Orally Administered Glucosylceramide Improves the Skin Barrier Function by Upregulating Genes Associated with the Tight Junction and Cornified Envelope Formation

Ritsuro IDETA,† Tomohiro SAKUTA, Yusuke NAKANO, and Taro UCHIYAMA

Shiseido Functional Food Research and Development Center, 2-12-1 Fukuura, Kanazawa-ku, Yokohama 236-8643, Japan

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Dietary glucosylceramide improves the skin barrier function. We used a microarray system to analyze the mRNA expression in SDS-treated dorsal skin of the hairless mouse to elucidate the molecular mechanisms involved. The transepidermal water loss of mouse skin was increased by the SDS treatment, this increase being significantly reduced by a prior oral administration of glucosylceramides. The microarray-evaluated mRNA expression ratio showed a statistically significant increase in the expression of genes related to the cornified envelope and tight junction formation when compared with all genes in the glucosylceramide-fed/SDS-treated mouse skin. We then examined the contribution of glucosylceramide metabolites to the tight junction formation of cultured keratinocytes. The SDS treatment of cultured keratinocytes significantly decreased the transepidermal electrical resistance, this decrease being significantly ameliorated in the presence of sphingosine or phytosphingosine, the major metabolites of glucosylceramide. These results suggest that an oral administration of glucosylceramide improved the skin barrier function by up-regulating genes associated with both the cornified envelope and tight junction formation.

Key words: glucosylceramide; sphingoid base; cornified envelope; tight junction; skin barrier

Amorphophallus konjac (A. konjac) is a perennial plant native to eastern Asia (from Japan and China, south to Indonesia). It forms a large corm which contains around 40% glucomannan gum,1) and is used as a popular health food in Japan, because it has almost no calories, but is very high in fiber content. A. konjac is also a rich source of glucosylceramides (GCs), which are structurally constituted by sphingoid bases, long-chain fatty acids and sugar moieties, and occur in animals, fungi and plants.2) GCs are essential structural components of mammalian cell membranes and are mostly found at the cell surface; they participate in such biological functions as immunomodulation3) and insulin resistance.4) They also serve to maintain the mammalian skin barrier function through their role as intracellular lipids.5) The skin barrier is essential for protecting against physical stimuli, thermal challenge, ultraviolet light (UV), chemical substances and microorganisms, as well as for preventing water loss.6) The barrier function is mainly localized in the stratum corneum (SC) which is formed in the outermost layer of the epidermis and consists of the cornified envelope (CE) and intercellular multilamellar lipids. CE forms a highly durable and flexible barrier7) comprising a 15-nm-thick structure composed of such insoluble proteins as involucrin, loricrin, and small proline-rich proteins that are covalently cross-linked by transglutaminases (TGases).8) SC intercellular lipids, which consist mainly of ceramide and such other components as cholesterol esters and free fatty acids, are also known to influence the skin barrier function. Maturation of CE is essential for a proper barrier function,9) and it is known that a decrease of ceramide causes impairment of the barrier function in human skin.10)

Tight junctions (TJs) in the granular layer of the epidermis also contribute to the skin barrier function11,12) by controlling the paracellular permeability of ions and water, as well as larger molecules. TJ is formed by a variety of proteins, including structural transmembrane components (claudins (Cldns), occludin, junctional adhesion molecules (JAMs) and tricellulin) and scaffolding proteins for undercoating TJ and for the assembly of transmembrane proteins (ZO-1, 2, 3, Mupp-1, Magi-1 and so on).13,14) as well as some protein complexes which regulate the set-up of the polarity, aPKC/Par3/Par6 complex and Crb3/Pals1/Patj complex.15,16) TJ and TJ proteins form zipper-like structures in epithelial cells which firmly fasten adjacent cells to each other, but divide between the apical space and basal space to control the paracellular passage of soluble factors. TJ proteins in mammalian skin contribute to various skin functions, including barrier formation, polarity, gene expression, proliferation, differentiation, and vesicular transport.14)

Interestingly, an oral intake of GC has been reported to improve the skin barrier function; for example, GC

† To whom correspondence should be addressed. Fax: +81-45-788-7284; E-mail: ritsuro.ideta@to.shiseido.co.jp

Abbreviations: AI, adherens junction; aPKC, atypical protein kinase C; CE, cornified envelope; Cldn, claudin; Cy, cyanine; dNHEK, normal human epidermal keratinocytes; GSEA, gene set enrichment analysis; JAM, junctional adhesion molecule; Magi, membrane-associated guanylate kinase; Mugu, multiple PDZ domain protein; NHEK, normal human epidermal keratinocytes; PAGE, parametric analysis of gene set enrichment; SC, stratum corneum; SD, standard deviation; Sprr, small proline-rich protein; TER, transepithelial electrical resistance; TEWL, transepidermal water loss; TGase, transglutaminase; UV, ultraviolet light; TJ, tight junction; ZO, zonula occludens
improved the recovery of SC flexibility and transepidermal water loss (TEWL) in acutely barrier-perturbed mice. A konjac extract, which contains GC, has also improved TEWL in healthy human subjects.

We performed a microarray analysis in this study to evaluate mRNA expression in the SDS-treated (barrier-perturbed) skin of GC-fed and control-fed mice in order to clarify the mechanism for the barrier-improving effect of orally administered GC. We evaluated two sets of genes associated with CE formation and TJ formation and function, and calculated their average induced expression ratio by GC feeding against the average expression ratio of all genes on the microarray. We also confirmed the contribution of GC metabolites to TJ formation by examining their effect on the transepithelial electrical resistance (TER) of normal cultured human epidermal keratinocytes.

Materials and Methods

Materials. Normal human epidermal keratinocytes (NHEK) were obtained from Kurabo Co. (Osaka, Japan). NHEKs were cultured in a low-Ca\(^2\+) (0.15 mM Ca\(^{2+}\)) medium, HuMedia-KG2 (Kurabo). Cells at passage three were used in this study.

Konjac extracts containing various concentrations of GC (12%, 66% and 100%) were presented by Unitika Limited (Osaka, Japan) and suspended in 1% of tragacanth gum (Wako Pure Chemicals Industries, Osaka, Japan) at respective final GC concentrations of 30, 165 and 250 g/mL.

Sphingoid bases, sphingosine and phytosphingosine, were purchased from Avanti Polar Lipids (Alabaster, AL, USA).

Animals. This study was approved by the ethics committee of Shiseido Research Center in accordance with the guidelines of the National Institute of Health. Hairless male mice (HR-1) were purchased from Hoshino Laboratory Animals (Ibaraki, Japan), and 5-week-old HR-1 were fed with the AIN-93G rodent diet (Oriental Shiseido Research Center in accordance with the guidelines of the manufacturer. Total RNAs were further purified by using an RNaseasy fibrous tissue mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. The RNA quantity and quality were evaluated by a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

cRNA amplification and labeling. Total RNA was amplified and labeled with Cyanine 5 (Cy5) and Cyanine 3 (Cy3) by using a Low RNA Input linear amplification kit (Agilent Technologies) according to the manufacturer’s instructions. RNAs for each sample were individually coupled to both Cy3 and Cy5 dyes so that a dye swap comparison could be made. Briefly, 500 ng of total RNA was reverse-transcribed to double-stranded cDNA by using a poly dt-T7 promoter primer and MMLV-RT enzyme. The cDNA products were used as templates for in vitro transcription to generate fluorescent cRNA by using T7 RNA polymerase and Cy5-labeled or Cy3-labeled CTP. Labeled cRNAs were purified by using Qiagen RNeasy mini spin columns and eluted in nuclease-free water. The cRNA quantity and cyanine incorporation were determined using the Nanodrop ND-1000 and Bioanalyzer instruments.

Hybridization of sample cRNAs and data processing. Two labeled cDNA samples, one from a GC-fed mouse and the other from a control-fed mouse, were combined. Each hybridization used 825 ng of labeled cRNAs that were mixed, fragmented, and hybridized at 65°C for 17 h to an Agilent 4 x 44 K Whole Mouse Genome microarray (Agilent 14868). After washing, the microarray was scanned with an Agilent DNA microarray scanner. Feature Extraction software version 9.1.3.1 (Agilent Technologies) was used to assess the fluorescent hybridization signals and to normalize the signals by using linear regression and a Lowess curve-fitting technique. The reproducibility and reliability of each microarray were assessed by using Quality Control report data in Feature Extraction.

Statistical analysis. The scanned data were analyzed by using Genespring® microarray data processing software (Agilent Technologies). Normalized signals were processed after first eliminating the flagged signals. Since some genes have multiple probes on the Agilent platform, gene-level expression values were calculated by using the “Gene-level experiment” function in Genespring. Expression values below 200 were floored to 200. The processed signal values for the GC-fed mice were divided by the processed signal values for the references, and these values were used as expression ratio data. Log base 2 of the expression ratio (log ratio) was used for further analysis, and Grubb’s method was used to eliminate outliers. The mean and standard deviation values for parent populations were calculated, and a t-test statistical analysis was performed between CE-related genes and overall genes and between TJ-related genes and overall genes by using the ZTEST function in Microsoft Excel (Microsoft, Redmond, WA, USA) according to the method described by Kim et al.\(^{20}\) Ingenuity Pathway database analyzing software (Ingenuity Systems, Redwood City, CA, USA) was used to search the knowledge-based relationships among genes and to prepare the figures. The data discussed in this study have been deposited in NCBI's Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO series accession no. GSE28086. Dunnett’s multiple-comparison test was used to evaluate the TEWL and TER data.

Transepithelial electrical resistance assay. NHEKs were seeded into 12-well Transwell-Clear polyester membrane inserts (Corning International, Akasaka, Japan) and cultured in a low-Ca\(^2\+) medium. The cells were then cultured at 80% confluence in a high-Ca\(^2\+) (1.80 mM) medium for 3 d to induce differentiation. The differentiated NHEKs (dNHEKs) were treated with 0.001% SDS for 3 h in order to inhibit the TJ function. After this SDS treatment, the cells were incubated for 2 h in the presence of sphingoid bases (sphingosine and phytosphingosine) at a concentration of 10\(^{-4}\) M, and their barrier function was measured with a Millicell-ERS instrument (Millipore, Billerica, MA, USA).

Results

Effect of dietary GC on TEWL of SDS-treated mouse skin

The mice were fed with different concentrations of konjac GC, the dorsal skin on one side was then treated
in the mice fed with 250 μg/mL of GC (Fig. 1).

Microarray results and differences of mean expression ratios of CE- and TJ-related genes from the mean expression ratios of all genes

Skin from the mice fed with or without 250 μg/mL of GC was used to obtain microarray data. Skin samples were obtained just before the SDS treatment (0 d), and after two SDS treatments (2 d). One sample for 0 d was lost during the hybridization procedure, so that, three replicates of the 0 d samples and four replicates of the 2 d samples were used for further analysis. We obtained respective gene level log ratio data for 14,518 and 14,547 genes from the 0 d and 2 d samples after data processing. The values for the average log ratio for all genes at 0 d (n = 3) and at 2 d (n = 4) were close to zero (Tables 1 and 2).

We selected 29 genes as a CE-related gene set. These included genes of the CE precursors, i.e., iorcin, involucrin, filaggrin, cystatin-A, elaﬁn, desmoplakin, envoplakin, periplakin, small proline-rich proteins (Sprr1a and 1b, Sprr2a1 to 2k and Sprr4), trichohyalin, keratin-1, 5 and 10, annexin 1, annexin 2, annexin 3, and S-100 proteins (S100a10 and S100a11). We also included genes for TGase-1 and 2, and sulphydryl oxidase, because they formed cross-links among the CE precursors. Caspase-14 also plays a role in CE maturation as it is involved in the cleavage of proﬁlaggrin into ﬁlaggrin units to stabilize keratin intermediate ﬁlaments. As shown in Table 1, the respective log ratios for iorcin, involucrin, ﬁlaggrin and TGase-1 at 2 d were 0.52, 0.41, 0.45 and 0.41. The respective mean log ratios for the CE-related genes at 0 d and 2 d were 0.02 and 0.27. We calculated the Z-score and p-value by applying the ZTEST function of Microsoft Excel to the 2 d data. The Z-score is the distance from a sample mean to the parent population mean in units of the standard error, and it can be used to estimate the probability of observing a sample mean by chance; for example, the probability of observing a Z-score over 1.96 is under 0.05. In this case, many CE-related genes showed increases, and the Z-score for the gene set was 8.26. The calculated p-value for the mean of CE-related genes with respect to the mean of all genes was extremely low (1.11 × 10^{-16}) (Table 1).

We also selected a set of 30 genes considered to be related to TJ formation and maintenance, including claudin-1, 3, 4, 5, 7, 8, 10, 12 and 23 from the claudin family, occludin, tricellulin, and junctional adhesion molecules (JAM1, 2 and 3), as transmembrane proteins in TJ. Several genes for cytoplasmic scaffolding proteins which undercoat and organize TJ proteins, i.e., ZO proteins (Tjp1, 2 and 3), multiple PDZ domain protein (Mupp-1) and membrane-associated guanylate kinase-1 (Magi-1), were also selected. We also included other genes known to be constitutively associated with TJ proteins and considered to be involved in the assembly and function of TJ, i.e., symplekin, members of the aPKC/Par3/Par6 complex (Prkci, Prkcz, Pard3, Pard6a, Pard6b and Pard6g), Crb3, Pals1/ Patj complex (Crb3, Mupp5 and Inadl), and afadin. Among these genes, claudin-1, 4, 10 and occludin showed relatively high log ratios (0.36, 0.35, 0.54, and 0.35, respectively). The respective mean log ratios for the set of 30 genes at 0 d and 2 d were 0.02 and 0.14. The respective Z-score for the gene set was 8.26. The calculated p-value for the mean of CE-related genes with respect to the mean of all genes was extremely low (1.11 × 10^{-16}) (Table 1).

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Discussion

We have already reported that an oral intake of a konjac extract containing GC improved the skin barrier condition in mice and humans. The increase in TEWL of SDS-treated (barrier-perturbed) mouse skin was significantly reduced by the konjac ingestion. A drink containing a konjac extract reduced TEWL on the cheek of healthy human volunteers in a randomized double-blind placebo-controlled trial. We examined in the present work the effects of dietary GC derived from konjac extracts on a mouse model of SDS-treated skin. GC at 250 μg/mL produced a significant improvement in TEWL, so we used this concentration for the subsequent experiments to elucidate the molecular mechanism for the improvement of skin barrier function by the oral GC treatment.

We hypothesized that the genes involved in the maintenance and formation of SC would be up-regulated by GC or its metabolites. Indeed, we have observed a significant increase of TGase-1 in the skin of GC-fed mice following barrier perturbation by UV exposure. TGase-1 is known to catalyze the formation of ester bonds between speciﬁc glutaminyl residues of involu-
corneocyte lipid envelope and thus contributes to mature lamellar membrane formation in SC and to skin barrier homeostasis. An increase of TGase-1 might therefore contribute to barrier recovery. However, many other genes may also be involved, including those associated with the production and assembly of CE components, and with their anchorage to the intracellular lipid layer. We also considered that genes related to TJ formation and TJ protein production in the epidermis might be relevant.

We therefore used a microarray assay to examine changes of mRNA expression in the skin of SDS-treated, GC-fed mice, and obtained normalized gene-level expression ratio data for GC-fed mice against control-fed mice. We used the Ingenuity Pathway analysis software with threshold values for the expression ratio (power of base 2 log ratio) of more than 1.1 and less than 0.9 to confirm no marked changes of expression level among the CE-related genes before the SDS treatment (0 d) (Fig. 3A). Six genes were up-regulated and 3 genes were down-regulated, the mean log ratio being 0.02 (Table 1). However, many CE-related genes seemed to be up-regulated after two SDS treatments (2 d) (Fig. 3B). Among these genes, those for the main components of CE (loricrin, filaggrin and involucrin) and the gene for CE maturation were relatively highly expressed, although the average log ratio for the 29 CE-related genes was not large (0.27). The result of the Z-test showed very high statistical significance (a very low p-value) (Table 1).

### Table 1. List of CE-Related Genes

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| **2d**      |           |                              |                                   |                      |
|             |           | 0.012                        | 1.016                             |                      |
|             |           | 0.993                        | 1.353                             |                      |
|             |           | 1.085                        | 1.310                             |                      |
|             |           | 0.950                        | 1.258                             |                      |
|             |           | 1.218                        | 1.568                             |                      |
|             |           | 1.078                        | 1.651                             |                      |
|             |           | 1.286                        | 1.513                             |                      |
|             |           | 1.107                        | 1.217                             |                      |
|             |           | 1.078                        | 1.434                             |                      |
|             |           | 1.010                        | 1.271                             |                      |
|             |           | 0.899                        | 0.906                             |                      |
|             |           | 0.999                        | 1.024                             |                      |
|             |           | 0.873                        | 1.254                             |                      |
|             |           | 0.946                        | 1.048                             |                      |
|             |           | 1.093                        | 1.066                             |                      |
|             |           | 1.065                        | 1.083                             |                      |
|             |           | 1.233                        | 1.160                             |                      |
|             |           | 1.077                        | 1.040                             |                      |
|             |           | 1.064                        | 1.019                             |                      |
|             |           | 1.000                        | 1.000                             |                      |
|             |           | 1.010                        | 1.123                             |                      |
|             |           | 1.078                        | 1.052                             |                      |
|             |           | 0.915                        | 1.338                             |                      |
|             |           | 0.710                        | 1.265                             |                      |
|             |           | 0.988                        | 1.324                             |                      |
|             |           | 0.989                        | 1.139                             |                      |
|             |           | 0.985                        | 1.156                             |                      |
|             |           | 1.021                        | 1.432                             |                      |

**Number of genes**: 29

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<th>p-value</th>
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Gene names, gene symbols, entre IDs and their functions are shown. The mean and SD of the gene-level log scale expression ratio (base 2) and expression ratio (GC/Control) under each experimental condition are shown (0 d, n = 3; 2 d, n = 4). The mean and SD of log ratios and ratios of overall genes (ALL) are indicated. Z-scores and Z-test p-values were calculated based on the log scale ratio. An asterisk indicates statistical significance in the Z-test (***, p < 0.001).
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</table>

Gene names, gene symbols, entre IDs and their functions are shown. The mean and SD of the gene-level log scale expression ratio (base 2) and expression ratio (GC/Control) under each experimental condition are shown (0 d, n = 3; 2 d, n = 4). The mean and SD of log ratios and ratios of overall genes (ALL) are indicated. Z-scores and Z-test p-values are calculated based on the log scale ratio. An asterisk indicates statistical significance in the Z-test (*** p < 0.001).
It is known that a gene set enrichment analysis (GSEA) and parametric analysis of gene set enrichment (PAGE) are useful to evaluate the significant biological changes of predefined gene sets in microarray data. Both methods are especially useful when the gene expression changes in a given array data set is minimal or moderate, and PAGE is more sensitive compared to GSEA because it uses Z-scores to evaluate the significance of the difference between the mean of the expression of the gene set and the mean of the parent population. We therefore used Z-scores and corresponding p-values to evaluate whether the average log ratio of the CE-related genes was increased or not. It seems likely that the oral GC administration did not simply induce several important genes for CE-formation, but rather induced a broad increase in expression of the CE-related genes which would be expected to speed up the barrier recovery in barrier-perturbed skin.

Among the 30 TJ-related genes, many appeared to be weakly induced, as in the case of the CE-related genes. However, the increase in genes of the claudin family and occludin, the major transmembrane component of TJ, were relatively higher (Fig. 3B). A Z-test result for the 2 d data of the TJ-related gene set showed a significant difference with a p-value of 9.93 × 10⁻⁶ (Table 2). It appears that GC administration enhanced TJ formation through a broad increase in the expression of TJ-related genes.

We performed a TER assay, which is routinely used to evaluate the TJ barrier function (intact TJ shows high TER and impaired TJ shows low TER), to confirm these findings experimentally. As shown in Fig. 2, the major sphingometabolites of GC, sphingosine and phytosphingosine, significantly improved the TER value in SDS-treated dNHEK cells, as we expected.

In conclusion, we examined the mechanism for the skin barrier improvement induced by dietary GC, and our results indicate that sphingoid-based metabolites derived from GC enhanced TJ formation by inducing the increased expression of genes related to TJ formation in the epidermis, as well as promoting barrier recovery by inducing an increased expression of those genes related to CE formation. These findings indicate that dietary GC can play an important role in maintaining a healthy skin barrier condition and in promoting recovery from an impaired skin barrier by enhancing CE and TJ formation at the gene expression level. We have recently shown the increased expression of Tgm-1 mRNA and claudin-1 and involcrin proteins in GC-fed barrier-perturbed mice skin after UV exposure (data are not

Fig. 2. Effects of the GC Metabolites on SDS-Treated dNHEK.

The TER value for dNHEKs is shown. Control (−) represents no SDS treatment, while control (+) represents the SDS treatment. Sphingosine and phytosphingosine represent cells treated for 72 h with a 10⁻⁶ M sphingoid base after the SDS treatment. All data are expressed as the mean ± SD (n = 4). An asterisk indicates statistical significance in Dunnett’s multiple-comparison test (**p < 0.01).

Fig. 3. Schematic Illustration of the Changes in Expression of CE-Related Genes and TJ-Related Genes on (A) Day 0 and (B) Day 2 Drawn by Using the Pathdesigner Function of IPA.

A red color represents genes up-regulated by more than 1.1 times in GC-fed mice against control mice. Green indicates genes down-regulated by below 0.9 times in GC-fed mice against control mice.
shown). These results also suggest the relationship between CE- and TJ-related genes in the dietary GC effect against skin barrier perturbation. Although the involvement of CE- and TJ-related genes in promoting barrier recovery by dietary GC was experimentally confirmed, it is also possible that other gene groups were involved. We have included afadin in this study as one of the TJ-related genes, because it directly binds to ZO-1 before the formation of TJ and is involved in the first step of recruitment for TJ on the apical side of the cells. Afadin is also essential to the formation of the adherens junction (AJ), and the formation of AJ is known to facilitate the assembly of TJ. Genes related to AJ formation such as those encoding cadherins, catenins and nectins may therefore also be involved in the promotion of barrier recovery by dietary GC. Indeed, weak up-regulation of many AJ-related genes was apparent in our microarray results (Table 3). It therefore appears that dietary GC promoted recovery of the skin barrier function by inducing relatively weak increases in expression of many genes related to the formation and maintenance of the skin barrier system. Although the changes in expression of individual genes were small, such a broadly based action might well explain the efficacy of an oral GC intake.

Acknowledgment

We greatly thank Dr. Jotaro Nakanishi for technical guidance and helpful discussions.

References

19) Uchiyama T, Nakano Y, Ueda O, Mori H, Nakashima M, Noda

### Table 3. List of AJ-Related Genes

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Log ratio (log2, GC/Control)</th>
<th>Gene expression ratio (GC/Control)</th>
<th>Description/Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdh1</td>
<td>cadherin 1/E-cadherin</td>
<td>0.067</td>
<td>0.312</td>
<td>1.048</td>
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<tr>
<td>Cdh3</td>
<td>cadherin 3/P-cadherin</td>
<td>0.189</td>
<td>0.285</td>
<td>1.140</td>
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<tr>
<td>Ctnn1</td>
<td>catenin, alpha 1</td>
<td>−0.045</td>
<td>0.083</td>
<td>0.969</td>
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<tr>
<td>Ctnn2</td>
<td>catenin, alpha 2</td>
<td>−0.484</td>
<td>0.000</td>
<td>0.715</td>
</tr>
<tr>
<td>Ctnnb1</td>
<td>catenin, beta 1</td>
<td>0.082</td>
<td>0.166</td>
<td>1.058</td>
</tr>
<tr>
<td>Ctnnd1</td>
<td>catenin, delta 1</td>
<td>0.000</td>
<td>0.039</td>
<td>1.000</td>
</tr>
<tr>
<td>Ctnnd2</td>
<td>catenin, delta 2</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Jup</td>
<td>junction plakoglobin</td>
<td>0.096</td>
<td>0.251</td>
<td>1.069</td>
</tr>
<tr>
<td>Pvl1</td>
<td>poliovirus receptor-related 1/nectin-1</td>
<td>−0.025</td>
<td>0.366</td>
<td>0.983</td>
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<tr>
<td>Pvl2</td>
<td>poliovirus receptor-related 2/nectin-2</td>
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<td>0.170</td>
<td>1.035</td>
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<tr>
<td>Pvl3</td>
<td>poliovirus receptor-related 3/nectin-3</td>
<td>−0.095</td>
<td>0.089</td>
<td>0.936</td>
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<tr>
<td>Pvl4</td>
<td>poliovirus receptor-related 4/nectin-4</td>
<td>−0.036</td>
<td>0.282</td>
<td>0.976</td>
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<tr>
<td>AF-6/Mllt4</td>
<td>Afadin/myeloid-lymphoid or mixed-lineage leukemia; translocated to 4</td>
<td>0.112</td>
<td>0.288</td>
<td>1.081</td>
</tr>
</tbody>
</table>

Gene names, gene symbols, entre IDs and their functions are shown. The mean and SD of the gene-level log scale expression ratio (base 2) and expression ratio under each experimental condition are shown (0d, n = 3; 2d, n = 4).