Original Article

Association of Gap Junction Beta 2 and Transglutaminase 1 Gene Expression with Canine Atopic Dermatitis

Gunnaporn Suriyaphol[1]* Sirin Theerawatanasirikul[2] Piyarat Chansiripornchai[3]

Abstract

Atopic dermatitis is a common pruritic inflammatory skin disease in humans and dogs. Cell junction and cornified envelope are groups of proteins that are crucial for the formation and stability of the skin barrier. The purpose of this study was to investigate gene expression in cell junction and cornified envelope groups in canine atopic dermatitis (CAD) in small dog breeds. Skin biopsy was performed from 10 lesional CAD, 9 non-lesional CAD cases and 11 normal dogs and subjected to quantitative reverse transcription-polymerase chain reaction. Several cell junction genes were evaluated, including claudin-1, occludin, zonula occludens-1 and -2, zonula occludens-1-associated nucleic acid binding protein, cingulin, gap junction beta 2 and e-cadherin together with transglutaminase 1, a cross-linker of the cornified envelope. An upregulation of gap junction beta 2 and transglutaminase 1 was significantly observed in the lesional skin. In conclusion, the present study demonstrates the expression of gap junction beta 2 and transglutaminase 1 in CAD. This is the first report on the association of cell junction and transglutaminase 1 genes with CAD.

Keywords: canine atopic dermatitis, cell junction, dog, gap junction beta 2, gene expression, transglutaminase 1

[1]*Biochemistry Unit, Department of Veterinary Physiology, Faculty of Veterinary Science, Chulalongkorn University, 39 Henri Dunant Rd., Pathumwan, Bangkok 10330, Thailand*

[2]*Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, 50 Ngamwongwan Rd., Chatuchak, Bangkok 10900, Thailand*

[3]*Department of Veterinary Pharmacology, Faculty of Veterinary Science, Chulalongkorn University, 39 Henri Dunant Rd., Pathumwan, Bangkok 10330, Thailand*

* ***Correspondence:*** *Gunnaporn.V@chula.ac.th*

Introduction

Atopic dermatitis (AD) or atopic eczema is a common multifactorial skin disease recognized in dogs (Hillier and Griffin, 2001) and human (Wadonda-Kabondo et al. 2003). Canine atopic dermatitis (CAD) is originally defined as "genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens" (Halliwell, 2006). For the canine atopic-like dermatitis, it is the CAD without IgE prerequisite. Both food-induced and non-food induced atopic dermatitis or canine atopic dermatitis senso strictu have been mentioned. (Favrot et al., 2010; Olivry, 2010).

CAD is associated with the defect of skin barrier which prevents allergens from penetrating the skin. A cell junction or intercellular bridge is a contact of adjacent cells or between a cell and the extracellular matrix especially in epithelial tissues, contributing the paracellular barrier and helping the paracellular transport. In vertebrate, cell junctions can be functionally classified into 4 types, including i.) anchoring junctions, ii.) gap or communicating or channel-forming junctions, iii.) tight or occluding junctions, and iv.) signal-relaying junctions (Alberts et al., 2007). Anchoring-type junctions function to hold cells and provide strong structural cohesion between adjacent cells in tissues that have to stand constant mechanical stress, e.g. skin and cardiac muscle in mouse and/or human (Kuwahara et al., 2001; Mezzano and Sheikh, 2012). Protein members in this group include cadherins (CDH), catenins, integrins, etc. Gap junctions are intercellular channels that allow communication of small molecules (metabolites, second messengers, and ions, up to a molecular weight of about 1000 daltons between cells) (Sosinsky and Perkins, 2000), leading to cell proliferation, cell death, tumor suppression, and action potential in several organs such as rat heart, mouse brain, chicken retina, and mouse skin (Becker et al., 2002; Clarke et al., 2006; Kretz et al., 2004; Teubner et al., 2001). There are several gap junction proteins such as gap junction alpha 1 protein (GJA1) or connexin 43 (Cx43) and gap junction beta 2 protein (GJB2) or Cx26 (Bruzzone, 2001). Tight junctions (TJs) are intercellular junctions that adhere two neighboring cells near the apical side of cells. A number of TJ proteins have been identified in mammalian epidermis including occludin (OCL), cingulin (CGN), zonula occludens-proteins (ZOs), claudins (CLDNs), and junctional adhesion molecules (JAMs) (Kirschner et al., 2010). CGN can form complexes with ZO-1, ZO-2 and JAM-1 (Guillemot and Citi, 2006). In skin diseases which are characterized by impaired skin barrier function, altered proliferation/differentiation of the epidermis and/or infiltration of inflammatory cells, alterations of the expression patterns of TJ genes were described such as the downregulation of CLDN1, CLDN3, and JAM-1 and upregulation of ZO-1 and OCLN in human psoriasis (Kirschner et al., 2009; Watson et al., 2007). Upregulation of ZO-1 and OCLN proteins in early stage of *Staphylococcus aureus* infection and upregulation of CLDN-1 and OCLN proteins in mouse skin exposed to UV radiation were also shown (Ohnemus et al., 2008). In atopic dermatitis, CLDN1 and CLDN23 expression were decreased in human atopic dermatitis (HAD) patients whereas GJB2 was inversely upregulated (De Benedetto et al., 2012). The association of TJ gene expression with CAD has not yet been demonstrated. The signal-relaying junctions such as chemical synapses and immunological synapses play an important role in relaying signals from cell to cell and the three groups mentioned previously also have signal functions (Alberts et al., 2007).

The cornified envelope (CE) is a protein complex layer beneath the plasma membrane of mammalian epidermis in terminal differentiation of the skin. It helps protect skin cells against water loss and infection and maintain epidermal structure integrity (Hohl, 1990; Reichert et al., 1993). Gene expression of a number of CE proteins, including involucrin (IVL), filaggrin (FLG) together with other epidermal differentiation and proliferation markers such as keratin 5 (KRT5) and KRT 10 have been reported in normal dog skin in different breeds and coat types (Theerawatanasirikul et al., 2012[a]) and in CAD (Theerawatanasirikul et al., 2012[b]; Theerawatanasirikul et al., 2012[c]). Transglutaminase 1 (TGM1) is an enzyme that facilitates cross-linking of the CE proteins in mature keratinocytes by catalyzing formation of ε-(γ-glutamyl)-lysine cross-links in proteins (Greenberg et al., 1991). However, the association of TGM1 and CAD has not yet been demonstrated. The objective of this study, therefore, was to characterize the gene expression patterns of *CLDN1, CGN, OCL, CDH1, GJB2* and *TGM1* genes in lesional atopic, non-lesional atopic and healthy canine skin by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The study would give better understanding of the CAD disease.

Materials and Methods

Animals: Nineteen atopic dogs from private small animal clinics, fulfilling at least 5 signs of the diagnostic criteria for CAD (Favrot et al., 2010; Olivry 2010), including onset of signs under 3 years of age, living mostly indoors, glucocorticoid-responsive pruritus, pruritus sine materia at onset, affected front feet and/or ear pinnae, and non-affected ear margins and/or dorso-lumbar area, were included in the study. This group comprised eleven Poodles (1 male and 10 females), six Shih tzus (5 males and 1 female) and two Pugs (1 male and 1 female), with a mean age of seven years (age ranging from 2–11 years). For the control group, there were eleven healthy dogs comprising seven Poodles (1 male and 6 females), three Shih tzus (1 male and 2 females) and one male Pug, with a mean age of seven years (age ranging from 1–10 years).

Skin biopsies and tissue samples: Skin specimens were all taken from the ventral area of each dog to minimize variations due to body location. Lesional samples of erythematous, macular-papular dermatitis and lichenification were selected from affected areas. Non-lesional samples were obtained from clinically unaffected skin of atopic dogs whereas control samples were from clinically normal dogs. Punch skin biopsies

(6 mm) were obtained after local anesthesia with 2% lidocaine and, then, sutured routinely. Subcutaneous fat was stripped off. The biopsy was immersed in RNALater solution (Life Technologies, Carlsbad, CA) overnight at 4°C and stored at -20°C until being processed for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). The sample collection and processing procedures were approved by the Chulalongkorn University Animal Care and Use Committee (CU-ACUC), Thailand.

RNA Extraction: The skin tissues in RNALater solution were disrupted in liquid nitrogen to maintain a low temperature. Total RNA was extracted from the skin tissues by homogenization with Trizol reagent (Life Technologies, Carlsbad, CA) and phenol/chloroform/isopropyl alcohol. Subsequently, genomic DNA traces were removed from the RNA with Turbo DNase (Ambion, Austin, TX) to purify the total RNA according to the instructions. The DNase-treated RNA quality and concentration were analyzed using a NanoDrop ND-1000 Spectrophotometer V3.7 (Thermo Fisher Scientific, Waltham, MA).

Quantitative reverse transcriptase PCR: SuperScript III First-strand synthesis system for RT-PCR (Life Technologies, Carlsbad, CA) was used to synthesize cDNA according to the manufacturer's instructions. Briefly, one microgram of RNA was reverse transcribed in a 20 µL reaction containing 50 ng random primers, 40U RNase inhibitor and 200U Superscript III enzyme. Rotor Gene 3000 Thermal Cycler (Qiagen, Hilden, Germany) was used to perform quantitative PCR. Except for the housekeeping gene (HKG), *RPS19*, which has been previously described as a suitable HKG for CAD (Schlotter et al., 2009; Theerawatanasirikul et al., 2012c), all primers were designed by the Primer 3 program version 0.4.0 (http://frodo.wi.mit.edu/). Primer pairs were sequenced for specificity and uniqueness in the dog genome (CanFam2.0, May 2005 assembly). The primers sequences, melting temperatures and amplicons are depicted in Table 1. PCR reactions were performed in a 10 µL volume containing 1x KAPA SYBR Fast qPCR Master Mix Universal (KAPA Biosystems, Cambridge, MA), 200 nM of each primer and the cDNA template. Thermal cycling conditions were as follows: 95 °C for 2 min for one cycle followed by 40 cycles at 95 °C for 3 s, 60 °C for 20 s and 72 °C for 1 s. Each reaction was performed in duplicate in 3 independent runs. Data from the FAM/SYBR channel operating at an excitation maximum of 495 nm and an emission maximum of 520 nm was evaluated. A melting curve analysis was used to determine the purity of the amplified products. Relative expression levels were analyzed by REST-384 (Relative Expression Software Tool) software. Standard curves were generated for each assay as previously demonstrated and threshold cycles of all targets in the test samples were normalized to the corresponding *RPS19* levels in the control samples (Pfaffl, 2001; Pfaffl et al., 2002; Theerawatanasirikul et al., 2012c).

Statistical analysis: The data was analyzed in REST 384 software, using a pair wise fixed reallocation randomization test to test for significance between groups. Results with a p value <0.05 were considered significant. Hierarchical clustering was performed to analyze the pattern of similarity in gene expression in lesional skin in order to identify gene clusters , using Multiexperiment Viewer (MeV) program, version 4.8 (Saeed et al., 2003; Saeed et al., 2006).

Table 1 Primers used in the present study. Those indicated are sequences, annealing temperatures in real-time PCR reactions and expected product sizes

Genes	Primers (5'to 3')	Amplicon (bp)	Accession number
CGN	Fwd 5'-agctcggatgaggagtttga-3' Rev 5'-agaggcaagcctgtctacca-3'	277	DQ910799
CLDN1	Fwd 5'-ggccactattggcatgaagt-3' Rev 5'-atgttgtttttcggggacag-3'	284	XM845155.2
CLDN23	Fwd 5'-gtggacgtggagctgtacc-3' Rev 5'-cggtgggtgtaccaggac-3'	293	XM003639565.1
CDH1	Fwd 5'-ggtgctcacatttcccagtt -3' Rev 5'-aaatgggcctttctcgtttt-3'	100	NM001197148.1
GJB2	Fwd 5'-aaatatgggccggatagacc-3' Rev 5'-tcccaagcaagctccttaaa-3'	180	NM001197148.1
OCLN	Fwd 5'-catggtgattgtggcttttg-3' Rev 5'-ggaggaggcatgtcttgtgt -3'	180	NM001003195.1
ZO-1	Fwd 5'-cggtaccagctcctctcttg-3' Rev 5'-cggtttggtggtctgaaagt-3'	153	NM001003140.1
ZO-2	Fwd 5'-caattcagcatcagcaagga-3' Rev 5'-gctcatccagctcattgtca-3'	198	NM001003204.1
ZONAB	Fwd 5'-cggttcatcgaaatccaact-3' Rev 5'-atggaacttcaggtgccttg-3'	234	AF171061.1
TGM1	Fwd 5'-gcaagaaggaagtggtgctc -3' Rev 5'-acggaaggtatgctgtttgg-3'	167	AF262219.1
RPS19	Fwd 5'-ccttcctcaaaaa/gtctggg-3' Rev 5'-gttctcatcgtagggagcaag-3'	95	XM533657

Results

For the qRT-PCR experiment, *GJB2 and TGM1* expressed upregulation in the lesional skin compared to the normal skin with fold changes of 8.081 ($p = 0.007$) and 3.859 ($p = 0.038$), respectively. *GJB2* also showed upregulation in the lesional skin compared to the non-lesional skin with a fold change of 5.757 ($p = 0.025$). Lower expression of *ZONAB* was observed in the non-lesional skin compared to the normal skin (Table 2) with a fold change of 0.379 (p=0.003). For the hierarchical clustering in the lesional skin, when compared with gene expression patterns of *FLG*, *IVL*, other *KRT*s in a previous report (Theerawatanasirikul et al., 2012[c]), *GJB2*, *TGM1*, and *KRT14* were clustered together in one sub-cluster and *KRT1*, *KRT5* and *KRT17* in the other. However both were placed in the same super-cluster with *IVL* and *FLG*, whereas *KRT10* and *KRT2*e were clearly different (Fig 1).

Discussion

In the present study, we demonstrate the gene upregulation of *GJB2* and *TGM1* in CAD and this is the first report on the an association of cell junction and transglutaminase 1 genes with CAD. Gap junction-mediated cell communication plays a role in maintaining a uniform epidermal thickness with the balance of cell proliferation and differentiation. Several reports showed the association of an increase in the number of keratinocytes and/or the thickening of the epidermis with the induction of GJB2 expression either at the mRNA or protein levels, including a lesion

Table 2 Fold change in gene-specific mRNA expression in CAD lesional, CAD non-lesional and control dog skin compared to *RPS19* expression. *GJB2* and *TGM1* express upregulation in lesional skin compared to normal skin. *GJB2* also shows upregulation in lesional skin compared to non- lesional skin. Lower expression of *ZONAB* is observed in non-lesional skin compared to normal skin

Gene	lesional vs. normal		non-lesional vs. normal		lesional vs. non-lesion	
	Fold change	*p* value*	Fold change	*p* value*	Fold change	*p* value*
CGN	0.711	0.653	1.927	0.314	0.431	0.261
CLDN1	1.330	0.664	2.077	0.178	0.640	0.534
CLDN23	0.665	0.693	0.704	0.632	0.946	0.954
CDH1	1.739	0.363	0.889	0.756	1.955	0.261
GJB2	8.081**	0.007	1.404	0.531	5.757**	0.025
OCLN	0.895	0.883	1.278	0.537	0.700	0.652
ZO-1	8.397	0.499	88.364	0.219	0.095	0.440
ZO-2	1.747	0.367	1.745	0.175	1.001	0.998
ZONAB	0.503	0.333	0.379**	0.003	1.327	0.709
TGM1	3.859**	0.038	2.342	0.073	1.648	0.439

* Pair wise fixed reallocation randomization test normalized by reference gene
** Significantly different at $p < 0.05$

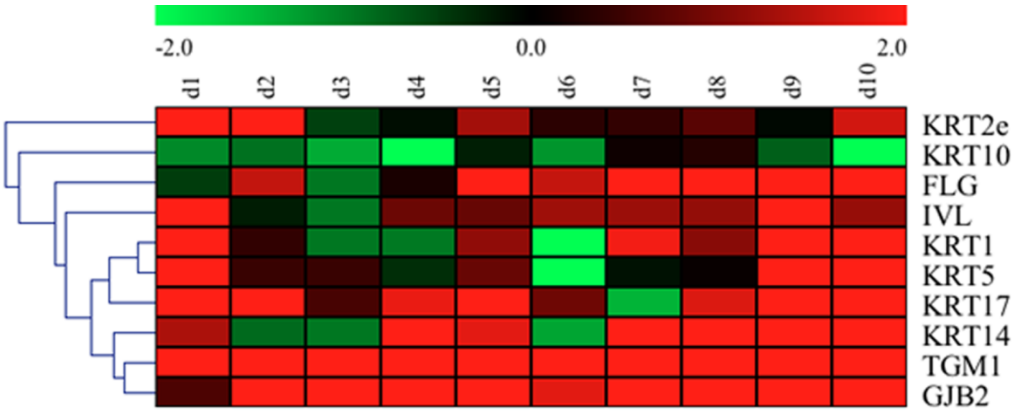


Figure 1 Hierarchical clustering of genes in lesional skin. d = dog

of human skin treated with retinoic acid (Masgrau-Peya et al., 1997), human psoriatic lesions (Hivnor et al., 2004; Labarthe et al., 1998; Lucke et al., 1999; Shaker and Abdel-Halim, 2012), human porokeratotic lesions (Hivnor et al., 2004), tape-stripped epidermis and viral warts (Lucke et al., 1999). In mouse epidermis, GJB2 expression rapidly increased in hyperproliferative wound epidermis (Djalilian et al., 2006; Goliger and Paul, 1995). In mouse skin papillomas, expression of both GJB2 and GJA1 was elevated in the proliferating neoplasms (Sawey et al., 1996). GJB2 was also upregulated in HAD (De Benedetto et al., 2012). In CAD, the hyperproliferation and aberrant keratinocyte differentiation resulted in the thickening of the epidermis (Theerawatanasirikul et al., 2012[b]). Since the hierarchical clustering showed the similarities in patterns of gene expression of *GJB2* and several *KRT*s associated with cell proliferation, it might indicate co-expression partners. Moreover, the upregulation of *GJB2* gene in this study corresponded to the abnormal characteristics of the skin similar to those in human and mouse (De Benedetto et al., 2012; Djalilian et al., 2006; Goliger and Paul, 1995; Hivnor et al., 2004). This congruence supports the use of dog as models to study human AD and other skin diseases that arise from the upregulation of this gene. GJB2 may also be used to determine therapeutic efficacy of drugs treating skin diseases related to cell proliferation as in human psoriasis (Shaker and Abdel-Halim, 2012). In addition, CAD was reported to be associated with the upregulation of a number of genes in a cornified envelope group, including *IVL* and *FLG* (Theerawatanasirikul et al., 2012[c]). Since TGM1 was the linker of the CE proteins, the upregulation of *TGM1* gene in lesional skin in the present study corresponded with the previous gene expression of the CE group. From the hierarchical clustering, *TGM1* expression was in the same supercluster with *IVL* and *FLG*. Since *TGM1* expression was not significantly observed in the non-lesional skin, the upregulated gene might reflect the clinical phenotypes, which should be further studied. *ZONAB* was found to be downregulated in the non-lesional skin and it also tended to show low expression in lesional skin. ZONAB has been reported to regulate epithelial cell proliferation in canine kidney and mammary cell lines (Sourisseau et al., 2006). However, we observed the lower expression of ZONAB in the non-lesional skin. Hence, the role of ZONAB in CAD is needed to be further investigated. Taken together, these studies establish an intriguing correlation between increased *GJB2* and *TGM1* expression and CAD, including the possible associations of *GJB2* and cell proliferation, and *TGM1* and CE proteins. For future work, since we observed the upregulation of GJB2 and TGM1 in CAD, the clinical application of GJB2 and TGM1 expression as potential diagnostic and/or therapeutic markers for CAD should be investigated.

Acknowledgements

We would like to express our thanks to Dr.Tossaporn Nakbed for sample collections. This study was supported by the Thailand Research Fund and the Office of the Higher Education Commission (Grant number: MRG5580144, 2012-2014).

References

Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P 2007. Cell Junctions, Cell Adhesion, and the Extracellular Matrix. In: Molecular Biology of the Cell. 5th ed. B Alberts, A Johnson, J Lewis, M Raff, K Roberts and P Walter (eds). New York: Garland Science. 1131-1204.

Becker DL, Bonness V, Catsicas M and Mobbs P 2002. Changing patterns of ganglion cell coupling and connexin expression during chick retinal development. J Neurobiol. 52: 280-293.

Bruzzone R 2001. Learning the language of cell-cell communication through connexin channels. Genome Biol. 2: REPORTS4027.

Clarke TC, Thomas D, Petersen JS, Evans WH and Martin PE 2006. The antiarrhythmic peptide rotigaptide (ZP123) increases gap junction intercellular communication in cardiac myocytes and HeLa cells expressing connexin 43. Br J Pharmacol. 147: 486-495.

De Benedetto A, Yoshida T, Kuo I, Rafaels N, Boguniewicz M, Hata T, Schneider L, Hanifin J, Gallo R, Leung D, Barnes K and Beck L 2012. Connexin-26 is upregulated in atopic dermatitis epidermis. J Invest Dermatol. 132: S52.

Djalilian AR, McGaughey D, Patel S, Seo EY, Yang C, Cheng J, Tomic M, Sinha S, Ishida-Yamamoto A and Segre JA 2006. Connexin 26 regulates epidermal barrier and wound remodeling and promotes psoriasiform response. J Clin Invest. 116: 1243-1253.

Favrot C, Steffan J, Seewald W and Picco F 2010. A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. Vet Dermatol. 21: 23–31.

Goliger JA and Paul DL 1995. Wounding alters epidermal connexin expression and gap junction-mediated intercellular communication. Mol Biol Cell. 6: 1491–1501.

Greenberg CS, Birckbichler PJ and Rice RH 1991. Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues. FASEB J. 5: 3071-3077.

Guillemot L and Citi S 2006. Cingulin, a Cytoskeleton-Associated Protein of the Tight Junction. In: Tight Junctions (Molecular Biology Intelligence Unit). L Gonzalez-Mariscal (ed). New York: Springer Science+Business Media. 54-63.

Halliwell REW 2006. Revised nomenclature for veterinary allergy. Vet Immunol Immunopathol. 114: 207-208.

Hillier A and Griffin CE 2001. The ACVD task force on canine atopic dermatitis (I): Incidence and prevalence. Vet Immunol Immunopathol. 81: 147-151.

Hivnor C, Williams N, Singh F, VanVoorhees A, Dzubow L, Baldwin D and Seykora J 2004. Gene expression profiling of porokeratosis demonstrates similarities with psoriasis. J Cutan Pathol. 31: 657-664.

Hohl D 1990. Cornified cell envelope. Dermatologica. 180: 201-211.

Kirschner N, Bohner C, Rachow S and Brandner JM 2010. Tight junctions: is there a role in dermatology? Arch Dermatol Res. 302: 483-493.

Kirschner N, Poetzl C, von den Driesch P, Wladykowski E, Moll I, Behne MJ and Brandner JM 2009. Alteration of tight junction proteins is an early event in psoriasis: putative involvement of proinflammatory cytokines. Am J Pathol. 175: 1095–1106.

Kretz M, Maass K and Willecke K 2004. Expression and function of connexins in the epidermis, analyzed with transgenic mouse mutants. Eur J Cell Biol. 83: 647–654.

Kuwahara M, Hatoko M, Tada H and Tanaka A 2001. E-cadherin expression in wound healing of mouse skin. J Cutan Pathol. 28: 191-199.

Labarthe MP, Bosco D, Saurat JH, Meda P and Salomon D 1998. Upregulation of connexin 26 between keratinocytes of psoriatic lesions. J Invest Dermatol. 111: 72–76.

Lucke T, Choudhry R, Thom R, Selmer IS, Burden AD and Hodgins MB 1999.Upregulation of connexin 26 is a feature of keratinocyte differentiation in hyperproliferative epidermis, vaginal epithelium, and buccal epithelium. J Invest Dermatol. 112: 354-361.

Masgrau-Peya E, Salomon D, Saurat JH and Meda P 1997. In vivo modulation of connexins 43 and 26 of human epidermis by topical retinoic acid treatment. J Histochem Cytochem. 45: 1207–1215.

Mezzano V and Sheikh F 2012. Cell-cell junction remodeling in the heart: possible role in cardiac conduction system function and arrhythmias? Life Sci. 9: 313-321.

Ohnemus U, Kohrmeyer K, Houdek P, Rohde H, Wladykowski E, Vidal S, Horstkotte MA, Aepfelbacher M, Kirschner N, Behne MJ, Moll I and Brandner JM 2008. Regulation of epidermal tightjunctions (TJ) during infection with exfoliative toxin-negative Staphylococcus strains. J Invest Dermatol. 128: 906–916.

Olivry T 2010. New diagnostic criteria for canine atopic dermatitis. Vet Dermatol 21: 123–126.

Pfaffl MW 2001. A new mathematical model for relative quantification in realtime RT-PCR. Nucleic Acids Res 29: e45.

Pfaffl MW, Horgan GW and Dempfle L 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30: e36.

Reichert U, Michel S and Schmidt R 1993. The cornified envelope: A key structure of terminally differentiating keratinocytes. In: Molecular Biology of the Skin: The Keratinocyte. M Darmon and M Blumenberg (eds). San Diego: Academic Press. 107-150.

Saeed AI, Bhagabati NK, Braisted JC, Liang W, Sharov V, Howe EA, Li J, Thiagarajan M, White JA and Quackenbush J 2006. TM4 microarray software suite. Methods Enzymol. 411: 134–193.

Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Sturn A, Snuffin M, Rezantsev A, Popov D, Ryltsov A, Kostukovich, E, Borisovsky I, Liu Z, Vinsavich A, Trush V and Quackenbush J 2003. TM4: a free, open-source system for microarray data management and analysis. BioTechniques. 34: 374–378.

Sawey MJ, Goldschmidt MH, Risek B, Gilula NB and Lo CW 1996. Perturbation in connexin 43 and connexin 26 gap-junction expression in mouse skin hyperplasia and neoplasia. Mol Carcinog. 17: 49–61.

Schlotter YM, Veenhof EZ, Brinkhof B, Rutten VP, Spee B, Willemse T and Penning LC 2009. A GeNorm algorithm-based selection of reference genes for quantitative real-time PCR in skin biopsies of healthy dogs and dogs with atopic dermatitis. Vet Immunol Immunopathol. 129: 115–118.

Shaker O and Abdel-Halim M 2012. Connexin 26 in psoriatic skin before and after two conventional therapeutic modalities: methotrexate and PUVA. Eur J Dermatol. 22: 218-224.

Sosinsky GE and Perkins GA 2000. Electron crystallographic methods for investigating gap junction structure. Methods. 20: 140-155.

Sourisseau T, Georgiadis A, Tsapara A, Ali RR, Pestell R, Matter K and Balda MS 2006. Regulation of PCNA and cyclin D1 expression and epithelial morphogenesis by the ZO-1-regulated transcription factor ZONAB/DbpA. Mol Cell Biol. 26: 2387-2398.

Teubner B, Odermatt B, Guldenagel M, Sohl G, Degen J, Bukauskas F, Kronengold J, Verselis VK, Jung YT, Kozak CA, Schilling K and Willecke K 2001. Functional expression of the new gap junction gene connexin47 transcribed in mouse brain and spinal cord neurons. J Neurosci. 21: 1117-1126.

Theerawatanasirikul S, Suriyaphol G, Thanawongnuwech R and Sailasuta A 2012a. Histologic morphology and involucrin, filaggrin, and keratin expression in normal canine skin from dogs of different breeds and coat types. J Vet Sci. 13: 163-170.

Theerawatanasirikul S, Sailasuta A, Thanawongnuwech R, Nakbed T, Charngkaew K and Suriyaphol G 2012b. Differential expression patterns of proteins involved in epidermal proliferation and differentiation in canine atopic dermatitis. Thai J Vet Med. 42: 287-296.

Theerawatanasirikul S, Sailasuta A, Thanawongnuwech R and Suriyaphol G 2012c. Alterations of keratins, involucrin and filaggrin gene expression in canine atopic dermatitis. Res Vet Sci. 93: 1287-1292.

Wadonda-Kabondo N, Sterne JA, Golding J, Kennedy CT, Archer CB and Dunnill MG 2003. A prospective study of the prevalence and incidence of atopic dermatitis in children aged 0-42 months. Br J Dermatol. 149: 1023-1028.

Watson RE, Poddar R, Walker JM, McGuill I, Hoare LM, GriYths CE and O'Neill CA 2007. Altered claudin expression is a feature of chronic plaque psoriasis. J Pathol. 212: 450–458.

บทคัดย่อ

ความสัมพันธ์ของการแสดงออกของจีนแก็ปจังชันเบต้า 2 และ ทรานส์กลูตามิเนส 1 กับโรคผื่นภูมิแพ้ผิวหนังในสุนัข

กรรณาภรณ์ สุริยผล[1*] ศิรินทร์ ธีระวัฒนศิริกุล[2] ปิยะรัตน์ จันทร์ศิริพรชัย[3]

โรคผื่นภูมิแพ้ผิวหนังเป็นโรคที่ทำให้ผิวหนังอักเสบและคันที่พบได้บ่อยทั้งในคนและสุนัข โปรตีนกลุ่มรอยต่อระหว่างเซลล์และกลุ่มคอร์นิไฟด์เอนเวลโลปมีความสำคัญในการสร้างผิวหนังและความอยู่ตัวของผิวหนัง วัตถุประสงค์ของการศึกษาครั้งนี้คือต้องการตรวจสอบการแสดงออกของจีนกลุ่มรอยต่อระหว่างเซลล์และจีนที่เกี่ยวข้องกับการสร้างคอร์นิไฟด์เอนเวลโลปในโรคผื่นภูมิแพ้ผิวหนังในสุนัขพันธุ์เล็ก โดยทำการตัดชิ้นเนื้อจากผิวหนังสุนัขที่มีรอยโรค 10 ตัว ไม่มีรอยโรค 9 ตัว เปรียบเทียบกับผิวหนังสุนัขปกติ 11 ตัว และนำชิ้นเนื้อทำปฏิกิริยาลูกโซ่พอลิเมอเรสเรียลไทม์แบบย้อนกลับ จีนกลุ่มรอยต่อระหว่างเซลล์ที่ศึกษาได้แก่ คลาวดิน-1 อ็อกคลูดิน โซนาอ็อกคลูเดนส์-1 และ -2 โซนาอ็อกคลูเดนส์-1 แอสโซสิเอดเต็ดนิวคลีอิกแอซิดบายดิงโปรตีน ซินกูลิน แก็ปจังชันเบต้า 2 และอีแคทฮีริน และได้ทำการศึกษาจีนทรานส์กลูตามิเนส 1 ซึ่งมีหน้าที่เชื่อมโปรตีนในกลุ่มคอร์นิไฟด์เอนเวลโลป จากการศึกษาพบการแสดงออกของจีนแก็ปจังชันเบต้า-2 และทรานส์กลูตามิเนส 1 เพิ่มขึ้นอย่างมีนัยสำคัญ ณ ผิวหนังที่มีรอยโรค สรุปได้ว่าการศึกษาครั้งนี้ได้รายงานการแสดงออกของจีนแก็ป-จังชันเบต้า 2 ทรานส์กลูตามิเนส 1 ในโรคผื่นภูมิแพ้ผิวหนังในสุนัข และได้แสดงความสัมพันธ์ระหว่างรอยต่อระหว่างเซลล์และทรานส์กลูตา-มิเนส 1 กับโรคผื่นภูมิแพ้ผิวหนังในสุนัขเป็นครั้งแรก

คำสำคัญ: โรคผื่นภูมิแพ้ผิวหนังในสุนัข รอยต่อระหว่างเซลล์ สุนัข แก็ปจังชั่นเบต้า 2 การแสดงออกของจีน ทรานส์กลูตามิเนส 1

*[1] หน่วยชีวเคมี ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **39** ถนนอังรีดูนังต์ ปทุมวัน กรุงเทพฯ **10330***

*[2] ภาควิชากายวิภาค คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ **50** ถนนงามวงศ์วาน จตุจักร กรุงเทพฯ **10900***

*[3] ภาควิชาเภสัชวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **39** ถนนอังรีดูนังต์ ปทุมวัน กรุงเทพฯ **10330***

****ผู้รับผิดชอบบทความ E-mail:** Gunnaporn.V@chula.ac.th*