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Identification of Coding Single-Nucleotide Polymorphisms in Human Taste Receptor Genes Involving Bitter Tasting

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T2Rs comprise a G-protein-coupled receptor superfamily that contains functionally defined bitter taste receptors. Here we report the tissue expressions and coding single-nucleotide polymorphisms (cSNPs) in human T2R genes (hT2R3, hT2R4, and hT2R5) on chromosome 7q31. We first demonstrated that hT2R3, hT2R4, and hT2R5 are actually expressed in the circumvallate papillae of the human tongue by reverse transcription–polymerase chain reaction (RT-PCR). We identified six cSNPs within the T2R receptor genes. The hT2R4 and hT2R5 contained four and one cSNPs that cause missense mutations, respectively, while hT2R3 included one silent nucleotide mutation. However, we could not find any nonsense mutations that resulted in a frameshift or a premature stop codon within the open reading frames. Genotype frequencies of each cSNP were in Hardy–Weinberg equilibrium. The identification of nucleotide diversity and amino acid polymorphisms in human T2R receptors could help clarify individual differences in the acceptability and sensitivity to bitter compounds.

Key Words: **human; tongue; bitter taste; taste receptor; multigene family; G-protein-coupled receptor (GPCR); single-nucleotide polymorphism (SNP); missense mutation; amino acid substitution.**

In mammals, there are five primary tastes: salty, sour, bitter, sweet, and umami (the taste of monosodium glutamate) (1, 2). These tastes are believed to be mediated by distinct cell surface receptors on taste receptor cells (TRCs) clustered in taste buds. Salty and sour tastes are simply transduced via cation channels, for which the genes have been identified (3–5). In contrast, bitter taste, along with sweet and umami tastes, is presumed to be mediated by guanine-nucleotide-binding protein (G protein)-coupled receptors (GPCRs) (1, 2). To date, many GPCRs have been reported as candidate taste receptors in humans: T1Rs, T2Rs, and a splicing variant of brain metabotropic glutamate receptor 4 (taste-mGluR 4) (6–12). Human T1R1 and T1R2 have been identified as human orthologs to rodent T1Rs expressed in the TRCs, but the ligand specificities are not yet known (6). T1R3, a new member of T1R family, is presumed to function as a sweet taste receptor in human, since the mouse ortholog is closely linked to *Sac* locus that controls the detection of saccharin, sucrose and other sweeteners in mice (11, 12). Human T2R/TRB (hT2R) receptors are a large GPCR multigene family of an estimated 40–80 receptors, one of which (hT2R4) has been shown to respond to bitter compounds, denatonium and 6-*n*-propyl-2-thiouracil (PROP) (8–10). In addition, human T2R1 and T2R16 (hT2R1 and hT2R16) genes map to locations implicated in human bitter perception (8, 9), suggesting that the hT2R family could be the likeliest GPCR family that function to recognize bitter substances in humans. Similarly, 25 mouse T2R orthologs (mT2Rs) localize with mouse chromosome 6, a genetic loci that control sensitivity to bitter compounds in mice: *Qui* (for Quinine) (13), *Rua* (raffinose undecaacetate) (14), *Cyx* (cycloheximide) (15), and *Soa* (sucrose octaacetate) (16). In *in vitro* assay, one of mT2R mapped to this region (mT2R5) displayed a strict specificity for cycloheximide with the range of affinity compatible with behavioral sensitivity (10). It is interesting that missense mutations were found in the sequences of T2R5 from mouse strains deficient in cycloheximide sensitivity (10). We reasoned that each human T2R gene comprises at least two alleles resulted from coding single nucleotide polymorphisms (cSNPs), missense mutations of which could change the sensitivity of receptor to a corresponding bitter compound. In the present study, we

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examined cSNPs of hT2R receptor genes expressed in the circumvallate papillae on the human tongue.

MATERIALS AND METHODS

Reverse transcription–polymerase chain reaction (RT-PCR) analysis. Total RNA was isolated from the circumvallate papillae of an adult human tongue containing circumvallate papillae. Then, 2 μg of total RNA was subjected to random-primed reverse transcription using SuperScript II (Life Technologies). Next, 1/10 of the sample was amplified by PCR for 35 cycles with the same oligonucleotide primers used for the PCR amplification from genomic DNA. As a negative control, total RNA isolated from the circumvallate papillae of a human tongue was treated in the same way, except that no reverse transcriptase was added. The PCR products obtained were separated by 1% agarose gel electrophoresis. The molecular identity and homogeneity of the resulting PCR products were checked by DNA sequencing.

DNA samples. Since T2Rs do not contain introns that interrupt coding regions (8), we used genomic DNAs for amplification. Genomic DNAs of 50 unrelated Japanese individuals were isolated from whole blood using QIAmp DNA Blood Mini kit (Qiagen). A Caucasian genomic DNA (Clontech) and the nucleotide sequences of the T2Rs in the GenBank database (GenBank Accession Nos. hT2R3:AF227130, hT2R4:AF227131, and hT2R5:AF227132), which are identical to those in *Homo sapiens* genomic clone 7q31.3-q32 (Accession No. AC004979), were used as references.

Polymerase chain reaction (PCR) procedures. We focused on a cluster of T2Rs on human chromosome 7q31.3-q32, which contains three full-length genes (hT2R3, hT2R4, and hT2R5). We designed two sense (S) and two antisense (AS) primers for PCR to amplify the full open reading frame (ORF) of the three hT2R genes as shown below.

T2R3-S1, AAGAGACAGGTACAGTGAAGCAACA
T2R3-AS1, CAGTCAGTGTTTCTCTCCTACTGAT
T2R3-S3, GCTCTACGACAGACTAAATTGGGCA
T2R3-AS3, CCTTGGAACAAGTGCCCAGGGACCA
T2R4-S1, ATGCTGGGAAAAATTAAAAAGGAGA
T2R4-AS1, AAAAGCCTATGATGTCAGATCAGCA
T2R4-S3, TGGCCCTTGATCATGAATGGCTCAT
T2R4-AS3, CAGAATGAACTGAAATTCAGGCACT
T2R5-S1, ACGGAAGGACGAGGCCAAATCCAGA
T2R5-AS1, TCTGTTGCTCATTCATTACATTGGT
T2R5-S3, CTGTTCAGTCTCGCTTGAAGACAGA
T2R5-AS3, GACATAGTATTGGGTTTTGATCCGT

We tested four combinations of sense and antisense primers (S1–AS1, S1–AS3, S3–AS1, and S3–AS3) for the respective T2R genes and used a primer combination (S1 and AS3 for all the T2Rs) to effectually amplify the full ORF of the T2Rs in this experimental study.

We carried out PCR in a total volume of 25 μl containing deoxynucleotides (0.2 mM of each; Applied Biosystem), primers (0.5 μM of each), GeneAmp PCR Gold buffer (containing 1.5 μM $MgCl_2$) (Applied Biosystem), 2 U AmpliTaq Gold DNA polymerase (Applied Biosystems) and genomic DNA (60 ng). The PCR conditions were follows: 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 80 s. The first denaturation step and the last extension step were 9 and 7 min, respectively. PCRs were performed at least three times per gene to avoid errors in the PCR steps. Then, PCR products were separated on a 1% agarose gel and purified.

DNA sequencing. Sequencing reactions were performed on PCR products in both directions using the specific primers used in the PCR amplification, and internal primers that were synthesized to ensure a complete read on both strands (S2 and AS2), with dye-terminators (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit; Applied Biosystem) on an ABI 3100 automated sequencer. In some cases, we subcloned the PCR products into a pGEM T vector (Promega), with prior purification, using a Takara Ligation Kit version 2 (Takara, Japan). We extracted the DNA from the subclones using a Qiagen Plasmid Mini kit (Qiagen) and sequenced using vector primers in both directions, in addition to the specific primers.

T2R3-S2, GCTCTTATCCTGTGGTAGTACCGCA
T2R3-AS2, ACCACAGAGTCCCAAGAACATGGAT
T2R4-S2, ACACTCAGTGTTTCTCCTGCTGA
T2R4-AS2, TGATGTGTTATTTCTCGTAGTCA
T2R5-S2, AGAAGATCACGACCTTCGAT
T2R5-AS2, TGTTTCCTTGGGGAGGATGA

Hardy–Weinberg equilibrium was examined using the χ^2 test (17).

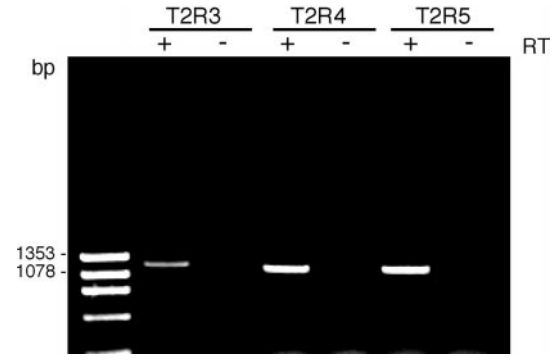

FIG. 1. The human T2R genes studied are expressed in the circumvallate papillae of the human tongue. RT, reverse transcription.

RESULTS AND DISCUSSION

Human T2Rs Studied Are Expressed in Circumvallate Papillae of the Human Tongue

Since T2R family belongs to multigene family that is thought to contain many pseudogenes, we first examined whether hT2Rs studied are actually expressed in the circumvallate papillae of the human tongue by RT-PCR in a human circumvallate papillae. Figure 1 shows that one transcript (about 1227-, 1179-, and 1169-bp fragments, corresponding to hT2R3, hT2R4, and hT2R5, respectively) was detected in human circumvallate papillae. No detectable signal was seen in the sample without reverse transcriptase treatment. This study for the first time directly demonstrates the expression of the human T2R genes in the circumvallate papillae of the human tongue, suggesting that all the genes are functional as taste receptors *in vivo.*

Identification of cSNPs in the T2R Genes

We analyzed the population variability of three hT2R receptor genes (hT2R3, hT2R4, and hT2R5) on human chromosomes 7q31.3-q32. We sequenced the T2Rs in the human genomic DNA samples from 50 unrelated Japanese individuals. As shown in Table 1,

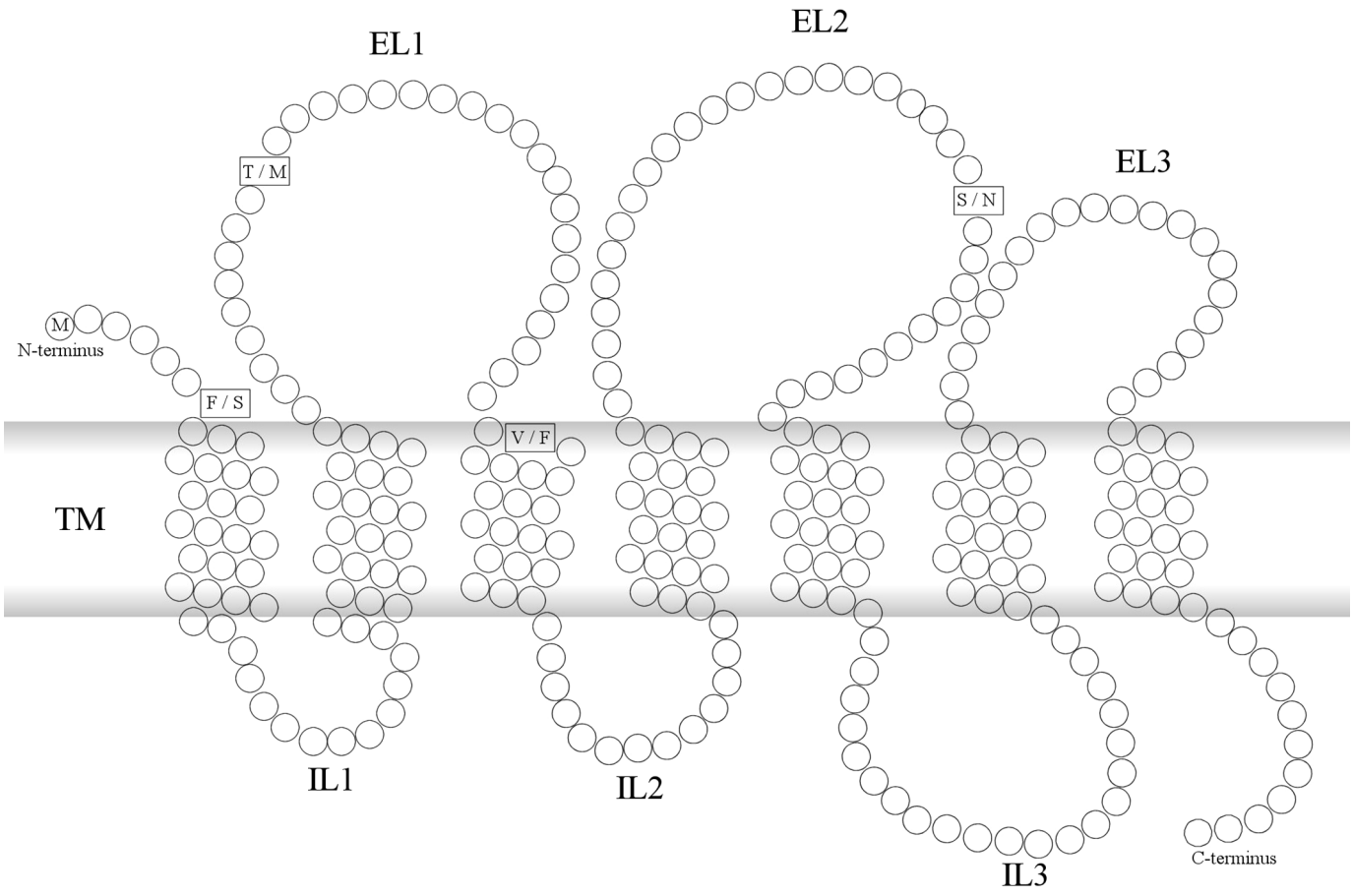

FIG. 2. Predicted transmembrane topology of hT2R4; amino acid substitutions by cSNPs are indicated. TM, transmembrane domain; NT, N-terminal; EL, extracellular loop, IL, intracellular loop.

we identified six cSNPs in these T2Rs. The hT2R3 was composed of 948 nucleotides and one cSNP was found at 807 (C or T), but was synonymous (silent). The former nucleotide pattern (C) occurred at a frequency of 37%, whereas the latter (T) at 63%. The hT2R4 was coded by 900 nucleotides and four cSNPs were detected at nucleotide positions 20 (T or C), 221 (C or T), 286 (G or C), and 512 (G or A). The former nucleotide (T, C, G, and G) in each position occurred at frequency of 37, 91, 23, and 24%, respectively, while the latter (C, T, C, and A) at 63, 9, 77, and 76%. All the four cSNPs resulted in amino acid substitutions at Phe7Ser (the former nucleotide pattern: phenylalanine and the latter: serine) in the N-terminal extracellular domain, Thr74Met in the first extracellular loop, Val96Phe in the third transmembrane domain and Ser171Asn in the second extracellular loop (Fig. 2). The hT2R5 comprised 900 nucleotides and one cSNP was detected at 77 (G or T) corresponding to an amino acid substitution at Ser26Ile in the first transmembrane domain. The former nucleotide pattern (G) accounted for 31%, while the latter (T) for 69%. However, we found no nonsense mutations carrying stop codons in the T2Rs examined.

The SNP data were subjected to a Hardy–Weinberg equilibrium analysis using χ^2 test for each of the polymorphic sites (Table 1). All the cSNPs were found to be at equilibrium ($P > 0.05$).

The present investigation demonstrated for the first time the individual variation of the genes coding taste receptors in humans. T2Rs are members of the 7 transmembrane domain, GPCR superfamily, as well as odorant receptors (ORs) and a candidate pheromone receptor expressed in vomeronasal organ (V1Rs) (2, 8). They are extremely diverse in amino acid sequences, and the differences are thought to be relevant to the recognition of structurally diverse substances (8, 18). In human, individual members of T2R family exhibit 40–80% amino acid identity. The hT2R genes examined in this study share approximately 45% amino acid identity with each other (45% for hT2R3/hT2R4 and hT2R3/hT2R5 and 44% for hT2R4/hT2R5), indicating that the gene cluster on 7q31.2-q32 contains highly divergent T2R receptors. To date, 12 human T2R receptor genes have been identified on three human chromosomes, 5p15, 12p13 and 7q31 (8), which are biased for their terminal bands. The locus at human chromo-

TABLE 1

The Features of SNPs in Three Human T2R Genes Clustered on Human Chromosome 7q31.3–32

	Nucleotide								Amino acid		
					Hardy–Weinberg equilibrium						
hT2R genes	Coding length	SNP location	Nucleotide	Observed	Expected	χ^2	Probability	GenBank	Amino acid	Substitution type	Domain
hT2R3	948	C807T	CC	8	6.85	0.49	0.48	C	Gly	Silent	El4
			CT	21	23.31				269		
			TT	21	19.85				Gly		
hT2R4	900	T20C	TT	8	6.85	0.49	0.48	T	Phe	Missense	NT
			CT	21	23.31				7		
			CC	21	19.85				Ser		
		C221T	CC	42	41.41	1.08	0.3	C	Thr	Missense	EL1
			CT	7	8.19				74		
			TT	1	0.41				Met		
		G286C	GG	3	2.65	0.08	0.77	G	Val	Missense	TM3
			GC	17	17.71				96		
			CC	30	29.65				Phe		
		G512A	GG	3	2.88	0.08	0.93	G	Ser	Missense	EL2
			GA	18	18.24				171		
			AA	29	28.88				Asn		
hT2R5	900	G77T	GG	5	4.85	<0.01	0.89	G	Ser	Missense	TM1
			GT	21	21.39				26		
			TT	24	23.8				Ile		

Note. TM, transmembrane domain; NT, N-terminal; EC, extracellular loop; IL, intracellular loop.

somes 5p15 and 7q31.1-3 are known to associated with the ability of PROP perception (8, 19), whereas the region at 12p13 is believed to be syntenous to the distal end of mouse 6 chromosome that includes *Cyx*, *Qui*, *Rua*, and *Soa* (8). Although there is no genomic information about the region at 7q31.3-q32, hT2R4 located at this region is the only human gene that veritably functions as bitter taste receptor *in vitro* (10).

In the present study, we identified six cSNPs within T2R receptor genes on human chromosome 7q31.3-q32. Interestingly, all the cSNPs except one in the hT2R3 were missense mutations that cause amino acid substitutions. Moreover, these were restricted in the predicted transmembrane segments and extracellular loops. In particular, the hT2R4, which has been shown to respond to denatonium and PROP (10), contains four cSNPs (Fig. 2). These were higher in frequency than those of the other taste receptor genes clustered on human chromosome 7. In many GPCRs with short N-termini like T2Rs, ORs, and V1Rs, ligand appears to bind in a pocket that is formed in the membrane by combination of the transmembrane domains (20). A single amino acid change in one transmembrane domain of a rodent OR has been shown to alter its odorant specificity (21). Furthermore, it has been recently demonstrated that cSNPs in the predicted extracellular loops and transmembrane domains of mouse T2R5 are associated with changes in bitter taste sensitivity to cycloheximide (cyx) (10). Thus, polymorphisms of the human T2R4 could also alter bitter sensitivity to the corresponding substances.

Mammals can recognize a large number of bitter compounds (22). In addition, its perception varies in individuals (23). The mutation of any gene in the T2R family does not appear to be critical for survival, but the diversity of each T2R resulting from mutations may contribute to the extension of acceptability to foods and to the enhancement of sensitivity to poisonous compounds. Further studies on polymorphisms in taste receptors will provide useful information on not only individual differences in the acceptability and sensitivity to bitter compounds, but also to elucidate the molecular mechanism, by which structurally diverse bitter substances are detected by receptors.

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