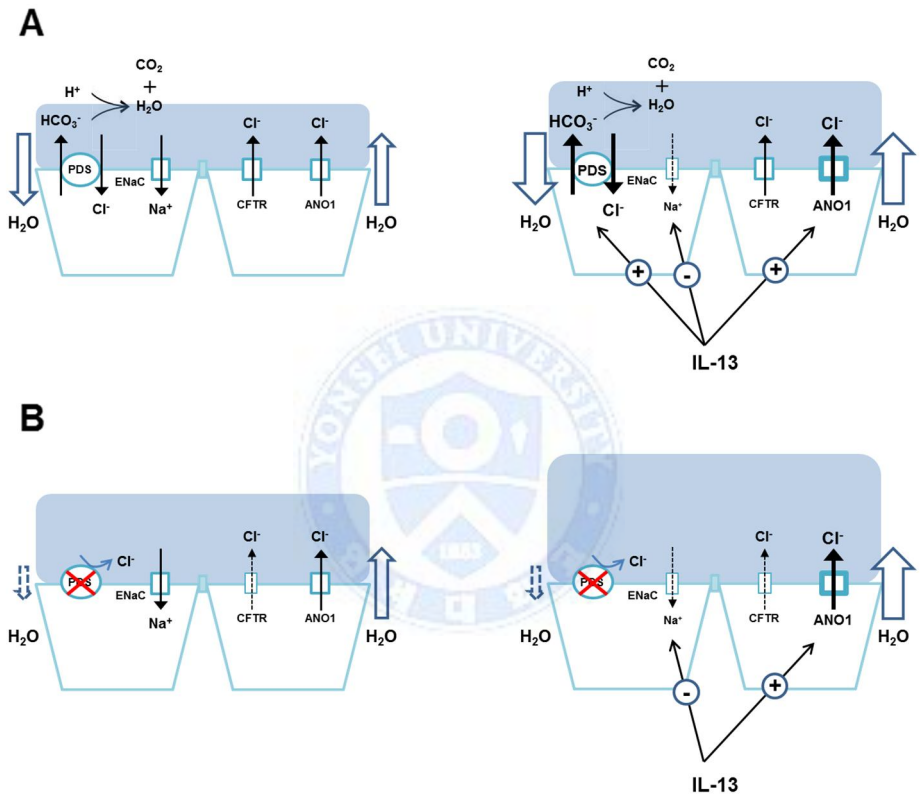

Figure 9. The hypothetical role of pendrin in the regulation of airway surface liquid (ASL). (A) Pendrin regulates ASL volume via Cl^- and subsequent water absorption. The secreted HCO_3^- will evaporate, thus leading to net anion movement into the cell. Under inflammatory conditions, up-regulated pendrin compensates for the over-secreted fluid caused by up-regulated Cl^- channels. (B) In pendrin-deficient airways, ASL thickness is

increased due to the lack of Cl^- absorption by pendrin under basal conditions and cannot compensate for the over-accumulated fluid.

IV. DISCUSSION

A recent report[13] indicates that pendrin is involved in the regulation of ASL thickness in mice. Although the ASL thickness did not differ in cultured tracheal epithelial cells between wild-type and pendrin-deficient mice under basal conditions, the ASL became much thicker in pendrin-deficient mice compared to the cells from wild-type mice after stimulation with an allergic cytokine.[13] However, pendrin regulates the pH of the ASL but has little effect on fluid secretion in Calu-3 cells, which are human serous airway epithelial cells.[24] Therefore, it was unclear whether pendrin regulates ASL volume in airway epithelia in humans. In our data, the expression pattern of ion transporters in airway epithelia was similar between the patients with *SLC26A4* mutations and controls except for the weak expression of CFTR in patients with *SLC26A4* mutations. However, the ASL layer was thicker in HNE cells from patients with *SLC26A4* mutations compared to controls. These data indicate that pendrin is involved in ASL volume regulation even in basal conditions. This finding is not consistent with data from the mouse airway. This discrepancy may stem from species differences in the role of ion transporters in airway epithelia. For instance, the expression patterns of ion channels are quite different between mice and humans as CFTR expression is barely detectable in mouse airway epithelia. Another possible reason for the

conflicting results between reports may be attributable to a methodological problem. Nakagami *et al.*[13] measured ASL thickness 3 min after PBS/dye loading, before ASL thickness had reached a steady state.

The mechanism by which pendrin regulates ASL is puzzling because this electro-neutral transporter cannot generate an osmotic gradient. One possible mechanism underlying this phenomenon is the evaporation of secreted $HCO3^-$, which results in net anion movement into the lumen and subsequent Na^+ secretion, via ENaC, to generate osmotic pressure **(Figure 9A)**. Interestingly, the difference of ASL height between normal and pendrin-deficient airway epithelia became much more prominent with IL-13 treatment. IL-13 induces airway fluid secretion and as a result, the ASL thickness is increased. In normal airway epithelial cells, the up-regulation of pendrin (~ 100 fold) following IL-13 treatment compensates for the thickened ASL via Cl^- absorption. However, presumably since this compensatory mechanism does not exist in the pendrin mutants, it results in an overaccumulation of ASL **(Figure 9B)**.

Pendrin seems to be not only involved in ASL regulation, but also mucus secretion. Enforced expression of pendrin in NCI-H292 cells or mouse airway epithelia induces the up-regulation of *MUC5AC*.[14] However, pendrin–deficient mice exhibit impaired airway hyperresponsiveness and eosinophilic inflammation, but not mucus production. Our data showed that *MUC5AC* was

expressed at very low levels in the nasal epithelia from *SLC26A4* mutants and was not up-regulated by IL-13 treatment, which contrasts with our findings in the cells of the controls. We also revealed that the goblet cell differentiation in basal condition and hyperplasia induced by IL-13 was minimal in the epithelia from patients with *SLC26A4* mutations. Furthermore, we demonstrated that the proportion of goblet cells in the nasal mucosa from patients with SLC26A4 mutation is much lower compared to that in controls. These data all together indicate that pendrin is a major participant in mucous cell differentiation and hyperplasia induced by allergic cytokines in airway epithelia. Although the exact role of pendrin in mucus secretion in the airways is unclear, pendrin may be involved in the release of inflammatory cytokines or may activate transcription factors for the mucin gene, leading to mucus secretion, as suggested by Rose & Voynow.[25]

An interesting phenomenon is the linkage between the pathogenesis of asthma and pendrin. Pendrin knockout mice show less of an allergic response to OVA stimulation than their wild-type counterparts.[13] Pendrin expression is up-regulated after IL-4 stimulation in cultured airway epithelia[16] and disease tissues, such as those from asthma, COPD, and rhinitis patients.[12,18,26] Moreover, patients with pendrin mutations had a tendency to be resistant to asthma.[14] Based on our data, we postulate that the increase in ASL thickness observed in the airways of pendrin-deficient patients under basal conditions

may decrease the chance of allergens coming into contact with the airway epithelia, and therefore hinder the initiation of allergic sensitization. Moreover, the dramatic increase in ASL thickness induced by allergic cytokines could further protect the airway epithelia against exposure to allergens in pendrin-deficient airways. Another possible mechanism explaining the lower incidence of asthma in patients harboring pendrin mutations is a defect in mucus secretion, although the exact molecular mechanism underlying this process is unclear.

In summary, we showed that the ASL layer was thicker in human airway epithelial cells from patients with *SLC26A4* mutation compared to controls, and this phenomenon was more prominent in allergic conditions. Pendrin seems to be essential in mucin expression and goblet cell development. These findings may explain the low incidence of allergic airway diseases in patients with *SLC26A4* mutation. Specific blockers targeting pendrin in the airway epithelium may represent promising candidate drugs for the treatment of allergic airway diseases such as asthma and allergic rhinitis.

V. **CONCLUSION**

In summary, we showed that the ASL layer was thicker in human airway epithelial cells from patients with *SLC26A4* mutation compared to controls, and this phenomenon was more prominent in allergic conditions. Pendrin seems to be essential in mucin expression and goblet cell development. These findings may explain the low incidence of allergic airway diseases in patients with *SLC26A4* mutation. Specific blockers targeting pendrin in the airway epithelium may represent promising candidate drugs for the treatment of allergic airway diseases such as asthma and allergic rhinitis.

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ABSTRACT (in Korean)

펜드린 결핍 인간 기도 상피세포에서 증가된 기도 표면 액체와 감소된 mucin 발현

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이현재

펜드린은 음이온 교환 단백이며 이것의 돌연변이는 난청을 유발한다고 알려져 있다. 그러나 최근 기도 표면 액체의 양과 mucin 발현의 조절에 펜드린의 역할을 밝혀내기 위한 펜드린의 발현과 천식 같은 기도 질환의 관한 연구가 진행되었다.

16 명의 펜드린 돌연변이를 가진 난청 환자와 17 명의 돌연변이를 가지고 있지 않은 환자로부터 얻은 인간 코 상피세포 배양하였다. 세포에 Interleukin 13 을 처리하여 점액 과분비를 유도하였다. 기도 표면 액체의 높이 측정과 실시간 중합효소 연쇄 반응을 이용하여 다양한 이온 운반체와 MUC5AC 를 발현량을 확인 하였다. pH 민감성 형광 프로브를 이용하여 음이온 교환 능력을 측정하였다. 배양된 세포와 하위 갑개 조직에서 과아이오딘산 시프 염색을 시행하였다. 펜드린 돌연변이 세포의 기도 표면 액체는 실험군의

기도 표면 액체보다 더 두꺼웠고, 이러한 차이는 Interleukin 13 을 처리하였을 때 현저하게 나타났다. 실험군에서 Interleukin 13 처리 후 음이온 교환 능력이 증가한 반면 돌연변이 군에서는 차이를 보이지 않았다. 배상세포 변질형성은 실험군에서 나타났지만 돌연변이에서는 나타나지 않았다. 게다가 과아이오딘산 시프 염색에 양성인 부분은 돌연변이 군에서 더 적게 나타났다.

펜드린은 기도 표면 액체의 양과 mucin 발현 조절에 중요한 역할을 한다. 기도에 존재하는 펜드린을 표적으로 하는 특이 차단제는 알레르기성의 기도 질환 치료제로의 가능성을 가질 것이다.

핵심되는 말: *SLC26A4*, 천식, 음이온 통로

PUBLICATION LIST

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