



The Role of Collagen VI $\alpha 6$ Chain Gene in Atopic Dermatitis

Hye Jung Jung, Won Il Heo¹, Kui Young Park¹, Mi-Kyung Lee¹, Ji Young Ahn, Mi Youn Park, Seong Jun Seo¹

Department of Dermatology, National Medical Center, ¹Department of Dermatology, Chung-Ang University Hospital, Seoul, Korea

Received April 21, 2021

Revised August 19, 2021

Accepted September 8, 2021

Corresponding Author

Seong Jun Seo
Department of Dermatology, Chung-Ang
University Hospital, 102 Heukseok-ro,
Dongjak-gu, Seoul 06973, Korea
Tel: +82-2-6299-1525
Fax: +82-2-6299-1718
E-mail: drseo@cau.ac.kr
<https://orcid.org/0000-0003-2915-839X>

Background: In a previous study, we carried out whole-exome sequencing to identify genetic variants associated with early onset atopic dermatitis (AD) in Koreans and found that collagen VI $\alpha 6$ chain (*COL6A6*) gene polymorphisms are associated. *COL6A6* is one of the chains that makes up the triple helix of collagen VI, and little is known about its role in AD.

Objective: To identify how *COL6A6* changes in AD and clarify its role.

Methods: Immunohistochemical staining for *COL6A6* was performed on tissues of AD, other skin diseases, and healthy controls. Human keratinocytes and fibroblasts were exposed to inflammatory cytokines and cultured to evaluate changes in *COL6A6* expression. *COL6A6* small interfering RNA (siRNA) was transfected into cells to identify the role of *COL6A6*.

Results: Total *COL6A6* mRNA was higher in AD than in controls. In AD tissues, *COL6A6* mRNA decreased significantly in the epidermis compared to controls, whereas *COL6A6* protein was increased in the dermis. In the cultured cells, *COL6A6* mRNA was suppressed in the epidermis by interleukin (IL)-4 and IL-13, whereas *COL6A6* protein was induced in the dermis. In the *COL6A6* siRNA-transfected keratinocyte, mRNA of *FLG*, *LOR*, and *CASP14* decreased compared to controls; in contrast, mRNA of *MMP1* increased.

Conclusion: The reduction of epidermal *COL6A6* due to the genetic mutation can cause skin barrier damage and it can contribute to the early onset of AD. *COL6A6* is induced by IL-4 and IL-13, and it may play a role in fibrotic remodeling and inflammatory processes, which are major features of AD.

Keywords: Atopic dermatitis, *COL6A6*, Collagen, Interleukin-4, Interleukin-13

INTRODUCTION

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease. Although the pathophysiology of AD is quite complex, it can be summarized as chronic inflammation caused by skin barrier impairment and immune dysregulation^{1,2}. Both genetic and environmental factors are involved in these abnormalities. The most well-known genetic abnormality in AD is the *FLG* mutation; filaggrin deficiency is associated with early onset and severe AD, greater allergen sensitization, and increased susceptibility to infections¹⁻³. However, in Koreans, the *FLG* mutation occurs only in 9.0% of the AD patients, which is much lower than in Irish (46%), Chinese (20.2%~31.4%), and Japanese (18.4%~26.7%) populations⁴⁻⁷.

Therefore, we wanted to investigate if other genetic factors besides *FLG* are involved in early onset AD in Koreans. In our previous study, whole-exome sequencing was performed in three families with early onset AD. The results showed that family-specific collagen VI $\alpha 6$ chain (*COL6A6*) gene polymorphisms are associated with early onset AD in Koreans. Three variants of the *COL6A6* gene appeared in all three families, and their frequency was higher in AD patients than in controls in a population-based case-control study⁸.

Collagen is an abundant, hard, insoluble, and fibrous protein present throughout the body, and mutations in collagen VI (*COL6*) cause myopathy^{9,10}. Collagen types I and III comprise most of the skin collagen content in humans, but *COL6* has not been extensively studied in dermatology^{9,11}. In addi-



tion, it is known that collagen abnormalities in the skin cause some bullous disorders, but little is known about effect of collagen on AD. Therefore, we aimed to determine how *COL6A6* changes in patients with AD and its role in AD.

MATERIALS AND METHODS

Study design

We aimed to determine whether the expression of *COL6A6* was altered in patients with AD. Accordingly, two experiments were conducted. First, immunohistochemistry (IHC) staining was performed on skin biopsies from patients with AD, psoriasis, papular urticaria, and pityriasis rosea, as well as controls, to compare the expression of *COL6A6* in each disease. Second, the *COL6A6* messenger RNA (mRNA) expression levels were compared by measuring fragments per kilobase of transcript per million (FPKM) in the tissues of AD patients and controls.

Next, we aimed to determine the factors affecting the change in *COL6A6* found in the epidermis of patients with AD. Changes in *COL6A6* mRNA expression were measured over time while culturing human keratinocytes with interleukin (IL)-4, IL-13, and thymic stromal lymphopoietin (TSLP) which are the representative cytokines involved in AD. Conversely, keratinocytes were also exposed to tumor necrosis factor (TNF)- α , which is involved in Th1 type immunity. To determine the effect of *COL6A6* on the epidermis, several differentiation markers such as FLG and IVL were measured using real-time polymerase chain reaction (PCR) after transfection of *COL6A6* small interfering RNA (siRNA) into keratinocytes.

We also aimed to identify the factors affecting the change in *COL6A6* found in the dermis of patients with AD. We measured changes in *COL6A6* expression by western blot analysis over time while culturing human fibroblasts with IL-4, IL-13, TNF- α , and TSLP. To determine the effect of *COL6A6* on the dermis, several protein levels were measured using real-time PCR after transfection of *COL6A6* siRNA into fibroblasts.

The present study protocol was reviewed and approved by the institutional review board of Chung-Ang University Hospital (IRB No. C2015258 [1716]). Informed consent was obtained from all participants when they were enrolled.

Immunohistochemical analysis

This analysis included 7 control subjects, 7 AD patients, 7

were fixed in 4% formaldehyde for two days and embedded in paraffin. The section of blocks was cut into thickness of 4 μ m using a microtome (Leica RM2125, Leica Biosystems, Heidelberg, Germany). For hematoxylin and eosin (H&E), Sirius red staining the sections were deparaffinized rehydrated, and washed with distilled water to allow the hydrophilic solution to penetrate. After tissue watering, H&E staining was performed to observe liver sample histological changes. For IHC analysis, the sections were deparaffinized, rehydrated, cooked in antigen retrieval solution (Abcam, Cambridge, UK), and dipped in 3% hydrogen peroxide solution for 30 minutes. The primary antibody used was anti-*COL6A6* (1:200, ab150926; Abcam) were then applied for 1 hour at room temperature, and the sections were incubated with secondary antibodies for 40 minutes. Immunoreactions were visualized with DAB staining, and the sections were counterstained with Mayer's hematoxylin. All data were normalized against the equivalent data in mice fed chow (control). Immunostaining was quantified using ImageJ software (ImageJ software, 1.52a; National Institutes of Health, Bethesda, MD, USA).

RNA sequencing

This investigation included 6 control subjects and 7 patients with AD. RNA quality was assessed by analysis of ribosomal RNA band integrity using an Agilent RNA 6000 Nano kit (Agilent Technologies, Santa Clara, CA, USA). Ahead of complementary DNA (cDNA) library construction, 2 μ g of total RNA and magnetic beads with Oligo (dT) were used to enrich poly (A) mRNA. The purified mRNAs were then disrupted into short fragments, and double-stranded cDNAs were immediately synthesized. The cDNAs were subjected to end-repair and, poly (A) addition, and were then connected with sequencing adapters using the TruSeq RNA sample prep Kit (Illumina, San Diego, CA, USA). Suitable fragments automatically purified using a BluePippin 2% agarose gel cassette (Sage Science, Beverly, MA, USA) were selected as templates for PCR amplification. The final library sizes and qualities were evaluated electrophoretically using an Agilent High Sensitivity DNA kit (Agilent Technologies), and the fragments were found to be between 350 and 450 bp. The library was sequenced using an Illumina HiSeq2500 sequencer (Illumina).

Cell cultures

Human epidermal keratinocytes, neonatal (HEKn) were ac-

quired from Invitrogen (Carlsbad, CA, USA) and were grown in EpiLife medium supplemented with human keratinocyte growth supplement (HKGS, Cascade Biologics; Invitrogen). HEK293 cells were incubated with recombinant human IL-4 (30 ng/ml), IL-13 (30 ng/ml), TNF- α (30 ng/ml), TSLP (10 ng/ml), and polyinosinic-polycytidylic acid (poly I:C; Calbiochem, Billerica, MA, USA) (4 μ g/ml) for 0, 2, 6, 12, and 24 hours. Fibroblast cells were maintained in HyClone Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Cell cultures were placed in an incubator with 5% CO₂ at 37°C.

Real-time quantitative PCR

Total RNA was extracted from cells using RiboExTM Trizol (GeneAll, Seoul, Korea), according to the manufacturer's instructions. RNA concentrations were measured using a Nanodrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the purity was determined by measuring the A260/A280 ratio. cDNA was generated by reverse transcription, using 1 μ g of purified RNA and the RevertAid First Strand cDNA Synthesis Kit (Applied Biosystems, Waltham, MA, USA; Thermo Fisher Scientific) and incubated for 1 hour at 42°C. Real-time quantitative PCR assays were performed using a QuantStudio 3 system (Applied Biosystems) using a PowerUp SYBR Green Master Mix (Applied Biosystems). All data were normalized to the housekeeping gene GAPDH. Relative quantitation was analyzed using the 2- $\Delta\Delta$ Ct method according to the manufacturer's instructions.

Small interfering RNA

HEK293 and human fibroblast cells were plated at a density of 2×10^5 cells/35-mm tissue culture dish. After 20 hours and 70%~80% confluence, the cells were transfected with siRNAs in serum-free medium using Lipofectamine 3000 (Life Technologies, Carlsbad, CA, USA). Then, 5 μ l of each siRNA stock solution (20 μ M) and the PLUS reagent (10 μ l) were mixed in Opti-MEM (85 μ l; Invitrogen) in a small sterile tube. After immediate mixing and incubation at room temperature for 15 minutes, the Lipofectamine 3000 reagent (4 μ l) in Opti-MEM (100 μ l) was added, and the mixture was left at room temperature for 15 minutes. Then, 0.8 ml of HEK293 and human fibroblast cells were added to generate the siRNA-lipid complex. The transfected HEK293 and human fibroblast cells with the decreased level of COL6A6 were further identified by qRT-PCR.

Statistical analysis

GraphPad Prism (v5; GraphPad Software, Inc., La Jolla, CA, USA) was used, and all data are expressed as mean $\pm$ standard error of the mean. Differences in results were analyzed using the Mann-Whitney U test. *p*-values <0.05 were considered statistically significant.

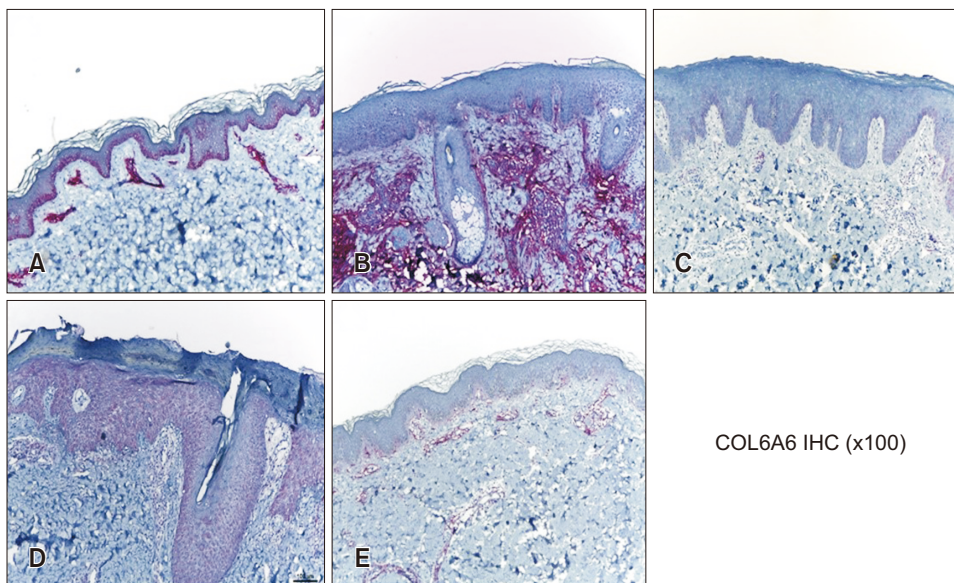


Fig. 1. (A) Immunohistochemical staining (IHC) of normal skin for COL6A6 protein expression (purple). (B) IHC of skin with atopic dermatitis shows reduced epidermal COL6A6 expression and increased dermal COL6A6 expression. (C) Skin with psoriasis shows reduced overall COL6A6 expression. (D) Skin with papular urticaria shows COL6A6 in all epidermal layers and reduced dermal COL6A6. (E) Skin with pityriasis rosea shows reduced epidermal COL6A6 expression. Scale bar 100 μ m, magnification $\times 100$. COL6A6: collagen VI $\alpha 6$ chain.

RESULTS

COL6A6 expression decreased in the epidermis of AD patients, while a significant increase was observed in the dermis

Seven healthy controls (age, 32.4 ± 4.0 years), 7 patients with AD (age, 26.2 ± 10.3 years), 7 psoriasis patients (age, 25.4 ± 29.0

years), 1 papular urticaria patient (age, 28.0 years), and 3 patients with pityriasis rosea (age, 28.0 ± 7.0 years) were enrolled for IHC analysis. *COL6A6* was observed around the vessels of the papillary dermis and the basal layer of the epidermis in normal skin. *COL6A6* expression was decreased in the epidermis of AD patients compared to that in the control group, while a significant increase and broad distribution were ob-

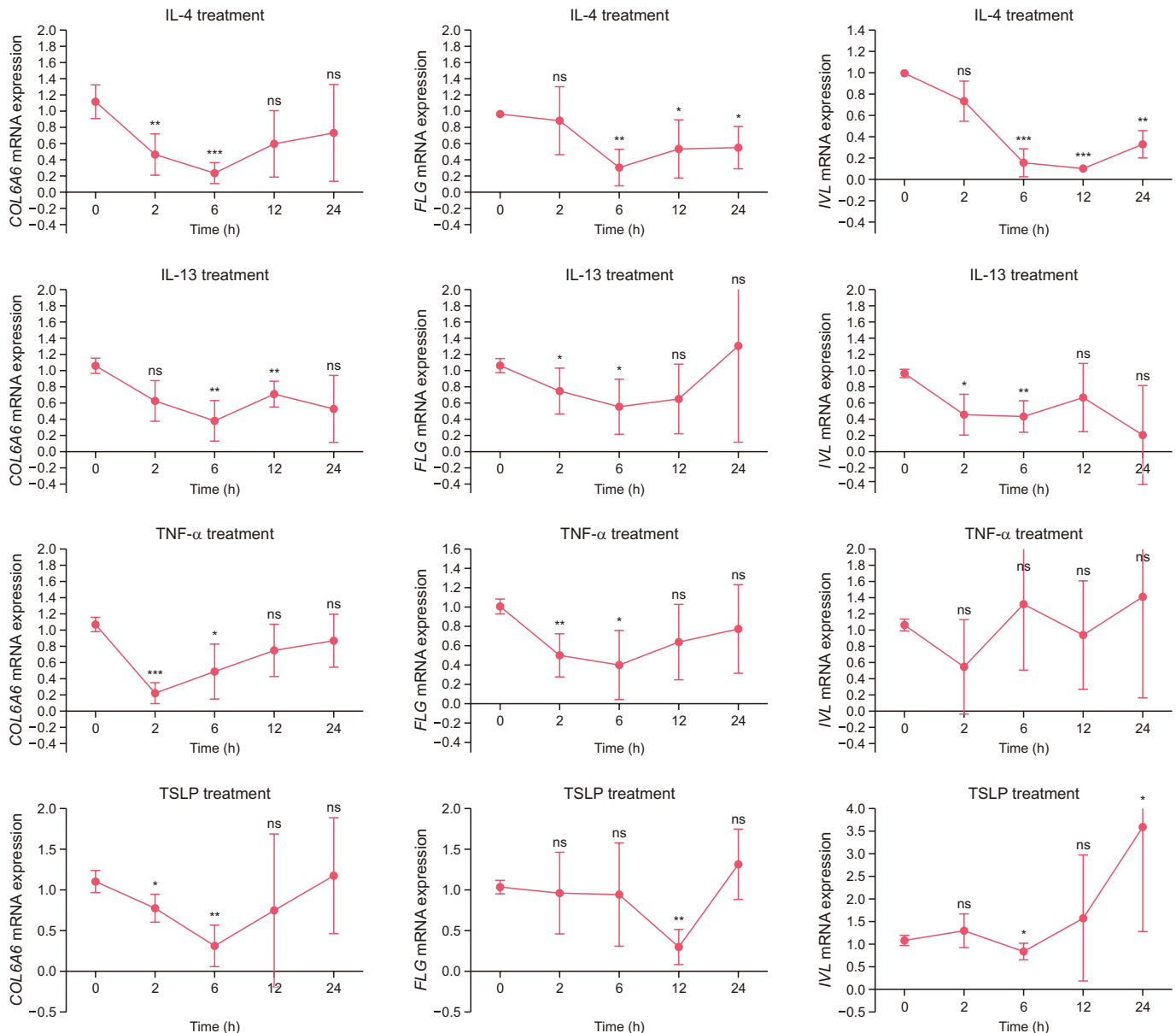


Fig. 2. *COL6A6* mRNA expression in human keratinocytes exposed to various inflammatory cytokines. After IL-4 and IL-13 treatment, mRNA expression of *COL6A6*, *FLG*, and *IVL* was suppressed. After $\text{TNF-}\alpha$ treatment, mRNA expression of *COL6A6* and *FLG* was suppressed but mRNA expression of *IVL* was unchanged. After TSLP treatment, mRNA expression of *COL6A6* and *FLG* was suppressed, but the expression of *IVL* mRNA was increased by three times compared to the baseline. *COL6A6*: collagen VI $\alpha 6$ chain, IL-4: interleukin-4, IL-13: interleukin-13, *FLG*: filaggrin, *IVL*: involucrin, $\text{TNF-}\alpha$: tumor necrosis factor- α , TSLP: thymic stromal lymphopoietin. All *p*-values less than *0.05, **0.01, or ***0.001 are summarized.

served in the dermis. IHC data from patients with psoriasis showed reduced overall COL6A6 expression. The sample from the patient with papular urticaria showed an increase in epidermal COL6A6 and a decrease in the amount of dermal COL6A6. Samples from patients with pityriasis rosea showed a decrease in the amount of epidermal COL6A6 (Fig. 1).

Total mRNA expression of COL6A6 increased in skin lesions of AD patients

RNA sequencing of COL6A6 mRNA was performed on skin biopsies. Six healthy controls (age, 22.2 ± 1.7 years) and 7 AD patients (age, 21.9 ± 1.1 years) were enrolled to obtain skin samples. The FPKMs of the control group and AD patient group were 1.257 and 20.949, respectively, and the difference between the two groups was statistically significant ($p=0.0225$). Based on this data, the mRNA expression of COL6A6 was 16.6 times higher in skin lesions of AD patients than in samples obtained from controls.

COL6A6 mRNA expression of human keratinocytes exposed to IL-4 and IL-13 was significantly suppressed

After IL-4 treatment, mRNA expression of COL6A6, FLG, and IVL was significantly suppressed by up to 80%, 60%, and

90%, respectively. After IL-13 treatment, the mRNA expression of COL6A6, FLG, and IVL was suppressed by up to 60%, 40%, and 60%, respectively. After TNF- α treatment, mRNA expression of COL6A6 and FLG was significantly suppressed by up to 80% and 60%, respectively, while the expression of IVL was unchanged. After TSLP treatment, COL6A6 and FLG mRNA expression was significantly suppressed by up to 60% and 70%, respectively, and the expression of IVL mRNA was increased by three times that of the baseline (Fig. 2).

Real-time PCR results of COL6A6 siRNA-transfected keratinocytes showed a reduced mRNA expression of FLG, LOR, and CASP14

In COL6A6 siRNA-transfected keratinocytes, the mRNA expression of FLG, LOR, and CASP14 was significantly suppressed compared to that of normal keratinocytes (Fig. 3).

COL6A6 expression of fibroblasts exposed to IL-4 and IL-13 was significantly increased

Expression of COL6A6 gradually and significantly increased after 24 hours of IL-4 and IL-13 treatments. TSLP did not affect COL6A6 expression. COL6A6 expression was significantly suppressed at 24 hours after TNF- α treatment. To confirm these results, TNF- α was applied in a concentration-

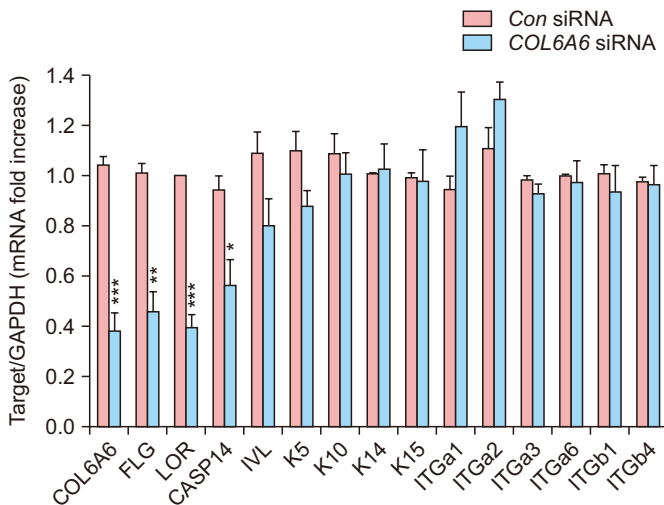


Fig. 3. Real-time PCR results of COL6A6 siRNA-transfected keratinocytes. In COL6A6 siRNA-transfected keratinocytes, expression of mRNA of FLG, LOR, and CASP14 was significantly suppressed. COL6A6: collagen VI α 6 chain, FLG: filaggrin, LOR: loricrin, CASP14: caspase 14, IVL: involucrin, K5: keratin 5, K10: keratin 10, K14: keratin 14, K15: keratin 15, ITGa1: integrin α 1, ITGa2: integrin α 2, ITGa3: integrin α 3, ITGa6: integrin α 6, ITGb1: integrin β 1, ITGb4: integrin β 4. All p -values less than *0.05, **0.01, or ***0.001 are summarized.

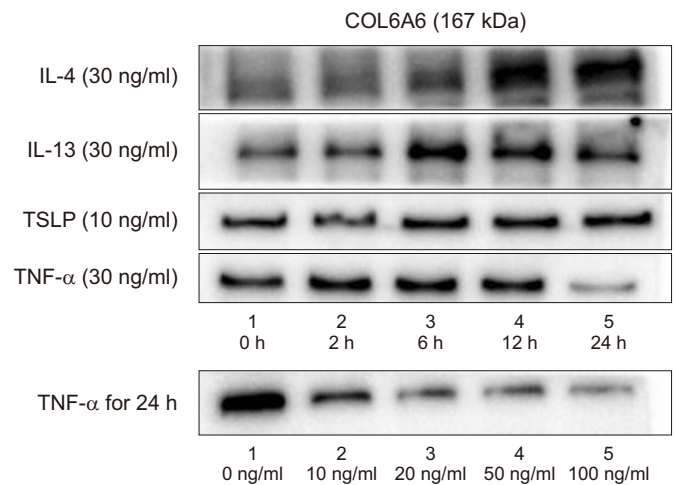


Fig. 4. COL6A6 expression in human fibroblasts exposed to various inflammatory cytokines. Expression of COL6A6 was increased for 24 hours with IL-4 and IL-13 treatment. TSLP did not affect COL6A6 expression. After TNF- α treatment, COL6A6 expression was suppressed in a concentration-dependent manner. COL6A6: collagen VI α 6 chain, IL-4: interleukin 4, IL-13: interleukin 13, TSLP: thymic stromal lymphopoietin, TNF- α : tumor necrosis factor- α .

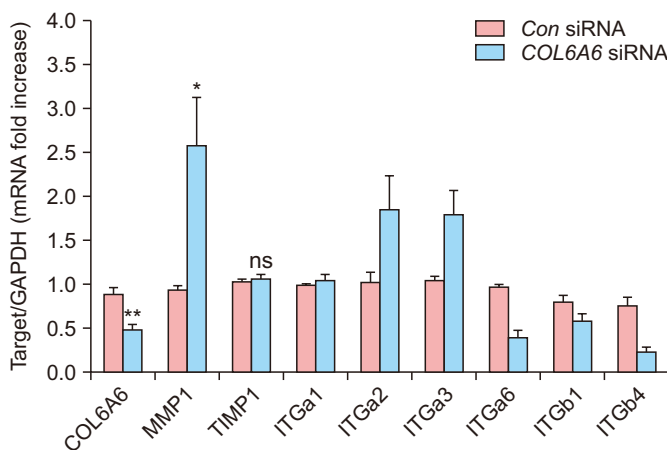


Fig. 5. Real-time PCR results of *COL6A6* siRNA-transfected fibroblasts. *COL6A6* siRNA-transfected fibroblasts showed a statistically significant increase in the production of *MMP1* mRNA. *COL6A6*: collagen VI $\alpha 6$ chain, *MMP1*: matrix metalloproteinase 1, *TIMP1*: tissue inhibitor of metalloproteinases, *ITGa1*: integrin $\alpha 1$, *ITGa2*: integrin $\alpha 2$, *ITGa3*: integrin $\alpha 3$, *ITGa6*: integrin $\alpha 6$, *ITGb1*: integrin $\beta 1$, *ITGb4*: integrin $\beta 4$. All *p*-values less than *0.05 or **0.01 are summarized.

dependent manner (0, 10, 20, 50, and 100 ng/ml) for 24 hours. The expression of *COL6A6* was suppressed as the concentration of TNF- α increased (Fig. 4).

Real-time PCR result of *COL6A6* siRNA-transfected fibroblasts showed a significant increase of *MMP1* mRNA

COL6A6 siRNA-transfected fibroblasts showed a statistically significant increase in the production of *MMP1* mRNA compared to that of normal fibroblasts (Fig. 5).

DISCUSSION

AD has a complex etiology. Although various mechanisms of AD have been studied, damage to the skin barrier, abnormal immune reactions, and inflammatory changes have been emphasized as important factors. As a result, little is known about involvement of the collagen. In particular, little is known about *COL6* because the skin is mostly comprised of collagen types I and III¹².

COL6 is composed of a triple helix in which $\alpha 1$ and $\alpha 2$ chains are combined with $\alpha 3$, $\alpha 4$, $\alpha 5$, or $\alpha 6$ ^{9,13}. Genes encoding $\alpha 4$, $\alpha 5$, and $\alpha 6$ chains are clustered together in chromosome 3q21¹³. In the skin, the $\alpha 5$ chain is mainly present in the

papillary dermis, and the $\alpha 6$ chain is mainly present around the blood vessels, nerve fibers, hair follicles, hypodermis, and basement membrane^{9,14}.

As mentioned earlier, in previous studies, we identified that polymorphisms in the *COL6A6* gene are common in Korean families with early onset AD. There have been some other reports suggesting a connection between AD and *COL6*. First, 3q21, where the genes of the *COL6* $\alpha 4$, $\alpha 5$, and $\alpha 6$ chains are located, is the major susceptibility locus of AD¹⁵. In addition, changes in *COL6A6* have been reported in AD-related transcriptome data studies¹⁶. Mutations in $\alpha 1$, $\alpha 2$, and $\alpha 3$ chains resulted in changes such as keloids, cigarette paper scars, dry skin, striae rubrae, and keratosis pilaris; in contrast, AD occurred in children with mutations in the $\alpha 6$ chain^{9,11,17,18}. As such, there is some evidence that *COL6A6* and AD are related, but no detailed studies have been conducted. This is the first full-scale study to date.

First, we wanted to know if there are any changes in *COL6A6* in AD. And whether these changes are characteristics that distinguishes it from other skin diseases. Therefore, IHC stain were performed after obtaining lesions of various skin diseases, and as a result, it was confirmed that a unique change in *COL6A6* in AD appeared.

This study confirmed the decrease in *COL6A6* in the epidermis of AD patients. Exposure of keratinocytes to IL-4 and IL-13 suppressed the expression of *COL6A6*. The exact mechanism and significance of the results on AD require additional research, but there is a study that may be helpful for understanding: Söderhäll et al.¹⁹ reported that the expression of collagen VI $\alpha 5$ chain (*COL6A5*) was reduced in the outer epidermis in AD patients. *COL6A5* has a von Willebrand factor type A domain (vWA), is involved in the binding of proteins and ligands, and plays a role in keratinocyte cohesion¹⁹. Accordingly, it has been argued that when *COL6A5* is reduced, the integrity of the epidermis decreases and the penetration of antigens through the skin increases^{19,20}. *COL6A6* exists in the same genetic locus as *COL6A5*, and *COL6A6* is collagen with vWA, an important component of the basal lamina. It is also involved in the binding of epithelial cells to fibronectin^{9,21,22}. Therefore, it is possible that reduced *COL6A6* has a similar effect as the reduction of *COL6A5* and more research is needed on that.

Experiments using *COL6A6* siRNA-transfected keratinocytes showed the suppression of *FLG*, *LOR*, and *CASPI4*

mRNA expression. Until now, the cause of reduction of filaggrin or loricrin in AD referred to *FLG* mutation, changes in skin pH, calcium gradient, and other factors²³. However, considering the results of this study and some reports that co-culture of keratinocytes with collagen promotes proliferation and differentiation of keratinocytes^{24,25}, it is possible that suppressed COL6A6 affects the expression of *FLG*, and *LOR*. In other words, the predominant TH2 cytokines in AD reduce the expression of COL6A6 in the epidermis, and the decrease in COL6A6 expression further decreases the expression of *FLG*, *LOR*, and *CASPI4*, which are important factors in the skin barrier. Therefore, it can be interpreted that a baby born with a mutation of the *COL6A6* gene is in a condition similar to a baby born with a *FLG* gene mutation because the skin barrier is not strong¹⁸. Therefore, it is worth conducting a more detailed study.

IL-4 and IL-13, representative inflammatory cytokines in AD, are known to promote fibrosis²⁴. IL-13 prevents fibroblasts from producing MMP, causing collagen to accumulate and promote the production of transforming growth factor β -1^{26,27}. IL-4 has been reported to be involved in fibroblast proliferation and collagen production^{27,28}. This study showed that the amount of COL6A6 in the dermis increased when fibroblasts were exposed to IL-4 and IL-13, and it can be considered that fibrotic cytokines promote COL6A6 production. These changes are not unique to skin fibroblasts. When conjunctival fibroblasts are exposed to Th2 cytokines, collagen production increases and the amount of MMP1 decreases²⁹.

The accumulated collagen in the dermis may primarily be a result of the disease, but it is questioned whether there is any further significance. Other studies investigated this matter, and the following studies provided insight.

Dendritic cells, which are important for immune reactions in the dermis, move across the extracellular matrix (ECM) and are, mainly composed of collagen1. Dendritic cell movement is influenced by the tissue origin of cells, the degree of maturity, and the three-dimensional structure of the ECM³⁰. Because changes in collagen cause changes in the three-dimensional structure of the dermis, it can affect the migration of dendritic cells. When the migration of dendritic cells changes, the binding between antigen-presenting cells and T cells also changes. In other words, the time required for the two cells to cause an immune response becomes too short or too long³¹. Considering the results of the aforementioned

study and the results of the present experiment, accumulated collagen is a result of AD, but there is a possibility that it may affect AD inflammation in a reversed manner.

The main limitation of this study was the small sample size. In addition, the expression of COL6A6 in the epidermis of AD patients was so low that protein expression could not be measured using western blot analysis. And when RNA sequencing of the entire lesion was initially performed, the epidermis and dermis were not separated. As the changes of COL6A6 in the epidermis and dermis are reversed, there is a limitation according to this.

In conclusion, AD patients with increased IL-4 and IL-13 levels showed reduced COL6A6 levels in the epidermis; *FLG*, *LOR*, and *CASPI4* mRNA levels decreased accordingly. The increased amount of COL6A6 in the dermis coincided with the decreased expression of MMP1. In addition to confirming these specific changes of COL6A6 in AD, this study suggests topics that are worth researching, such as how changes in COL6A6 occur and how COL6A6 affects AD.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

FUNDING SOURCE

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. 2019R1A2C1090226).

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Hye Jung Jung, <https://orcid.org/0000-0003-0995-5711>
 Won Il Heo, <https://orcid.org/0000-0003-3220-2275>
 Kui Young Park, <https://orcid.org/0000-0001-5965-1754>
 Mi-Kyung Lee, <https://orcid.org/0000-0003-1824-476X>
 Ji Young Ahn, <https://orcid.org/0000-0002-6766-9978>
 Mi Youn Park, <https://orcid.org/0000-0002-1824-8309>
 Seong Jun Seo, <https://orcid.org/0000-0003-2915-839X>

REFERENCES

- David Boothe W, Tarbox JA, Tarbox MB. Atopic dermatitis: pathophysiology. *Adv Exp Med Biol* 2017;1027:21-37.
- Malik K, Heitmiller KD, Czarnowicki T. An update on the pathophysiology of atopic dermatitis. *Dermatol Clin* 2017;35:317-326.
- Kim BJ, Wang HY, Lee H, Lee SY, Hong SJ, Choi EH. Clinical characteristics and genetic variations in early-onset atopic dermatitis patients. *Ann Dermatol* 2019;31:286-293.
- Li K, Seok J, Park KY, Yoon Y, Kim KH, Seo SJ. Copy-number variation of the filaggrin gene in Korean patients with atopic dermatitis: what really matters, 'number' or 'variation'? *Br J Dermatol* 2016;174:1098-1100.
- Li K, Oh WJ, Park KY, Kim KH, Seo SJ. FLG mutations in the East Asian atopic dermatitis patients: genetic and clinical implication. *Exp Dermatol* 2016;25:816-818.
- Park KY, Li K, Seok J, Seo SJ. An analysis of the filaggrin gene polymorphism in Korean atopic dermatitis patients. *J Korean Med Sci* 2016;31:1136-1142.
- Lee J, Jang A, Seo SJ, Myung SC. Epigenetic regulation of filaggrin gene expression in human epidermal keratinocytes. *Ann Dermatol* 2020;32:122-129.
- Heo WI, Park KY, Jin T, Lee MK, Kim M, Choi EH, et al. Identification of novel candidate variants including *COL6A6* polymorphisms in early-onset atopic dermatitis using whole-exome sequencing. *BMC Med Genet* 2017;18:8.
- Cescon M, Gattazzo F, Chen P, Bonaldo P. Collagen VI at a glance. *J Cell Sci* 2015;128:3525-3531.
- Lebbink RJ, de Ruiter T, Adelmeijer J, Brenkman AB, van Helvoort JM, Koch M, et al. Collagens are functional, high affinity ligands for the inhibitory immune receptor LAIR-1. *J Exp Med* 2006;203:1419-1425.
- Tagliavini F, Pellegrini C, Sardone F, Squarzone S, Paulsson M, Wagener R, et al. Defective collagen VI $\alpha 6$ chain expression in the skeletal muscle of patients with collagen VI-related myopathies. *Biochim Biophys Acta* 2014;1842:1604-1612.
- Mempel M, Schmidt T, Boeck K, Brockow K, Stachowitz S, Fesq H, et al. Changes in collagen I and collagen III metabolism in patients with generalized atopic eczema undergoing medium-dose ultraviolet A1 phototherapy. *Br J Dermatol* 2000;142:473-480.
- Fitzgerald J, Rich C, Zhou FH, Hansen U. Three novel collagen VI chains, $\alpha 4(VI)$, $\alpha 5(VI)$, and $\alpha 6(VI)$. *J Biol Chem* 2008;283:20170-20180.
- Gara SK, Grumati P, Squarzone S, Sabatelli P, Urciuolo A, Bonaldo P, et al. Differential and restricted expression of novel collagen VI chains in mouse. *Matrix Biol* 2011;30:248-257.
- Lee YA, Wahn U, Kehrt R, Tarani L, Businco L, Gustafsson D, et al. A major susceptibility locus for atopic dermatitis maps to chromosome 3q21. *Nat Genet* 2000;26:470-473.
- Ghosh D, Ding L, Sivaprasad U, Geh E, Biagini Myers J, Bernstein JA, et al. Multiple transcriptome data analysis reveals biologically relevant atopic dermatitis signature genes and pathways. *PLoS One* 2015;10:e0144316.
- Hunter JM, Ahearn ME, Balak CD, Liang WS, Kurdoglu A, Corneveaux JJ, et al. Novel pathogenic variants and genes for myopathies identified by whole exome sequencing. *Mol Genet Genomic Med* 2015;3:283-301.
- Sabatelli P, Gara SK, Grumati P, Urciuolo A, Gualandi F, Curci R, et al. Expression of the collagen VI $\alpha 5$ and $\alpha 6$ chains in normal human skin and in skin of patients with collagen VI-related myopathies. *J Invest Dermatol* 2011;131:99-107.
- Söderhäll C, Marenholz I, Kerscher T, Rüschenhoff F, Esparza-Gordillo J, Worm M, et al. Variants in a novel epidermal collagen gene (*COL29A1*) are associated with atopic dermatitis. *PLoS Biol* 2007;5:e242.
- Harazin M, Parwez Q, Petrasch-Parwez E, Epplen JT, Arinir U, Hoffjan S, et al. Variation in the *COL29A1* gene in German patients with atopic dermatitis, asthma and chronic obstructive pulmonary disease. *J Dermatol* 2010;37:740-742.
- Groulx JF, Gagné D, Benoit YD, Martel D, Basora N, Beaulieu JF. Collagen VI is a basement membrane component that regulates epithelial cell-fibronectin interactions. *Matrix Biol* 2011;30:195-206.
- Keene DR, Engvall E, Glanville RW. Ultrastructure of type VI collagen in human skin and cartilage suggests an anchoring function for this filamentous network. *J Cell Biol* 1988;107:1995-2006.
- Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M, et al. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009;129:1892-1908.
- Matsuura-Hachiya Y, Arai KY, Muraguchi T, Sasaki T, Nishiyama T. Type IV collagen aggregates promote keratinocyte proliferation and formation of epidermal layer in human skin equivalents. *Exp Dermatol* 2018;27:443-448.
- Zhou T, Wang N, Xue Y, Ding T, Liu X, Mo X, et al. Electrospun tilapia collagen nanofibers accelerating wound healing via inducing keratinocytes proliferation and differentiation. *Colloids Surf B Biointerfaces* 2016;143:415-422.
- Oh MH, Oh SY, Yu J, Myers AC, Leonard WJ, Liu YJ, et al. IL-13 induces skin fibrosis in atopic dermatitis by thymic stromal lympho-

- poietin. *J Immunol* 2011;186:7232-7242.
27. Van Linthout S, Miteva K, Tschöpe C. Crosstalk between fibroblasts and inflammatory cells. *Cardiovasc Res* 2014;102:258-269.
28. Berroth A, Kühnl J, Kurschat N, Schwarz A, Stäb F, Schwarz T, et al. Role of fibroblasts in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 2013;131:1547-1554.
29. Leonardi A, Cortivo R, Fregona I, Plebani M, Secchi AG, Abatan-gelo G. Effects of Th2 cytokines on expression of collagen, MMP-1, and TIMP-1 in conjunctival fibroblasts. *Invest Ophthalmol Vis Sci* 2003;44:183-189.
30. Gunzer M, Friedl P, Niggemann B, Bröcker EB, Kämpgen E, Zänker KS. Migration of dendritic cells within 3-D collagen lattices is dependent on tissue origin, state of maturation, and matrix structure and is maintained by proinflammatory cytokines. *J Leukoc Biol* 2000;67:622-629.
31. Dustin ML, de Fougères AR. Reprogramming T cells: the role of extracellular matrix in coordination of T cell activation and migration. *Curr Opin Immunol* 2001;13:286-290.