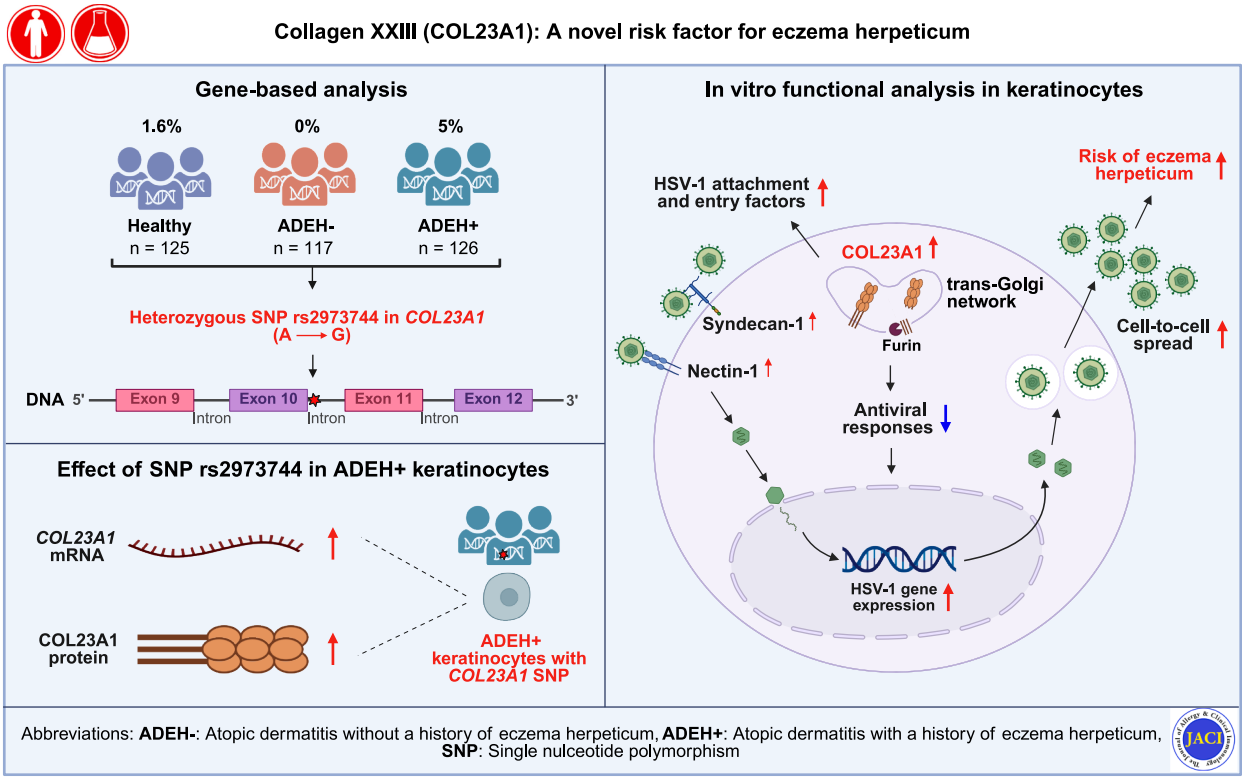
Collagen XXIII (COL23A1): A novel risk factor for eczema herpeticum

Shruti Chopra, PhD, Jana Zeitvogel, PhD, Stephan Traidl, MD, MSc, Ilona Klug, CTA, Elke Rodriguez, PhD, Inken Harder, MSc, et al

GRAPHICAL ABSTRACT



Capsule summary: The single nucleotide polymorphism rs2973744 in *COL23A1* was identified as a novel risk factor for eczema herpeticum, and it was discovered that elevated COL23A1 levels promote herpes simplex virus 1 infection in keratinocytes, which could be exploited as a therapeutic target for eczema herpeticum.

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Collagen XXIII (COL23A1): A novel risk factor for eczema herpeticum

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Background: Eczema herpeticum (EH) is a potentially life-threatening disseminated skin infection caused by herpes simplex virus (HSV) in a subset of patients with atopic dermatitis (AD). The occurrence of EH in a subset of patients with AD and its frequent recurrence imply the importance of genetic factors in its pathogenesis.
Objective: We sought to identify novel genetic risk factors for EH and to study their impact on HSV-1 infection.
Methods: Using whole-exome sequencing we identified a heterozygous single nucleotide polymorphism (SNP) in the *COL23A1* gene (encoding collagen type XXIII alpha 1 chain or COL23A1) that was associated with EH and validated it by PCR in a larger cohort. We studied the effect of upregulated *COL23A1* expression on HSV-1 infection in primary keratinocytes and HaCaT cells and performed bulk RNA sequencing to address the underlying mechanism.
Results: Primary keratinocytes derived from patients with EH carrying this heterozygous SNP rs2973744 had elevated *COL23A1* mRNA and protein levels as well as an increased susceptibility to HSV-1. Increasing the COL23A1 levels experimentally enhanced HSV-1 infection in human keratinocytes. *COL23A1* overexpression elevated syndecan-1 and nectin-1 levels on the cell surface, which are HSV-1 attachment and entry factors, respectively, and downregulated genes involved in antiviral responses, such as *IL1R1*, *IL32*, *TLR4*, *IRF1*, *S100A9*, *C3*, and *CFH*.
Conclusions: The SNP rs2973744 enhances COL23A1 expression in keratinocytes derived from patients with AD and a history of EH. Upregulation of COL23A1 promotes HSV-1 infection presumably by upregulating the HSV-1 attachment and entry factors syndecan-1 and nectin-1 on the cell surface and attenuating antiviral responses of keratinocytes. (J Allergy Clin Immunol 2025;156:1247-59.)

Key words: *Atopic dermatitis, eczema herpeticum, HSV-1,* COL23A1*, keratinocytes*

Atopic dermatitis (AD) is a common chronic inflammatory skin disease that affects 20% of children and 2% to 5% of adults worldwide.[1-3] It is a multifactorial disease influenced by genetic, immunologic, and environmental factors. Patients suffering from AD have an impaired skin barrier with type 2–dominated immune responses and heightened allergen sensitization. This presumably renders them more susceptible to bacterial and viral infections, including infections with herpes simplex virus (HSV).[4-7] About 67% and 13% of humans worldwide are estimated to be infected with HSV-1 and HSV-2, respectively; however, most of them develop no or only minor symptoms.[8] HSV-1 primarily causes oral lesions, whereas HSV-2 predominantly leads to genital lesions.

HSV-1 and HSV-2 are enveloped double-stranded DNA viruses that infect epithelial cells of skin, mucosa, or cornea. HSV-1 enters keratinocytes either by direct fusion with the plasma membrane or after endocytosis by fusion with endosomal membranes.[9,10] Initially, HSV-1 and HSV-2 attach to the cell surface via interactions between the viral glycoprotein gC or gB and heparan sulfate proteoglycans such as syndecan-1 or chondroitin proteoglycans on the cell surface.[11] HSV-1 binds heparan sulfate proteoglycans more efficiently than HSV-2. Subsequent entry requires the interaction of viral glycoprotein gD with one of the major HSV-1 receptors on host cells—nectin-1, herpesvirus entry mediator (HVEM), or 3-*O* sulfated heparan sulfate.[12] HSV-1 and HSV-2 interact with nectin-1 in a highly conserved, nearly identical manner. Because of differences in the N-termini of their gD proteins, HSV-2 can use nectin-2 as entry receptor, whereas HSV-1 generally cannot. After fusion of the viral envelope with a host membrane, incoming capsids are transported to the nucleus where the HSV-1 genome is transcribed in a cascade-like manner starting with immediate-early genes, followed by early and late genes.[13,14] Products of immediate-early genes are required for the transcription of early genes, which then encode proteins required for viral DNA synthesis. The products encoded by immediate-early and early genes are further used for efficient late gene transcription. After primary infection of epithelial cells, HSV-1 spreads to neurons and establishes a lifelong latent infection in trigeminal or dorsal root ganglia. On reactivation, it can be transported back to the original dermatome in the skin or the mucosa.[15-18]

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Abbreviations used
AD: Atopic dermatitis
ADEH+: Patients with atopic dermatitis with a history of eczema herpeticum
ADEH−: Patients with atopic dermatitis without a history of eczema herpeticum
COL23A1: Collagen type XXIII alpha 1 chain
EH: Eczema herpeticum
FC: Fold change
FI: Furin inhibitor I
GFP: Green fluorescent protein
hpi: Hours postinfection
HSV: Herpes simplex virus
HVEM: Herpesvirus entry mediator
MOI: Multiplicity of infection
pfu: Plaque-forming unit
RNA-seq: RNA sequencing
SNP: Single nucleotide polymorphism

In a subgroup of patients with AD, HSV can cause severe disseminated infections, referred to as eczema herpeticum (EH).[7,19] EH is a dermatological emergency, because it can lead to life-threatening encephalitis or even death if left untreated.[20] Approximately 22% of moderately to severely affected patients with AD report at least 1 episode of EH in their lifetime.[7,19,21] Notably, more than 50% of these individuals suffer from recurrent episodes. These findings indicate that genetic predispositions contribute to EH development.

Previous studies identified several genetic factors associated with EH.[22] Particularly, the R501X mutation in filaggrin, an important structural protein involved in skin barrier function, increased the risk of developing EH by 3-fold in patients with AD.[23,24] Single nucleotide polymorphisms (SNPs) in *CLDN1* and *CLDN16* encoding the tight junction proteins claudin-1 and claudin-16, and in *SIDT2* and *RBBP8NL* encoding SID1 transmembrane family member 2 and RBBP8 N-terminal like, are also associated with an increased risk of EH in patients with AD, and silencing *CLDN1*, *SIDT2*, or *RBBP8NL* increases HSV-1 infection.[25-27]

Because these EH-associated SNPs do not explain all EH cases, we performed comparative whole-exome sequencing in patients with AD without a history of EH (ADEH−) or those with a history of EH (ADEH+) and healthy controls to search for further genetic variants associated with EH. We discovered that a novel heterozygous SNP in *COL23A1*, rs2973744, significantly associated with EH development.

COL23A1 encodes collagen type XXIII alpha 1 chain (COL23A1), a type II transmembrane protein, which is located in its full-length form (75 kDa) in the lipid rafts of the cell membrane or is cleaved by furin or other proteases in the Golgi/trans-Golgi network. The resulting ectodomain (60 kDa) is secreted from the cells into the extracellular matrix.[28,29] The SNP rs2973744 in *COL23A1* caused thymine to cytosine substitution at a splice donor site of the transcript allele between exons 10 and 11, which led to COL23A1 upregulation and expression of a differentially processed form of COL23A1 in keratinocytes derived from patients with EH. In a HaCaT model, elevated COL23A1 expression enhanced cell surface syndecan-1 and nectin-1 levels while reducing the expression of genes associated with effective antiviral responses, such as *IL1R1*, *IL32*, *TLR4*, *IRF1*, *S100A9*, *C3*, and *CFH*. These changes most likely increased HSV-1 gene expression and the formation of infectious centers and may render patients with AD carrying the SNP more susceptible to EH.

METHODS

Study subjects

The recruitment of ADEH+ and ADEH− patients was carried out at the Department of Dermatology and Allergy of Hannover Medical School, Hannover, Germany. AD was diagnosed in the patients according to Hanifin and Rajka criteria.[30] The Ethics Committee of Hannover Medical School (no. 2857-2015 and 8733_BO_S_2019) approved this study, and all study participants provided written informed consent. DNA samples from ADEH+ and ADEH− patients were derived from the GENEVA cohort, Biobank of the University Hospital Schleswig-Holstein, Kiel, Germany.

Whole-exome sequencing

Whole-exome sequencing was performed at the Research Core Unit Genomics facility of Hannover Medical School. The analysis involved 9 patients with a history of EH and first-degree healthy relatives of 7 patients. The SureSelect^{XT} Target Enrichment System (Agilent, Santa Clara, Calif) and a NextSeq (Illumina, San Diego, Calif) sequencing device were used to perform sequencing. TaqMan-PCR (Applied Biosystems, Foster City, Calif) was used to reanalyze candidate variants in an age/sex-adjusted manner on 3 larger cohorts, comprising 117 ADEH+ and 117 ADEH− patients (GENEVA cohort and RESIST HSV/AD cohort) and 118 healthy controls (POPGEN cohort).[31,32] The detailed methods are described in this article's Methods section in the Online Repository at www.jacionline.org.

Cell culture

Primary hair keratinocytes were derived from the outer root sheath of the hair follicle, as previously described.[33] The primary foreskin keratinocytes were isolated from the foreskin of anonymous children undergoing surgery, as described.[34] Informed consent for experiments involving primary foreskin keratinocytes was not required because German laws consider anonymized leftover human tissue after surgery as discarded material. The detailed methods for culturing primary keratinocytes and HaCaT cells are described in this article's Online Repository.

Viruses

We used parental HSV1(17$^+$)Lox (HSV-1), HSV1(17$^+$)Lox-$_{pMCMV}$GFP (green fluorescent protein) (HSV1-GFP for short), or HSV1(17$^+$)Lox-$_{pMCMV}$mChe (HSV1-Che for short) expressing soluble GFP or mCherry under the control of the murine cytomegalovirus major immediate-early promoter, respectively.[35-37] The detailed methods are described in this article's Online Repository.

HSV-1 infection

To reach 80% confluency of cells on the day of infection, keratinocytes were seeded 1 day before infection. Next day, cells were washed with PBS (PAN Biotech, Aidenbach, Germany), and keratinocyte growth medium 2 (KGM2; PromoCell, Heidelberg, Germany) containing HSV-1 inoculum was added onto cells for 1 hour and incubated at 37°C and 5% CO_2. After the 1-hour incubation, the medium containing the virus was replaced with fresh KGM2.

A further incubation of 20 hours was carried out at 37°C and 5% CO_2. The virus strain and multiplicity of infection (MOI) used were assay-dependent and are described in the respective sections. HSV-1 infection was performed as described, unless stated otherwise.

mRNA isolation and quantitative RT-PCR

Keratinocytes were lysed, RNA was isolated, and cDNA was prepared. LightCycler480 SYBR Green I Master (Roche Molecular Biochemicals, Manheim, Germany) was used to perform quantitative real-time PCR of the target genes, as described in this article's Online Repository. Primer sequences are provided in Table E1 (in the Online Repository available at www.jacionline.org).

Flow cytometry

Keratinocytes infected with HSV1-GFP or HSV1-Che were trypsinized, and GFP and mCherry expressions were analyzed by flow cytometry (CytoFLEX; Beckman Coulter, Brea, Calif). Cell surface levels of COL23A1, syndecan-1, nectin-1, HVEM, and E-cadherin were measured by flow cytometry, as described in this article's Online Repository.

Protein lysate preparation and Western blotting

Proteins isolated from cell lysates were loaded onto 4% to 20% SDS-polyacrylamide gels (Bio-rad, Hercules, Calif), proteins were transferred to polyvinylidene fluoride membranes (Immobilon-P, Merck, Darmstadt, Germany), and COL23A1 and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were labeled with specific antibodies, as detailed in this article's Online Repository.

Furin inhibitor I

Primary foreskin keratinocytes were preseeded to reach 80% confluency on the day of treatment. Furin inhibitor I (FI; 75 μM), decanoyl-Arg-Val-Lys-Arg-chloromethylketone (Dec-RVKR-CMK; Bachem, Bubendorf, Switzerland), was added to the cells and incubated for 24 hours at 37°C and 5% CO_2. The next day, cells were either used to analyze COL23A1 surface expression using flow cytometry or infected with HSV1-GFP (0.75 plaque-forming unit [pfu]/cell) to analyze HSV-1 susceptibility.

Overexpression of *COL23A1*

Control lentiviral particles (no. LPP-NEG-Lv205-100) encoding enhanced GFP and a puromycin resistance cassette and lentiviral particles encoding *COL23A1* (no. LPP-Y2210-Lv205-100), in addition, were purchased from GeneCopoeia (Rockville, Md) and transduced in HaCaT cells. The detailed methods are described in this article's Online Repository.

HSV-1 cell-to-cell spread analysis

HaCaT cells were infected with HSV1-Che at MOIs of 0.001, 0.01, and 0.05 pfu/cell. At 20 hours postinfection (hpi), infectious centers were visualized using the BioTek Cytation 5 Cell Imaging Multimode Reader (Agilent, Santa Clara, Calif), and their number, size, and mCherry intensity were quantified using the CellProfiler software (Broad Institute, Cambridge, Mass).[38] The detailed methods are described in this article's Online Repository.

TABLE I. Frequency of the heterozygous SNP rs2973744

ADEH+ (n = 126)	5.0%
ADEH− (n = 117)	0.0%
Healthy controls (n = 125)	1.6%
χ^2 (degrees of freedom)	6762.2
P value	.034
P value summary	$P < .05$

HSV-1 temporal gene expression analysis

HaCaT cells were seeded to reach 100% confluency on the day of infection. Cells were infected with 5 pfu/cell of HSV-1 for 1 hour at 37°C and 5% CO_2. After 2, 4, 6, and 8 hpi, cells were lysed and RNA was isolated.

Bulk RNA-sequencing data analysis

Control and *COL23A1* overexpressing HaCaT cells were infected with 5 pfu/cell of HSV-1. At 2 and 6 hpi, RNA and cDNA were prepared from cells as mentioned before. Bulk RNA sequencing (RNA-seq) was performed at the Research Core Unit Genomics facility of Hannover Medical School. The bulk RNA-seq data have been uploaded to the publicly accessible Gene Expression Omnibus repository (www.ncbi.nlm.nih.gov/geo; series accession no. GSE278244). The detailed methods are described in this article's Online Repository.

Statistical analysis

Data were assessed for normality by the Shapiro-Wilk test, and subsequently the 2-tailed paired *t* test (parametric test), the Mann-Whitney *U* test, or the 1-sample Wilcoxon test was used to compare group means or medians. Statistical analyses and plots were generated using GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, Calif). Asterisks in the figures indicate *P* values ($*P < .05$; $**P < .01$; $***P < .001$). Box and whisker plots represent the 10th to 90th percentiles.

RESULTS

A novel SNP in *COL23A1*, rs2973744, is associated with EH

To identify novel genetic risk factors for EH, we performed next-generation whole-exome sequencing of 9 ADEH+ patients and their respective first-degree relatives. Three of the 9 ADEH+ patients and 1 of the unaffected healthy relatives were carriers of the heterozygous SNP rs2973744 in the gene *COL23A1* located on chromosome 5q35. None of these 3 ADEH+ patients with the SNP rs2973744 carried the variant rs61816761 (FLG R501X), rs142171036 (SIDT2), or rs200738153 (RBBPP8NL). Association between EH and the *COL23A1* rs2973744 variant was validated in a larger cohort comprising 117 ADEH+ and 117 ADEH− patients (GENEVA cohort and RESIST HSV/AD cohort) and 118 healthy controls (POPGEN cohort).[31,32] DNA was obtained from each group and TaqMan-PCR was used to investigate the occurrence of this variant. Taken together, the SNP rs2973744 occurred in 5% of ADEH+ patients, 1.6% of healthy donors, and 0% of ADEH− patients, thus showing that rs2973744 is significantly associated with EH in this group of patients with AD (χ^2 test: $P = .034$; Table I).

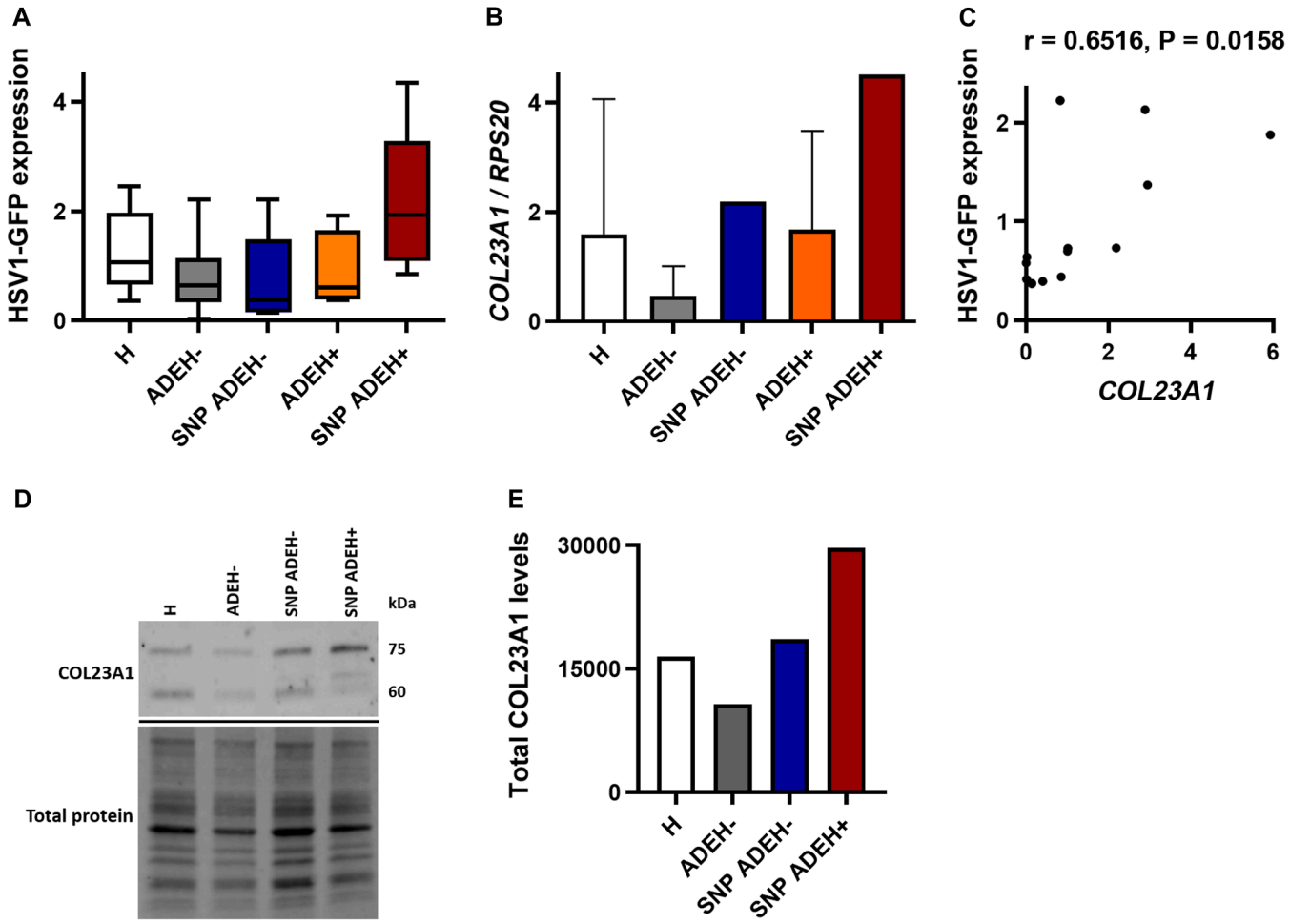

FIG 1. Primary keratinocytes derived from an ADEH+ patient are more susceptible to HSV-1 and express more *COL23A1* mRNA and total protein levels than those from ADEH− patients. **A,** Primary hair keratinocytes isolated from healthy donors (H; n = 5), patients with AD without a history of EH (ADEH−; n = 4) and with the rs2973744 SNP (SNP ADEH−; n = 1), and patients with AD with a history of EH (ADEH+; n = 2) and with the rs2973744 SNP (SNP ADEH+; n = 1) were infected with 0.75 pfu/cell of HSV1-GFP. At 20 hpi, HSV1-GFP expression was measured by flow cytometry. Data were normalized to the mean of infected ADEH− cells (floating bar graph, median; n = 1-4 independently repeated experiments). **B,** *COL23A1* mRNA levels in primary hair keratinocytes (from Fig 1, *A*) were quantified by quantitative RT-PCR and normalized to the housekeeping gene *RPS20* (*black bars*, median; n = 1). **C,** Correlation between *COL23A1* mRNA levels and HSV-1 susceptibility. **D,** Total COL23A1 protein levels in primary hair keratinocytes derived from 1 healthy, ADEH−, SNP ADEH−, and SNP ADEH+ donors were visualized using Western blot in comparison with total protein. **E,** COL23A1 protein levels visualized in Fig 1, *D*, were quantified and normalized to total protein levels (column bar graph, median; n = 1).

Keratinocytes with SNP rs2973744 are more susceptible to HSV-1 infection

Because epidermal keratinocytes are the major entry portal for HSV-1 infections of the skin,[14] we investigated the susceptibility of primary keratinocytes isolated from hair follicles of healthy donors, ADEH− patients, or ADEH+ patients to a recombinant HSV-1 strain expressing GFP as a reporter (HSV1-GFP). HSV1-GFP expression was higher in keratinocytes from an ADEH+ patient with the *COL23A1* SNP than in keratinocytes derived from healthy donors, ADEH− patients without or with the *COL23A1* SNP, and ADEH+ patients without the SNP (Fig 1, *A*). To investigate whether COL23A1 expression could affect HSV-1 susceptibility, we measured the *COL23A1* transcript and protein levels in different groups. Primary keratinocytes isolated from the ADEH+ patient carrying the SNP rs2973744 exhibited the highest levels of *COL23A1* mRNA and protein (Fig 1, *B*, *D*, and *E*). Interestingly, there was a positive correlation between *COL23A1* mRNA levels and HSV-1 susceptibility (Fig 1, *C*; Pearson correlation coefficient = 0.65; $P = .016$). In keratinocytes harboring the SNP, the 75-kDa protein was more strongly expressed than the 60-kDa form, whereas in healthy keratinocytes the 60-kDa protein was more prominent than the 75-kDa form. Moreover, keratinocytes with the SNP rs2973744 expressed another protein variant with an intermediate apparent molecular weight, which was not observed in the keratinocytes lacking the SNP. These data suggest that the SNP at the splice donor site leads to differentially expressed proteins, possibly because of alternative splicing of the different *COL23A1* mRNAs. The increased COL23A1 levels in keratinocytes from an ADEH+ donor with the SNP rs2973744 correlated with a more efficient HSV-1 infection, suggesting that this SNP in conjunction with other genetic factors associated with EH increases COL23A1 expression and contributes to the heightened susceptibility to HSV-1.

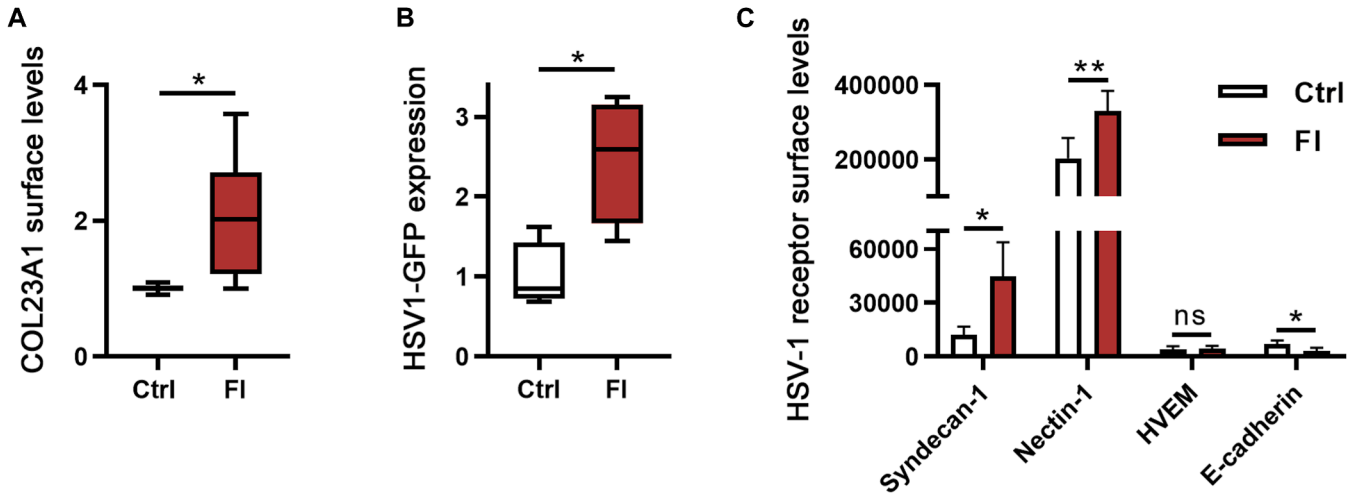

FIG 2. Furin inhibition increases COL23A1 levels on the surface of keratinocytes and enhances their susceptibility to HSV-1. **A,** Primary foreskin keratinocytes were stained for COL23A1 after 20 hours in the absence (Ctrl) or presence (FI) of FI (75 μM). COL23A1 surface levels were analyzed by flow cytometry and normalized to isotype control (n = 6 different donors). Statistical significance was analyzed with paired *t* test (SD = 0.8742; SEM = 0.3569; $*P < .05$). **B,** Primary foreskin keratinocytes were preincubated with FI as described in Fig 2, *A*, and then infected with 0.75 pfu/cell of HSV1-GFP for 20 hours in the absence of FI. The effect of FI treatment on HSV1-GFP expression was measured by flow cytometry. Data were normalized to the mean of infected Ctrl cells (Box and whisker plots represent 10th-90th percentile; n = 4 different donors). Statistical significance was analyzed with paired *t* test (SD = 0.5185; SEM = 0.2592; $*P < .05$). **C,** Primary foreskin keratinocytes were preincubated with FI as described in Fig 2, *A*. Syndecan-1, nectin-1, HVEM, and E-cadherin surface levels were analyzed by flow cytometry and normalized to isotype control (n = 4 different donors). Statistical significance was analyzed with paired *t* test ($*P < .05$; $**P < .01$). *NS,* Not significant.

Increased cell surface expression of *COL23A1* in primary keratinocytes correlates with increased susceptibility to HSV-1

Cultured cells synthesize full-length COL23A1 (75 kDa), which localizes to the plasma membrane, and a secreted ectodomain (60 kDa) derived from cleavage primarily by the protease furin in the Golgi/trans-Golgi network.[29,39] To test whether blocking the shedding of COL23A1 affects HSV-1 infection, we pretreated keratinocytes with FI and infected them with HSV1-GFP in the absence of this inhibitor. FI significantly increased the cell surface level of COL23A1 (Fig 2, *A*) and HSV1-GFP expression in primary keratinocytes (Fig 2, *B*) by 2- and 2.5-fold, respectively, indicating that an increased surface expression of COL23A1 at the plasma membrane promoted HSV-1 infection. Elevated COL23A1 levels at the cell surface may stabilize HSV-1 attachment factors and viral receptors and thereby increase HSV-1 susceptibility. Notably, blocking furin cleavage significantly increased cell surface levels of syndecan-1 and nectin-1 but had no effect on HVEM levels and decreased the cell surface levels of the cell adhesion molecule E-cadherin (Fig 2, *C*).

Stable overexpression of *COL23A1* in HaCaT cells enhanced HSV-1 susceptibility, cell-to-cell spread, and gene expression

To analyze the impact of elevated COL23A1 levels on HSV-1 infection, we stably overexpressed *COL23A1* in the human keratinocyte cell line HaCaT. After transduction with *COL23A1* encoding but not with empty (Ctrl) lentiviral vectors, HaCaT cells overexpressed *COL23A1* at the mRNA and protein level (Fig 3, *A* and *B*). *COL23A1* overexpression but not lentiviral transduction alone significantly increased infection with a recombinant HSV-1 strain expressing mCherry as a reporter (HSV1-Che) by about 1.6-fold (Fig 3, *C*).

Within 20 hours of infection at an MOI of 1 pfu/cell, HSV-1 released from the initially infected cells had spread to neighboring cells resulting in a second, if not a third, infection cycle. To investigate whether the higher expression of HSV-1–mediated Che in cultures of cells overexpressing *COL23A1* was due to an increased HSV-1 cell-to-cell spread, we infected the cells with HSV1-Che at very low MOIs and quantified the number, size, and intensity of infectious centers. Interestingly, the numbers of infectious centers were increased approximately 2-fold, whereas their size and their mCherry expression levels were slightly but significantly increased in *COL23A1* overexpressing HaCaT cells (Fig 3, *D-G*). These data suggest that increased amounts of COL23A1 enhanced both initial HSV-1 infection of HaCaT cells and cell-to-cell spread.

To determine at which stage *COL23A1* overexpression affects HSV-1 infection, we infected control and *COL23A1* overexpressing HaCaT cells with HSV-1 and investigated viral gene expression. At 6 and 8 hpi, mRNA expression levels of 2 immediate-early (*UL54* and *US12*), 2 early (*UL23* and *UL42*), and 2 late (*UL19* and *UL27*) genes were either significantly upregulated or increased by trend (Fig 4, *A-F*), suggesting that *COL23A1* overexpression increased the expression of HSV-1 genes of all 3 kinetic classes.

COL23A1 overexpression enhances cell surface syndecan-1 and nectin-1 levels on HaCaT cells

Because *COL23A1* overexpression already increased HSV-1 immediate-early gene expression, we measured cell surface levels of the HSV-1 attachment factor syndecan-1, a heparan sulfate

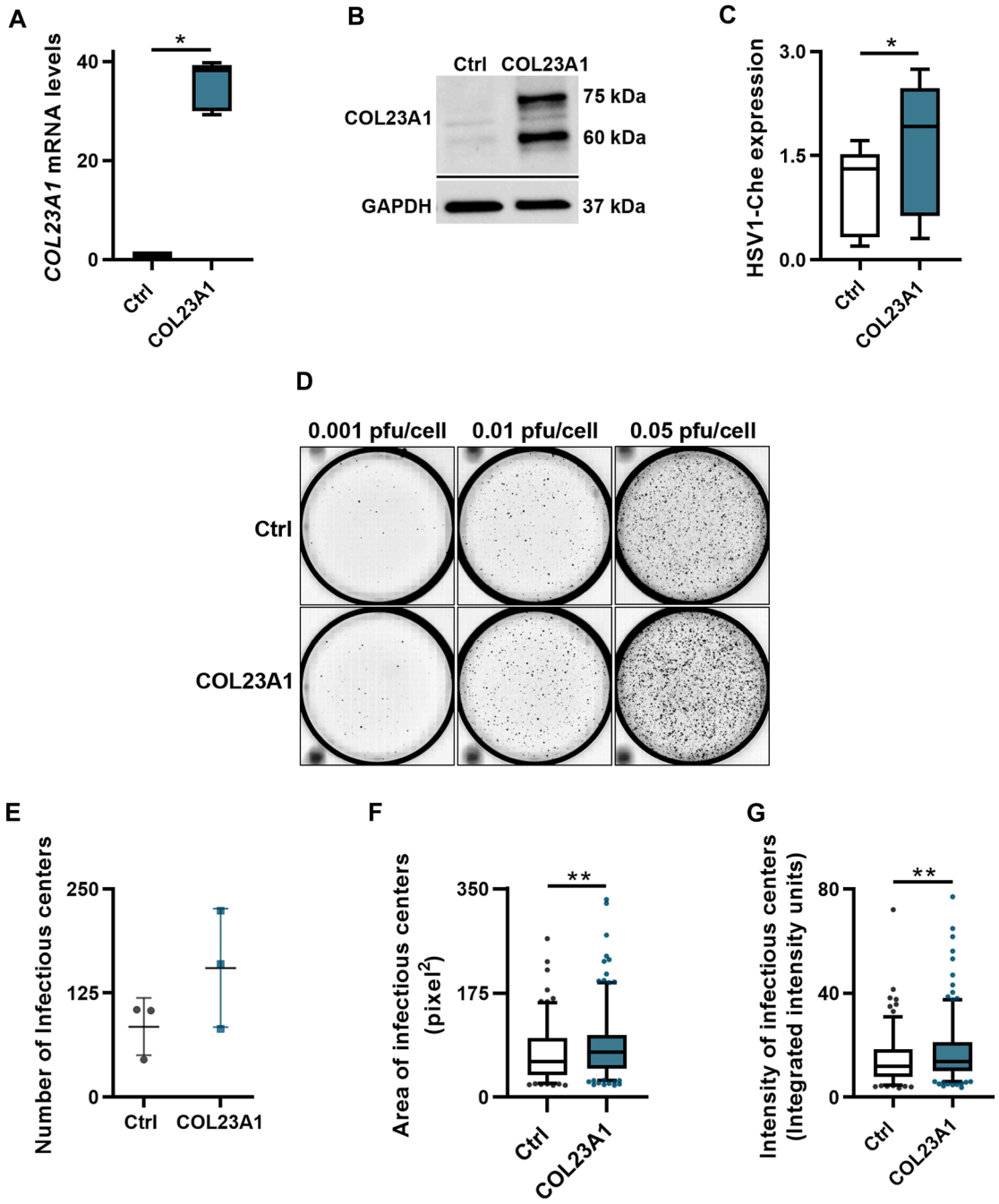

FIG 3. *COL23A1* overexpression increases HSV-1 susceptibility and cell-to-cell spread in HaCaT cells. **A** and **B,** *COL23A1* overexpression was confirmed by quantifying *COL23A1* mRNA levels by quantitative RT-PCR and normalizing to the housekeeping gene *RPS20* (box and whisker plots represent 10th-90th percentile; n = 6 independently repeated experiments) (Fig 3, *A*) (statistical significance was analyzed with the 1-sample Wilcoxon test), and COL23A1 protein levels using Western blot in comparison with the housekeeping protein GAPDH (n = 4 independently repeated experiments) (Fig 3, *B*). **C,** Control and *COL23A1* overexpressing HaCaT cells were infected with 1 pfu/cell of HSV1-Che. At 20 hpi, mCherry expression was measured by flow cytometry and normalized to the mean of infected control cells (box and whisker plots represent 10th-90th percentile; n = 5 independently repeated experiments). Statistical significance was analyzed with paired *t* test (SD = 0.3603; SEM = 0.1611; $*P < .05$). **D,** Control and *COL23A1* overexpressing cells were infected with 0.001, 0.01, or 0.05 pfu/cell of HSV1-Che. mCherry signals were visualized by automated microscopy (n = 3 independently repeated experiments). **E-G,** Quantitation of the number of infectious centers (Fig 3, *E*; scatter dot plot, mean ± SD) and area and integrated intensity (Fig 3, *F* and *G*; box and whisker plots represent 10th-90th percentile; n = 45-224 infectious centers at MOI 0.01) was carried out using the CellProfiler software. Statistical significances were analyzed using the Mann-Whitney test (Fig 3, *F*, median, 59.67, 75.25; Fig 3, *G*, median, 11.94, 13.64; $**P < .01$; $*P < .05$). *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase; *NS*, not significant. Ctrl represents HaCaT cells stably transduced with control lentiviral particles, and COL23A1 represents HaCaT cells stably transduced with *COL23A1* lentiviral particles.

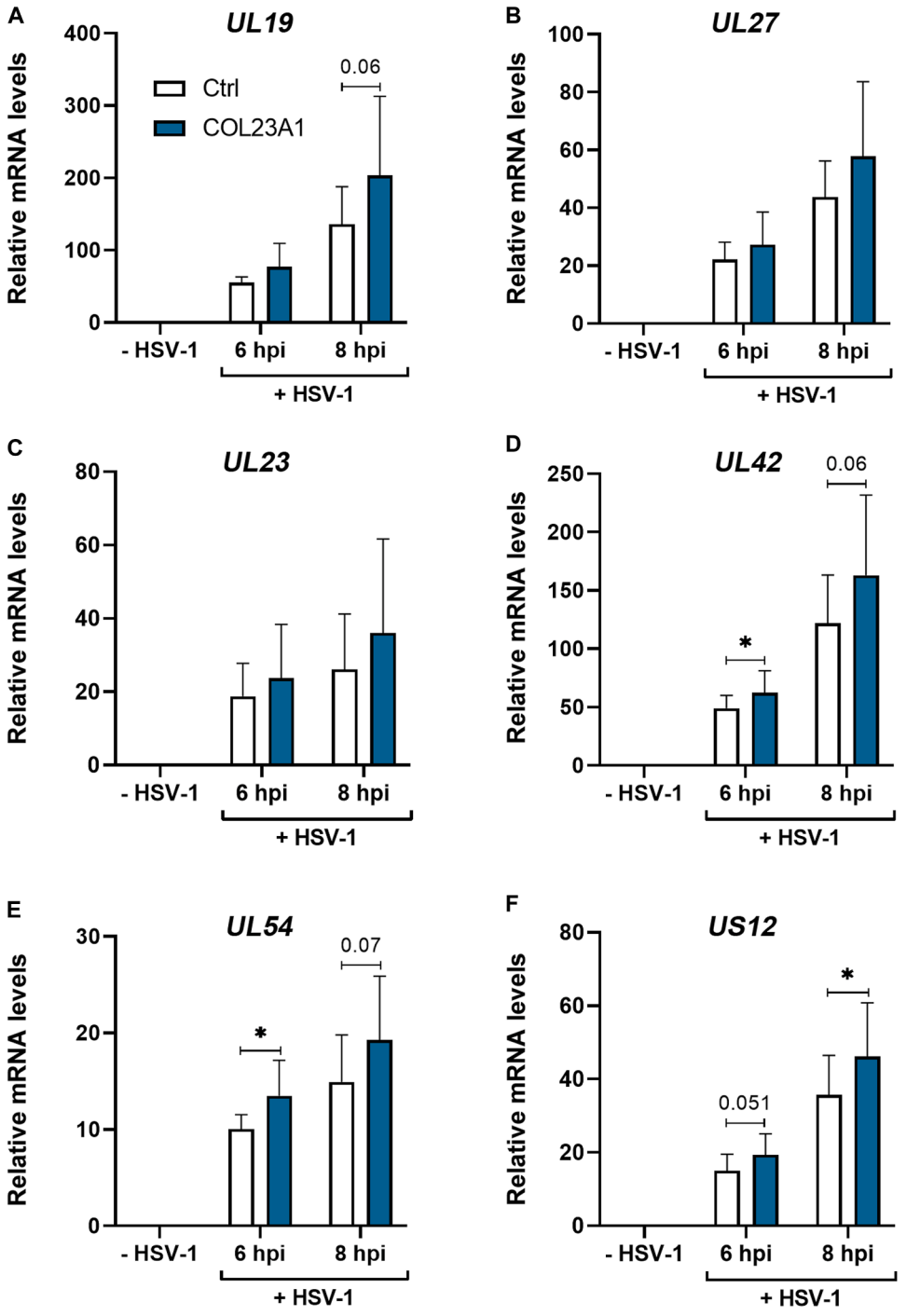

FIG 4. HSV-1 gene expression is more efficient in *COL23A1* overexpressing HaCaT cells. **A-F,** Control and *COL23A1* overexpressing HaCaT cells were infected with 5 pfu/cell of HSV-1. *UL19, UL27, UL23, UL42, UL54,* and *US12* mRNA levels were measured at 6 and 8 hpi by quantitative RT-PCR and normalized to the housekeeping gene *RPS20* (*black bars*, mean ± SD; n = 5 independently repeated experiments). Statistical significance was analyzed by paired *t* test (**P* < .05).Ctrl represents HaCaT cells stably transduced with control lentiviral particles, and COL23A1 represents HaCaT cells stably transduced with *COL23A1* lentiviral particles.

proteoglycan, and the entry receptors nectin-1 and HVEM in control and *COL23A1* overexpressing HaCaT cells. The impact of *COL23A1* overexpression on cell surface levels of syndecan-1, nectin-1, and HVEM depended on the confluency of cells (data not shown). At 80% confluency, although the cell surface levels of HVEM did not change, syndecan-1 and nectin-1 levels

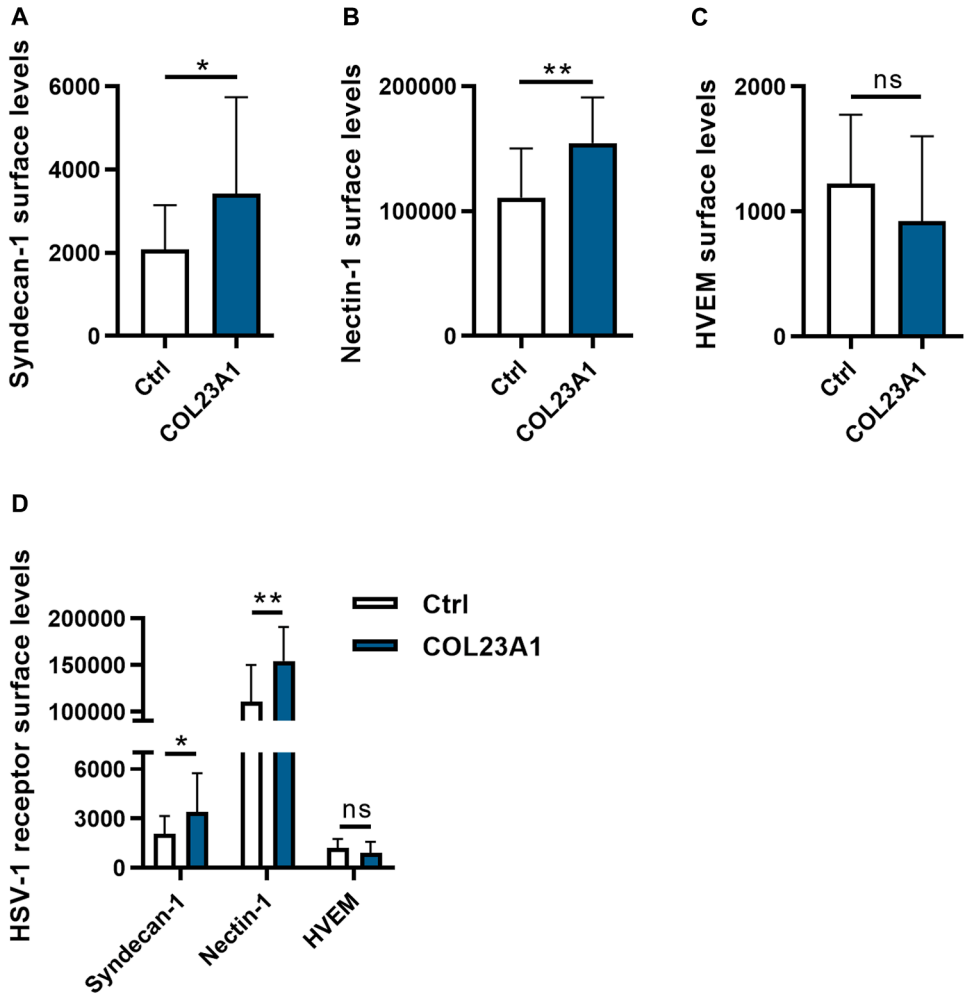

FIG 5. HSV-1 attachment factor syndecan-1 and HSV-1 receptor nectin-1 are expressed at higher levels in *COL23A1* overexpressing HaCaT cells. **A-D,** Control and *COL23A1* overexpressing HaCaT cells were immunostained for syndecan-1, nectin-1, and HVEM. The geomean fluorescence intensities were analyzed by flow cytometry and normalized to isotype control (cell surface levels). Mean ± SD of at least 5 independent experiments each involving 2 to 3 technical replicates (n = 14-15). Statistical significance was tested with paired *t* test (syndecan-1 and nectin-1) or the Wilcoxon matched-pairs signed rank test (HVEM) (**P* < .05; ***P* < .01). *NS,* Not significant. Ctrl represents HaCaT cells stably transduced with control lentiviral particles, and COL23A1 represents HaCaT cells stably transduced with *COL23A1* lentiviral particles.

significantly increased in *COL23A1* overexpressing cells by approximately 64% and 39%, respectively. This increase in HSV-1 attachment and entry factors may allow HSV-1 particles to bind and enter more efficiently, and thereby enhance viral gene expression and subsequent cell-to-cell spread (Fig 5, *A-D*).

COL23A1 overexpression in HaCaT cells dampens the expression of immune and inflammatory response genes

To analyze the effect of *COL23A1* overexpression on the transcriptome, we infected control or *COL23A1* overexpressing HaCaT cells with HSV-1 and subjected them to bulk RNA-seq. We detected 225 differentially expressed genes (adjusted $P \leq .05$) in pooled HSV-1 infected and uninfected *COL23A1* overexpressing HaCaT cells as compared with control cells, of which 189 genes had a $\log_2$ fold change [$\log_2$(FC)] value of greater than 0.5 or less than −0.5 (volcano plot; Fig 6, *A*). As expected, the highest change was observed in *COL23A1* expression, with a value of 8.04 $\log_2$(FC) (accession no. GSE278244). Interestingly, several genes involved in inflammatory and innate or adaptive immune responses such as *IL1R1*, *IL32*, *TLR4*, *CFH*, *C3*, *S100A9*, and *IRF1* were significantly downregulated, whereas *ADAM23*, *TNC*, and *SPINK5*, genes associated with chronic inflammatory skin diseases, were significantly upregulated (Fig 6, *B*).[40-45] The Kyoto Encyclopedia of Genes and Genomes and the Gene Ontology pathway analyses highlight a significant suppression of genes mediating immune and inflammatory responses, cytokine production and defense response mechanisms, as well as the activation of extracellular matrix assembly and stimulation of TGF-β activation and production on *COL23A1* upregulation (see Fig E1 in this article's Online Repository at www.jacionline.org). Quantitative RT-PCR confirmed the downregulation of several host genes involved in an effective immune response and upregulation of those associated with inflammatory

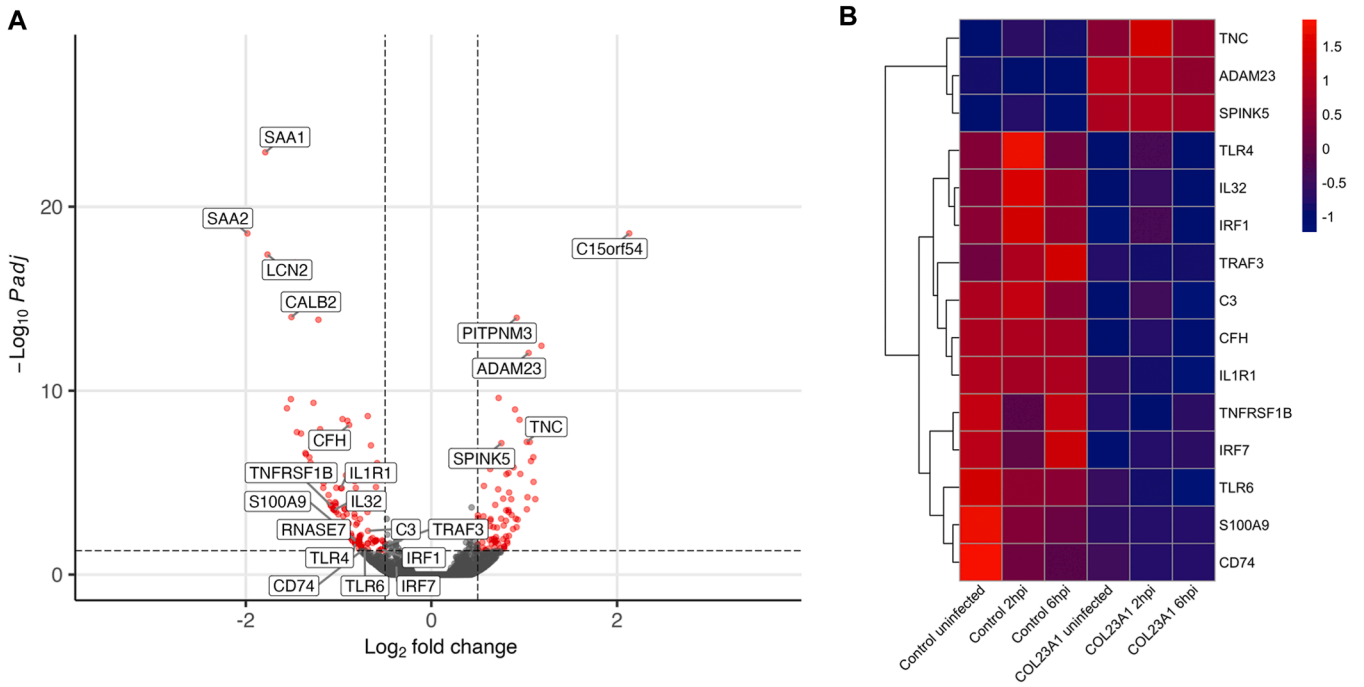

FIG 6. Global transcriptomic changes after *COL23A1* overexpression in HaCaT cells using bulk RNA-seq. HaCaT control and *COL23A1* overexpressing cells were infected with 5 pfu/cell of HSV-1, and RNA was isolated at 2 and 6 hpi. **A,** Bulk RNA-seq revealed 189 differentially expressed genes (adjusted $P \leq .05$) with $\log_2$(FC) of greater than 0.5 or less than −0.5, as depicted in *red*. **B,** Heatmap illustrating the differential expression of selected genes involved in immune response or AD in control and *COL23A1* overexpressing HaCaT cells.

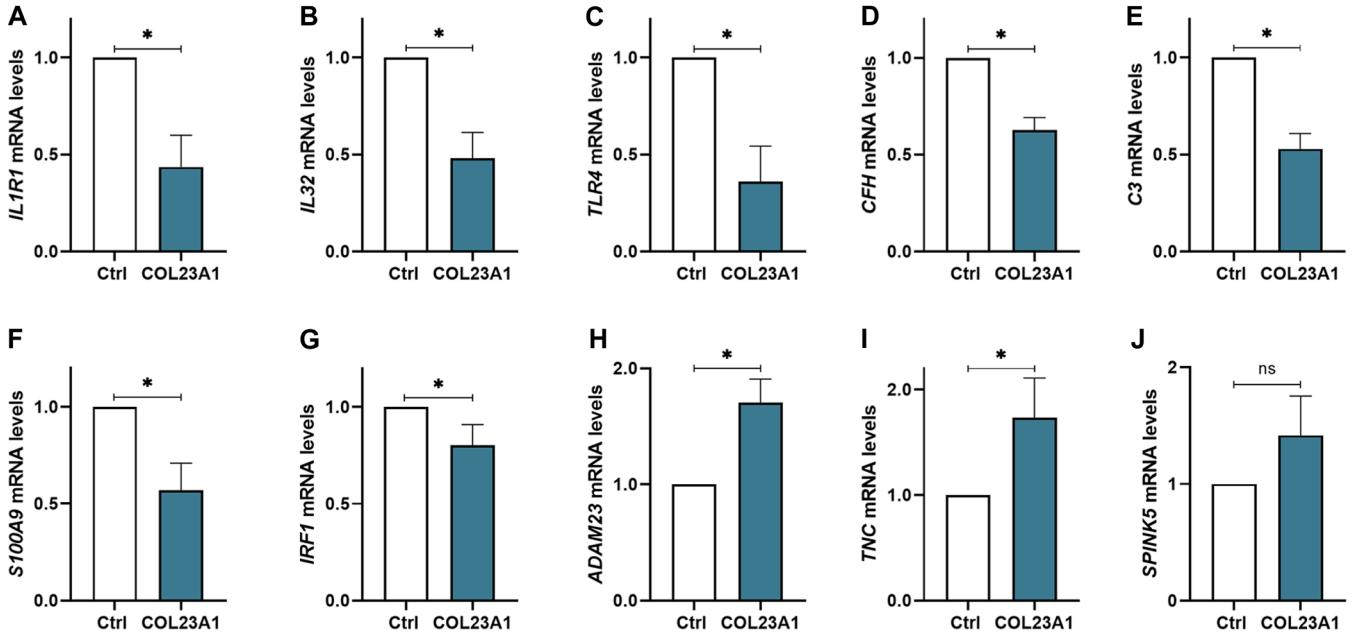

FIG 7. Confirmation of hits revealed in bulk RNA-seq data using quantitative RT-PCR. **A-F,** *IL1R1*, *IL32*, *TLR4*, *CFH*, *C3*, *S100A9*, *IRF1*, *ADAM23*, *TNC*, and *SPINK5* mRNA levels were measured in uninfected control and *COL23A1* overexpressing cells by quantitative RT-PCR and normalized to the housekeeping gene *RPS20* (*black bars*, mean ± SD; n = 6 independently repeated experiments). Statistical significances were analyzed using the 1-sample Wilcoxon test (*$P < .05$). *NS*, Not significant. Ctrl represents HaCaT cells stably transduced with control lentiviral particles, and COL23A1 represents HaCaT cells stably transduced with *COL23A1* lentiviral particles.

skin diseases in uninfected *COL23A1* overexpressing cells as compared with control cells (Fig 7, *A-J*). These findings indicate that the overexpression of *COL23A1* diminishes immune and inflammatory responses, thereby allowing HSV-1 to infect keratinocytes more efficiently.

DISCUSSION

Previous studies identified various genetic risk factors involved in EH using GeneChip profiling, genotyping, targeted resequencing approaches, and whole-genome sequencing.[21,23,46-50] In our study, we observed a significant novel association of SNP rs2973744, affecting *COL23A1* in 5% of ADEH+ patients. The SNP rs2973744 is located at the splice donor site of an exon-intron boundary in the *COL23A1* gene. SNPs at splice donor sites often lead to exons skipping or generation of differentially spliced transcripts.[51,52] Interestingly, an SNP at a splice donor site in the thrombopoietin (*THRO*) gene increased thrombopoietin serum levels in patients with hereditary thrombocythemia because of a more efficient translation of the resulting transcripts.[53] COL23A1 has 3 isoforms resulting from alternative splicing (Human Protein Atlas).[46,47] Here, we show that the heterozygous SNP in *COL23A1* results in a novel, differentially processed form of COL23A1 and, in the presence of other risk factors for EH, elevates transcript and protein levels.

A subgroup of patients with AD is more prone to severe HSV-1 infections resulting in EH.[7,19,21] In line with that, we show here that primary keratinocytes isolated from an ADEH+ patient carrying the SNP rs2973744 were more susceptible to HSV-1 than those from ADEH− patients. Moreover, the *COL23A1* expression was increased in these patient-derived keratinocytes. Consistent with this, we found a positive correlation between *COL23A1* mRNA levels and HSV-1 susceptibility across all donors. These findings suggest that higher levels of COL23A1 correspond to higher susceptibility to HSV-1. Notably, the effect of the SNP rs2973744 on increasing the HSV-1 susceptibility in keratinocytes derived from ADEH− patients was less pronounced, suggesting that additional risk factors for EH play a significant role in enhancing HSV-1 susceptibility, with *COL23A1* SNP contributing to this effect.

The neurotransmitter serotonin upregulates the expression of *COL23A1* in macrophages,[48] and preliminary data from our laboratory suggest that the T_H2 cytokines IL-4 and IL-13 may also increase the expression of *COL23A1* (data not shown).

COL23A1 is expressed in skin, brain, lung, cornea, tendon, and kidney and its expression is upregulated in several cancers.[39,49,50,54,55] Currently, there are no studies demonstrating an association of *COL23A1* with AD or EH. However, another collagen, *COL6A6*, is upregulated in the dermis of nonlesional skin in both ADEH− and ADEH+ patients compared with healthy controls.[56] Class A macrophage scavenger receptor, a protein distantly related to COL23A1, was reported to increase HSV-1 susceptibility in keratinocytes by enhancing virus binding to host cells, whereas another study showed that it is not required for HSV-1 entry in the epidermis or into dermal fibroblasts.[57,58] In this context, our study is the first to investigate the role of COL23A1 in HSV-1 infection of keratinocytes.

It was reported that in cultured primary keratinocytes, COL23A1 is undetectable on the cell surface because of cleavage of the full-length protein by furin.[39] Here, we also detected only low amounts of COL23A1 on the cell surface of primary foreskin keratinocytes. To study the effect of increased membrane levels of COL23A1 on HSV-1 infection, we reduced the shedding of COL23A1 by inhibiting furin. HSV-1 proteins do not contain furin cleavage sites; however, furin plays a crucial role in the maturation and activation of envelope proteins of many other viruses.[59,60] Nevertheless, subsequent viral infection of keratinocytes was conducted in the absence of FI to prevent any potential effects on HSV-1. Furin inhibition increased the cell surface expression of the HSV-1 attachment factor syndecan-1 and the HSV-1 receptor nectin-1 as well as the susceptibility of keratinocytes to HSV-1, possibly by enhancing COL23A1 levels on the cell surface. However, we cannot rule out the possibility that furin inhibition also increased the susceptibility to HSV-1 by interfering with the proteolytic maturation of other host factors.[59,60]

As a more specific approach to study the effect of COL23A1 in HSV-1 infection, we overexpressed *COL23A1*. Because primary foreskin keratinocytes did not proliferate after transduction, we used HaCaT cells instead. Consistent with the FI experiment, increased COL23A1 levels significantly enhanced the susceptibility of HaCaT cells to HSV-1. However, the underlying mechanisms between these 2 approaches may differ. In addition, *COL23A1* overexpression increased HSV-1 cell-to-cell spread and the expression of immediate-early, early, and late genes, suggesting that it promotes HSV-1 infection at or upstream of immediate-early gene transcription. Increased cell surface syndecan-1 and nectin-1 levels suggest that *COL23A1* overexpression may enhance HSV-1 binding and internalization and consequently the number of viral genomes available for transcription. Some collagens can directly bind to syndecan-1.[61] In addition, syndecan-1 enhances the binding of collagen to integrin $\alpha2\beta1$, a known receptor for COL23A1.[62] This suggests that COL23A1 may also interact with syndecan-1 directly or indirectly and thereby stabilize syndecan-1 on the plasma membrane by preventing its internalization and degradation. Type XIII collagen, another transmembrane collagen, colocalizes with E-cadherin, suggesting that type XIII is very likely to be closely associated with adherens junctions.[63] This suggests that COL23A1 may also be associated with adherens junctions, stabilize nectin-1 on the cell surface, and thereby increase HSV-1 susceptibility.

As revealed by transcriptome analysis, *COL23A1* overexpression led to the downregulation of several genes, such as *IL1R1*, *IL32*, *TLR4*, *CFH*, *C3*, *S100A9*, and *IRF1*, which are involved in the immune and inflammatory response of keratinocytes. Transmembrane collagens such as COL23A1 may interact with immune receptors on the surface of the host cell and alter their ability to sense viral infection and initiate downstream gene expression. Such interaction could suppress the activation of antiviral transcription factors such as IRF3 or nuclear factor-κB. Interestingly, upregulation of *ADAM23*, *TNC*, and *SPINK5* was also observed in these cells. ADAM23 belongs to the disintegrin and metalloprotease domain family, which has recently been discussed as a potential biomarker for psoriasis.[45] TNC is an extracellular matrix protein that is significantly upregulated in the lesional skin of patients with AD.[42,43] SPINK5 is essential for maintaining epidermal barrier integrity and regulating the proteolysis of the extracellular matrix. Moreover, *SPINK5* polymorphisms are associated with AD across various ethnicities.[40,41,44]

Our data show that elevated COL23A1 levels promote more efficient HSV-1 infection of keratinocytes presumably by enhancing their syndecan-1 and nectin-1 surface expression as well as attenuating their immune and inflammatory response. Consistently, ADEH+ patients carrying the heterozygous SNP rs2973744 are predisposed to develop severe HSV-1 infections. Early screening for the SNP rs2973744 in patients with AD

with recurrent HSV-1 infections might help to prevent disseminated HSV-1 infections through awareness and early therapeutic intervention as well as by guiding the decision between therapeutic options. Patients with moderate to severe AD with a genetic predisposition to develop disseminated HSV infection should rather be treated with biologics such as dupilumab, which decreases the EH risk, than with systemic Janus kinase inhibitors, which increase the risk of herpes virus infections.[64,65] Additional research is needed to further dissect the role of the *COL23A1* SNP rs2973744 in EH and to explore its potential as a therapeutic target.

DISCLOSURE STATEMENT

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Clinical implications: The SNP rs2973744 in *COL23A1*, which is associated with increased COL23A1 levels, could be used to identify patients with AD at risk of developing EH.

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