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Characterization of Vomeronasal Receptor Class 2 in Danio rerio

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ABSTRACT

The best three distinct families of putative pheromone receptors have been identified in the vomeronasal organ (V1Rs, V2Rs and V3Rs). All are G protein-coupled receptors but are only distantly related to the receptors of the main olfactory system, highlighting their different role. **Objective:** To characterize the Vomeronasal receptor 2 gene family in Zebra Fish (*Danio rerio*). **Methods:** Extensive survey was done to choose top V2R genes. Different software and tools were used to characterize those genes including Eggnog 2.0, MAFFT, ITOL, Weblogo and SOSUI Signal. **Results:** In order to get insights into this gene family in Zebra fish, we performed an extensive survey of V2R derived datasets. We identified 62 genes distributed among *Danio rerio* encoding putative vomeronasal proteins. V2R gene family was found to be highly conserved in this study by using Weblogo. It evolved at the level of eukaryotes. The V2R is involved mainly in olfaction. **Conclusions:** The basic repertoire of V2R genes seems to be larger for most of the species including *Danio rerio* and gene duplication still plays a role in lineage-specific increases in diversity. V2R gene family is very ancient, has high duplicability suggesting its essentiality.

INTRODUCTION

Evolution of pheromones have been seen in all phylum of animals to indicate sex and dominance position and to explain traditional social and sexual behaviour among individuals of the same species. The vomeronasal organ (VNO), a chemosensory organ situated at the base of the nasal septum, is thought to be the primary mechanism in mammals that detects these chemical signals. Different pheromones or odorant molecules stimulate olfaction in vertebrates, and the main sensory neurons in the olfactory epithelium recognize these molecules through receptors they have expressed [1]. The olfactory epithelium of fish has been demonstrated to express receptors from the C family of GPCRs [2, 3]. The solitary olfactory organ of the fish has a subpopulation of microvillous sensory neurons that express members of the olfactory C family GPCRs, in contrast to ciliated sensory neurons that express members of the OR family of odorant receptors [4, 5]. Significantly, two orthologous receptors from the zebrafish and goldfish, designated as receptor 5.24 and receptor ZO6, respectively, are activated by amino acids [5, 6], which are strong food signals in fish [7, 8]. These findings suggest that the olfactory C family GPCRs could function as a family of amino acid-sensing receptors in teleost fish. Olfaction is essential to vertebrates' daily activities, including prey identification, predator avoidance, mating, and territoriality [9]. The main olfactory system (MOS) and the vomeronasal system (VNS) are the two separate nasal olfactory systems found in the majority of terrestrial animals [10, 11]. The metabotropic glutamate receptors (mGluR), extracellular calcium sensing receptors (CaSR), and GABA-B receptors are all members of the GPCR "C family," which also contains the V2R receptors [12]. The major criteria for ligand binding are found in the long N-terminal extracellular domain of members of this receptor family [13, 14]. Around 60 V2R genes are encoded by the genomes of the mouse and rat, respectively [15]. These receptors are expressed in the subclass of Go-expressing neurons in a manner that complements V1R/Gi expression

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[16, 17]. Zebra fish, or *Danio rerio*, are frequently employed as model organisms for genetic and developmental research. Developmental biology, cancer, toxicity, reproductive studies, teratology, genetics, neuroscience, environmental sciences, stem cell and regenerative medicine, and evolution have all benefited from research on *Danio rerio*. Because of its fully sequenced genome, established genetic background, readily observable and testable developmental characteristics, availability of well-characterized mutations, quick embryogenic development, and big, robust, and transparent embryos, it is frequently employed as an experimental model. As a result, it is crucial to characterise V2R genes in *Danio rerio* and to conduct a phylogenetic and evolutionary investigation.

METHODS

Orthologs of vomeronasal receptors were searched by using eggNog version 2.0 and vomeronasal 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 vomeronasal receptor in Danio rerio was studied and the receptor genes were found in species that were more closely related to it. All this was done on PubMed. 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 V2R gene. eggNOG (stands for evolutionary genealogy of genes: Non-supervised Orthologous Groups) is a database of orthologous groups of genes. The orthologous groups are explained with functional description lines (sourced by identifying a common denominator for the genes rely on their different annotations), with functional categories (i.e., came from the original COG/KOG categories) [18]. 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. All the sequences in newick format were pasted in the query box and were uploaded. A tree was constructed by using ITOL. 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. Weblogo gives an idea of the conserved amino acid sequences. NCBI protein database was used to get the protein sequences. TopPred was used for the prediction of topology of Vomeronasal receptor. SOSUI database was used to see, if this protein is a soluble protein and signal peptide or not.

RESULTS

We have characterized the genes by using EggNog and found 62 V2R genes. We have searched V2R genes in 4 more species (Mus musculus, Oryctolagus cuniculus and Xenopus laevis and Xenopus tropicalis). We took only few representative V2R genes from other species as ingroup for comparison. T2R was taken as outgroup from 4 different species (Danio rerio, Mus musculus, Xenopus tropicalis and Oryctolagus cuniculus)(Figure 1A and Table 1) By using eggNOG version 2.0, it was revealed that V2R has 1563 proteins in 34 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. BLAT results of V2R also confirm that its gene is duplicable and has multiple copies in each species(Figure 1B).

Figure 1: (A) Evolution of V2R genes as seen by eggNOG (B) Duplicability of V2R genes

Species	V2R Genes
Danio rerio	55
Mus musculus	7
Xenopus tropicalis	4
Xenopus laevis	2
Oryctolagus cuniculus	5

Table 1: V2R Genes in different species

Figure 2 shows evolutionary analysis of V2R genes in form of a phylogenetic tree created by ITOL. V2R gene family was found to be highly conserved in this study. It evolved at the level of eukaryotes. The V2R is involved mainly in olfaction. In the phylogenetic trees, outgroup has been observed as a separate branch in all trees and has no link with the other branches which shows that V2R and T2R are quite different phylogenetically and it serves as a control here which confirms that our phylogenetic analysis is correct, otherwise T2R could be in between the V2R genes.

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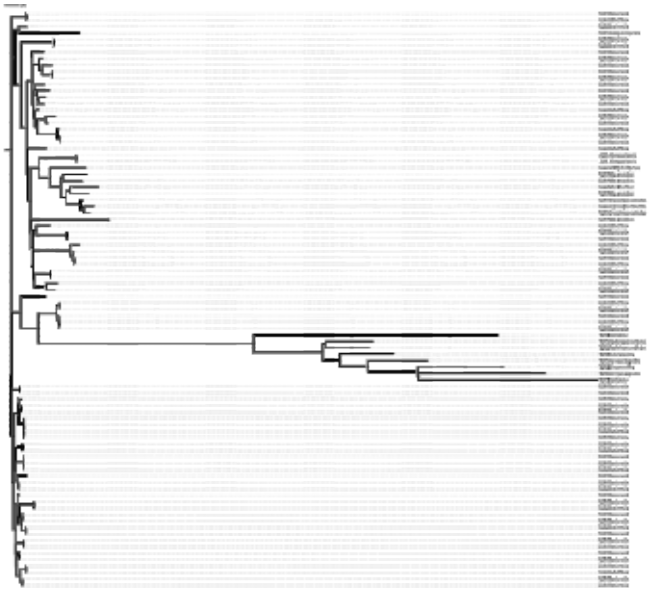

Figure 2: Phylogenetic tree showing V2R in Danio rerio (73 genes). Ingroups and outgroups are also seen here

Figure 3 shows generation of sequence Logo for V2R gene family in Danio rerio generated by Weblogo that shows highly conserved amino acid sequences in this family indicative of its conservation throughout evolution and history.

Figure 3: Generation of sequence Logo for V2R gene family in Danio rerio

Transmembrane topology of the vomeronasal receptor in Danio rerio was predicted by using the database TMHMM (Figure 4A). It showed that this receptor has 7 transmembrane domains and length of protein sequence is approximately 850-900. Exp number of AAs in TMHs is the expected number of amino acids in transmembrane helices. If this number is larger than 18 it is very likely to be a transmembrane protein (or have a signal peptide). If the whole sequence is labelled as inside or outside, the prediction is that it contains no membrane helices. Figure 4B shows another graphical diagram made by using database TOPPRED (http://mobyle.pasteur.fr) and the transmembrane topology of V2R in Danio rerio as predicted by it also indicates that 7 it contains 7 helices which start from amino acid sequence 600 till 900.

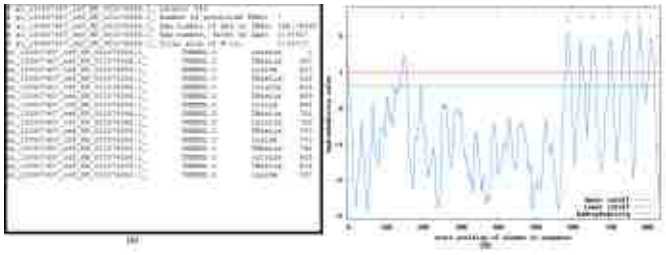

Figure 4: (A) Transmembrane domains and length of protein sequence (B) Transmembrane topology of V2R in *Danio rerio* by TopPred

SOSUI signal result showed that V2R in *Danio rerio* is a soluble protein and the amino acid has no signal peptide. Table 2 reveals the amino acid sequence, length of the amino acid sequences in each transmembrane domain, initiation and termination of each domain, type of the protein structure which is primary in all the helices.

No.	N-Terminal	Transmembrane region	C-terminal	Type	Length
1	588	ISLTTASLLGSCICSAVVVIFA	609	Primary	22
2	625	SFLLLVSLKLCFLCVLLFIGOPQ	647	Primary	23
3	659	GISFVLCISSILVKTMVVIAVFK	681	Primary	23
4	702	TVLVLTALQVVICAVWLTNA	721	Primary	20
5	746	VGFAMLLGYIGILAAVSFLLAFL	768	Primary	23
6	779	AKFITFSMLIFCAVWIAFVPAYV	801	Primary	23
7	813	IFAILASSFGLLAAIFAPKCYII	835	Primary	23

Table 2: Sequence of Amino Acid

DISCUSSION

By utilising EggNog to explore zebrafish draught genome sequences, we were able to identify the V2R gene repertoire in our work, which is consistent with the earlier findings [18]. Our findings give a broad overview of the fish V2R gene repertoire. In zebra fish, we discovered 55 putatively functioning V2R genes. The V2R family of chemical receptor genes is thought to be the most variable family of genes in fishes. The V2R gene family has a similarly wide range of sizes in animals. 61 and 57 functional V2R genes, respectively, have been reported in the mouse and rat [15], but no functional V2R genes have been discovered in humans or other primates [19, 20]. EggNog results showed that V2R 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,

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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 making sequence Logos of V2R sequences. Web Logos also confirmed out hypothesis and these sequences were found highly conserved among all the species throughout evolution. eggNOG and BLAT results also indicated that V2R gene is duplicable gene, which means that it has multiple copies within same organism. 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 shows that there are many copies of V2R genes in all these species. In the phylogenetic trees, outgroup has been observed as a separate branch in all the three trees and has no link with the other branches which shows that V2R and T2R are quite different phylogenetically and it serves as a control here which confirms that our phylogenetic analysis is correct, otherwise T2R could be in between the V2R genes. Ingroups were V2R genes of 4 different species i.e., *Xenopus laevis, Xenopus tropicalis, Mus musculus and Oryctolagus cuniculus*. These ingroups were present among the V2R of *Danio rerio. Danio rerio* is a fish and after *Danio rerio* we can see the V2R of amphibian and then mammals. It shows that during evolution first V2R of fishes evolved and then amphibians and at last mammals. Genes of one class or species can be seen clustered together. Web Logo shows that the sequences of V2R proteins in all the species is highly conserved throughout evolution. It is evident from the bold and big size letters. The bigger the letter is, the more conserved it is. Receptor of the V2R 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 V2R has 7 transmembrane helices and has approximately 850-900 amino acid sequences. This was also showed that it has 7 helices. V2R is a soluble protein with no signal peptide. Table 2 indicates length of amino acid sequences in all the helices, start and end point of each helix. Hydropathy plot of V2Rreceptor shows cross sections of 7 transmembrane helices, its hydrophobic and polar regions [18-20].

CONCLUSIONS

Here, we present a thorough examination of the OlfC receptor family, a collection of C family GPCRs expressed in the olfactory system of zebrafish. This family, which by evolutionary study differs from other C family GPCRs, consists of sixty-two complete genes.

Conflicts of Interest

The authors declare no conflict of interest.

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