

Letter to the Editor

The broad spectrum of interepithelial junctions in skin and lung

To the Editor:

Asthma and atopic dermatitis represent the biggest group of chronic noncontagious diseases in children and adults with the largest burden on health care costs. The better understanding of the molecular mechanisms has opened a way for the development of many novel treatment modalities.^{1,2} Two recent articles published in the *Journal of Allergy and Clinical Immunology* demonstrated the disruption of epithelial barrier function of keratinocytes in the skin of patients with atopic dermatitis and bronchial epithelial cells in the lungs of asthmatic patients.^{3,4} These studies suggest that tissue integrity is disturbed in patients, and allergens, bacterial toxins, and other particles are able to penetrate the epidermis and the lung epithelium, where they may activate the immune system leading to severe chronic inflammation in both diseases. Therefore, paracellular sealing of keratinocytes and bronchial epithelial cells appears to be very important to prevent the infiltration of the dermis and submucosa by factors that induce allergic inflammation.

There are several types of cell-cell adhesion and sealing complexes in between epithelial cells: tight junctions (TJs),

adherens junctions, gap junctions, and desmosomes (Fig 1, A). Main contributors to the barrier function of epithelia are TJs. These form the most apical cell-cell adhesion complexes regulating the paracellular flux of water and ions as well as of larger compounds.⁵ They are able to regulate the apical-basolateral differentiation of epithelial cells. TJs are composed of the claudin family, junctional adhesion molecules, marvel family members, and adaptor proteins (Fig 1, B). In humans, the claudin family has 24 members; however, claudin-13 is not expressed at all.⁶

In the study by Xiao et al,⁴ the circumferential band of TJs was shown to be disrupted in asthma biopsies and in bronchoepithelial cell cultures. Although the authors did not find any differences in the mRNA level, the protein expression of zonula occludens-1 was significantly reduced and occludin showed a trend of downregulation in bronchoepithelial cell cultures.⁴ Bronchial epithelial cells from asthmatic patients cultured on air-liquid interfaces showed a decreased transepithelial resistance than did cells from healthy individuals, indicating a reduced barrier function in asthma. This defective barrier function in asthma could be restored by epithelial growth factor.⁴ Cell cultures exposed to cigarette smoke extracts exert an impaired barrier function that can be restored by the addition of epithelial growth factor.⁴ In the study by De Benedetto et al,³ the expression of claudin-1 and claudin-23 was reduced

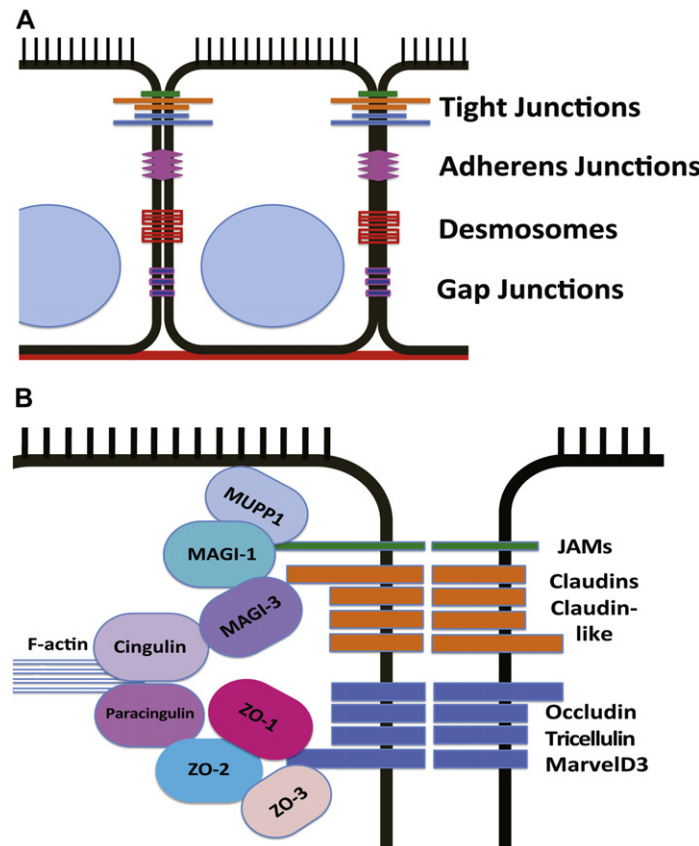


FIG 1. Junctional complexes of epithelial cells and protein composition of TJs. **A**, Scheme of the junctional complexes in epithelial cells: TJs, adherens junction, desmosomes, and gap junctions. **B**, Protein composition of TJ: transmembrane proteins include junctional adhesion molecules (JAMs), claudin family, Marvel family members, and adaptor proteins. MAGI, Membrane-associated guanylate kinase inverted; MUPP1, multi-PDZ domain containing protein 1; ZO, zonula occludens.

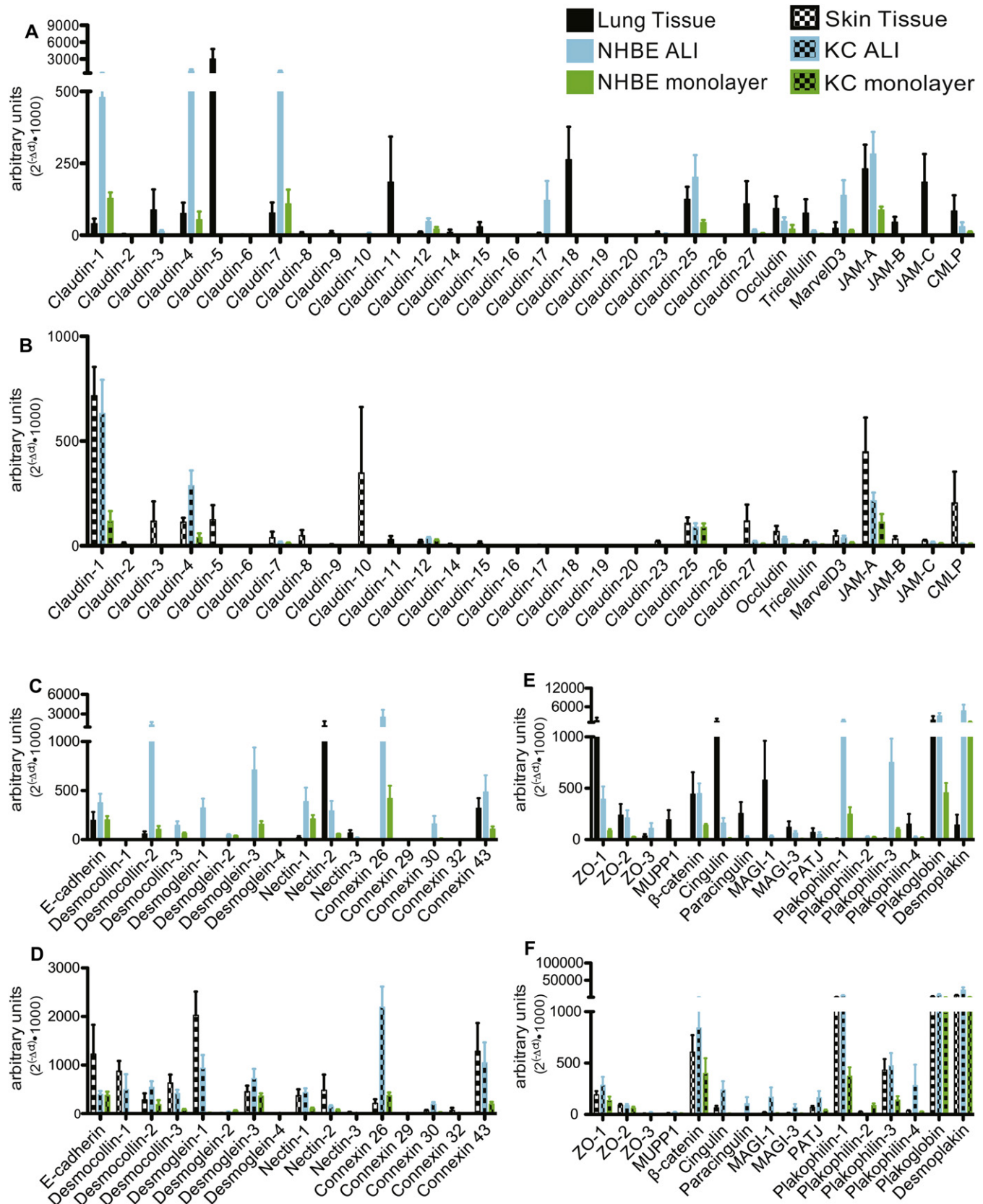


FIG 2. Taqman low-density array microfluidic card mRNA expression profile of TJs in the skin and lung tissue, ALI, and monolayer cultures of NHBE cells and primary KC lines. mRNA expression of transmembrane TJ proteins (A and B), of other cell junctions, such as desmosomes, adherens junctions, and gap

in nonlesional atopic dermatitis skin. Silencing of claudin-1 led to a reduction in transepithelial resistance. This study suggests that the reduced expression of claudin-1 in atopic dermatitis epidermis is an important factor of an impaired barrier function.³

Here, we hypothesize that the whole picture of interepithelial junction molecule expression could be more complicated than suggested before.

To perform fully detailed analyses of interepithelial junction expression, we developed a low-density array microfluidic card (Applied Biosystems, Carlsbad, Calif) containing all known junctional and associated proteins that are expressed in the epidermis and epithelia. In addition to TJ proteins, desmosomes, gap junctions, and adherens junction genes were included in the analysis (see the **Methods** section in this article's Online Repository at www.jacionline.org). We analyzed lung and skin biopsies, primary keratinocytes, and normal human bronchial epithelial cells either grown as monolayers or in air-liquid interphase (ALI) cultures⁷ for the expression of junctional proteins (see the **Methods** section).

In skin and lung tissue and primary isolated keratinocyte and normal human bronchial epithelial cells, there are commonly expressed junctional proteins, such as claudin-1, claudin-4, claudin-12, and claudin-25, which is also known as claudin domain containing 1.⁸ It also appears that there are several differently expressed junctional and adaptor proteins between the skin and lung. In lung tissue, claudin-18 ($P = .02$) and JAM-C ($P = .03$) are uniquely present (Fig 2, A). In comparison, skin tissue uniquely expressed claudin-8 ($P = .05$) and claudin-10 ($P = .02$) (Fig 2, B). Skin tissue expressed connexin-26 ($P = .02$), -30 ($P = .02$), -32 ($P = .06$), desmocollin-1 ($P = .02$), desmocollin-3 ($P = .02$), desmoglein-1 ($P = .02$), desmoglein-3 ($P = .02$) (Fig 2, D), and their corresponding adaptor proteins plakophilin-1 ($P = .02$) and plakophilin-3 ($P = .02$) (Fig 2, F). For comparison, lung tissue expressed relatively low levels of gap junction and desmosomes and some are fully not detectable (Fig 2, C). Normal human bronchial epithelial cells cultured in ALI expressed claudin-17 ($P = .03$) (Fig 2, A). In contrast, primary keratinocyte in ALI cultures expressed uniquely desmocollin-1 ($P = .02$) (Fig 2, D). Keratinocyte ALIs did not express any TJ proteins in addition to the common junctional proteins observed in skin biopsies and monolayer cultures (Fig 2, B). Detailed statistical analysis is shown in Table E1 (in the Online Repository available at www.jacionline.org).

Our data demonstrate that monolayer cell cultures express a similar profile of junction molecules compared with ALI cultures, however, with relatively low levels. After apicobasal differentiation in ALIs, more junctional molecules are expressed (Fig 2). Interestingly, whole tissues expressed a much broader profile of junctional molecules. The main reason behind having additional junctional mRNAs appearing in the whole tissue of lung and skin can be that other cell types, such as endothelial cells, fibroblasts in both, and smooth muscle cells in the lung, are also expressing junctional molecules. ALIs represent here a more cell-type-specific junctional molecule expression pattern.

After performing the detailed expression analyses of interepithelial barrier molecules, we realized that it is essential to study the TJs in a broader way. Although some are dominantly expressed, the determination of one or two junctional molecules, especially TJs, may not represent the whole picture. There are distinct and overlapping patterns of TJs and other cell-cell adhesion molecules expressed in the skin and lung epithelia, which implicate a highly regulated and complex pattern. Each cell type shows its own, unique expression profile reflecting its specialization related to its function within the organism. In addition, we have to take into account that TJs are not uniquely expressed in epithelial cells but are also found and regulated in other cells, especially in endothelium. Furthermore, other mesenchymal tissues also express them as recently observed in smooth muscle cells in asthma.⁹ The number of different junctional proteins, which are expressed, interact in multiple ways in every cell type to form appropriate cell-cell contacts and tissue integrity. Our data demonstrated here suggest that the picture of the junctional apparatus in each cell type and tissue is distinct, and the regulation of epithelial integrity might be more complex than being considered so far.

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junctions (C and D), and of junctional adaptor proteins (E and F). Lung tissue, $n = 5$; skin tissue, $n = 4$; NHBE ALI, $n = 4$; KC ALI, $n = 4$; NHBE monolayer, $n = 4$; and KC monolayer, $n = 4$, were investigated. Mean $\pm$ SEM is displayed. *CMLP*, CXADR-like membrane protein (ASAM); *JAM*, junctional adhesion molecules; *KC*, keratinocyte; *MAGI*, membrane-associated guanylate kinase inverted; *MUPP1*, multi-PDZ domain containing protein 1; *NHBE*, normal human bronchial epithelial; *PATJ*, inAD-like protein (INADL); *ZO*, zonula occludens.

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METHODS

Skin and lung tissue

mRNAs from skin biopsies from healthy volunteers were used in the study of the University of Tartu. Lung material was obtained from necropsies in the University of Zurich Department of Pathology. Ethical permissions were obtained from the Ethical Review Commission on Human Research of the University of Tartu, Estonia, and Canton of Zurich, Switzerland.

Cell culture of keratinocytes and normal human bronchial epithelial cells

Primary keratinocytes (Promocell, Heidelberg, Germany) were cultured in keratinocyte-serum-free medium (Invitrogen, Grand Island, NY) reconstituted with 5 ng/mL human recombinant epidermal growth factor, 50 µg/mL bovine pituitary extract, and 2 µmol/L L-glutamine (all from Gibco Life Technologies, Corning, NY). Monolayers of healthy normal human bronchial epithelial (NHBE) cells (Lonza, Allendale, NJ) were cultured in bronchial epithelial cell basal medium (BEBM) reconstituted with bronchial epithelial cell growth medium (BEGM) Bulletkit (Lonza), ALIs of healthy NHBE cells were cultured in transwells (Corning Costar, Corning, NY) with BEBM reconstituted with BEGM Bulletkit (Lonza) without triiodothyronine mixed 1:1 with Dulbecco modified Eagle medium (Gibco), and the ALIs of keratinocytes were cultured in transwells (Costars) with the corresponding medium mixed 1:1 with Dulbecco modified Eagle medium (Gibco), adapted from Lin et al.⁷ ALI cultured cells were used for mRNA isolation after they reached the highest transepithelial resistance, usually at day 5 of culturing at ALI. Transepithelial resistance was measured by using

Millicell-Electrical Resistance System (Millipore, Billerica, Mass). Monolayer cells were used for mRNA isolation after they reached almost 100% confluence.

mRNA analysis by Taqman low-density array microfluidic cards

We chose 62 predesigned probes representing the junctional apparatus of epithelial cells from the Applied Biosystem database (Table E2). The probes were spanning an exon-exon junction and amplify an amplicon length of maximal 200 nt. The housekeeping gene *GAPDH* was taken as reference gene. mRNA was extracted by using the RNeasy mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Reverse transcription was accomplished with the use of reverse transcription reagents containing random hexamers (Fermentas, Hilden, Germany). Total RNA (400 ng) was measured per microfluidic card in 7900HT Fast Real-Time PCR System (Applied Biosystems). Arbitrary units were calculated with the following formula: arbitrary units = $2^{(-\Delta Ct)} \times 1000$.

Statistics

Lung tissue (n = 5), skin tissue (n = 4), NHBE ALI (n = 4), keratinocyte ALI (n = 4), NHBE monolayer (n = 4), and keratinocyte monolayer (n = 4) were investigated. Statistics have been calculated with Graphpad Prism 5.0c. Mean ± SEM is displayed in the figures. Differences between tissue/ALI, tissue/monolayer, and ALI/monolayer of skin and lung and differences between lung/skin, NHBE/keratinocyte monolayers, and NHBE/keratinocyte ALIs were calculated by using the Mann-Whitney *U* test and are displayed in Table E1. *P* < .05 is significant.

TABLE E1. Statistical analysis of the microfluidic card mRNA expression array*

B	Lung tissue (n = 5)	NHBE ALI (n = 4)	NHBE monolayer (n = 4)	Lung tissue	Lung tissue	NHBE ALI	Skin tissue (n = 4)	Skin tissue	KC ALI (n = 4)
	Skin tissue	KC ALI	KC monolayer	NHBE ALI	NHBE monolayer	NHBE monolayer	KC ALI	KC monolayer	KC monolayer (n = 4)
A									
Cingulin	—	—	—	—	0.02	0.03	—	0.03	0.03
Claudin-1	0.02	—	—	—	0.03	0.03	—	0.03	0.03
Claudin-10	0.02	0.03	—	—	—	—	0.03	0.03	—
Claudin-11	—	—	—	0.02	0.02	—	0.03	0.03	—
Claudin-12	—	—	—	0.02	—	—	—	—	—
Claudin-14	—	—	—	—	—	—	—	—	—
Claudin-15	—	—	—	0.02	0.03	—	—	—	—
Claudin-16	—	—	—	—	—	—	—	—	—
Claudin-17	—	0.03	—	0.03	—	0.03	—	—	—
Claudin-18	0.02	—	—	0.02	0.02	—	—	—	—
Claudin-19	—	—	—	—	—	—	—	—	—
Claudin-2	—	—	—	—	—	—	—	—	—
Claudin-20	—	—	—	—	—	—	—	—	—
Claudin-23	—	—	—	—	—	—	—	—	—
Claudin-25	—	—	—	—	—	—	—	—	—
Claudin-26	—	—	—	—	—	—	—	—	—
Claudin-27	—	—	—	—	—	—	—	0.03	—
Claudin-3	—	—	—	—	0.02	0.03	0.03	0.03	—
Claudin-4	—	—	—	0.03	—	0.03	—	—	0.03
Claudin-5	—	—	—	0.02	0.02	—	0.03	0.03	—
Claudin-6	—	—	—	—	—	—	—	—	—
Claudin-7	—	0.03	0.03	0.03	—	—	—	—	—
Claudin-8	0.05	—	—	—	—	—	0.03	0.03	—
Claudin-9	—	—	—	—	—	—	—	—	—
CLMP	—	—	—	—	—	—	0.03	0.03	—
Conexin-26	0.02	—	—	—	—	—	0.03	0.03	—
Connexin-29	—	—	—	0.02	0.02	—	—	—	0.03
Connexin-30	0.02	—	—	0.02	0.02	—	0.03	—	0.03
Connexin-32	—	—	—	—	—	—	—	—	0.03
Connexin-46	—	—	—	—	—	—	—	—	—
Desmocollin-1	0.02	0.02	0.03	—	—	—	—	0.03	0.03
Desmocollin-2	0.03	—	—	0.02	—	0.03	—	—	—
Desmocollin-3	0.02	—	—	0.02	0.02	—	—	0.03	0.03
Desmoglein-1	0.02	—	0.03	0.02	—	0.03	—	0.03	0.03
Desmoglein-2	—	—	—	0.02	0.02	—	—	—	0.03
Desmoglein-3	0.02	—	—	0.02	0.02	—	—	—	—
Desmoglein-4	—	—	—	—	—	—	—	—	—
Desmoplakin	0.02	—	—	0.02	0.03	—	—	0.03	0.03
E-cadherin	—	—	—	—	—	—	—	—	—
JAM-A	—	—	—	—	—	0.03	—	—	—
JAM-B	—	—	—	0.02	0.02	—	0.03	0.03	—
JAM-C	0.03	0.03	0.03	0.02	0.02	—	—	—	—
MAGI-1	—	—	—	—	0.02	0.03	0.03	0.03	0.03
MAGI-3	—	0.03	—	—	—	0.03	0.03	0.03	—
MARVELD3	—	—	—	0.03	—	0.03	—	—	0.03
MUPPI	—	—	—	0.02	0.02	—	—	—	—
Nectin-1	0.02	—	—	0.02	0.02	—	—	0.03	0.03
Nectin-2	—	—	—	—	0.02	0.03	—	—	—
Nectin-3	—	—	—	—	0.02	—	0.03	0.03	—
Occludin	0.02	—	—	—	—	—	—	0.03	0.03
Paracingulin	—	—	—	—	0.02	0.03	0.03	0.03	0.03
PATJ	—	—	0.03	—	—	—	—	—	—
Plakophilin-1	0.02	—	—	0.02	0.02	0.03	—	0.03	0.03
Plakophilin-2	—	—	—	—	—	—	—	0.03	0.03
Plakophilin-3	0.02	—	—	0.02	0.02	—	—	—	—
Plakophilin-4	—	—	—	—	—	—	—	0.03	—
Plakoglobin	—	—	—	—	—	—	—	0.03	0.03

(Continued)

TABLE E1. (Continued)

B	Lung tissue (n = 5)	NHBE ALI (n = 4)	NHBE monolayer (n = 4)	Lung tissue	Lung tissue	NHBE ALI	Skin tissue (n = 4)	Skin tissue	KC ALI (n = 4)
A	Skin tissue	KC ALI	KC monolayer	NHBE ALI	NHBE monolayer	NHBE monolayer	KC ALI	KC monolayer	KC monolayer (n = 4)
Tricellulin	—	—	—	—	—	—	—	0.03	0.03
ZO-1	—	—	—	—	—	—	—	—	—
ZO-2	—	—	—	—	—	0.03	—	—	—
ZO-3	—	0.03	—	—	—	0.03	—	0.04	0.03
β-catenin	—	—	—	—	—	0.03	—	—	—

JAM, Junctional adhesion molecule; *KC*, keratinocyte; *MAGI*, membrane-associated guanylate kinase inverted; *MUPPI*, multi-PDZ domain containing protein 1; *ZO*, zonula occludens.

*Differences between tissue/ALI, tissue/monolayer, and ALI/monolayer of skin and lung and differences between lung/skin, NHBE/KC monolayers, and NHBE/KC ALIs were calculated by using the Mann-Whitney *U* test. *P* < .05 is significant; —, not significant.

TABLE E2. The microfluidic card mRNA expression array analyzing the junctional apparatus of epithelial cells*

Gene symbol	Gene name	Assay ID	Gene symbol	Gene name	Assay ID
<i>OCN</i>	Occludin	Hs00170162_m1	<i>GJB2</i>	Connexin-26	Hs00955889_m1
<i>F11R</i>	JAM-1	Hs00170991_m1	<i>GJB6</i>	Connexin-30	Hs00917676_m1
<i>JAM2</i>	JAM-2	Hs00221894_m1	<i>GJC3</i>	Connexin-29	Hs01384570_m1
<i>JAM3</i>	JAM-3	Hs00230289_m1	<i>CDH1</i>	E-cadherin	Hs01023895_m1
<i>MARVELD2</i>	Tricellulin	Hs00376394_m1	<i>PVRL1</i>	Nectin-1	Hs01591978_m1
<i>CLDN1</i>	Claudin-1	Hs01076359_m1	<i>PVRL2</i>	Nectin-2	Hs01071562_m1
<i>CLDN2</i>	Claudin-2	Hs00252666_s1	<i>PVRL3</i>	Nectin-3	Hs00210045_m1
<i>CLDN3</i>	Claudin-3	Hs00265816_s1	<i>DSG1</i>	Desmoglein-1	Hs00355084_m1
<i>CLDN4</i>	Claudin-4	Hs00976831_s1	<i>DSG2</i>	Desmoglein-2	Hs00170071_m1
<i>CLDN5</i>	Claudin-5	Hs00533949_s1	<i>DSG3</i>	Desmoglein-3	Hs00170075_m1
<i>CLDN6</i>	Claudin-6	Hs00607528_s1	<i>DSG4</i>	Desmoglein-4	Hs00698286_m1
<i>CLDN7</i>	Claudin-7	Hs00600772_m1	<i>DSC1</i>	Desmocollin-1	Hs00245189_m1
<i>CLDN8</i>	Claudin-8	Hs00273282_s1	<i>DSC2</i>	Desmocollin-2	Hs00951428_m1
<i>CLDN9</i>	Claudin-9	Hs00253134_s1	<i>DSC3</i>	Desmocollin-3	Hs00170032_m1
<i>CLDN10</i>	Claudin-10	Hs00199599_m1	<i>MPDZ</i>	MUPP1	Hs00187106_m1
<i>CLDN11</i>	Claudin-11	Hs00194440_m1	<i>TJP1</i>	ZO-1	Hs01551876_m1
<i>CLDN12</i>	Claudin-12	Hs01082669_m1	<i>TJP2</i>	ZO-2	Hs00910541_m1
<i>CLDN14</i>	Claudin-14	Hs00273267_s1	<i>TJP3</i>	ZO-3	Hs00274276_m1
<i>CLDN15</i>	Claudin-15	Hs00204982_m1	<i>CGN</i>	Cingulin	Hs00430426_m1
<i>CLDN16</i>	Claudin-16	Hs00198134_m1	<i>CGNL1</i>	Paracingulin	Hs00262671_m1
<i>CLDN17</i>	Claudin-17	Hs01043467_s1	<i>MAG1</i>	MAGI-1	Hs00191026_m1
<i>CLDN18</i>	Claudin-18	Hs00212584_m1	<i>MAG3</i>	MAGI-2	Hs00326365_m1
<i>CLDN19</i>	Claudin-19	Hs00381204_m1	<i>INADL</i>	PATJ	Hs00195106_m1
<i>CLDN20</i>	Claudin-20	Hs00378662_m1	<i>MARVELD3</i>	MARVELD3	Hs00369354_m1
<i>CLDN23</i>	Claudin-23	Hs01013638_s1	<i>JUP</i>	Plakoglobin	Hs00158408_m1
<i>CLDND1</i>	Claudin-25	Hs00219886_m1	<i>DSP</i>	Desmoplakin	Hs00189422_m1
<i>TMEM114</i>	Claudin-26	Hs00418203_m1	<i>PKP1</i>	Plakophilin-1	Hs00240873_m1
<i>C1orf91</i>	Claudin-27	Hs00963921_m1	<i>PKP2</i>	Plakophilin-2	Hs00428040_m1
<i>ASAM</i>	CMLP	Hs00293345_m1	<i>PKP3</i>	Plakophilin-3	Hs00170887_m1
<i>GJA1</i>	Connexin-43	Hs00748445_s1	<i>PKP4</i>	Plakophilin-4	Hs00269305_m1
<i>GJB1</i>	Connexin-32	Hs00939759_s1	<i>CTNBN1</i>	B-catenin	Hs00355049_m1

JAM, Junctional adhesion molecule; *MAGI*, membrane-associated guanylate kinase inverted; *MUPP1*, multi-PDZ domain containing protein 1; *ZO*, zonula occludens.

*The assay ID identifies the predesigned array from the database of Applied Biosystems. These 62 mRNAs were measured in the Tagman low-density array microfluidic cards.