

Differential Expression of Taste Receptors in Tongue Papillae of DBA Mouse

Ha-Jung Choi¹, Young-Kyung Cho^{1,2}, Ki-Myung Chung^{1,2} and Kyung-Nyun Kim^{1,2*}

¹Department of Physiology and Neuroscience, college of Dentistry, Gangneung-Wonju National University

²Research Institute of Oral Sciences, Gangneung-Wonju National University

(received February 02, 2016; revised March 06, 2016; accepted March 07, 2016)

The tongue has 4 kinds of papillae, which are filiform, fungiform (FU), foliate (FO) and circumvallate papilla (CV). Tongue papillae except filiform papilla include taste buds. The papillae differ in taste sensitivities, likely due to differential expression of taste receptors. In this study, we evaluated differences in the expression levels of taste receptors in FU, FO and CV.

Male DBA2 mice, 42-60 days old, were used in the study. Messenger RNAs were extracted from the murine epithelial tissues including FU, FO and CV. Cloned DNAs were synthesized by reverse transcription. Quantitative PCRs (qPCRs) were performed to determine mRNA expression levels of taste receptors.

Results of qPCR revealed that the relative expression levels and patterns were different among FU, FO and CV. All three type 1 taste receptors were expressed FU, FO and CV at varying relative expression levels. All 35 kinds of type 2 taste receptors showed higher expression in FO and CV than in FU. *Tas2r108* and *Tas2r137* showed the two highest expression levels in all tested papillae. The differential expression levels and patterns of taste receptors

among the three papillae could contribute to the different physiological sensitivities by tongue areas.

Additional studies such as *in situ* hybridization or taste receptor cell activity recording is necessary to elucidate the functional relationship between expression levels of taste receptors and taste sensitivity.

Key words: taste receptors, fungiform papilla, foliate papilla, circumvallate papilla, qPCR

Introduction

Taste is essential to the survival of the animals and to the maintenance of the quality of life. Most mammals feels sweet, bitter, sour, salty and umami tastes[1]. Tongue has four type papillae : filiform, fungiform (FU), foliate (FO) and circumvallate papilla (CV). Taste buds exist in three papillae except the filiform papilla. FU is distributed in the anterior two-thirds of the tongue, FO located on the posterior lateral edges of the tongue, and CV is aligned along the posterior border of the tongue[2].

Taste buds of the mammal were composed of three types of spindle-shaped cells and one type cuboid stem cells. The taste signals, which are detected by the taste receptor cells, are delivered into the brain along a taste pathway[3].

Type 1 taste bud cells are the most abundant cells in taste buds. Type 1 taste bud cells have large granules of 100-400nm diameters and irregular shape of the nucleus[4]. Type 1 taste bud cells express glutamate aspartate transporter

*Correspondence to: Kyung-Nyun Kim, Department of Physiology and Neuroscience, College of Dentistry, and Research Institute of Oral Sciences, Gangneung-Wonju National University, 7, Jukheon-gil, Gangneung, 25457, Korea.
Tel.: +82-33-640-2450, E-mail: knkim@gwnu.ac.kr
ORCID: 0000-0001-5429-1358

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

(GLAST), indicating that they may be involved in glutamate uptake[5]. The renal outer medullary potassium channels (ROMK) is also expressed in type 1 taste bud cells. A potassium channel may be involved in potassium homeostasis [6], and it would be involved in salty taste transduction[7]. Type 1 taste cells would be considered as glial cells in neural tissues, and they were called supporting cells.

Type 2 taste bud cells usually located in edge of the taste buds, and are less than 20% of taste bud cells. Type 2 taste bud cells did not have the conventional synapse structures[8], and express specific taste G protein linked receptors for sweet, bitter and/or umami tastes[9]. Because they serves to detect taste substances with receptors, they are called receptor cells,[10].

Type 3 taste bud cells possess only 5-7% in taste buds cell numbers. Type 3 taste bud cells are the only cells that they have the synapses between primary afferent gustatory nerves. This cells are called presynaptic cells[11]. Recently, it was reported that they might involve in sour taste transduction [12].

Taste receptors are the kinds of G-protein coupled receptors (GPCRs), GPCRs are the largest family of receptors that are present on cell membrane proteins belong to group across the cell membrane seven times. GPCRs act indirectly through the process of activating the G protein to regulate the activity of cell membrane target protein. Taste receptors activate the phospholipase C (PLC) and the resulting production of inositol trisphosphate (IP₃) induced the release of calcium ions from the intracellular calcium ion storage and the inflow of calcium ions by opening of transient receptor potential channel M5 (TrpM5). After all tastes signals are converted to depolarization, the increase of intracellular calcium ions elicit the release of neurotransmitters, then the signals can be passed to taste nerves[13].

Taste receptors can be divided into type 1 taste receptors (T1Rs) and type 2 taste receptors (T2Rs). Type 1 taste receptors consist of three kinds of protein, T1R1, T1R2, and T1R3 [14], T1R2 and T1R3 proteins make up heterodimer to form sweet taste receptor, T1R1 and T1R3 proteins do heterodimer to form umami receptor [9,15].

Type 2 taste receptor genes are known to exist the 35 kinds in mouse and 25 kinds in human[16,17]. Recently, it was discovered that taste receptors were expressed in other area apart from the oral cavity. Tizzano *et al*[18] reported that type 2 taste receptors were expressed in airway of genetically

engineered mice and rat by the reverse transcription polymerase chain reaction (RT-PCR) and *in situ* hybridization. Singh *et al*[19] reported that the type 2 taste receptors were expressed in the brain of rats by immunohistochemistry and RT-PCR. Kwoen[20] reported that taste receptors were expressed in the submandibular, sublingual, and lacrimal glands employing the RT-PCR. Also Nishijima *et al*[21] also reported that the existence of taste buds and their nerve fibers in the larynx of rats by immunohistochemical study.

Sweetness and umami selecting nutritional substances is sensitive in anterior tongue, but bitterness restricting the intake of probable toxic substances is sensitive in posterior tongue.

Even the sensitivity differences in different tongue area were well known, however, the systemic study of the differences of expression levels of taste receptor genes has not been reported yet. In this study, the expression levels and expression patterns of the taste receptor genes in tongue papillae were examined by qPCR.

Materials and Methods

Animal

This study was approved (GWNU-2013-12) in Gangneung-Wonju National University Animal Experiment Ethics Commission, and was carried out and supervised by the Commission. DBA/2 male mice of 42-60 days old were used, because DBA/2 mice(Oriental Bio, Republic of Korea) are sensitive to bitter taste[22], and bitter taste receptors were well detected in our previous experiments[20].

Separation of mouse taste buds

After sacrificing the animal by cervical dislocation, tongue was immediately excised. The isolated tissues were stored in HEPES buffered Tyrode solution (140 mM NaCl, 5 ml KCl, 1 mM CaCl₂, 1mM MgCl₂·6H₂O, 10mM HEPES, 5mM glucose, 5mM pyruvate, pH7.4). For tissue sampling, the enzyme cocktail solution containing collagenase A (boehringer ingelheim, Germany, 1 mg / ml) and, dispase II (boehringer ingelheim, Germany, 0.25 mg / ml), trypsin inhibitor (1 mg / ml) in Ca²⁺-free HEPES buffered Tyrode solution(140 mM NaCl, 5 ml KCl, 2 mM EGTA, 5 mM glucose, 10 mM HEPES, 5 mM pyruvate, pH 7.4) were injected beneath the papillae. After 30 minutes treatment at 37 °C, the epithelial tissues including papillae were peeled off. CV and FO were

dissected from the peeled off posterior tongue epithelium. FUs were collected about 300 each with glass micro-pipette from the peeled off anterior tongue epithelium.

RNA preparation

From the collected taste epithelial tissues, the RNA was extracted with RNeasy Micro Kit (Qiagen, USA). RNA extraction procedure is RLT buffer including guanidine thiocyanate and β -mercaptoethanol 100: 1 ratio into a mixed solution mixed by centrifugation, and then 70% ethanol in a 1: 1 mix. After mounting it in the prepared column, and washed with RW1 buffer containing ethanol. DNase I incubation mix into a column placed in 15 minutes at room temperature. After washing the RW1 buffer containing ethanol, and again washed with RPE buffer. And RNA dried by centrifugation, washed with 80% ethanol, dried and put the RNase-free water to extract the RNA. RNA is prepared and stored in the next experiment at -20°C .

DNA synthesis

From extracted RNA, the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan) was used to synthesize cDNA. cDNA is then denatured for 5 minutes at 65°C by preparing RNA from $0.5\mu\text{g}$. And DN Master Mix, Nuclear free water containing RNase inhibitor to denaturing RNA mix incubated for 5 minutes at 37°C . Reverse Transcriptase, RNase inhibitor, and then mixed with oligo dT primer and RT Master Mix containing the incubated for 15 minutes at 37°C again. In 98°C , 5 minutes to stop the reaction by heating. The synthesized cDNA was kept until the next step at -20°C .

Primer

In this study, we used primers that used the RT-PCR in kwoen[20].

Quantitative polymerase chain reaction (qPCR)

100ng cDNA and primers (Table 1 and 2) that specific to taste receptor genes confirmed single PCR in Kwoen[20] were mixed KOD SYBR® qPCR Mix (TOYOBO, Japan). After a denaturation at 95°C with 3 seconds, process at 95°C with 5 seconds, at 58°C with 30 seconds, it is repeated 60 cycles. At 95°C with 10 seconds, 65°C at 95°C to 5°C interval undergo a 5 second process to draw a melt curve. All the process was repeated at least twice (type 1 taste receptor) or three times (type 2 taste receptors) to check reproducibility.

Reagents

All reagents were purchased from Sigma (U.S.A.) except specifically marked.

Data analysis

All results were calculated by the relative value to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Statistical significance between each group was tested using the variance analysis (ANOVA test).

Results

Type 1 taste receptor

The expressions of type 1 taste receptors relative to that of the GAPDH in FU, FO, CV were different (Fig 1).

The expression of type 1 taste receptors relative to that of the GAPDH were $5.68 \times 10^{-6} \sim 1.5 \times 10^{-2}$ folds in FU, $3.0 \times 10^{-6} \sim 1.17 \times 10^{-4}$ folds in FO, and $4.55 \times 10^{-6} \sim 7.18 \times 10^{-4}$ folds in CV respectively.

It showed that three type 1 taste receptors were differently expressed among papillae, but there was no significant ($p = 0.153$). In addition, there was no significant differences in the

Table 1. DNA sequences of specific primers, expected products sizes (PS) and annealing temperatures (AT) for detecting the mRNA expression of GAPDH and type 1 taste receptors by qPCR

Gene	Gene bank number	Primer sequence(forward/reverse)	PS(bp)	AT($^{\circ}\text{C}$)
Tas1r1	NM_031867	AGTTCATCTAGACATAAATAAGACAAAAAT/ GTACCTTGGTAGAAAACCTTGAAGATG	581	50
Tas1r2	NM_031873	AAGCATCGCCTCCTACTCC/ GGCTGGCAACTCTTAGAACAC	114	58
Tas1r3	NM_031872	GAAGCATCCAGATGACTTCA/ GGGAACAGAAGGACACTGAG	283	58
GAPDH	NM_017008	TGGGGTGATGCTGGTGCTGA/ CGCCTGCTTACCACCTTCT	500	60

Table 2. DNA sequences of specific primers, expected products sizes (PS) and annealing temperatures (AT) for detecting the mRNA expression of type 2 taste receptors by qPCR

Gene	Gene bank number	Primer sequence(forward/reverse)	PS(bp)	AT(°C)
Tas2r102	NM_199153	TCTGGCTTGCCACAACCTCTC/ GATCAGCTCGGTCCACATTG	242	55
Tas2r103	NM_053211	TGCAACATGCCTCAGTATCT/ CCTCAGCCACAATATCACAG	595	50
Tas2r104	NM_207011	CTAAGATTAACCTTTATTCTCACTGGCTTAG/ AATATAAGATAAAGAGGATGATAAATGCAA	600	50
Tas2r105	NM_020501	GATAGCCAATTTTTCCGACT/ ACCTTTACAAAGGCTTGCTT	549	50
Tas2r106	NM_207016	CTAGAAATATGTATATTGTTATCTGAATGA/ CCAAAGTGAAATAATTAATAGGAAG	515	50
Tas2r107	NM_199154	ATTACTTACATATGGGTATTTCTCAATCAC/ AACCAGTTATAAAGAGCAGTTTATTTTGT	538	50
Tas2r108	NM_020502	TTGTGTTGGAAGTTTCTGGA/ GGAGGGTAAGCAGCAGTAAT	563	50
Tas2r109	NM_207017	GTGTCTGGTTTGCTACATGC/ GCAATGAAAGGAGGTTTCTT	511	50
Tas2r110	NM_199155	GATGCAGGTCAATGCCAAAC/ GATCGGCACCTCAGACAATG	279	55
Tas2r113	NM_207018	TGCTCATCTTCTCCCTGTGG/ CCAGAGCCCAGACAAACAAA	213	55
Tas2r114	NM_207019	TTGCTGTCTCCTGTCAACAT/ ACAAGTTGCTTTCTGGGATT	556	50
Tas2r115	NM_207020	ATAAGCAATCATTTCAGTATATGGT/ GATGGAAGGAAAAATCATTATAGTA	558	50
Tas2r116	NM_053212	GGTGCCTACAGGTTACATTTATAG/ ATAGAAAGCTGTGTGTTATTCAAAA	521	50
Tas2r117	NM_207021	AGATTTAACTACAAGTTCAAGGATG/ ATTAAGACAGACAGAATAAAAAATGG	531	50
Tas2r118	NM_207022	GGTATCAGTCGTCTGGGAGT/ CACAGTGCCAATAAAGGAGA	520	50
Tas2r119	NM_020503	CACTGTTGCTGTTCTGCTCT/ ATCGAGTGTAAGGAGGGGTA	252	50
Tas2r120	NM_207023	TTGTGATTTTCTGGGATCG/ TTTGCAAGGCCTTTATGTGG	300	55
Tas2r121	NM_207024	CCATTTCAAGAATGGTGTTG/ CCCCTCTAGAATTGAGATGC	501	50
Tas2r122	NM_001039128.1	CTACAAGACGTTAAGTTACTCTAAGAA/ TATAATATATGATGAAGAACAAAAGGA	527	50
Tas2r123	NM_207025	TCAGCAATGGGTATTTTCTG/ AGCAGGAAGGAGAACACAGT	518	50
Tas2r124	NM_207026	ACTAATTATATGGGTTAAAGATCAACTAAT/ AGAAGAGACAGGAAATATATAGTATAGAGC	555	50
Tas2r125	NM_207027	TGGGCAGCTATTATCATTGT/ CAACATCCGTACATCTTTGG	600	50
Tas2r126	NM_207028.1	GTTCTGTATCAAGATTGCTAACTTCTC/ GTACAGACATGCAAAAAGTAAAGTATAA	521	50
Tas2r129	NM_207029	TCAAATACTCACAGCCTTGG/ CATCTGCAAGGACATCTCTG	545	50
Tas2r130	NM_199156	ATACTACCTTAATGCTTGTAGCTGT/ CTTTGCATCTCAAAGTAGAGTTTAT	504	50
Tas2r131	NM_207030	TGAGAGGTGTGCTTGTGTT/ AGAGGATCAGCTCTTGAAGC	549	50
Tas2r134	NM_199158	TTCTGTCTGCATGGAATAGC/ AAGATGAGGAAGAAGGCAAG	536	50
Tas2r135	NM_199159	TTGGAATGTCAGTGGGAATA/ GTTGCTGGCAGAACTGAGTA	280	50
Tas2r136	NM_181276	ATGTAGGTTTGTATTTCCAAGTAAG/ TGTAATCTGATATGATTAGCAACAG	528	50
Tas2r137	NM_001025385	ATTCTGCCCTCACTAGAAGC/ TCAGCACTCTGATCTCTGGA	217	50
Tas2r138	NM_001001451	CCTGCCTCAGTCTCCTCTAC/ CCCACATTGCAGAAGATAAA	268	50
Tas2r139	NM_181275	TTATATAGAGTCTCAAATGTAAGTTTTGTA/ ATTTAAAATGTAGAGAAAGAGAAAACA	501	50
Tas2r140	NM_021562	TAGAAATCATTATAGGGTGTTTAGG/ AAGGGTATGAATGTGAATATAGTGT	529	50
Tas2r143	NM_001452	GAATGGCTTCATGATGATTG/ CAGGAAGAAAGGAGTGAACC	505	50
Tas2r144	NM_001453	ACCTCTGTTTCTTCTGATG/ AGATGAACATGGTGCTGAAA	565	50

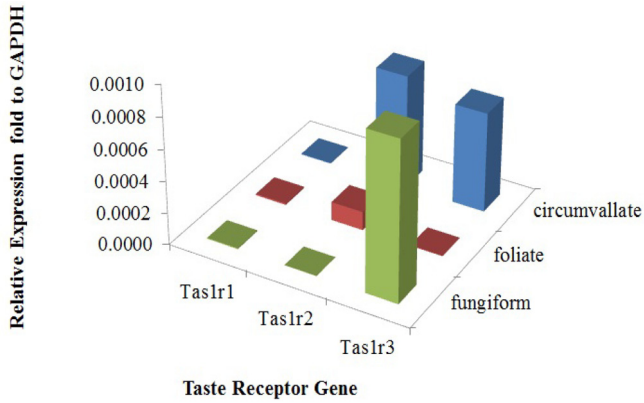


Fig. 1. Relative expression levels of T1Rs to that of GAPDH in epithelium including fungiform, foliate and circumvallate papilla.

expression of type 1 taste receptors each disc ($p = 0.861$).

Type 2 taste receptor

The expressions of type 2 taste receptors relative to that of the GAPDH in FU, FO, CV were different (Fig 2).

The expressions of type 2 taste receptors relative to that of the GAPDH were $8.5 \times 10^{-8} \sim 3.7 \times 10^{-1}$ folds in FU, $2.0 \times 10^{-9} \sim 2.0$ folds in FO and $1.3 \times 10^{-7} \sim 5.8$ folds in CV, respectively (Fig 2).

The expression levels of type 2 taste receptors in FO and CV were higher than that in FU (Table 3). In all tested taste papillae, *Tas2r108* and *Tas2r137* were shown the most two highest expression levels (Table 3, Fig 2). *Tas2r108*

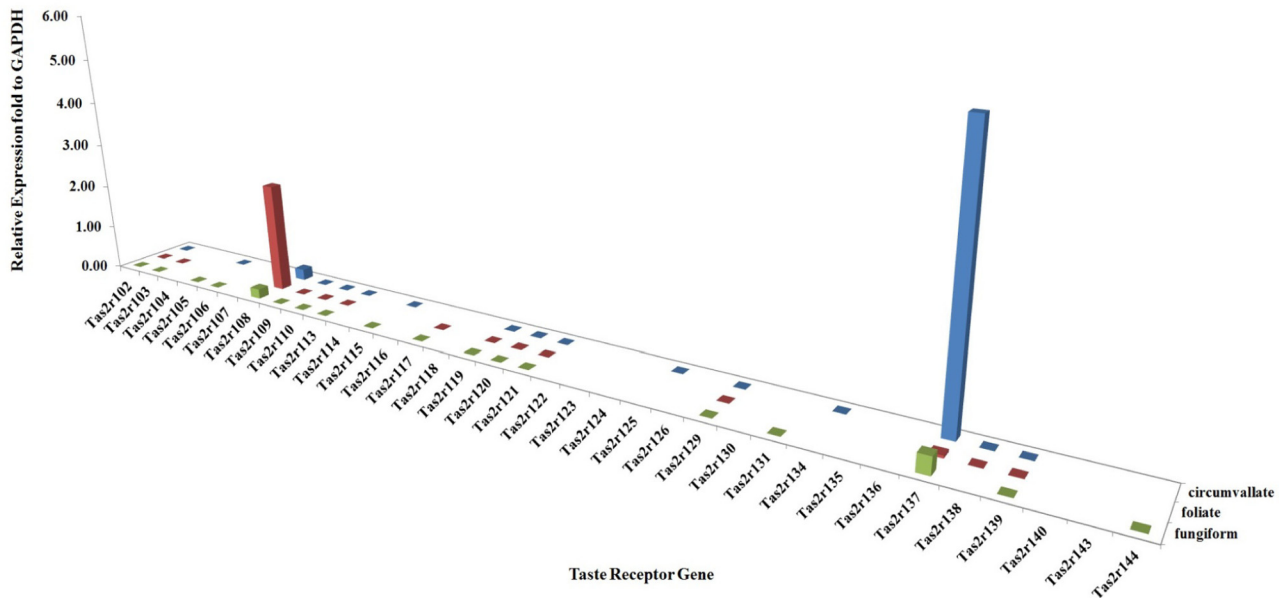


Fig. 2. Relative expression levels of T2Rs to that of GAPDH in epithelium including fungiform, foliate and circumvallate papilla.

Table 3. Relative expression levels of T1Rs in epithelium including fungiform, foliate and circumvallate papilla (Mouse #3). ($\times 10^{-7}$)

Gene	fungiform papilla	foliate papilla	circumvallate papilla
Tas1r1	56.79	90.36	45.50
Tas1r2	21.08	1170	7180
Tas1r3	150600	30.07	6288

expression levels were $2.1 \times 10^{-1} \sim 2.4$ folds to that of GAPDH, *Tas2r137* was expressed in a range of $5.6 \times 10^{-2} \sim 5.8$ folds to that of GAPDH.

The expression levels of type 2 taste receptors in taste papillae were significantly different ($p < 0.05$). However, the expression levels among papillae were not statistically different.

Discussion

Taste is chemical sensation felt mainly in the oral cavity and is detected by the taste buds of the tongue papillae. Tongue has 4 kinds of papillae which are filiform papilla, fungiform (FU), foliate (FO) and circumvallate papilla (CV)[2].

FU is present in anterior tongue, approximately the 0.5mm diameter and the bell shaped. FU sends tastes signals to

central nervous system through the chorda tympani nerves. FO is located parallel to the back edge of the tongue, next to the mandibular molar. The taste signals from FU passes through both the glossopharyngeal nerves and chorda tympani nerves. Human CV is present in a V-shape side of the root of the tongue, the diameter of 2-8mm. Murine CV is present on the center of posterior border of tongue. CV sends tastes signals only through the glossopharyngeal nerve[23].

The taste sensitivities are different from area by area of tongue. The posterior of the tongue is sensitive to bitter, the anterior of the tongue is sensitive to sweet, salty, sour.

Tastes is converted through sensory afferent gustatory from papilla, gustatory nerve projected to the dorsal part of nucleus tractus solitaries (NTS). The thalamic taste area, VPMpc, projects to the primary taste cortex, which forms the rostral part of the frontal operculum and adjoining insula. The main gustatory information is projected to the cerebral cover parts of the prefrontal cortex and the temporal island cortex[24].

Type 1 taste receptors exist the 3 kinds in mammals including mouse and human. Type 1 taste receptors of the 3 kinds form a heterodimer act receptors of different taste. The T1R2 and T1R3 heterodimer is taking action sweet receptors. Sweetness is evoked by nutrients of most carbohydrates. The T1R1 and T1R3 heterodimers act umami receptors. Umami taste is elicited also another nutrients of amino acid, glutamic acid[9,15,25]. Glutamate is rich components of fermented peptides, such as soy bean sauce, fish sauce, and cheese.

Type 2 taste receptors play the bitter taste receptor. Bitterness has a function that limits the intake of probable toxic molecules, such as various materials, including alkaloids. Type 2 taste receptors are reported 35 kinds in mouse and 25 kinds in human[16,17]. Type 2 taste receptor cells is characterized by expression of taste-specific G-protein, α -gustducin or PLCb2[17].

In principle, the PCR is repeated replication of the DNA template using a DNA polymerase. The specific DNA polymerization is triggered by the specific reciprocal nucleotide sequence on both ends of the DNA template to be replicated. Double strands of the DNA template are separated and independent for each replication phase. Final products of PCR can be identified through separation by gel electrophoresis and dye staining. However, PCR can only check whether the desired DNA is present or not, it is very difficult to know how much does the target gene express.

Quantitative PCR is a method for measuring the amount of

PCR product amplified in every PCR cycle. And In this study, we used the SYBR green as a fluorescent tracer. Because the SYBR green can emit the fluorescence with any DNA bases, the primers for qPCR should be specific. The primers used in this experiments were very specific to every taste receptor genes and others[20]. Thus the qPCR results from this study were considered as the reflection of

Table 4. Relative expression of T2Rs in epithelium including fungiform, foliate and circumvallate papilla. ($\times 10^{-7}$)

Gene	fungiform papilla	foliate papilla	circumvallate papilla
Tas2r102	6.10	67.99	1.27
Tas2r103	29.4	0.02	*
Tas2r104	*	*	*
Tas2r105	23100	*	238
Tas2r106	0.96	*	*
Tas2r107	*	*	*
Tas2r108	2140000	24300000	2450000
Tas2r109	9.78	221	7650
Tas2r110	7810	14700	2690
Tas2r113	153	6560	11100
Tas2r114	*	*	*
Tas2r115	773	*	150
Tas2r116	*	*	*
Tas2r117	669	9190	*
Tas2r118	*	*	*
Tas2r119	14600	6450	37400
Tas2r120	5750	75300	56900
Tas2r121	55.1	3280	173
Tas2r122	*	*	*
Tas2r123	*	*	*
Tas2r124	*	*	*
Tas2r125	*	*	0.68
Tas2r126	*	*	*
Tas2r129	177	3290	4050
Tas2r130	*	*	*
Tas2r131	16.2	*	*
Tas2r134	*	*	1.92
Tas2r135	*	*	*
Tas2r136	*	*	*
Tas2r137	3710000	559000	5800000
Tas2r138	*	62.1	34500
Tas2r139	142	62500	348
Tas2r140	*	*	*
Tas2r143	*	*	*
Tas2r144	0.85	*	*

* means that the relative levels of T2Rs were 10^{-7} less than that of GAPDH.*

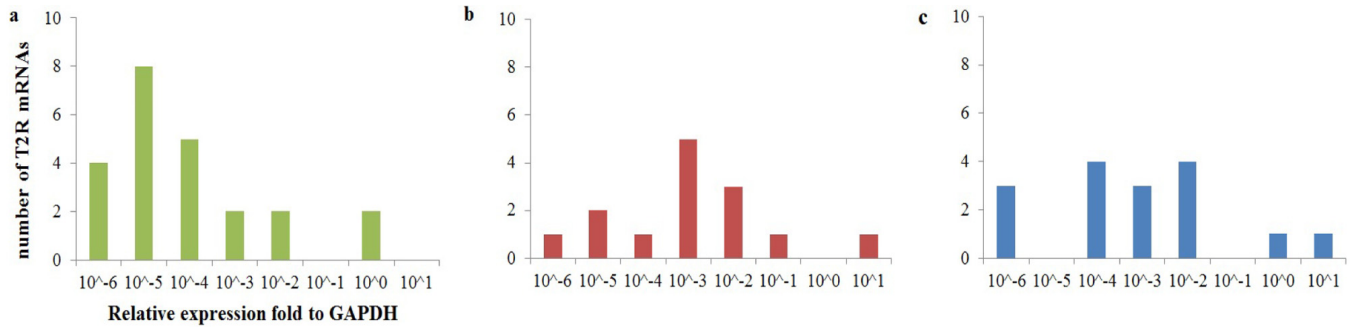


Fig. 3. Distribution of expression levels of T2Rs mRNAs in epithelium including (a) fungiform, (b) foliate and (c) circumvallate papilla.

expression levels of those genes.

Result of the qPCR, FU, FO, CV were expressed 35 kinds type 2 taste receptor and three type 1 taste receptor. *Tas1r3* is the component of both sweet and umami taste receptors, it would be expressed more than others, however, in this experiment *Tas1r2* was expressed more. This result might be due to taste specificity of strain of mice used. DBA2 mice might be functionally *Tas1r2* sweetness is much more important physiologically than the rich more than the expression used in the study was *Tas1r3*. Also DBA2 mice might be sensitive bitter taste but less sensitive umami taste. Because expression levels of taste receptor mRNA were 10^{-2} ~ 10^{-6} times to that of GAPDH, the possibilities are low, this may be experiment error. Most sweet stimuli were similar, nerve recordings of some sweet stimuli were significantly larger in the chorda tympani (CT) nerve than in the glossopharyngeal (NG) nerve[26]. However, analysis variance results did not show significant difference in the papilla. It may be due to small sample size.

In the case of type 2 taste receptor, FO and CV showed higher expression levels than FU expression levels (Table 4, Fig 2 and 3). This result is reflected that FO and CV are distributed to posterior of tongue which more sensitive to bitter taste. In particular, the expression levels of *Tas2r108* and *Tas2r137* are frequently than the other type 2 taste receptors (Fig 2). If you check the relative expression of the GAPDH of type 2 taste receptor, while the majority of type 2 taste receptor is showing less expression than GAPDH (Fig 3). *Tas2r108* and *Tas2r137* are similar to GAPDH, or showed much expression (Fig 2). Ligands of *Tas2r108* are denatonium, quinine, colchicine, diphenidol, caffeine, dapson and Ligand of *Tas2r137* is known as chloroquine[27,28]. *Tas2r108* acts take a wide range of groups for detecting various bitter taste

substances[29]. *Tas2r108* is commonly expressed, but it is not known that many expressed *Tas2r137*, it is very interesting. The type 2 taste receptor, except for *Tas2r108* and *Tas2r137* can be considered some of the possibilities for a very small, but important physiologically. Type 2 taste receptor relative to their particular taste buds can only express a note of this expression is less likely. Unlike type 1 receptor, type 2 taste receptors are expressed in other organ as well as taste bud.

Distribution analysis results showed that the type 2 taste receptors are a significant difference in the papilla. However, the expression between the taste receptors, between the papilla was confirmed that there are no significant differences. It may be due to small sample. Therefore, it is necessary to increase the number of sample used study.

Differences in taste sensitivity of the tongue would be related to specific areas of the tongue taste threshold. Also, more expression of *Tas2r108* is considered that *Tas2r108* has a lot of ligand as it is known in the prior, more expression of *Tas2r137* yet be studied.

Through qPCR it was able to determine the expression pattern of the three groups taking taste papillae. Confirmed that 3 kinds of type 1 group and 35 kinds of type 2 group, but because not all of the expression in the papilla not make a significant difference, it is necessary to obtain a significant difference by additional research.

Acknowledgements

The work is supported by Basic science Research Program through the National Research Foundation in Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2013R1A1A2008424)

References

1. Scott K. Chemical senses: taste and olfaction. In: Squire LR, Berg D, Bloom FE, du-Lac S, Ghosh A, Spitzer NC, editors. *Fundamental neuroscience*. Oxford: Elsevier Inc; 2012. pp. 513-530
2. Royer SM, Kinnamon JC. Ultrastructure of mouse foliate taste buds: Synaptic and nonsynaptic interactions between taste cells and nerve fibers. *J Comp Neurol*. 1988;270:11-24. DOI:10.1002/cne.902700103
3. Lindemann B. Chemoreception: tasting the sweet and the bitter. *Current Biol*. 1996;6:1234-1237. DOI: 10.1016/S0960-9822(96)00704-X
4. Murray RG. The mammalian taste bud type III cell: a critical analysis. *J Ultrastruct Mol Struct Res*. 1986;95:175-188. DOI: 10.1016/0889-1605(86)90039-X
5. Lawton DM, Furness DN, Lindemann B, Hackney CM. Localization of the glutamate-aspartate transporter, GLAST, in rat taste buds. *Eur J Neurosci*. 2000;12:3163-3171. DOI: 10.1046/j.1460-9568.2000.00207.x
6. Dvoryanchikov G, Sinclair MS, Perea-Martinez I, Wang T, Chaudhari N. Inward rectifier channel, ROMK, is localized to the apical tips of glial-like cells in mouse taste buds. *J Comp Neurol*. 2009;517:1-14. DOI: 10.1002/cne.22202
7. Vandenbeuch A, Clapp TR, Kinnamon SC. Amiloride-sensitive channels in type I fungiform taste cells in mouse. *BMC Neurosci*. 2008;9:1. DOI: 10.1186/1471-2202-9-1
8. Farbman AI, Hellekant G, Nelson A. Structure of taste buds in foliate papillae of the rhesus monkey, *Macaca mulatta*. *Am J Anat*. 1985;172:41-56
9. Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. Mammalian sweet taste receptors. *Cell*. 2001;106:381-390. DOI: 10.1016/S0092-8674(01)00451-2
10. DeFazio RA, Dvoryanchikov G, Maruyama Y, Kim JW, Pereira E, Roper SD, and et al. Separate populations of receptor cells and presynaptic cells in mouse taste buds. *J Neurosci*. 2006;26:3971-3980. DOI: 10.1523/JNEUROSCI.0515-06.2006
11. Fujimoto S, Ueda H, Kagawa H. Immunocytochemistry on the localization of 5-hydroxytryptamine in monkey and rabbit taste buds. *Acta Anatomica*. 1987;128:80-83. DOI: 10.1159/000146320
12. Chaudhari N, Roper SD. The cell biology of taste. *J Cell Biol*. 2010;190:285-296. doi: 10.1083/jcb.201003144
13. Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, and et al. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell*. 2003;112:293-301. doi:10.1016/S0092-8674(03)00071-0
14. Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, Zuker CS. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell*. 1999;96:541-551. doi:10.1016/S0092-8674(00)80658-3
15. Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS. An amino-acid taste receptor. *Nature*. 2002;416:199-202. doi:10.1038/nature726
16. Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS. A novel family of mammalian taste receptors. *Cell*. 2000;100:693-702. doi:10.1016/S0092-8674(00)80705-9
17. Wong GT, Gannon KS, Margolskee RF. Transduction of bitter and sweet taste by gustducin. *Nature*. 1996;381:796-800. DOI: 10.1038/381796a0
18. Tizzano M, Cristofolletti M, Sbarbati A, Finger TE. Expression of taste receptors in solitary chemosensory cells of rodent airways. *BMC Pulmonary Medicine*. 2011;11:3. DOI: 10.1186/1471-2466-11-3
19. Singh N, Vrontakis M, Parkinson F, Chelikani P. Functional bitter taste receptors are expressed in brain cells. *Biochem Biophys Res Commun*. 2011;406:146-151. doi:10.1016/j.bbrc.2011.02.016
20. Kwoen SB. *Physiology of Taste Receptors in Exocrine Glands*. Ph.D., Gangneung-Wonju National University, Gangneung, Korea, 2012
21. Nishijima K, Atoji Y. Taste buds and nerve fibers in the rat larynx: an ultrastructural and immunohistochemical study. *Arch Histol Cytol*. 2004;49:195-209. DOI: 10.1679/aohc.67.195
22. Boughter JD, Raghow S, Nelson TM, Munger SD. Inbred mouse strains C57BL/6J and DBA/2J vary in sensitivity to a subset of bitter stimuli. *BMC Genet*. 2005;6:36. DOI: 10.1186/1471-2156-6-36
23. Witt M, Reutter K, Miller IJ Jr. Morphology of peripheral taste system. In: Doty RL, editor. *Handbook of olfaction and gustation*. vol.2. New Jersey: John Wiley&Sons, Inc; 2003. pp. 651-678.
24. Roll ET, Scott TR. Central taste anatomy and neurophysiology. In: Doty RL, editor. *Handbook of olfaction and gustation*. vol.2. New Jersey: John Wiley&Sons, Inc; 2003. pp. 679-706
25. Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. Human receptors for sweet and umami taste. *Proc Natl Acad Sci USA*. 2002;99:4692-4696. doi: 10.1073/pnas.072090199
26. V Danilova, G Hellekant. Comparison of the responses of the chorda tympani and glossopharyngeal nerves to taste stimuli in C57BL/6J mice. *BMC Neurosci*. 2003;4:1. DOI: 10.1186/1471-2202-4-5
27. Foster SR, Porrello ER, Purdue B, Chan HW, Voigt A, Frenzel S, and et al. Expression, Regulation and putative nutrient-sensing function of taste GPCRs in the heart. *PLoS ONE*. 2013;8:e64579. DOI: 10.1371/journal.pone.0064579
28. Grassin-Delyle S, Abrial C, Fayad-Kobeissi S, Brollo M, Faisy C, Alvarez JC, and et al. The expression and relaxant effect of bitter taste receptors in human bronchi. *Respir Res*. 2013;14:134. DOI: 10.1186/1465-9921-14-134
29. Foster SR, Blank K, See Hoe LE, Behrens M, Meyerhof W, Peart JN, Tomas WG. Bitter taste receptors agonists elicit G-protein-dependent negative inotropy in the murine heart. *The FASEB Journal*. 2014;28:4497-4508. doi: 10.1096/fj.14-256305