The Chemosensory Receptor Repertoire of a True Shark Is Dominated by a Single Olfactory Receptor Family

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Accepted: January 6, 2019

Abstract

Throughout the animal kingdom chemical senses are one of the primary means by which organisms make sense of their environment. To achieve perception of complex chemosensory stimuli large repertoires of olfactory and gustatory receptors are employed in bony vertebrates, which are characterized by high evolutionary dynamics in receptor repertoire size and composition. However, little is known about their evolution in earlier diverging vertebrates such as cartilaginous fish, which include sharks, skates, rays, and chimeras. Recently, the olfactory repertoire of a chimera, elephant shark, was found to be curiously reduced in odorant receptor number. Elephant sharks rely heavily on electroreception to localize prey; thus, it is unclear how representative their chemosensory receptor repertoire sizes would be for cartilaginous fishes in general. Here, we have mined the genome of a true shark, Scyliorhinus canicula (catshark) for olfactory and gustatory receptors, and have performed a thorough phylogenetic study to shed light on the evolution of chemosensory receptors in cartilaginous fish. We report the presence of several gustatory receptors of the TAS1R family in catshark and elephant shark, whereas TAS2R receptors are absent. The catshark olfactory repertoire is dominated by V2R receptors, with 5–8 receptors in the other three families (OR, ORA, TAAR). Species-specific expansions are mostly limited to the V2R family. Overall, the catshark chemosensory receptor repertoires are generally similar in size to those of elephant shark, if somewhat larger, showing similar evolutionary tendencies across over 400 Myr of separate evolution between catshark and elephant shark.

Key words: catshark, Scyliorhinus canicula, chimera, phylogeny, evolution, taste receptor.

Main Text

Catshark Possess Five of the Six Major Vertebrate Chemosensory Receptor Families

Bony vertebrates exhibit four major families of olfactory receptors (OR, TAAR, ORA, V2R) and two gustatory GPCR families, TAS1R and TAS2R (Bachmanov and Beauchamp 2007). We have performed a recursive search in the preliminary draft genome of catshark, Scyliorhinus canicula to delineate its complete chemosensory receptor repertoire, using representative protein sequences from all six families in several species as initial queries. Phylogenetic analysis was performed using a maximum likelihood approach, for details see Materials and Methods.

For all OR families catshark as well as elephant shark genes could be identified, with the exception of T2R receptors. Since the closely related ORA receptors were present, it is unlikely that t2r genes were not found for technical reasons. We conclude that T2R receptors are absent in both species, and possibly in all cartilaginous fish, for elephant shark consistent with earlier observations (Grus and Zhang 2009).

Our analysis identified between 5 and 40 receptors per chemosensory receptor family in catshark (table 1). Additionally, we found some new TAARs, ORs, V2Rs, and TAS1R gene sequences in the elephant shark genome beyond those previously published (Grus and Zhang 2009; Nimura 2009b; Venkatesh et al. 2014). In total, the catshark...
The Catshark Chemosensory Receptor Repertoire Is Dominated by V2Rs

In bony fish and tetrapods ORs constitute the dominant chemosensory family (Niimura and Nei 2006). It had therefore been surprising, when only six or genes were reported in the elephant shark genome (Venkatesh et al. 2014), but it had been unclear, how representative this reduced repertoire was for cartilaginous fish in general and true sharks in particular. Here, we report one additional gene in elephant shark (table 1). This is considerably less than even the sea lamprey OR repertoire sizes (Niimura and Nei 2006) suggesting that gene birth events are comparatively rare in jawless and cartilaginous fish chemosensory receptor families compared with the bony fish lineage, and in particular its tetrapod branch.

Two to Three Gustatory tas1r Genes Present in the MRCA of Cartilaginous and Bony Vertebrates

The gustatory tas1r genes are close relatives of the olfactory v2r genes and, like these, belong to class C GPCRs, which are characterized by a large, extracellular N-terminus and a characteristic six exon structure (Sainz et al. 2001). The mammalian taste receptor 1 (TAS1R) family is best understood. It comprises three members TAS1R1, TAS1R2, and TAS1R3 (Voigt et al. 2012), which hetero-oligomerize to TAS1R1/TAS1R3 and TAS1R2/TAS1R3, functioning as umami and sweet taste receptor, respectively (Zhao et al. 2003). Teleost fish possess the direct orthologs of TAS1R1 and TAS1R3, but jawless fish (Libants et al. 2009), thus the origin of the family appears to be in the most recent common ancestor (MRCA) of jawed fish.

Phylogenetic analysis shows a small subgroup of catshark and elephant shark v2r genes orthologous to zebrafish V2R-like OlfCa1 and OlfCb1 (fig. 1). We therefore suggest to name these genes as v2rl, V2R-like. The v2rl subgroup is most closely related to type 1 taste receptors, TAS1Rs, from which they segregate with maximal branch support (fig. 1a). We report five such genes for catshark and three for elephant shark (fig. 1b). The maximal branch support within the V2RL clade allows the deduction of two ancestral v2rl genes already in the MRCA of cartilaginous and bony fish. Subsequently, small gene expansions specific to the cartilaginous lineage generated the extant v2rl gene numbers, which are considerably larger than present in zebrafish (two genes, olfCa1, olfCb1) and mammals (one gene, gprc6).

The main group of V2Rs is most closely related to the calcium-sensing receptor (CaSR), from which it segregates with maximal branch support (fig. 1a). There are 35 catshark genes in this group and nearly the same number (34) of elephant shark genes (table 1). Interestingly these numbers are reached by several species-specific gene duplications generating subclades of up to 7 catshark and 12 elephant shark genes, which just happen to result in a very similar total number. In several cases, direct orthologs of catshark and elephant shark V2Rs are observed, for example, V2R2 (fig. 2).

The most basal gene, Sc-V2R1, Cm-V2R1, is orthologous to zebrafish OlfCc1 and the mammalian V2R2 subfamily (fig. 2). It may serve as coreceptor in zebrafish and mouse (Martini et al. 2001; DeMaria et al. 2013) and it will be interesting to investigate, whether such a function might also be conserved in cartilaginous fish. The second most basal gene, Sc-V2R2, Cm-V2R2, is orthologous to all remaining mouse v2r genes (a single clade), but appears to have been lost in zebrafish (fig. 2). The remaining catshark/elephant gene expansion is intermingled with six zebrafish clades comprising 1–19 olfC genes, suggesting a similar number of ancestral v2rl genes in the MRCA of cartilaginous and bony fish, all of which appear to have been lost in tetrapods (fig. 2).

## Table 1

| Gene Family | No. of Genes in Catshark | No. of Genes in Elephant Shark | No. of Genes in Mouse | No. of Genes in Zebrafish |
|-------------|--------------------------|---------------------------------|------------------------|---------------------------|
| OR          | 8 (1)                    | 7                              | 1037                   | 154                       |
| TAA         | 5                       | 5                              | 15                     | 112                       |
| ORAV1R      | 6                       | 4                              | 211                    | 7                         |
| V2R         | 35                      | 34                             | 121                    | 58                       |
| V2RL        | 5                       | 3                              | 0                      | 2                         |
| TAS1R       | 6                       | 4                              | 3                      | 4                         |
| T2R         | 0                       | 0                              | 33                     | 4                         |

Note: —Total gene numbers are given, in parentheses the number of pseudogenes.

aAdditional genes identified (elephant shark, three TAA, four TAS1R, one OR, two V2R, two V2RL) compared with previously published numbers (Niimura 2009b; Venkatesh et al. 2014).

bRefer to genes newly identified here (catshark). Superscripts refer to

aNiimura (2009a).

bHussain et al. (2009).

cSaraiva and Korsching (2007).

dHussain et al. (2009).

eSaraiva and Korsching (2007).

fAhuja et al. (2018).

gAlioto and Ngai (2006).
Fig. 1.—Two to three ancestral \textit{tas1r} genes already present in the MRCA of cartilaginous and bony vertebrates. Phylogenetic tree of TAS1Rs (orange), V2RLs (grey) and V2Rs of catshark and elephant shark. CaSR and TAS1Rs as outgroups for V2Rs and V2RLs respectively. (a) All gene groups are shown in collapsed representation to emphasize the basal nodes. Note that \textit{v2rl} genes are the sister group to TAS1Rs and CaSR represents the sister group for the main group of V2Rs. The phylogenetic tree was generated using a maximum likelihood method (PhyML-aLRT) with SPR setting for tree optimization and chi square-based aLRT for branch support (given as percentage). Note the maximal branch support for all nodes. (b) The TAS1R and V2RL node of (a) shown in detail. Branch support shown as percentage. Branches are color-coded for catshark (red) and elephant shark (blue) along with zebrafish (yellow), mouse (brown), spotted gar (cyan), and \textit{Latimeria} (orange). Transparent grey circles denote the clades corresponding to the three predicted ancestral \textit{tas1r} genes in the MRCA of cartilaginous and bony vertebrates. Two new \textit{v2rl} genes were found in elephant shark (purple spades).

Fig. 2.—The catshark chemosensory receptor repertoire is dominated by V2Rs. Largest family of V2Rs in catshark comprising of thirty-five genes in catshark (red) were compared with thirty-four elephant shark V2Rs (blue) along with zebrafish (yellow), and mouse (brown). The most basal gene, Sc-V2R1, Cm-V2R1, is orthologous to zebrafish OlfCc1 and the mammalian V2R2 subfamily. The phylogenetic tree was generated as described in figure 1 and branch support is given as percentage. Sequences were named according to named orthologs or closest paralogs from other species otherwise according to phylogenetic relationship. Sequences are named \textit{a}, \textit{b} where exon 3 and exon 6 might be derived from the same gene. New genes in elephant shark are marked with purple spades.

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have expanded TAS1R2 to 2–3 genes (Ishimaru et al. 2005). Interestingly, the TAS1R2/TAS1R3 hetero-oligomers of teleosts also react to amino acids, not to sugars like their mammalian counterparts (Oike et al. 2007). In Latimeria, one TAS1R1, two TAS1R2, and two TAS1R3 have been described (Picone et al. 2014). The evolutionary relationships of these genes are not clear so far, because the TAS1R repertoire of earlier-diverging species has not been available so far.

Here, we identified in total six tas1r genes in the catshark genome, and four in the elephant shark. We assume these numbers to be final for both catshark and elephant shark, because the current genomic coverage is 200× and 19.25×, respectively (Wyffels et al. 2014). All elephant shark tas1r genes possess orthologs in catshark. Interestingly, one of the catshark tas1r genes, Sc-TAS1R3, appears to have been lost in elephant shark. Furthermore, catshark TAS1R7 and TAS1R8 appear to result from a gene duplication within the true shark lineage, because elephant shark has a single gene, TAS1R7, in this subnode. All confirmed TAS1R candidates show the characteristic exon structure (data not shown), although due to the preliminary nature of the genomic assembly not all six exons could be identified in each case.

The phylogeny shown here allows some conclusions concerning the origin and relationship of mammalian and teleost TAS1R receptors. Mouse and zebrafish TAS1R3 possess a direct ortholog in catshark, Sc-TAS1R3 (fig. 1b), suggesting this gene to be already present in the MRCA of cartilaginous and bony vertebrates, whereas TAS1R1 and TAS1R2 appear to have originated in a duplication event within the bony lineage (fig. 1b). In the 420 Myr since divergence of chimeras and true sharks (Heinicke et al. 2009) the evolutionary dynamic has been very small (three gene birth event in catshark, two in elephant shark, all except one in the MRCA of true sharks and chimeras), which parallels the slow evolution of this family in bony fish and tetrapods. This is very different from the evolutionary history of the closely related V2Rs, which often exhibit species-specific repertoires (Hashiguchi and Nishida 2006). The intermingling of cartilaginous fish TAS1Rs with bony fish TAS1Rs in the phylogenetic tree (fig. 1b) allows to estimate the number of ancestral TAS1Rs in the MRCA of cartilaginous and bony vertebrates. The most parsimonious explanation of the observed tree assumes a gene loss event for bony fish in the Sc-TAS1R7, eight subclade, which results in a prediction of three ancestral tas1r genes in the MRCA of cartilaginous and bony vertebrates (fig. 1). The origin of the TAS1R family cannot be exactly deduced, but should have happened within the jawed lineage, since TAS1Rs were not found in lamprey (Grus and Zhang 2009).

Small Repertoires for OR, TAAR, and ORA Receptor Families in Catshark and Elephant Shark

OR genes are the largest gene family in bony vertebrates (Niimura and Nei 2006), but have only undergone very limited gene expansion in cartilaginous fish (table 1; fig. 3). In mammals, class I and class II ORs have been distinguished, with class I orthologous to a zebrafish subfamily of five genes, and class II possessing a single zebrafish ortholog, Dr3OR5.4. Both classes exhibit a single catshark ortholog gene, Sc-OR1 and Sc-OR2, respectively (fig. 3) suggesting the origin of these two genes in the MRCA of cartilaginous and bony fish. Three more zebrafish genes or subclades are orthologous to a catshark and/or elephant shark gene, suggesting in total the presence of at least five or genes in the MRCA of cartilaginous and bony fish, of which elephant shark appears to have lost two genes and catshark one. Thirty-two putatively functional OR genes were identified from the sea lamprey genome (Niimura 2009b), whereas in elephant shark we identified seven ORs (Cm-OR1 and Cm-OR3-8). Previously in elephant shark or8 and or1 gene have been reported as real ORs but others, or3-7 as nonORs (Niimura and Nei 2006; Venkatesh et al. 2014). Four elephant shark or genes have a direct ortholog in catshark, that is, for these four gene pairs not a single gene birth or death event happened in the last 420 Myr (Heinicke et al. 2009) another gene is a singleton in elephant shark (Cm-OR7), but has undergone a single duplication in catshark, resulting in Sc-OR7, Sc-OR8 (fig. 3). Overall the evolutionary dynamics of the OR family appear to be extremely limited in cartilaginous fish, in stark contrast to the very dynamic evolution in bony vertebrates.

The TAAR family is large in teleost fish, of medium size in tetrapods, and was reported as just two genes in elephant shark, based on analysis of an initial assembly (Hussain et al. 2009). We found five taar genes for elephant shark (Cm-Taar1a-Cm-Taar4) and report a similar size of five genes for catshark (Sc-Taar1a-Sc-Taar4), see table 1. Figure 4 shows the phylogeny of representative TAARs from zebrafish, mouse, frog, catshark, and elephant shark. This RphOgene clusters TAAR into three monophyletic groups. The most basal group tar1b and tar4 genes is clearly clustered separately from others. However, two of these genes in catshark (tar1 3 and tar4 4) and one in elephant shark (tar1 4), do not exhibit the characteristic TAAR motif present in TM7 (Hussain et al. 2009). Since they are a sister group to the validated taar genes (taar1a-1b), which do possess the motif, we refer to them as taar-like genes (tar1). There is a clear ortholog relationship between cartilaginous taar1 and teleost taar1 genes. taar 3-4 genes of sharks are more similar to vertebrate taar 2-4. This could point to the retention of ancestral characteristics by taar2-4.

The V1R/ORA gene family also shows opposing evolutionary characteristics in tetrapods versus teleosts. Here, the tetrapod families can be very large, but the teleost family is highly conserved, with 6–7 genes in many species (Zapilko and Korsching 2016). We identified six ora genes in catshark, and confirmed four ORAs for elephant shark (table 1). This conforms to the general tendency for catshark receptor repertoires to be somewhat larger than those of elephant shark.
Nevertheless, catshark has lost one of the genes present in elephant shark, ORA1, consistent with both gene birth and gene death events sculpting the ORA repertoire in catshark. Interestingly, this is the gene giving rise to all of tetrapod ORAs (fig. 5). The remaining three elephant shark genes possess direct orthologs in catshark, whose different terminal branch lengths suggest individually different evolutionary rates for these three genes (fig. 5). All of these genes are lost in tetrapods, with the exception of a single Xenopus gene, ORA15. Furthermore, we identified three additional ora genes in catshark that cluster with teleost ora5 and ora6. The absence of such genes in lamprey (Grus and Zhang 2009) and elephant shark (Venkatesh et al. 2014), confirmed here, had raised doubts as to the evolutionary origin of ORA5-6 compared with ORA1-4, whose orthologs are present in elephant shark. Now it can be concluded that the ancestral gene of the ORA5/6 clade was already present in the MRCA of cartilaginous fish and bony fish.

Taken together, all three families (OR, TAAR, ORA) show only minor gene birth and death events in a shark and a chimeric species, in stark contrast to the evolutionary dynamics of these families in bony vertebrates. Thus, in sharks these three
receptor families seem to play a much reduced role in olfaction as compared with bony vertebrates. In contrast, the shark V2R family exhibits extensive gene birth events very similar to the evolutionary characteristics of V2Rs in bony vertebrates, consistent with the hypothesis that odor detection in both true sharks and chimeras depends heavily on the V2R family.

Thus, one may expect odor detection via V2Rs to help in food localization. The large evolutionary divergence of 420 Myr notwithstanding, both catshark and elephant shark are benthic predators of small invertebrates (Cox and Francis 1997; Valls et al. 2011), consistent with an important role of V2Rs in prey detection.

The olfactory organ of elephant shark has not been described so far, and together with the known specialization in electroception of this species (Didier 1995; Lisney 2010) this raised doubts how representative the OR repertoire of this species might be. Catshark, on the other hand, exhibit a complex olfactory organ (Theisen et al. 1986) and do not appear as specialized for electroception as elephant shark. The overall similarity of the chemosensory repertoires of catshark and elephant shark we describe here suggests now that the elephant shark repertoire is no outlier. The slightly larger chemosensory receptor repertoire of catshark is consistent with a somewhat larger dependence on olfaction for catshark.

Materials and Methods

In order to delineate the olfactory and gustatory genes, scaffolds (sf., see supplementary data set S1, Supplementary Material online) from the draft of the catshark genome (to be published elsewhere) and recent elephant shark genome (Venkatesh et al. 2014) were obtained by genome-wide searches using TBlastN with the representative TAS1R, TAS2R, OR, TAAR, ORA, and V2R sequences from mouse, frog, elephant shark, Latimeria and zebrafish as queries, and recursively in follow-up searches. For TAS1R phylogeny we additionally searched for spotted gar sequences. Homology regions above 200 amino acid length were considered further. Splicing predictions were made by comparing related protein sequences to genomic DNA sequences with the online-tool GeneWise (Birney et al. 2004). Sequence data used in this article are included in supplementary file (data set S2), Supplementary Material online. Sequences were aligned with MAFFT7 (Katoh and Standley 2013) an online version of the multiple alignment tool MAFFT (Katoh et al. 2002) using the E-INS-I strategy with the default parameters. Clustal Omega (Sievers et al. 2011) was also used for alignment. The multiple sequence alignment was edited using Gap Strip Squeeze to remove regions with gaps in over 90% of sequences (https://www.hiv.lanl.gov/content/sequence/GAPSTREEZE/gap.html, last accessed January 22, 2019).

The phylogenetic trees were calculated using a Maximum likelihood algorithm, PhyML-aLRT with SPR setting for tree optimization and chi square-based aLRT (Guindon et al. 2010) for branch support on Phylogen server available online (Sanchez et al. 2011). Branch support above 80% was considered significant. TAS1R, CasR, nonOR rhodopsin-like GPCR genes, htr, and T2Rs of zebrafish, mouse, human and latimeria served as outgroups for V2RL, V2R, OR, TAAR, ORA, and V2R sequences from mouse, frog, elephant shark, Latimeria and zebrafish as queries, and recursively in follow-up searches. For TAS1R phylogeny we additionally searched for spotted gar sequences. Homology regions above 200 amino acid length were considered further. Splicing predictions were made by comparing related protein sequences to genomic DNA sequences with the online-tool GeneWise (Birney et al. 2004). Sequence data used in this article are included in supplementary file (data set S2), Supplementary Material online. Sequences were aligned with MAFFT7 (Katoh and Standley 2013) an online version of the multiple alignment tool MAFFT (Katoh et al. 2002) using the E-INS-I strategy with the default parameters. Clustal Omega (Sievers et al. 2011) was also used for alignment. The multiple sequence alignment was edited using Gap Strip Squeeze to remove regions with gaps in over 90% of sequences (https://www.hiv.lanl.gov/content/sequence/GAPSTREEZE/gap.html, last accessed January 22, 2019).

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set S3), Supplementary Material online. Trees were drawn using FigTree (http://tree.bio.ed.ac.uk/software/figtree/, last accessed January 22, 2019). Newly predicted genes were named according to previously named orthologs or closest paralogs from other species, starting with more basal genes. Gene with one or more stop codons was labelled as pseudogene. One or gene may either represent pseudogenes or databank inaccuracies due to the preliminary assembly (fig. 2, supplementary data set 1, Supplementary Material online). Fifteen genes are full or nearly full length (above 700 aa), three are partial (between 550 and 700 aa), fifteen and nine sequences were restricted to one of the large exons (exon 3 and exon 6, respectively). In those cases, where exon 3 and exon 6 might be derived from the same gene, we distinguished with a letter, for example, Sc-V2R2a and Sc-V2R2b.

**Supplementary Material**

Supplementary data are available at Genome Biology and Evolution online.

**Acknowledgments**

We thank the Genoscope-Centre National de Séquençage (Evry, France) for sequencing the catshark draft genome used in this work, Hélène Mayeur (UMR 7232, Observatoire Océanologique, Banyuls sur Mer, France) and Jean-Marc Aury (Genoscope) for assembling these data. This work was supported by the German Science foundation (grant KO 1046/7-1 to S.I.K.).

**Author Contributions**

The experiments were conceived by S.F., S.M., and S.I.K., designed and performed by K.S. and A.S.S. Illustrations were drafted by K.S. and S.I.K. Data analysis was done by K.S., A.S.S., and K.S., S.F. and S.I.K. wrote the paper.

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Associate editor: Martin Embley