DOI: https://doi.org/10.54393/pbmj.v5i11.829

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 5, Issue 11 (November 2022)

Original Article

Characterization of Taste Receptor Class 2 genes in Mouse [Mus musculus]

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ARTICLE INFO

Key Words:
Mus musculus, Taste Receptor Class, T2R, Mouse, Bioinformatics

How to Cite:
Asjad Riaz, M. ., Anwar, T. ., & Sarwar, S. . (2022). Characterization of Taste Receptor Class 2 genes in Mouse [Mus musculus]: Characterization of Taste Receptor Class 2 genes in Mouse. Pakistan BioMedical Journal, 5(11). https://doi.org/10.54393/pbmj.v5i11.829

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Received Date: 1st November, 2022
Acceptance Date: 28th November, 2022
Published Date: 30th November, 2022

ABSTRACT

Because the amino acid sequence of mouse specie is closely related to human genes therefore, we use it as a role model for doing research related to human genome in improving the standards of life. The T2R receptors belong to the C family of GPCRs, which includes the calcium-sensing receptor (CaSR). All are G protein-coupled receptors but here we are only highlighting the different roles of distantly related receptors of the main taste bud system. **Objective:** To characterize and analyse the Taste Receptor Class 2 genes in mouse [*Mus musculus*]. **Methods:** In order to get insights into this gene family in mouse, we performed an extensive survey of taste receptor derived datasets. We identified 500 genes distributed among mouse encoding putative taste receptor proteins. **Results:** We characterised 61 vomeronasal type 2 receptor genes in *Mus musculus*. T2R gene family was found to be highly conserved in this study by using Weblogo tool. Also, a broad view of GABA inhibitory taste buds was observed. It evolved at the level of eukaryotes. The T2R is involved mainly in taste sensation. We also see protein-protein interaction using string database. **Conclusions:** The basic repertoire of T2R genes seems to be larger for most of the species including mouse and gene duplication still plays a role in lineage-specific increases in diversity. T2R gene family is very ancient, has high duplicability suggesting its essentiality as well as high protein interaction for TsR2 gene.

INTRODUCTION

Receptors are primary structures found within or on the surface of cells that bind to particular substances and have particular effects on cells. In essence, it is the process through which bodily signals are received. These are simply the chemicals that may be entrenched in a cell's cytoplasm or present on its cell membrane [1]. Chemical inputs from the environment are translated by taste receptor cells, which then communicate this information to the neurological system. A connection between receptor signalling and membrane potential in taste cells always follows taste transduction [2]. The metabotropic glutamate receptor (mGluR), extracellular calcium sensing receptor (CaSR), and GABA-B receptors are all members of the GPCR "C family," which also contains T2R receptors [3]. The major criteria for ligand binding are found in the lengthy N-terminal extracellular domain of members of this receptor family [4, 5]. A total of 60 T2R genes are encoded by the mouse genome [6]; these receptors are expressed in the subclass of Gαo-expressing neurons in a manner that complements T1R/Gαi expression [7, 8]. Due to their closely comparable genomes, mice are most frequently utilized as model organisms to research human disease [9]. It is not only challenging but also impossible to analyse the human genome in the same manner as we do *Mus musculus*. *Mus musculus* has been worthwhile due to these outstanding traits, which have led to significant advancements in the domains of developmental biology, cancer, toxicology, reproductive studies, teratology, genetics, neuroscience, environmental sciences, and stem cell research. Because its genome is fully sequenced, well-established, readily

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observable, and testable, it is frequently employed as an experimental model. It is unclear how bitter taste perception has changed during mammalian evolution. A genetic method of answering this question is now possible because to recent discovery of the bitter taste receptor (T2R) genes. According to estimates, there are between 36 and 41 mouse and rat T2R genes spread over 3 chromosomes. On mouse chromosome 6, known bitter loci are home to all but two T2R genes. T2R genes are found in clusters on chromosomes in both humans and mice, and they are genetically connected to loci that are involved in reactions to diverse bitter chemicals [10]. The discovered human T2R genes are homologous to mouse chromosome 6 and 15 as they are located on chromosome 7q31, 5p15 and 12p13 [7, 11]. *Mus musculus* T2R genes, however, have not been thoroughly investigated and characterized. In the current investigation, we searched T2R-related sequences from commercial and public databases and looked at the expression and operation of novel and orphaned T2Rs in the tissues and cells of *Mus musculus.*

METHODS

Orthologs of Taste Receptors Class 2 genes were searched by using EggNOG version 2.0 and TR2 receptor genes were found in 15 species of vertebrates. Multiple sequence alignment was performed for subsequent computational analysis. PubMed was used for literature survey. Phylogenetic evolution of Taste receptor in Mouse [*Mus musculus*] was studied and the receptor genes were found in species that were more closely related to it. All this was done on Pub Med. Several databases were used for the evaluation of Taste Receptors Class 2 genes including its phylogeny, evolution, protein interaction with other receptors of taste, characterization of genes, also gene expression of mouse taste receptors, its sequence and overall data available concerning mouse T2Rs. EggNog version 2.0 (http://eggnog.embl.de/version_2/) was used to get the multiple sequence alignment and to get information about duplicability of T2R genes [12]. Phylogenetic tree was constructed by using MAFFT online version 6.0.(http://mafft.cbrc.jp/alignment/software/). For this purpose, first of all sequences of all the proteins including ingroups, outgroups and candidate proteins were pasted in FASTA format in the query box. Then Phylogenetic tree was obtained and NEWICK format was also obtained which was used to make tree in ITOL database to make phylogenetic tree again to compare the results. Interactive Tree of Life (ITOL)(http://itol.embl.de/) is an online tool for the expression and manipulation of phylogenetic trees. All the sequences in NEWICK format were pasted in the query box and were uploaded. A tree was constructed. Weblogo version 3.I (http://weblogo.berkeley.edu/logo.cgi) was used to create sequence logos. Multiple Sequence Alignment of all the sequences of ingroups, outgroups and candidate proteins taken from MAFFT was pasted in the query box to get the Weblogo results. Weblogo gives an idea of the conserved amino acid sequences. NCBI protein database was used to get the protein sequences. Literature survey was performed using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/). Pathways analysis was performed by KEGG Pathway. KEGG Pathway mapping is the process to map molecular datasets, especially large-scale datasets in genomics, transcriptomics, proteomics, and metabolomics, to the KEGG pathway maps for biological interpretation of higher-level systemic functions. STRING was used for protein-protein interaction analysis.

RESULTS

We characterized the genes by using eggNOG and found 47 T2R genes which is approximately in accordance with the previous studies (Figure 1). We also searched T2R genes in 3 more species (*Oryctolagus cuniculus*, *Xenopus laevis* and *Xenopus tropicalis*). We took only few representative T2R genes from other species as ingroup for comparison. T2R was taken as outgroup from 4 different species (*Mus Musculus*, *Danio rerio*, *Xenopus slurana tropicalis* and *Oryctolagus cuniculus*). By using eggNOG version 2.0, it was revealed that T2R has 47 proteins in 44 species which means that multiple genes for this receptor are present in each of these specie which are encoding several proteins. So, the gene for this receptor is duplicable. BLAST results of T2R also confirm that its gene is duplicable and has multiple copies in each species. We found 47 proteins in 44 species.

Figure 1: Evolution of T2R Gene

Figure 2 showed 47 mouse genes forms a cluster with common origin. These genes may have species-specific activities that are different from those of other groups since they are most likely the result of duplications that occurred after the primate-rodent separation. Data also imply that other mammals, besides mammals, had species-specific duplications.

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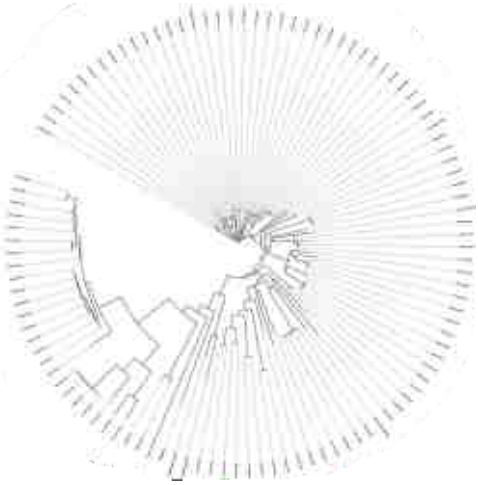

Figure 2: Phylogenetic tree (circular) showing T2R in Mus Musculus

Tree formed by KEGG is shown in Figure 3. The tree showed pathways how genes are involved in pathways involved in taste transductions i.e., salty, sweet, bitter, sour etc. T2R genes are involved in bitter taste transduction. Their activation transduces calcium signalling pathway that indirectly activates TRPM5 which releases cation that led to bitter taste feeling(Figure 3).

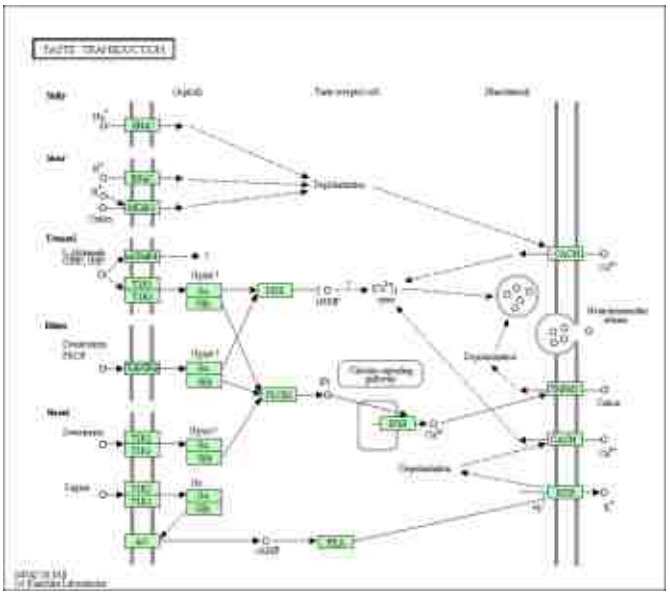

Figure 3: Tree formed by KEGG Pathways

A member of T2R gene family was chosen for protein interaction analysis. Enrichment score threshold of 0.5 was chosen. Tas2r104 showed interactions with Tas1r2 with enrichment score: 0.624. Tas1r1 with enrichment score: 0.619. TAS1R1is involved in umami taste response and stimulus. Enrichment score for Pkd1l3 was 0.591. It may function as a sour taste receptor by joining forces with PKD1L3 to generate a calcium channel in gustatory cells, although it's in vivo role in sour taste perception is unknown and may be indirect. The enrichment score for Tas1r3 was 0.586. TAS1R2/TAS1R3 can detect a variety of artificial and natural sweeteners. The selectivity and specificity of genes can be considerably impacted by changes in sequences across and within species. Taste receptor cell gene 1 showed enrichment score of 0.552 (Figure 4).

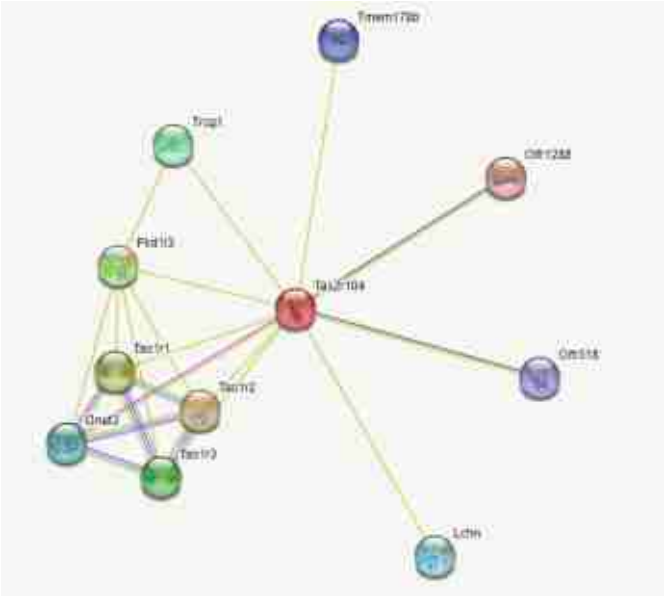

Figure 4: Protein-Protein interaction by STRING

DISCUSSION

In this investigation, utilising eggNOG version 2 to scan mouse genome sequences, we were able to identify the T2R gene repertoire in the mouse (Mus musculus), which was consistent with earlier findings [13]. Our findings offer a broad overview of the mouse T2R gene repertoire. In Mus musculus, we discovered 61 putatively functional T2R genes. The T2R family of chemical receptor genes is thought to be the most variable family of genes in fishes. Mammals exhibit a considerable degree of variety in the sizes of the T2R gene family. In comparison to humans and other primates, which lack functioning T2R genes, the mouse and rat have 61 and 57 functional T2R genes, respectively [13]. It is believed that the T2R chemical receptor family is the most varying gene family in amphibians. 55 putatively functional T2R genes have been identified in zebra fish [15]. eggNOG results (Figure 1) showed that T2R gene was evolved at the level of eukaryotes. So, it means that it is not a new gene but was present much earlier. It is present in metazoans, vertebrates and mammals too. It was not present in bacteria. Its, appearance in during evolution and presence in primitive to advance animals i.e.; from eukaryotes to mammals) suggest that its role is very basic and essential for organisms. It also gives us an idea about its conservation throughout evolution and it was confirmed by

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making sequence Logos of T2R sequences. Web Logos also confirmed out hypothesis and these sequences were found highly conserved among all the species throughout evolution. eggNOG and BLAST results also indicated that T2R gene is duplicable gene as also shown by GO *et al.*, which means that it has multiple copies within same organism [16]. If one of the gene copies is mutated then this effect will be compensated by the other gene copies present. So, the lethal effects will not occur and the organism will carry its normal functions. Table 1 show that there are many copies of T2R genes in all these species. In the phylogenetic trees, out-group has been observed as a separate branch in all the two trees (Figure 2) and has no link with the other branches which showed that T2R and T1R2 are quite different phylogenetically as shown by Ishimaru *et al.*, and it serves as a control here which confirms that our phylogenetic analysis is correct [17], otherwise T1R2 could be in between the T2R genes. In-groups were T2R genes of 5 different species i.e., *Danio rerio, Rattus norvegicus, Xenopus laevis, Oryctolagus cuniculus and Monodelphis domestica*. These ingroups were present among the T2R of *Mus musculus*. *Danio rerio* is a fish and after *Danio rerio* we can see the T2R of amphibian and then mammals (Figure 2). It showed that during evolution first T2R of fishes evolved and then amphibians and at last mammals. Genes of one class or species can be seen clustered together. Web Logo showed that the sequence of T2R proteins in all the species is highly conserved throughout evolution. It was evident from the bold and big size letters. The bigger the letter is, the more conserved it is. Receptor of the T2R protein is G-protein coupled receptor. We wanted to see that how many helices this receptor has; are these helices transmembrane, present inside or outside the cytoplasm and how many amino acid sequences it has. These all are collectively called as transmembrane topology which indicated that GPCR of T2R has 9 transmembrane helices and has approximately 850-900 amino acid sequences [18-20]. .

CONCLUSIONS

We have mentioned here a detailed analysis of the taste receptor family – the repertoire of C family GPCRs expressed in the Mus musculus taste receptor system. 92 intact genes comprise this family, which by phylogenetic analysis is distinct from other C family GPCRs. The major repertoire of T2R genes looks to be huge enough for most of the species including Mus musculus and gene duplication. All this still plays an important role in lineage-specific increases in diversity. T2R gene family is very ancient, has high duplicability suggesting its essentiality.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

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