



Title	TAS2R expression profile in brown adipose, white adipose, skeletal muscle, small intestine, liver and common cell lines derived from mice
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4

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15

16 1. Introduction

17 Taste 2 receptor (TAS2R) describes a family of one type of G-protein coupled receptor
18 that perceives bitter taste in the oral cavity. Bitter compounds that bind to TAS2Rs expressed on
19 gustatory cells activate these receptors, and the co-expressed G-proteins subsequently send the
20 signal through downstream effectors. This signaling leads to the release of calcium ions from the
21 endoplasmic reticulum to the cytosol, resulting in sodium ion influx through transient receptor
22 potential cation channel subfamily M member 5 (TRPM5). The influx depolarizes the gustatory
23 cells and causes the release of ATP, which transmits the bitter taste signal to the central nervous
24 system (Roper, 2013; Travedi, 2012).

25 Currently, 25 TAS2Rs have been identified in humans (Shi, Zhang, Yang, & Zhang,
26 2003) and 35 in mice (Chandrashekar, Hoon, Ryba, & Zuker, 2006). Each TAS2R recognizes a
27 range of compounds with different specificity, enabling the detection of a wide range of bitter
28 compounds (Lossow et al., 2016; Meyerhof et al., 2009). For example, human TAS2R
29 (hTAS2R)10 recognizes at least 20 bitter compounds including caffeine and quinine.

30 Over the last decade, much attention has been paid to the identification of TAS2R
31 expression and function in a wide variety of extraoral tissues: brain, upper airway, heart, liver,
32 testis (Freund & Lee, 2018); adipose tissue (Amisten et al., 2015); stomach (Liszt et al., 2017);
33 small intestine (Xie et al., 2018); and colon (Rozenfurt et al., 2006). TAS2Rs are recognized as
34 the receptor associated with repelling, excreting and decomposing of toxins. Moreover, TAS2Rs
35 expressed in the bordering tissues that separate the internal and external environments of the body
36 have been shown to possess analogical functions. For example, TAS2R38 expressed in the upper
37 airways detects acyl-homoserine lactone secreted from Gram-negative bacteria. This detection
38 induces calcium-dependent nitric oxide production, resulting in the stimulation of mucociliary
39 clearance function and has direct antibacterial effects (Lee et al., 2012). TAS2R43 expressed in
40 the stomach promotes the secretion of gastric acid in response to caffeine, which presumably
41 enhances the decomposition of toxins (Liszt et al., 2017). Furthermore, Deshpande et al. reported

42 that inhaled bitter tastants improved airway obstruction in mouse model of asthma (Deshpande et
43 al. 2010). Since several members of TAS2Rs (TAS2R105, 107, 119, 121, 123, 126 and 134) are
44 expressed in rodent airway (Tizzano, Cristofolletti, Sbarbati, & Finger, 2011), these TAS2Rs are
45 presumed to be involved in the improvement of asthma. In contrast, TAS2Rs expressed in internal
46 tissues are unlikely to function to repel and exclude toxic compounds; rather, these TAS2Rs would
47 be expected to respond to bitter compounds that have already been ingested. Expression of
48 TAS2Rs in internal tissues may have a different physiological function entirely.

49 Several reports suggest a role for TAS2R in metabolic functioning (Avau et al., 2015).
50 The association of genetic variations of TAS2R38 with obesity development was implied in a
51 cohort study (Ortega et al., 2016), and a TAS2R haplotype was shown to influence glucose
52 homeostasis, linking it with a diagnosis of type 2 diabetes (Dotson et al., 2008). In addition,
53 quinine, a well-known bitter compound, promotes the adipogenesis of 3T3-L1 adipocyte cells via
54 TAS2R (Ning, He, Shi, & Yang, 2016).

55 On the basis of the above facts, we hypothesized that extraoral TAS2Rs may be involved
56 in the regulation of nutrient metabolism. And as an entryway to this research, we analyzed the
57 expression profiles of multiple TAS2R genes in extraoral tissues that are related to the metabolism
58 of nutrients. The identification of TAS2R expression in extraoral tissues such as adipose tissue
59 and skeletal muscle will enable the exploration of the relationship between metabolic regulation
60 and TAS2R using specific ligands.

61 In this study, the gene expression profile of TAS2R in five extraoral tissues (brown
62 adipose, white adipose, skeletal muscle, small intestine and liver) was analyzed. Analysis of
63 guanine nucleotide-binding protein alpha subunit (Gnat) was accompanied since this protein
64 plays an important role in bitter taste transduction. In anticipation of future in vivo experiments,
65 we chose to use mouse tissues for this study. Furthermore, if the same TAS2Rs as mice tissues
66 were expressed in corresponding cell lines, the cell lines would be a useful model for exploring
67 the function of TAS2Rs. Thus, gene expression profiles of TAS2R in mouse 3T3-L1 cells and

68 C2C12 cells, commonly used models of adipocytes and myocytes, respectively, were analyzed to
69 facilitate future studies on the relationship between TAS2R and nutrient metabolism.

70 **2. Materials and Methods**

71 2.1 Cell culture

72 The 3T3-L1 (JCRB9014) and C2C12 (RCB0987) cell lines were obtained from the
73 JCRB Cell Bank (Tokyo, Japan) and RIKEN BRC (Saitama, Japan), respectively. Cells were
74 cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum
75 (FBS) at 37°C in a humidified atmosphere of 10% CO₂ until reaching an adequate confluency.

76

77 2.1.1 Differentiation of 3T3-L1 cells

78 Adipocyte differentiation was induced 1 day after 3T3-L1 cells reached confluence (day
79 0) by changing the medium to 10% FBS/DMEM supplemented with 0.5 mM 3-isobutyl-1-
80 methylxanthine, 0.25 µM dexamethasone, and 5 µg/mL insulin (differentiation medium). To
81 enhance the differentiation, 2 days and 4 days after induction (day 2, day 4), the medium was
82 changed to 10% FBS/DMEM supplemented with 10 µg/mL insulin (insulin medium). At day 6
83 and day 8, the medium was changed to 10% FBS/DMEM. Total RNA was extracted on day 0 and
84 day 10.

85

86 2.1.2 Differentiation of C2C12 cells

87 Myotube differentiation was induced 1 day before C2C12 cells reached confluence (day
88 0) by changing the medium to 2% horse serum (HS)/DMEM. The medium was changed to 2%
89 HS/DMEM every 2 days until sufficient differentiation was reached. Total RNA was extracted on
90 day 0 and day 8.

91

92 2.2 RNA preparation

93 Male C57BL/6 mice (age 8 weeks) total RNA (three independent samples, each from

94 individual mice) derived from brown adipose, white adipose, muscle, small intestine and liver,
95 was purchased from Genostaff Co. (Tokyo, Japan). For cell lines, the total RNA was extracted
96 using the ReliaPrep™ RNA Cell Miniprep System (Promega KK, Tokyo) followed by the DNase
97 treatment using AccuRT Genomic DNA Removal Kit (Applied Biological Materials Inc.)
98 according to the manufacturer's protocol. The quantity of total RNA was determined
99 spectrophotometrically. The RNA integrity was evaluated qualitatively from the intensities of the
100 28S and 18S ribosomal RNA bands following denaturing agarose gel electrophoresis.

101

102 2.3 Reverse transcription PCR

103 Reverse transcription was performed using ReverTra Ace qPCR RT Master Mix with
104 gDNA Remover (Toyobo, Osaka, Japan) according to the manufacturer's instructions. No reverse
105 transcription control experiments were also performed. PCR was carried out in 0.2-mL PCR tubes
106 in a total volume of 25 μ L containing 2 μ M primer, 2 μ L cDNA, and 12.5 μ L Go Taq G2 Hot
107 Start Polymerase Green Mastermix (Promega KK, Tokyo). Primers were designed using the
108 National Center for Biotechnology Information primer designing tool Primer BLAST and are
109 presented in Supplemental Table S1. Cycling conditions were as follows: 95°C for 2 min,
110 followed by 35 cycles of 95°C for 30 s, 60°C for 30 s (or 65°C for 30 s in the case of TAS2R102,
111 109, 118, 138, 140 and 143), and 72°C for 60 s for TAS2R genes, or 30 cycles of 95°C for 30 s,
112 60°C for 30 s and 72°C for 60 s for β -actin, Gnat1, Gnat2 and Gnat3; each was followed by a
113 final elongation step at 72°C for 5 min. β -actin expression was used as an internal control.

114

115 2.4 Electrophoresis

116 PCR products were examined by standard agarose gel electrophoresis (2% agarose, 40
117 mM Tris-acetate and 1 mM EDTA) with visualization of DNA by fluorescent dye (GelGreen™
118 Nucleic acid gel stain, Biotium Inc. or Midori Green Xtra, NIPPON Genetics Co.) Each DNA
119 band was converted into numerical form and quantified by ImageJ software and rated as follows.

120 ○ : Band intensity>0.1, ◦ : Band intensity between 0.1-0.01 (The band intensity of β -actin=1).

121 Gene expression analysis was repeated at least twice to confirm the replicability. The
122 representative results are shown in the Table 1 and Supplementary figures S1-S7.

123

124 **3. Results**

125 3.1 TAS2R expression in extraoral tissues

126 The gene expression profiles of TAS2Rs in extraoral tissues were investigated by
127 reverse transcription PCR using total RNA isolated from brown adipose, white adipose, skeletal
128 muscle, small intestine and liver (Table 1). Three individually prepared samples of each tissue
129 were amplified, and the products visualized following agarose gel electrophoresis. The expression
130 of each TAS2R gene was compared with the band intensity for β -actin and rated (blank, small
131 circle, or large circle which is from a single representative sample (n=1)).

132 Individual TAS2R expression was detected in at least 2 out of 3 samples of each tissue
133 as follows: 9 members of TAS2R in brown adipose (mTAS2R108, 110, 118, 126, 134, 135, 137,
134 140, 143), 11 members in white adipose (mTAS2R108, 113, 118, 119, 126, 135, 137, 138, 140,
135 143, 144), 8 members in skeletal muscle (mTAS2R108, 126, 134, 135, 137, 140, 143, 144), 10
136 members in small intestine (mTAS2R108, 109, 119, 126, 135, 137, 138, 140, 143, 144), and 8
137 members in liver (mTAS2R108, 109, 126, 130, 135, 137, 138, 143). Similarly, Gnat1 was detected
138 in white adipose, small intestine and liver, Gnat2 was detected in all tissues we analyzed, Gnat3
139 was detected in small intestine.

140

141 3.2 TAS2R expression in cell lines

142 Adipose tissue and skeletal muscle expressed a variety of TAS2Rs. To begin exploring
143 the possible functions of TAS2R expression in these tissue types, we chose the convenience of a
144 cell line-based approach. It is possible that cell lines and tissue-derived cells express different

145 members of TAS2R. To minimize this concern, the expression of TAS2R was investigated in cell
 146 lines corresponding to mice adipocytes and myocytes.

147 Table 1. Gene expression of TAS2Rs in five extraoral tissues of mice and two common cell lines
 148 derived from mice, before and after differentiation.

Genes	Mice extraoral tissues															Cell lines			
	Brown Adipose			White Adipose			Skeletal Muscle			Small Intestine			Liver			3T3-L1		C2C12	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	BD	AD	BD	AD
Gnat1	○			○	○	○				○	○	○	○	○	○				
Gnat2	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Gnat3										○	○	○							
mTAS2R102		○			○				○										
mTAS2R103	○														○				
mTAS2R104						○			○										
mTAS2R105						○								○					
mTAS2R106			○					○											
mTAS2R108	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
mTAS2R109		○			○				○				○	○	○	○			
mTAS2R110	○		○			○													
mTAS2R113	○			○	○	○			○										○
mTAS2R115								○							○				
mTAS2R116				○															
mTAS2R118	○	○		○	○	○	○			○									
mTAS2R119	○			○		○	○			○		○							
mTAS2R120																			○
mTAS2R121																		○	
mTAS2R122						○					○								○
mTAS2R123						○	○												
mTAS2R125			○		○														
mTAS2R126	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
mTAS2R130														○	○	○			
mTAS2R134	○	○	○		○		○	○											
mTAS2R135	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
mTAS2R137	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
mTAS2R138				○	○	○				○	○	○	○	○	○	○			
mTAS2R139				○														○	
mTAS2R140	○	○	○	○	○	○		○	○		○	○		○					
mTAS2R143	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
mTAS2R144			○	○		○		○	○		○	○		○	○				

149 BD: Before differentiation; AD: After differentiation; ○ : Band intensity>0.1, Band intensity between 0.1-0.01 (The band intensity
 150 of β-actin = 1). TAS2R107, 114,117, 124, 129, 131 and 136 were not detected. Each circle is from a single representative sample
 151 (n=1).
 152

153 The 3T3-L1 and C2C12 cell lines are widely used as models of white adipose cells and
 154 skeletal muscle cells, respectively. Since these cell lines can be stimulated to differentiate under
 155 established conditions, we examined the mRNA expression of TAS2R both before and after the

156 differentiation (Table 1). In 3T3-L1 cells, mTAS2R108, 121, 126, 135, 137, 143 were found to be
157 expressed in undifferentiated state, mTAS2R108, 126, 135, 137, 139, and 143 were expressed in
158 differentiated state. In C2C12 cells, mTAS2R108, 126, 135, 137 and 143 were expressed under
159 both states. Gnat2 was detected under both states in 3T3-L1 and C2C12 cells, but Gnat1 and
160 Gnat3 were not observed in any conditions.

161

162 **4. Discussion**

163 4.1 Comparison of extraoral TAS2R expression with previous studies_

164 TAS2R expression was previously reported by Prandi et al. in mouse small intestine and
165 liver (2018). In their report, small intestine expressed TAS2R108, 119, 126, 129, 135, 137, 138
166 and 143, while liver expressed TAS2R108, 126, 135, 137, 138 and 143. In contrast, in our study
167 of small intestine, we did not detect the expression of TAS2R129, but did detect the expression
168 of three additional TAS2R genes: 109, 140 and 144. Moreover, in the liver, we detected additional
169 expression of TAS2R109 and 130. In addition, according to Mouse Gene Expression Database
170 (MXD), liver, duodenum, ileum and jejunum expressed TAS2R108 and 138, jejunum expressed
171 TAS2R119, small intestine epithelium expressed Gnat3, corresponded with the expression profile
172 in this study. In contrast, we did not detect the expression of TAS2R118, which is confirmed the
173 expression in the database. These differences might be explained by the mouse strain (C57BL/6
174 in this study and C57BL/6J in Prandi et al.), as there are different perceptions of bitter taste
175 between individuals (Wooding, 2006). In addition, TAS2R expression is known to vary in
176 response to environmental factors such as secretions from parasites (Luo et al., 2019) and smoking
177 habit (Aoki, Takao, Takao, Koike, & Suganuma, 2014). Thus, the mouse strain and/or the
178 environmental conditions for breeding mice may have influenced these differences in TAS2R
179 expression profiles. Also, TAS2R might be transiently expressed due to these environmental
180 factors. However, our results do not address expressional variability, thus it is still unclear how
181 representative these data are for any related model. Since we have not confirmed the existence of

182 TAS2R proteins, all these points require investigation in future research.

183 This study is the first to report on TAS2R expression in mouse white and brown adipose
184 tissue and skeletal muscle. TAS2R expression patterns in human adipose and airway smooth
185 muscle have been previously reported. Amisten et al. showed that TAS2R3, 7, 14, 19, 20, 31, 43,
186 45 and 46 were expressed in human adipose tissue (2015), while Deshpande et al. showed that
187 TAS2R1, 3, 4, 5, 8, 9, 10, 13, 14, 19, 20, 30, 31, 42, 45, 46 and 50 were expressed in human
188 smooth muscle (2010). The data from Genotype-Tissue Expression (GTEx) also shows the
189 expression of TAS2Rs in these tissues (hTAS2R3,4,5,10,13,14,40,42,43,46,50,60 in human
190 adipose tissue, hTAS2R3,4,5,10,13,14,43,46,50 in human skeletal muscle). However, it is
191 difficult to directly compare these expression profiles given the differences in human and mouse
192 TAS2Rs.

193 mTAS2R108, 126, 135, 137, 143, which we confirmed their expression in tissues and
194 cell lines, have corresponding orthologue in hTAS2R except for mTAS2R143 (Lossow et al.,
195 2016). TAS2R108, 126, 135, 137 are the orthologue of hTAS2R4, 41, 60, 3. The ligands for each
196 orthologue pairs have been previously examined, but some of the pairs have no common ligand.
197 For example, mTAS2R108/hTAS2R4 shares 30% of the tested bitter compounds as a common
198 ligand, and the pairs of mTAS2R126/hTAS2R41 and mTAS2R137/hTAS2R3 do not share a
199 ligand. Thus, the orthologue of human and mouse TAS2R do not recognize same ligand making
200 it difficult to compare.

201 Although difficult to compare, the common expression of TAS2Rs in adipose and
202 muscle of both species suggests the existence of an unknown role of this receptor in those tissues.
203 It is also noteworthy that Gnat2, one of the downstream signaling proteins of TAS2R(Liszt et al.,
204 2017), is expressed in adipose, skeletal muscle and their corresponding cell lines, which may
205 support that TAS2Rs function in these tissues and cells.

206

207

208 4.2 Speculation of the TAS2R function in liver, muscle and adipose tissues

209 A glimpse of the possible receptor functions of TAS2Rs in liver, muscle and adipose
210 tissues is given by the biological effects of their respective ligands. Several ligands have been
211 identified for receptors TAS2R108, 126, 135, 137 and 143, which are commonly expressed in
212 liver, muscle and adipose tissues (Lossow et al., 2016). Among these ligands, the effects of
213 quinine (ligand for TAS2R108, 126, 137), denatonium benzoate (TAS2R135), allyl isothiocyanate
214 (TAS2R135), salicylic acid (TAS2R135), and epicatechin (TAS2R126) may offer clues as to the
215 functions of TAS2Rs in these tissues. Oral administration of quinine is reported to decrease body
216 weight without a change in energy intake in obese mice (Avau et al., 2015). In the same report,
217 denatonium benzoate also reduced body weight but was accompanied by decreased energy intake.
218 Allyl isothiocyanate, the organosulfur compound in radish and wasabi, increased glucose uptake
219 in insulin-resistant C2C12 myotubes and reduced diet-induced obesity in mice fed a high-fat diet
220 (Ahn, Lee, Im, Jung, & Ha, 2014). Salicylic acid, an active metabolite of aspirin with bitter taste,
221 is known to lower blood glucose levels in diabetic patients (Rumore & Kim, 2010). Epicatechin,
222 the flavonoid contained in green tea and cocoa, reduces body weight gain and improves insulin
223 sensitivity in mice fed a high-fat diet (Cremonini, Bettaieb, Haj, Fraga, & Oteiza, 2016; Hoek-
224 van den Hil et al., 2015). Additionally, epigallocatechin 3-*O*-gallate, the major flavonoid in green
225 tea, activated TAS2R144 expressed in white adipose tissue and skeletal muscle, and has been
226 widely explored for its anti-obesity or anti-diabetes effects (Eng, Thanikachalam, & Ramamurthy,
227 2018; Suzuki, Pervin, Goto, Isemura, & Nakamura, 2016). All of these effects are related to
228 metabolic function. Although some of the molecular mechanisms for these metabolic effects have
229 been revealed, the direct target of the above bitter compounds is often unclear. Therefore, as
230 hypothesized earlier, the expression of TAS2R in metabolically important tissues including the
231 liver, skeletal muscle and adipose tissues, may imply a connection between TAS2R and
232 metabolism. It is also noteworthy that human adipose tissue is known to express several members
233 of TAS2R that recognize quinine (TAS2R7, 14, 43 and 46) or denatonium benzoate (TAS2R43

234 and 46) as a ligand, implying that human TAS2Rs may possess metabolic functions.

235

236 4.3 Cell lines as a model to study TAS2R functions

237 Comparing the expression profile of TAS2R in tissues and cell lines, Gnat2,
238 mTAS2R108, 126, 135, 137, and 143 were expressed at relatively higher levels in both tissues
239 and cell lines. Whereas, in 3T3-L1 cells, we did not detect Gnat1, mTAS2R113, 118, 119, 138,
240 140 and 144, which are expressed in white adipose tissues. In C2C12 cells, we did not detect
241 mTAS2R134, 140 and 144, which are expressed in skeletal muscle tissues.

242 Environmental factors might have influenced this result, similarly to that described for
243 the difference between the Prandi et al. (2018) report and our study regarding TAS2R expression
244 in small intestine and liver tissue. Cell lines are cultured in media that are largely different from
245 the conditions of the mouse tissues showing differential expression of TAS2Rs. Another
246 consideration is the result of immortalization. Martin et al. reported that TAS2Rs are expressed in
247 human epithelial ovarian and prostate cancer cells, influencing cell survival (2019). Genetic
248 variations in TAS2R3 and 4 have been reported to modify papillary carcinoma risk (Choi et al.,
249 2018). Abnormal cell growth is a characteristic of cancer cells; thus TAS2R expression might
250 have been influenced during the construction of these cell lines.

251 Although the profiles and levels of TAS2R gene expression in cell lines and mouse
252 tissues are somewhat different, 3T3-L1 and C2C12 cells should be a useful tool to explore the
253 functions of TAS2Rs.

254

255 **5. Conclusions**

256 We have investigated the expression profile of TAS2R in multiple extraoral tissues that
257 are closely related to metabolism, namely brown and white adipose, skeletal muscle, small
258 intestine and liver. Several Tas2Rs are expressed in these five metabolically meaningful tissues
259 and previous studies imply that TAS2Rs might possess functions to regulate metabolism.

260 However, the connection of TAS2Rs to metabolism is still unclear and further considerations are
261 required to clarify the involvement of TAS2Rs in metabolism. Additionally, we discovered that
262 8 members of TAS2Rs express in 3T3-L1 and 6 members of TAS2Rs express in C2C12 cells
263 which expressions are observed in corresponding tissues. The TAS2R expression profile in these
264 cell lines support their use as model cells for exploring the function of TAS2R expression in
265 white adipose and skeletal muscle.

266

267 **Declaration of competing interest**

268 The authors declare that they have no known competing financial interests or personal
269 relationships that could have appeared to influence the work reported in this paper.

270

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278

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