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REVIEWS

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Functional and genomic comparative study of the bitter taste receptor family TAS2R: Insight into the role of human TAS2R5

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Abstract

Bitterness is perceived in humans by 25 subtypes of bitter taste receptors (hTAS2R) that range from broadly tuned to more narrowly tuned receptors. hTAS2R5 is one of the most narrowly tuned bitter taste receptors in humans. In this study, we review the literature on this receptor and show there is no consensus about its role. We then compare the possible role of hTAS2R5 with that of the proteins of the TAS2R family in rat, mouse, and pig. A phylogenetic tree of all mammalian TAS2R domain-containing proteins showed that human hTAS2R5 has no ortholog in pig, mouse, or rat genomes. By comparing the agonists that are common to hTAS2R5 and other members of the family, we observed that hTAS2R39 is the receptor that shares most agonists with hTAS2R5. In mouse, some of these agonists activate mTas2r105 and mTas2r144, which are distant paralogs of hTAS2R5. mTas2r144 seems to be the receptor that is most similar to hTAS2R5 because they are both activated by the same agonists and have affinities in the same range of values. Then, we can conclude that hTAS2R5 has a unique functional specificity in humans as it is activated by selective agonists and that its closest functional homolog in mouse is the phylogenetically distant mTas2r144.

KEYWORDS

agonist assays, bitter taste receptor, hTAS2R5

1 | BACKGROUND

The first step in taste sensing takes place in the mouth. The predominant consensus holds that there are five basic tastes—sweet, bitter, salty, sour, and "umami".[1] It remains a matter of debate whether fatty acids elicit an independent taste perception.[2] At the molecular level, taste perception depends on several G-protein-related receptors

Abbreviations: EC_{50}, half-maximum effective concentration; EGCG, epigallocatechin gallate; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; hTAS2R, human bitter taste receptors; hTAS2R39, human bitter taste receptor 39; hTAS2R5, human bitter taste receptor 5; hTAS2Ri, human bitter taste receptor "i"; mTas2r105, mouse bitter taste receptor 105; mTas2r144, mouse bitter taste receptor 144; PGG, pentagalloylglucose, a hydrolyzable tannin; SNPs, single nucleotide polymorphisms; TAS1R, family 1 of taste receptors; TAS2R, family 2 of taste receptors.

and ionic channels located in the gustatory papillae on the tongue.[3] Sour and salty tastes are sensed via ion channels and signal that dietary acid is present in spoiled foods or salts essential for maintaining the water balance of the body, respectively. Sweet or umami tastants that help to identify energy-rich nutrients are sensed via heterodimers of subtypes of the family 1 of taste receptors (TAS1R). Bitter tastants that may indicate potentially harmful compounds are identified by different subtypes of the family 2 of taste receptors (TAS2R). Beyond the tongue, an extra-oral expression of taste receptors has been reported, recently in other areas of the human body (these are known as ecnomotopic receptors[4]). They are found in the gastrointestinal (GI) tract, adipose tissue, the airways, and the pancreas.[5] Their role in these locations is far from clear. Since most of the available studies about the expression of ecnomotopic receptors are based mainly on RNA expression instead of protein expression, their function in these tissues remains vastly obscure.

Bitterness protects individuals from consuming unhealthy natural products.[6] However, not all toxins are bitter and, likewise, not all bitter compounds are toxic.[7] Many bitter compounds have health benefits and it has been suggested that healthier diets have a higher component of bitter-tasting ingredients, including bitter vegetables.[8] It is believed that the receptive ranges of bitter taste receptor repertoires match the profiles of bitter chemicals that species encounter in their diets. Humans and mice genomes contain pairs of phylogenetically orthologs[9,10] bitter receptor genes that have been conserved throughout evolution. In humans, bitterness is sensed by 25 subtypes encoded by 25 functional TAS2R loci, which reside on chromosomes 5, 7, and 12.[3,4,11] In addition to these genes, humans also carry 11 TAS2R pseudogenes.[12] Mice have 35 genes and 5 pseudogenes on chromosomes 5, 6, and 15.[13] Lossow and col have demonstrated that mouse taste 2 receptors, like their human counterparts, vary greatly in their breadth of tuning, ranging from very broadly to extremely narrowly tuned receptors. However, when compared with humans, mice have fewer broadly tuned receptors and a considerable number of narrowly tuned receptors, which supports the idea that a large receptor repertoire is based on which specialized receptors evolve. Lossow and colleagues have also shown that orthologs bitter taste receptors have distinct agonist profiles. Species-specific gene expansions have enabled further diversification of bitter substance recognition spectra.[14]

Human TAS2R5 is one of the most narrowly tuned bitter taste receptors in humans. Di Pizio et al. first described TAS2R5 and TAS2R49 as the only specialized TAS2Rs activated by exclusive bitter compounds: 1,10-Phenanthroline and cromolyn, respectively.[15] TAS2R49 seems to have a physiological role in the upper airways. Notably, the only compound that activates it is a common drug for treating asthma.[16] For TS2R5 there is no consensus about its role. In this paper, we review the possible role of TAS2R5 in the human body, construct a phylogenetic tree to identify its orthologs in other species commonly used as a model for human pre-clinical studies, and the structural similarity with other receptors based on common agonists.

2 | METHODS

2.1 | Systematic review

The electronic databases Web of Science, Scopus, and Pubmed were searched for relevant studies using the keywords TAS2R5 AND T2R5 (until March 2021). We set redundancy between journal articles and reviews as exclusion criteria. Out of a total of 58 results, 45 studies were selected to investigate the state of art on the function of human TAS2R5.

2.2 | Transcriptomics from The Human Protein Atlas

Tissue microarray data was downloaded from the publicly available The Human Protein Atlas (http://www.proteinatlas.org). The data consist of the consensus transcript expression levels (NX value) in 62 tissues based on transcriptomics data from three different studies: HPA, GTEx, and FANTOM5. This data are based on The Human Protein Atlas version 20.1 and Ensembl version 92.38.

2.3 | Genomic analyses of TAS2R

We downloaded the peptide sequences from all the genomes available in version 102 of the Ensembl database (http://www.ensembl.org/) of human (*Homo sapiens*: GRCh38.p13), pig (*Sus scrofa*: Sscrofa11.1), mouse (*Mus musculus*: GRCm38.p6), rat (*Rattus norvegicus*: Rnor_6.0), platypus (*Ornithorhynchus anatinus*: mOrnAna1.p.v1), opossum (*Monodelphis domestica*: ASM229v1), Tasmanian devil (*Sarcophilus harrisii*: mSarHar1.11), common wombat (*Vombatus ursinus*: bare-nosed_wombat_genome_assembly), hyrax (*Procavia capensis*: proCap1), hedgehog (*Erinaceus europaeus*: eriEur1), American black bear (*Ursus americanus*: ASM334442v1), dog (*Canis lupus familiaris*: CanFam3.1), alpaca (*Vicugna pacos*: vicPac1), vaquita (*Phocoena sinus*: mPhoSin1.pri), sperm whale (*Physeter catodon*: ASM283717v2), goat (*Capra hircus*: ARS1), cow (*Bos taurus*: ARS-UCD1.2), American beaver (*Castor canadensis*: C.can_genome_v1.0), mouse lemur (*Microcebus murinus*: Mmur_3.0), and macaque (*Macaca mulatta*: Mmul_10).

From this dataset, we selected the TAS2R proteins in these species using the Hidden Markov Models (HMM)-search software HMMER 3.3[17] and the Pfam model PF05296 for the TAS2R domain, obtained from version 31 of the Pfam database.[18] We used the gathering threshold (GA) as the search parameter in *hmmscan*.

The TAS2R domains of the retrieved sequences ($n = 300$) were aligned using the multiple alignment program MAFFT 7 and the algorithm E-INS-I.[19] Poorly aligned positions were removed from the alignment using trimAL with the automated1 algorithm.[20] From the resulting TAS2R protein alignment, we constructed a phylogenetic tree using the IQ-TREE 2.0.3 software, under the Jones-Taylor-Thornton substitution model with 4 Γ rate categories and empirical state frequencies (selected with the ModelFinder IQ-TREE module). Statistical supports were obtained from 1000 ultrafast bootstrap iterations.[21] The resulting phylogenetic tree was mid-point rooted using the R phangorn 2.53 library,[22] and visualized using the phytools 0.6–60[23] and ape 5.3 libraries (plot.phylo).[24] Groups of orthologs genes from our phylogenetic analysis were identified with *Possvm* software.[25] Finally, we compared the amino acid identities and similarities (with BLOSUM62z matrix) between all TAS2R sequences from the untrimmed multiple sequence alignments, using the dist.alignment function in the seqinr 4.2-5 R library.[26]

3 | RESULTS AND DISCUSSION

3.1 | What we can learn from the current literature

The human TAS2R5 gene is located on chromosome 7q31.3-q and encodes the bitter taste receptor. Its expression pattern has been summarized in "The human protein atlas" (Figure 1). The consensus dataset showed that the highest mRNA expression is in the skin, epididymis, cerebellum, and ovary. It is based on the quantification of this mRNA either by Taqman probes,[27,28] or specifically designed primers[29] (recruited in Supporting Information Table S1). There is no data based on protein quantification.

Although it is highly expressed in skin, there is little data on the role it plays. A positive correlation has been reported between increasing age and TAS2R5 gene expression in not-sun-exposed skin (suprapubic) (as well as human TAS2R4, TAS2R14, and TAS2R20).[29] No correlation has been reported in sun-exposed skin. TAS2R5 is expressed in human myometrial cells. Specific stimulation with 1,10-phenanthroline dose-dependently suppressed the oxytocin-induced contraction of the myometrium.[30] In the brain, TAS2R5 has been related to Parkinson's disease. TAS2R5 and TAS2R50 are downregulated in premotor and Parkinsonian stages in the frontal cortex area 8 in the brains of patients with Parkinson's disease.[28] Taste function-related genes, including TAS2R5 and TAS2R3, might also be state-specific mania markers in bipolar disorder.[31] Recently, TAS2R5 together with TAS2R4, TAS2R14, and TAS2R39 expression, was confirmed by immunohistochemistry to be present in human choroid plexus epithelial cells, which suggests that TAS2Rs play an active role at the human blood-cerebrospinal fluid barrier as surveyors of the bloodstream and cerebrospinal fluid compositions.[32]

Specific stimulation has shown that TAS2R5 is related to the relaxation of human bronchi.[27] The selective agonist of TAS2R5 (1,10-phenanthroline) induced the relaxation of human bronchi, whereas the TAS2R7 agonist cromoglycate and malvidin-3-glucoside were ineffective up to 10 mM and 30 μM, respectively. Phenanthroline-induced

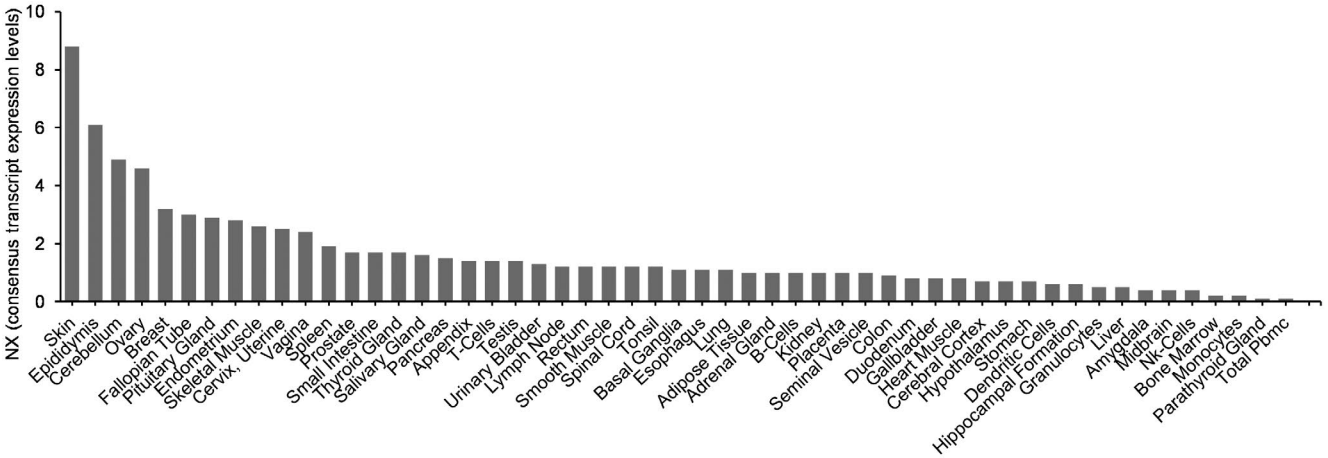

FIGURE 1 The Human Protein Atlas transcriptomic data for TAS2R5 https://www.proteinatlas.org/ENSG00000127366-hTAS2R5/tissue

relaxation at concentrations as low as 10 μM, 10-fold lower than the threshold concentration in a study performed with HEK cells, suggesting the involvement of TAS2R5.[5]

In the gastrointestinal tract, TAS2R5 has been co-localized in the human enteroendocrine L cell, where GLP-1 secretion is stimulated by 1,10-phenanthroline.[33] Moreover, bitter agonists selective to TAS2Rs such as TAS2R5 and TAS2R10 have stimulated ghrelin secretion in fundic cells.[34]

As observed for other bitter taste receptors, several single nucleotide polymorphisms (SNPs) in TAS2R5 may affect bitter taste sensitivity.[35] The 4 SNPs across TAS2R3, TAS2R4, and TAS2R5 are located close to each other on chromosome 7, forming a haplotype in our sample. The TGAG homozygotes perceived twice as much bitterness as CCGT homozygotes, while the heterozygotes exhibited intermediate values. Within the CCGT/TGAG haploblock, 2 of the polymorphisms encode amino acid substitutions in TAS2R4 and TAS2R5. It is unclear from the present data whether the variation in coffee bitterness seen for this haplotype results from the functional alteration of T2R5 or influences on transcription that alters the expression.[36] Additionally, recent studies in this allele have revealed that TGAG homozygotes show a significantly lower body mass index.[37] The bitterness of alcohol is known to be the major taste component that influences alcohol consumption.[38] Choi et al showed an association between the TAS2R5 rs2227264 variant and total alcohol intake.[39] The TAS2R5 rs2227264 variant also influenced rice wine drinking status and the rs1859646 SNP is located in the intron of the TAS2R5 gene.[40] In the elderly, Mikołajczyk-Stecyna et al.[35] showed an association between the SNPs of the TAS2R5 gene and the frequency of grapefruit intake, and between the simultaneous effects of polymorphisms within TAS2R3 and TAS2R5 and the frequency of eating Brassica vegetables in general. Moreover, the SNPs of the selected TAS2R genes may be associated with the lipid profile, serum level of glucose, and CRP, depending on the frequency of consumption of particular bitter-tasting items.[35]

Understanding how similar human TAS2R5 is to the other members of the TAS2R protein family and how it is related to other bitter taste receptors in other animal models could provide important insight into its function and specificity. Moreover, since TAS2R5 is a membrane protein that needs external stimulation to be effective at modifying cell function, the information available from the study of its agonists and those in common with its closely-related receptors would increase understanding of its specificity and its role in the organism. Thus, the similarity of TAS2R5 with other TAS2Rs and information about the agonists of these related receptors will be discussed below.

3.2 | Phylogenetic study of human TAS2R5

To gain insight into the TAS2R5 function by finding its closest related proteins, either a human paralog or an ortholog from another species, we constructed a phylogenetic tree with the proteins containing the TAS2R domain in humans and other mammals (Figure 2). Among the species selected were pig, mouse, and rat because a considerable number of studies have been made on their perception of bitterness and they have often been used as animal models for human studies. The other mammalian species were selected to ensure a taxonomically balanced sampling.

We identified 24 human TAS2R genes and 276 homologs in 19 other mammalian genomes. Our phylogenetic analysis identified groups of orthologs genes (OGs, Figure 2) and ascertained the evolutionary relationships between homologs in human and other animal models (Table 1).

We found that, of the 24 subtypes of human TAS2R, 8 had orthologs in pig, mouse, and rat, 13 had orthologs in rodents (mouse and rat) but not pig, and only TAS2R9 had a pig ortholog and no rodent orthologs. Only TAS2R5 and TAS2R8 have no orthologs in pig, mouse, or rat. However, rather than being human-specific, we find that both of these genes belong to ancient orthologs groups that include homologs from multiple placental mammals and were secondarily lost in rodents (OG2 and OG3 in Figure 2).

An examination of our phylogenetic reconstruction also supports an early origin for TAS2R5 and TAS2R8, in spite of their restricted taxonomic distribution in extant genomes. According to our phylogenetic tree, the closest paralogs of TAS2R5 genes are in either the TAS2R4 or TAS2R38 orthologs groups. Both these groups contain marsupial and placental homologs, thus pointing to an equally early origin for TAS2R5. Similarly, TAS2R8 has a well-supported sister-group relationship to a large clade that contains many orthologs groups including marsupials and placental mammals. This topology indicates that it, too, emerged early in mammalian evolution in spite of its currently limited taxonomic distribution (only in primates, pig, and *Ursus americanus* in our dataset).

Our phylogenetic analysis also allows us to identify cases of species- or taxa-specific diversifications of the TAS2R gene complement. For example, seven human genes (TAS2R19, TAS2R20, TAS2R30, TAS2R31, TAS2R43, TAS2R46, TAS2R50) emerged via primate-specific paralogy events within OG20. Similarly, we find rodent-specific duplications within OG21, which contains 10/11 recent paralogs in rat and mouse genomes, respectively. By

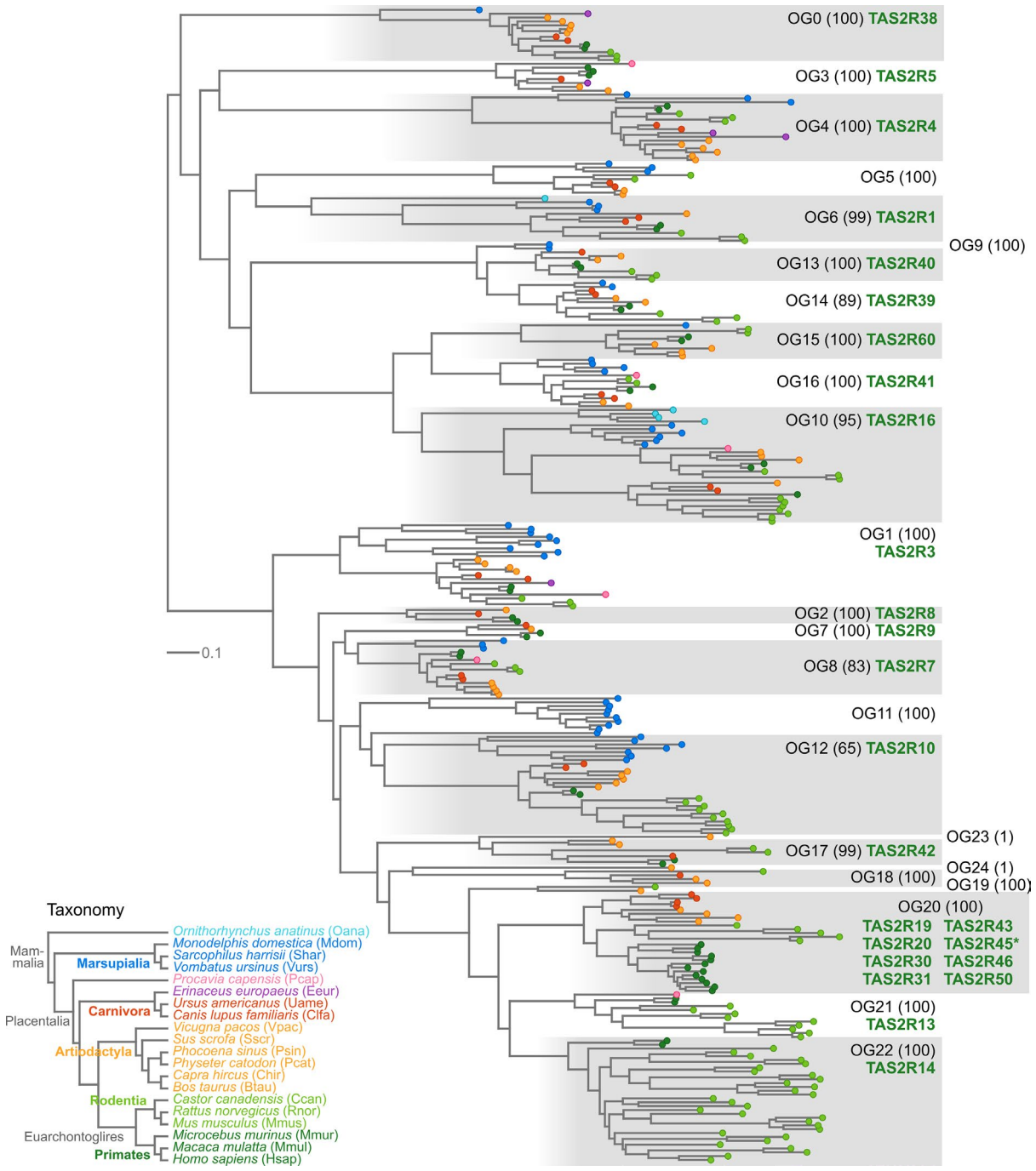

FIGURE 2 Phylogenetic tree of TAS2R. Species are colored according to their taxonomic families. Orthologs groups (OG) are formed by a set of genes from the different studied species that descend from the same common ancestor. OG are listed in grey with their statistical support in parenthesis. For each OG, only the human TAS2Rs are highlighted in dark green. *Although TAS2R45 is listed as a pseudogene and, therefore, not included in our dataset, we have added it to this figure because it is very similar to the human TAS2R in the OG20 orthologs groups

contrast, other orthologs groups have less dynamic evolutionary histories and have only one homolog per sampled species (e.g., TAS2R5).

It is worth noting that previous studies[14,41–45] have listed 25 TAS2R genes in humans, of which our survey recovers 24. The only missing gene is TAS2R45, which is currently annotated as an unprocessed pseudogene in the Ensembl database (http://www.ensembl.org/). TAS2R45 is located in the genome next to its close paralogs TAS2R30, TAS2R43, and TAS2R46, which all emerged during the recent primate-specific diversification of OG20 homologs (Figure 2). We have highlighted TAS2R45 with an asterisk (*) in the figures of the human TAS2R subtypes to reflect its pseudogene annotation.

TABLE 1 Orthologs groups and gene presence in selected model species

Orthologs groups (human and pig genes in bold UPPER CASE, rodent genes lower case)	Orthologs group	Human	Pig	Mouse	Rat
TAS2R1, Tas2r119	OG6	●		●	●
TAS2R3, Tas2r147	OG1	●	●	●	●
TAS2R4, Tas2r108	OG4	●	●	●	●
TAS2R5	OG3	●			
TAS2R7, Tas2r130	OG8	●	●	●	●
TAS2R8	OG2	●			
TAS2R9	OG7	●	●		
TAS2R10, Tas2r104, Tas2r105, Tas2r106, Tas2r107, Tas2r114	OG12	●	●	●	●
TAS2R13, Tas2r13, Tas2r102, Tas2r121, Tas2r124	OG21	●		●	●
TAS2R14, Tas2r103, Tas2r109, Tas2r110, Tas2r113, Tas2r115, Tas2r116, Tas2r117, Tas2r123, Tas2r125, Tas2r129, Tas2r140	OG22	●		●	●
TAS2R16, Tas2r118, Tas2r134, Tas2r143	OG10	●	●	●	●
TAS2R19, **TAS2R20**, **TAS2R30**, **TAS2R31**, **TAS2R43**, **TAS2R45**,[a] **TAS2R46**, **TAS2R50**, Tas2r120, Tas2r136	OG20	●		●	●
TAS2R38, Tas2r138	OG0	●	●	●	●
TAS2R39, Tas2r139	OG14	●	●	●	●
TAS2R40, Tas2r144	OG13	●		●	●
TAS2R41, Tas2r126	OG16	●	●	●	●
TAS2R42, Tas2r131 (Mmus), Tas2r145 (Rnor)	OG17	●		●	●
TAS2R60, Tas2r135	OG15	●	●	●	●
Tas2r7l	OG5				●
TAS2R? (Not annotated), Tas2r122	OG18		●	●	
TAS2R? (Not annotated)	OG24		●		

[a]Although TAS2R45 is listed as a pseudogene and, therefore, not included in our dataset, we have added it to this figure because it is very similar to the human TAS2R in the OG20 orthologs groups.

We studied the percentages of amino acid sequence identity and similarity between all human TAS2Rs to gain further insight into how they are related (Supporting Information Figure S1A,B). The most similar paralog in the human genome to TAS2R5 (OG3) both in terms of sequence identity and BLOSUM62 similarity, was the phylogenetically distant TAS2R7 (30% identity, 68% similarity; OG8). This proximity is due to the fact that TAS2R7 is a relatively slow-evolving orthologs group, as attested by the short stem branch in the gene tree (which reflects amino-acid substitutions per alignment position; Figure 2).

In contrast, TAS2R8, the only other TAS2R without orthologs in rodents or pig, is more similar to its phylogenetically closer paralogs TAS2R7, TAS2R9, and TAS2R10, with identity percentages ranging between 38% and 47% and similarity percentages ranging between 70% and 75%.

We also observe that the human paralogs of OG20 share high identity and similarity percentages, in line with their relatively recent, primate-specific origin. Other pairs of highly similar paralogous sequences were TAS2R39 and TAS2R40 (57% identity and 79% similarity), and TAS2R13 and TAS2R14 (50% identity and 75% similarity).

Table 2 summarises the phylogenetic relationships between human TAS2R proteins and all their homologs in pig, mouse, and rat with an identity percentage higher than 50%. We observe that human TAS2R5 and TAS2R8 do not have any homologs in these species. Moreover, although we previously showed that TAS2R29, TAS2R30, TAS2R42, TAS2R46, and TAS2R50 have rodent orthologs, the percentage of shared identity is low. We found that, although they do not belong to the same orthologs groups, pig TAS2R9 is highly similar to human TAS2R7 and that mouse Tas2R144 is similar to human TAS2R39. We also found that both human TAS2R39 and TAS2R40 are orthologs to pig TAS2R39.

The phylogenetic study shows that the TAS2R repertoires of rodents and humans (which share 15 out of 21 orthologs groups) are more similar than those of humans and pigs (which share 10 out of 21), in line with the closer phylogenetic relationship between humans and rodents. Furthermore, although TAS2R5 has orthologs in

TABLE 2 Human bitter taste receptors (TAS2R) and homologous genes with >50% aminoacidic identity in *Rattus norvegicus*, *Mus musculus*, and *Sus scrofa*

Human	Pig		Mouse		Rat	
Gene	**Gene**	**Identity (%)**	**Gene**	**Identity (%)**	**Gene**	**Identity (%)**
TAS2R1			Tas2r119	51.7	Tas2r119	51.5
TAS2R3	TAS2R3	71.5	Tas2r137	63.9	Tas2r137	64.2
TAS2R4	TAS2R4	70.6	Tas2r108	67.0	Tas2r108	64.6
TAS2R5						
TAS2R7	TAS2R7	73.1	Tas2r130	68.3	Tas2r130	69.2
	TAS2R9 (#)	50.8				
TAS2R8						
TAS2R9	TAS2R9	70.4				
TAS2R10	TAS2R10	69.1	Tas2r104	55.3	Tas2r104	54.3
			Tas2r105	54.4	Tas2r105	55.0
			Tas2r106	57.4	Tas2r106	54.4
			Tas2r107	56.9	Tas2r107	58.2
			Tas2r114	57.0	Tas2r114	55.0
TAS2R13			Tas2r102	51.0	Tas2r13	50.0
			Tas2r121	57.3	Tas2r102	50.7
					Tas2r121	56.3
TAS2R14			Tas2r116	51.2	Tas2r125	50.6
			Tas2r125	50.6		
TAS2R16	TAS2R16	61.5	Tas2r118	53.3	Tas2r118	52.2
TAS2R19						
TAS2R20			Tas2r120	50.8		
TAS2R30						
TAS2R31			Tas2r120	51.2		
TAS2R38	TAS2R38	67.5	Tas2r138	65.3	Tas2r138	65.3
TAS2R39	TAS2R39	70.7	Tas2r139	55.1	Tas2r139	53.2
			Tas2r144 (#)	51.3		
TAS2R40	TAS2R39	56.1	Tas2r144	65.8	Tas2r144	64.3
TAS2R41	TAS2R41	70.6	Tas2r126	68.7	Tas2r126	69.7
TAS2R42						
TAS2R43			Tas2r120	50.2		
TAS2R45[a]			Tas2r120	50.2		
TAS2R46						
TAS2R50						
TAS2R60	TAS2R60	66.1	Tas2r135	58.2	Tas2r135	57.9

Note: For each human gene, all listed homologous genes in other species are orthologs, unless otherwise indicated (#).

[a]Although TAS2R45 is listed as a pseudogene and therefore not included in our dataset, we have added it to this figure because it is very similar to the human TAS2R in the OG20 orthologs groups.

mammals, it does not have a close human paralog or an ortholog in pig, mouse, or rat. Although they are not phylogenetically close paralogs, TAS2R7 is the closest homolog of human TAS2R5 in terms of sequence identity and similarity.

3.3 | Search for functional homologs of human TAS2R5

In this section, we will evaluate how TAS2R5 and the homologs identified to interact with their agonists. By so

TABLE 3 Agonists of human TAS2R5 that bind to other human (hTAS2R) or mouse TA2R (mTas2r)

Bitter taste receptors	hTAS2R5	hTAS2R7	hTAS2R39	mTas2r105	mTas2r144
Agonists shared with human TAS2R5	12	3	6	4	5
1,10-Phenanthroline	✓			✓	✓
Sucralose	✓	✓	✓	✓	✓
Epicatechin	✓		✓	✓	✓
Denatonium saccharide	✓		✓	✓	✓
Punicalagin	✓	✓			
PGG (Pentagalloylglucose)	✓		✓		
Procyanidin B2G	✓		✓		
EGCG	✓		✓		✓
Procyanidin B1	✓	✓			
Procyanidin C2	✓				
Procyanidin B4	✓				
Procyanidin B7	✓				
Azathioprine			✓		
Acetaminophen			✓		
Denatonium benzoate			✓		
Chloroquine			✓		
Chlorpheniramine			✓		
Pyrocatechin			✓		
Diphenidol			✓		

doing, we will gain further insight into how TAS2R5 is similar to the other receptors in terms of functionality.

According to the initial classification of human bitter taste receptors proposed by Di Pizio et al.[15] TAS2R14 and TAS2R46 are among the most promiscuous receptors and TAS2R13 and TAS2R50 are two of the most selective. In contrast, in the section above, we observed that both TAS2R13 and TAS2R14 share considerable similarities to the cluster of paralogs that include TAS2R46 and TAS2R50. Therefore, the information we obtain from the agonists seems to be more useful than the similarity for elucidating the function of the receptors.

Di Pizio et al.[15] first reported in 2015 that human TAS2R5 (hTAS2R5) is one of two specialized TAS2Rs activated by exclusive bitter compounds, although they concluded that bigger screenings with more bitter compounds were needed to fully comprehend the binding activity of TAS2Rs. Subsequently, Soares et al.[42] studied the response of human receptor isoforms to condensed tannins. They tested the ability of each agonist to increase cytosolic calcium levels in HEK cells expressing different TAS2R isoforms. They found that (−)-epicatechin, procyanidin trimer C2 and PGG (pentagalloylglucose, a hydrolyzable tannin) can bind to hTAS2R5. Although (−)-epicatechin and PGG also had the ability to bind TA2R4 (hTAS2R4) and TAS2R39 (hTAS2R39), C2 was specific for hTAS2R5.[42] They suggested that the catechol or galloyl group (which has only one more hydroxyl group than catechol) is a critical (but not essential) feature for the interaction of polyphenol compounds with hTAS2R5. In subsequent studies, they included the following in the list of agonists for hTAS2R5: Procyanidin B1, B4, B2g, and EGCG as condensed tannins and punicalagin as hydrolyzable tannin.[43] Previous studies in the same system showed that hTAS2R5 can also be stimulated by denatonium saccharide and sucralose,[14] and specifically stimulated by 1,10-phenanthroline.

Although it was first classified into the specific group,[15] to date hTAS2R5 has shown that it can be activated by twelve molecules (Table 3). Most of these compounds can also activate other human and mouse receptors[14] (Table 3). The other human receptors that have agonists in common with hTAS2R5 are human TAS2R7 (hTAS2R7) and hTAS2R39. hTAS2R7 shares three agonists with hTAS2R5, sucralose, punicalagin, and procyanidin B1. Interestingly, hTAS2R39 shares six agonists with hTAS2R5. To compare the sensitivity of these two human isoforms to stimulation by a common agonist, we used EC_{50} in Table 4, when available. Procyanidin B2g, PGG, and EGCG showed no preference to binding to any of them. According to these data, only (−)-epicatechin showed a higher affinity for

TABLE 4 EC50, or dose used to activate, for shared agonists of hTAS2R5 and hTAS2R39

	Dose that activates cytosolic calcium increase (mM)		
Agonists	**hTAS2R5**	**hTAS2R39**	**References**
Denatonium saccharide	3	3	[14]
Sucralose	30	30	[14]
	EC50 (μM)		
Agonists	**hTAS2R5**	**hTAS2R39**	**References**
Procyanidin B2G	6.29 ± 3.22	9.11 ± 6.05	[43]
PGG (pentagalloylglucose)	≥8.5	≥6.6	[42]
EGCG	12.30 ± 3.63	8.50 ± 2.84	[38]
Epicatechin	3210.0 ± 42.0[a]	3800.0 ± 200.0[b]	[42]

Values with a different superscript letter are significantly different ($p < .05$). Values with ≥ are estimates because the dose-response curves did not saturate.

hTAS2R5 than for hTAS2R39, since the EC_{50} is lower for hTAS2R5. These data are not available for denatonium saccharide or sucralose, but they need doses close to the epicatechin dose to stimulate both receptors (on the millimolar scale). On the contrary, punicalagin, procyanidin B1, 1,10-phenanthroline, and procyanidin C2, B4, and B7 are hTAS2R5 agonists, assayed on hTAS2R39 and which do not activate it, so they are selective agonists for human hTAS2R5. There are also five different hTAS2R39 agonists that did not activate hTAS2R5.

In the search for close-to-human species, some bitter sensing studies have been performed in pig,[46,47] mouse, and rat but the only data available on agonist binding to Tas2r is in mouse.[14] Two mouse receptors are candidates for being functional homologs of hTAS2R5 (Table 3): Tas2r105 (mTas2r105) and Tas2r144 (mTas2r144). These two mouse receptors share the same four agonists with hTAS2R5: sucralose, epicatechin, denatonium saccharide, and 1,10-phenanthroline. In addition, mTas2r144 shares one more agonist with hTAS2R5: epigallocatechin gallate (EGCG). Table 5 compares the sensibility of these two mouse receptors to the agonists they have in common with hTAS2R5. The data does not enable a good comparison to be made since EC_{50} is not available for all the cases. As suggested by Lossow et al.[14] mTas2r105 seems to be more sensitive than hTAS2R5 to most of the agonists, but mTas2r144 shares more agonists with hTAS2R5. Most of the shared agonists with hTAS2R5 are common to both hTAS2R39 and the mouse receptors mTas2r104 and mTas2r144, while sucralose is the only agonist common to hTAS2R7 and these mouse receptors.

However, 1,10-phenanthroline, which binds mTas2r105 and mTas2R144 is not an agonist for hTAS2R39. Whether procyanidin B2g and PGG, two of the agonists shared by hTAS2R5 and hTAS2R39, also bind the mouse receptors has yet to be determined. Interestingly, the few studies available on mouse Tas2r144 reveal that, like hTAS2R5, it is expressed in the choroid plexus epithelial cells and may play a role in detecting alterations of the cerebrospinal fluid.[48,49] Moreover, its expression is regulated by female sex hormones, which corroborates the hypothesis of the importance of bitter taste receptors in the female reproductive system.

Finally, we studied the structural similarity and identity of the receptors that are functionally similar to hTASR5: hTAS2R7, hTAS2R39, mTas2r105, and mTas2r144 (Table 6). We observe that while hTAS2R39 shares the most agonists with hTAS2R5, it is less similar to it than hTAS2R7. Likewise, although we have previously seen that mTas2r144 seems to function more similarly to hTAS2R5, mTas2r105 is more structurally similar to it. In fact, considering the phylogenetic tree (Figure 2), mTas2r105 is much further than mTas2r144 from hTAS2R5, and hTAS2R7 is much further than hTAS2R39 from hTAS2R5. These observations indicate that the evolutionary history of this gene family does not recapitulate functional similarities between its members, in agreement with the findings by Lossow and colleagues.[14] Therefore, according to current data, structurally similar TAS2Rs are not the closest functional homologs of hTAS2R5. So, to date, the selective stimulators of hTAS2R5 in humans are procyanidin B4, B7,[43] procyanidin C2, and 1,10-phenanthroline, and the closest functional homolog of hTAS2R5 in mouse is mTas2r144. Although these agonist-response studies have enabled us to gain knowledge about hTAS2R5 functionality, it should be noted that they have been performed *in vitro* with HEK293T-Gα16gust44 cells transfected with the receptor without addressing whether these compounds are bioavailable after intake and whether they can reach the ectopic locations where TAS2R5 is expressed other than the tongue.[14,41–43] In addition, in the agonist-response

TABLE 5 EC50, or dose used to activate, for shared agonists of hTAS2R5, mTas2r105, and mTas2r144

	Dose that activates cytosolic calcium increase (mM)			
Agonists	**hTAS2R5**	**mTas2r105**	**mTas2r144**	**References**
1,10-Phenanthroline	0.1–1	1	1	[14,41]
Epicatechin		1	1	[42]
EGCG	12.30 ± 3.63	–	0.01	[43]
Denatonium saccharide	3	–	–	[14]
Sucralose	30	–	–	[14]
	EC_{50} (μM)			
Agonists	**hTAS2R5**	**mTas2r105**	**mTas2r144**	**References**
Epicatechin	3210.0 ± 42.0	–	–	[42]
Denatonium saccharide	–	0.59 ± 0.21	0.25 ± 0.01	[14]
Sucralose	–	4.9 ± 1.7	50	[14]

TABLE 6 Matrix of identity (up, normal case) and similarity (down, bold case) percentages in human and mouse TAS2R that have agonists in common with human hTAS2R5

	hTAS2R5	**hTAS2R7**	**hTAS2R39**	**mTas2r105**	**mTas2r144**
hTAS2R5		29	24	27	23
		50	**47**	**48**	**25**
hTAS2R7	29		30	41	27
	50		**50**	**58**	**47**
hTAS2R39	24	30		27	49
	47	**50**		**48**	**64**
mTas2r105	27	41	27		24
	48	**58**	**48**		**45**
mTas2r144	23	27	49	24	
	25	**47**	**64**	**45**	

studies, it is not considered if the compounds after ingestion may elicit different effects by interacting with enzymes and receptors other than TAS2Rs. For instance, 1,10-phenanthroline, even though it is an agonist for hTAS2R5 but not any other human TAS2R, has other physiological functions since it inhibits zinc metalloproteases, which has made it an interesting therapeutic candidate for its antimicrobial and antitumor activities.[50] Therefore, further research is needed to gain knowledge about which compounds may target ectopic hTAS2R5 selectively without eliciting any other effect in the body and use it with a therapeutic purpose.

4 | CONCLUSION

Our bibliographic review has suggested that hTAS2R5 has several roles. Besides its role in specific taste perception, hTAS2R5 may play a role in the aging of the skin, unlinked to exposure to the sun. In the brain, it seems that it is required to protect against Parkinson's disease and it is involved in bipolar pathology. It may also play a role in pregnancy, and participate in controlling enteroendocrine secretions and bronchodilation processes. Our comparative genomics study has not shed light on its possible role since it has no close paralog or ortholog, which highlights its uniqueness. Our functional study showed that it can be activated by several agonists and is less selective than initially described. It also showed that its closest functional homolog is mTas2r144. Although it is currently known that both hTAS2R5 and mTas2r144 are involved in the female reproductive system and the monitoring of cerebrospinal fluid, more research needs to be done to fully elucidate their role in the body.

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DISCLOSURES

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Carme Grau-Bové participated in the design of the study, performed the systematic review, and drafted the manuscript. Xavier Grau-Bové carried out sequence alignments and helped draft the manuscript. Ximena Terra participated in data analysis. Santi Garcia-Vallve participated in the genomic analysis. Esther Rodríguez-Gallego helped draft the manuscript. Raúl Beltran-Debón participated in the systematic review. M. Teresa Blay participated in data analysis. Montserrat Pinent participated in the design of the study and critically revised the manuscript. Anna Ardévol conceived and coordinated the study.

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