The dopamine receptor D_5 gene shows signs of independent erosion in toothed and baleen whales

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Abstract

To compare gene *loci* considering a phylogenetic framework is a promising approach to uncover the genetic basis of human diseases. Imbalance of dopaminergic systems is suspected to underlie some emerging neurological disorders. The physiological functions of dopamine are transduced via G-protein-coupled receptors, including DRD_5 which displays a relatively higher affinity towards dopamine. Importantly, DRD_5 knockout mice are hypertense, a condition emerging from an increase in sympathetic tone. We investigated the evolution of DRD_5, a high affinity receptor for dopamine, in mammals. Surprisingly, among 124 investigated mammalian genomes, we found that Cetacea lineages (Mysticeti and Odontoceti) have independently lost this gene, as well as the burrowing *Chrysochloris asiatica (*Cape golden mole). We suggest that DRD_5 inactivation parallels hypoxia-induced adaptations, such as peripheral vasoconstriction required for deep-diving in Cetacea, in accordance with the convergent evolution of vasoconstrictor genes in hypoxia-exposed animals. Our findings indicate that Cetacea are natural knockouts for DRD_5 and might offer valuable insights into the mechanisms of some forms of vasoconstriction responses and hypertension in humans.

Introduction

Dopamine is a neurotransmitter essential for brain function, regulating various physiological processes including locomotion, cognition, and neuroendocrine functions (Hollon et al., 2002; Ott & Nieder, 2018). Dopamine molecular actions are transduced *via* a specific group of G-protein coupled receptors entailing two major classes: DRD_1-like and DRD_2-like receptors (Beaulieu & Gainetdinov, 2011; Opazo et al., 2018). While DRD_1-like receptors stimulate cAMP production postsynaptically, DRD_2-like receptors inhibit cAMP production both pre and postsynaptically (Beaulieu & Gainetdinov, 2011). The genomic structure of the underlying genes is also distinct, with DRD_1-like receptors yielding single exon coding regions (Beaulieu & Gainetdinov, 2011). DRD_{1-2} receptor classes have diversified in vertebrate evolution most likely as a result of genome duplications (Opazo et al., 2018). Interestingly, agonist and antagonist amino acid site conservation suggests evolutionary stasis of dopaminergic pathways (Opazo et al., 2018). Among DRD_1-like receptors, the DRD_5 subtype displays distinctive features, namely a relatively higher affinity towards dopamine, a putative agonist-independent activity and low level, yet widespread, brain expression (Beaulieu & Gainetdinov, 2011; Ciliax et al., 2000; Sunahara et al., 1991; Tiberi & Caron, 1994). Nonetheless, the DRD_5 seems to display distinct regional and cellular distribution patterns in the brain, when compared to the DRD_1 and DRD_2 subtypes, with protein enrichment detected in the cerebral cortex, hippocampus and basal ganglia (Ariano et al., 1997; Ciliax et al., 2000). Peripheral expression has also been found in the adrenals (Dahmer & Senogles, 1996), kidney (Sanada et al., 2000) and gastrointestinal tract (Mezey et al., 1996). Despite the association with schizophrenia (Muir et al., 2001), attention-deficit/hyperactive disorder (Daly et al., 1999) and substance abuse (Vanyukov et al., 1998), gene targeting studies revealed that DRD_5 knock-out mice develop hypertension, showing increased blood pressure from 3 months of age (Hollon et al., 2002). This hypertensive phenotype appears to result from a central nervous system defect, leading to an increase in sympathetic tone and, consequently, vasoconstriction (Hollon et al., 2002). Besides neuronal impairment, DRD_5 disruption was also suggested to increase the expression of the prohypertensive Angiotensin II Type 1 Receptor (AT_1R), involved in renal salt balance, blood pressure and vasoconstriction (Li et al., 2008). In fact, renal dopamine was suggested to promote salt excretion, counter-regulating AT_1R and lowering hypertensive states (Hong et al., 2017; Li et al., 2008; Zeng et al., 2005).

Whole genome sequencing has greatly expanded our capacity to comprehend evolutionary history, the role of adaptation or the basis for phenotype differences across the tree of life. Multi-genome comparisons have also been powerful to recognize the molecular basis of human diseases, a field named as phylomedicine (Emerling et al., 2017; Kumar et al., 2011; Miller & Kumar, 2001; Springer & Murphy, 2007). Here we investigate the evolution of DRD_5 in mammalian species. By analyzing 124 genomes covering 16 orders, we show that independent coding-debilitating mutations occurred in the ancestors of Mysticeti, of *Physeter catodon* (sperm whale) and the remaining Odontoceti, strongly suggesting that DRD_5 is non-coding in Cetacea. Reductive episodes have been widely documented across the tree of life contributing to organismal divergence and physiological and morphological adaptation to environmental cues (Albalat & Canestro, 2016; Braun, 2003; Jeffery, 2009; Olson, 1999). In agreement, gene loss mechanisms seem pervasive in lineages that endured drastic habitat transitions in the course of evolution, such as Cetacea, entailing niche-specific adaptations (Huelsmann et al., 2019; Lachner et al., 2017; Lopes-Marques et al., 2019b; McGowen et al., 2014; Nery et al., 2014; Sharma et al., 2018; Strasser et al., 2015). Thus, our findings suggest that these species are natural KOs for this dopamine receptor and might offer valuable insights into the mechanisms of some forms of essential hypertension.

Materials & Methods

To manually infer the coding status of DRD_5 genes, the following strategy was used: first, gene orthology was assessed *via* synteny analysis, to clarify cases where gene annotation was not found, as well as to define genomic regions to be posteriorly collected for gene annotation (see supplementary figure 1). Next, open reading frame (ORF) manual annotation was performed to detect eventual ORF-abolishing mutations. At least one mutation being posteriorly validated using unassembled genomic sequencing reads available at the NCBI (National Center of Biotechnology Information) Sequence Read Archive (SRA) database. Finally, dN/dS analyses were also conducted to further investigate the inactive status of DRD_5 in Cetacea (see below).

Synteny analysis

To build the synteny maps for the DRD_5 gene *locus* in Cetacea and *Hippopotamus amphibius* (hippopotamus) several annotated Cetacea genome assemblies were inspected and

scrutinised using the NCBI browser, namely *Orcinus orca* (killer whale; GCF_000331955.2), *Lagenorhynchus obliquidens* (Pacific white-sided dolphin; GCF_003676395.1), *Tursiops truncatus* (common bottlenose dolphin; GCF_001922835.1), *Delphinapterus leucas* (beluga whale; GCF_002288925.1), *Neophocaena asiaeorientalis asiaeorientalis* (Yangtze finless porpoise; GCF_003031525.1), *Lipotes vexillifer* (Yangtze River dolphin; GCF_000442215.1), *Physeter catodon* (sperm whale; GCF_002837175.1) and *Balaenoptera acutorostrata scammoni* (minke whale; GCF_000493695.1). *Bos taurus* (cattle; GCF_002263795.1), a fully terrestrial relative of extant cetaceans, was used as reference. Next, (1) in genome assemblies with annotated DRD_5, the following procedure was used: five protein-coding genes, upstream and downstream of the DRD_5 gene, and from the same strand, were collected; (2) if DRD_5 gene annotations were not present, the genomic *locus* was retrieved using *Bos taurus* (cattle) DRD_5 flanking genes as reference. The selected anchoring genes to search upstream and downstream DRD_5 neighbouring genes were CPEB2 and OTOP1, respectively. Regarding *Hippopotamus amphibius* (hippopotamus), the synteny map was built via blast searches against the assembled, fragmented and not annotated genome of the same species, available at NCBI (GCA_002995585.1). *Bos taurus* (cattle) DRD_5 flanking genes were used as reference; using the discontiguous megablast task from blastn, the best blast hit (highest alignment identity and query coverage) was retrieved and the coordinates of the alignment in the target genome carefully inspected. The *Hippopotamus amphibius* (hippopotamus) synteny map was then built by sorting the genes according to the subject alignment coordinates within genes, aligning at the same genomic scaffold.

Sequence retrieval and gene annotation

The NCBI 'low-quality protein' (LQ) tag marks RefSeq sequences that have been modified in order to correct open reading frame (ORF) indels present in the source genomic sequence, which may arise from genome assembly errors; or, in some cases correspond to real sequence modifications leading to shifts in the predicted ORF, resulting in non-conserved, or even nonsense codons. For this reason, to clarify the functional status of DRD_5 in species presenting LQ tag for this gene, the corresponding genomic regions were directly collected from NCBI.

Concerning species with no annotated DRD_5 genes two scenarios were identified: (1) annotated genomes excluding DRD_5 gene annotation and (2) un-annotated genomes. Regarding

the first case (i.e. the cetaceans *Lipotes vexillifer,* the Yangtze River dolphin and *Balaenoptera acutorostrata scammoni*, the minke whale, as well as other non-cetacean mammals), the DRD_5 genomic *locus* was retrieved using, as reference, annotated DRD_5 flanking genes, from phylogenetically related species (*Bos taurus* (cattle) for cetaceans, regarding non-cetacean mammals see Supplementary Table 1). If the genomic *locus* exhibited severe genomic fragmentation (presence of Ns), hindering the retrieval using neighbouring genes, blastn searches were conducted against the Whole Shotgun Contigs dataset of the corresponding species *via* discontiguous megablast task, using as query the DRD_5 coding sequence of the same reference species (Supplementary Table 1). The genomic sequence corresponding to the blast hit with the highest alignment identity and query coverage was selected.

For species with no annotated genomes (i.e. *Balaenoptera bonaerensis* (Antarctic minke whale)*, Eschrichtius robustus* (grey whale)*, Balaena mysticetus* (bowhead whale)*, Sousa chinensis* (Indo-pacific humpbacked dolphin)*,* as well as *Hippopotamus amphibius* (hippopotamus)) genomic sequences were retrieved through blastn searches in the corresponding genome assembly using the *Bos taurus* (cattle) DRD_5 coding sequence as query. For each species, the best genomic scaffold corresponded to the blast hit with the highest query coverage and identity value. Due to the presence of a fragmented genomic region in the DRD_5 gene annotation of *T. truncatus* (common bottlenose dolphin), the same blast search procedure was carried out for this species, using as target the Whole Genome Shotgun contig dataset of the same species; subsequent gene annotation was performed in the retrieved genomic contig.

Collected genomic sequences were further imported into Geneious Prime 2019 (www.geneious.com) and the DRD_5 gene coding sequences manually annotated for each species. Briefly, using the built-in map to reference tool with the highest sensibility parameter selected, the reference single-exon DRD_5 gene, 3' and 5' UTR flanked, was mapped against the corresponding genomic sequence of the in-study species and aligned regions carefully screened for ORF abolishing mutations including frameshift mutations and in-frame premature stop codons. For Cetacea and *Hippopotamus amphibius* (hippopotamus) DRD_5 gene annotation*, Bos taurus* (cattle) DRD_5 was selected as reference. Regarding non-cetacean mammals DRD_5 annotation, different references were chosen according to the phylogenetic relationships between reference and test species (Supplementary Table 1). Finally, the identified mutations were next validated using unassembled sequencing reads retrieved from at least two independent genomic

NCBI sequence read archive (SRA) projects (when available). Briefly, blastn searches were conducted against the selected SRA projects (Supplementary Materials 1, 2 and 3) using as query the nucleotide sequence containing the mutation. Blast hits were imported into Geneious Prime 2019 and mapped against the manually annotated sequences, using the built-in map to reference tool, to confirm the presence of the identified mutation.

Phylogenetic and dN/dS analyses

The predicted cetacean and *Hippopotamus amphibius* (hippopotamus), DRD_5 coding sequences, as well as the DRD_5 coding sequences of *Homo sapiens* (human) and *Bos taurus* (cattle) available at NCBI were translation aligned in Geneious Prime 2019 using the Blosum62 substitution matrix. The alignment (1440 bp) was manually inspected and predicted DRD_5 coding sequences involved in alignment suffered prior inspection, with frameshift insertions and deletions being omitted and stop codons recorded as missing. The alignment was posteriorly exported for phylogenetic analysis. A Maximum likelihood phylogenetic tree, performed in PhyML3.0 server (Guindon et al., 2010), was produced with the best sequence evolutionary model being determined using the built-in smart model selection (HKY85 +G) (Lefort et al., 2017). Node support was inferred based on 100 bootstrap replicates. The resulting phylogenetic tree newick file is available as supplementary data (Supplementary File 1) and was subsequently imported for visualisation in FigTree (Supplementary Material 4) (Rambaut & Drummond, 2012).

PAML 4.6 (Yang, 2007) was used to estimate the ratio (ω) of the nonsynonymous substitution rate (dN) to the synonymous substitution rate (dS) using the produced phylogenetic tree (Supplementary Material 4). Codeml with the branch model was implemented to estimate dN/dS ratios (ω) for seven different branch categories: Mysticeti, Odontoceti, Functional, a category corresponding to the common cetacean branch, other concerning the stem Mysticeti branch, an additional one regarding the stem Odontoceti branch (excluding *Physeter catodon,* the sperm whale) and finally, a category corresponding to the *Physeter catodon* (sperm whale) ancestor branch. Mysticeti and Odontoceti branch categories comprised all predicted Mysticeti and Odontoceti DRD_5 sequences, respectively. The Functional branch category comprised *Hippopotamus amphibius* (hippopotamus), *Bos taurus* (cattle) and *Homo sapiens* (human) DRD_5

coding sequences. For each of the seven different branch categories, likelihood ratio tests (LRT) (following χ^2 distribution) were conducted to compare the estimated ratio (ω) of the nonsynonymous substitution rate (dN) to the synonymous substitution rate (dS) determined by PAML (the alternative hypothesis), with the expected ratio (ω) of the nonsynonymous substitution rate (dN) to the synonymous substitution rate (dS) according to a neutral model of evolution (ω = 1) (the null hypothesis). All PAML analyses were run with the CodonFreq set to 3 and codon sites with ambiguous data (including gaps and missing data) were included in the analyses.

Results

To examine the annotation tags and distribution of the DRD_5 gene across mammals, 119 annotated mammalian genomes available at NCBI were scrutinized for the presence of DRD_5 gene annotation and each respective protein product description screened for the 'low-quality protein' (LQ) tag. This examination resulted in 10 species presenting the DRD_5 LQ tag, including *Ovis aries* (sheep), *Phascolarctos cinereus* (koala), *Bison bison bison* (plains bison), *Myotis davidii* (vesper bat), *Ochotona princeps* (American pika) and 5 cetacean species. The latter included *Lagenorhynchus obliquidens* (Pacific white-sided dolphin), *N. a. asiaeorientalis* (Yangtze finless porpoise), *D. leucas* (beluga whale), *Physeter catodon* (sperm whale) *and Orcinus orca* (killer whale). Each genomic sequence corresponding to the DRD_5 LQ annotations was examined and the coding sequence (CDS) manually predicted (Lopes-Marques et al., 2017). Given the prominence of DRD_5 LQ annotations in Cetacea we scrutinized other cetacean species with available, but unannotated genomes, *Balaenoptera bonaerensis* (Antarctic minke whale), *Eschrichtius robustus* (gray whale), *Balaena mysticetus* (bowhead whale), *S. chinensis* (Indo-Pacific humpback dolphin), or with annotated genomes lacking DRD_5 annotations: *Lipotes vexillifer* (Yangtze River dolphin) and *Balaenoptera acutorostrata scammoni* (minke whale) (Supplementary Figure 1). Additionally, *T. truncatus* (bottlenose dolphin), presenting a seemingly intact DRD_5 gene annotation, without the 'low-quality protein' tag (LQ), as well as *Hippopotamus amphibius* (common hippopotamus) predicted DRD_5 coding sequence, representing the closest extant lineage of Cetacea, were equally inspected. Other mammals with annotated genome without DRD_5 gene annotation were also scrutinised, namely: *Microcebus*

murinus (gray mouse lemur), *Jaculus jaculus* (lesser Egyptian jerboa), *Chrysochloris asiatica* (Cape golden mole), *Erinaceus europaeus* (western European hedgehog), *Elephantulus edwardii* (Cape elephant shrew) and *Condylura cristata* (star-nosed mole). In total, 124 mammalian species were inspected and an in-depth description regarding analysed species list and genomic sequences accession numbers are available at Supplementary Table 2. The Supplementary Figure 2 presents a multiple translation alignment of the NCBI non-LQ tagged mammalian DRD_5 orthologous sequences. The alignment also includes the predicted *Hippopotamus amphibius* (hippopotamus) DRD_5 sequence and excludes *T. truncatus* (common bottlenose dolphin) DRD_5 sequence, afterwards demonstrated to contain inactivating mutations (see below). The examined sequences exhibit a substantial degree of conservation (average pairwise identity of over 80%), with minor variation in the expected protein size. The protein sequence conservation is particularly noticeable at the c-terminus (Supplementary Figure 2).

ORF disrupting mutations of DRD_5 in Cetacea

For cetacean species with annotated genomes, we started by examining the DRD_5 gene *locus*, including neighbouring genes, to verify and elucidate the orthology of the annotated and non-annotated genes and outline the genomic regions to be inspected (Supplementary Figure 1). All analysed *loci* were found to be conserved, including in both *Lipotes vexillifer* (Yangtze River dolphin) and *Balaenoptera acutorostrata scammoni* (minke whale), which lacked previous DRD_5 gene annotations (Supplementary figure 1). Subsequent manual annotation of all collected cetacean genomic sequences revealed DRD_5 gene erosion across all analysed species, except in *Balaenoptera acutorostrata scammoni* (minke whale), for which the DRD_5 coding status could not be accessed due to fragmentation of the 5' end of the respective genomic region (presence of sequencing gaps (Ns)). In detail, a conserved 2 nucleotide deletion was detected for the full set of Odontoceti examined species, except for *Physeter catodon* (sperm whale) that presented a premature stop codon near the middle of the gene and a single nucleotide insertion close to the end of the gene (Figure 1). The 2-nucleotide deletion alters the reading frame, leading to a drastic change in downstream amino acid composition. Additionally, non-conserved mutations were found in *Lipotes vexillifer* (Yangtze River dolphin), which presented 2 premature stop codons, *N. a. asiaeorientalis* (Yangtze finless porpoise) that presented a premature stop codon close to the middle of the ORF, and *D. leucas* (beluga whale) with a single nucleotide deletion near the 5'

end of the gene (Figure 1). *D. leucas* (beluga whale) presumed DRD_5 sequence presented another noticeable feature. A massive and abrupt alignment identity decrease observed when aligning *Bos taurus* (cattle) DRD_5 gene against the genomic target region of *D. leucas* (beluga whale). The alignment identity drop was noted approximately in the middle of the complete alignment length for this species, suggesting that the DRD_5 gene sequence is interrupted, and further supporting pseudogenization in this species. Regarding Odontoceti, at least one ORF-abolishing mutation was validated using available genomic Sequence Read Archives (SRAs) experiments for all studied species, excluding *S. chinensis* (Indo-pacific humpbacked dolphin) and *Lipotes vexillifer* (Yangtze River dolphin) for which no genomic sequencing runs were available at the NCBI SRA database (Supplementary Material 1).

Regarding the Mysticeti suborder, a conserved single nucleotide deletion was detected in all species except *Balaenoptera acutorostrata scammoni* (minke whale) (Figure 1). A non-conserved 2 nucleotide insertion was found also found in *Balaenoptera bonaerensis* (Antartic minke whale) and an insertion of one nucleotide was detected at *Eschrichtius robustus* (grey whale) near the 5' end of the DRD_5 sequence (Figure 1). Again, at least one ORF-abolishing mutation was validated using genomic SRA experiments for all analysed species (Supplementary Material 1). Importantly, no conserved mutations were detected between most Odontoceti, *Physeter catodon* (sperm whale, Odontoceti) and Mysticeti lineages, suggesting that DRD_5 three pseudogenization events occurred independently after their evolutionary divergence (Figure 1). To increase the robustness of our analysis, we further scrutinized the genome of the extant sister clade of the Cetacea, the Hippopotamidae and were able to predict a fully coding sequence for DRD_5 in *Hippopotamus amphibius* (hippopotamus), supporting the loss of DRD_5 after Cetacea diversification.

dN/dS analyses support independent inactivation events of DRD_5 in Cetacea lineages

To further strengthen the mutational analyses, we next carried out a dN/dS approach. The dN/dS analyses for species with functional DRD_5 gene (Functional branches category) rejected the null hypothesis ($\omega = 1$, $p = 0.0300$, $< .05$), confirming that, as expected, these have evolved under purifying selection ($\omega < 1$), favoring the conservation of DRD_5 in the tested lineages (Table 1). In contrast, concerning Mysticeti and Odontoceti branch categories, the performed analyses failed to reject the null hypothesis ($p = 0.692$ for Odontoceti branches category and $p =$

0.291 for Mysticeti branches category respectively) suggesting that DRD_5 evolved neutrally in both lineages, following a gene inactivation event (Table 1). Moreover, evidences of purifying selection were found for the common Cetacean branch category ($\omega = 0.135$, $p = 0.001$), suggesting the existence of a functional DRD_5 in the common ancestral to all cetaceans (Table 1). Purifying selection was also detected for the Odontoceti stem branch category (excluding *Physeter catodon*, the sperm whale) ($\omega = 0.213$, $p = 0.045$). In contrast the *Physeter catodon* (sperm whale) ancestor branch category, does not present a dN/dS ratio (ω) statistically significantly different from 1 ($\omega = 0{,}320$, $p = 0.460$) (Table 1). Together with the mutational evidences (Figure 1), these results suggest two independent inactivation events within the Odontoceti. Regarding the Mysticeti stem branch category, selection analysis showed evidence of neutral selection ($\omega = 1.174$, $p = 0.905$) (Table 1). However, mutational evidence supports a conserved inactivation event in the common ancestor of Mysticeti. Taken together, our results support three independent DRD_5 inactivation events in Cetacea.

Other mammalian species displaying DRD_5 LQ tags have a coding gene

Initial analysis revealed the presence of at least one ORF-abolishing mutation in *Ovis aries* (sheep), *Phascolarctos cinereus* (koala), *Bison bison bison* (plains bison) and *Ochotona princeps* (American pika). These are in some cases suggestive of gene inactivation and not sequencing artefacts (e.g. Emerling et al., 2017; Lopes-Marques et al., 2017). Manual annotation, including SRA validation, unveiled sequencing reads supporting the absence of disruptive ORF mutations, rebutting each inactivation mutation and suggesting that DRD_5 is, in fact, coding in these species (Supplementary Material 2). Regarding *Myotis davidii* (vesper bat), the fragmentation of the genomic region (Ns) flanked by upstream and downstream DRD_5 neighbouring genes impeded us to infer the DRD_5 coding status in this species. Interestingly, regarding *Ochotona princeps* (American pika) mutational SRA validation, two scenarios were observed: approximately 50% of aligned reads supported the presence of a premature stop codon in the DRD_5 gene of this species, with the remaining set of aligning reads supporting the absence of a premature stop codon in the same species. This suggest that an allelic pseudogenization event might have occurred in this species.

Chrysochloris asiatica presents a non-functional DRD_5 gene

Next, we examined other mammalian species with annotated genome yet lacking DRD_5 gene annotations. Results were inconclusive regarding the coding status of *Microcebus murinus* (gray mouse lemur), *Condylura cristata* (star-nosed mole), *J. jaculus* (lesser Egyptian jerboa) and *Erinaceus europaeus* (western European hedgehog) DRD_5, due to the fragmentation (Ns) of the genomic region flanked by the upstream and downstream DRD_5 neighbouring genes, and the unavailability of whole genome shotgun contigs spanning our target gene. For *Elephantulus edwardii* (Cape elephant shrew) we were able to deduce a fully DRD_5 coding sequence. Curiously, *Chrysochloris asiatica* (Cape golden mole) presented a single nucleotide insertion in the 5' end of the gene (validated by genomic SRA, see Supplementary Material 3), suggesting that DRD_5 might be pseudogenized in this species.

Discussion

The rise of large-scale genomic sequencing projects has emphasized the role of gene loss as a potent driver of evolutionary change: underlying phenotypic adaptations or neutral regressions in response to specific environmental cues and niches (Albalat & Canestro, 2016; Braun, 2003; Jebb & Hiller, 2018; Jeffery, 2009; Olson, 1999; Sadier et al., 2018; Sharma et al., 2018; Somorjai et al., 2018). By comparing 124 mammalian genomes, we document three independent erosion events of a dopamine receptor, DRD_5, in Cetacea lineages. Although the current analysis is highly dependent of the quality of genome sequencing projects and their assembly, the conserved mutational profile of DRD_5 gene inactivation within Cetacea lineages further strengthens our findings.

Dopamine, a neurotransmitter and signalling molecule, is involved in distinct functions both in the central nervous system and peripheral tissues: including movement, feeding, sleep, reward, learning and memory as well as in the regulation of olfaction, hormone pathways, renal functions, immunity, sympathetic regulation and cardiovascular functions, respectively (Beaulieu & Gainetdinov, 2011). More specifically, the disruption of DRD_5-dependent pathways in rodents was shown to increase blood pressure and sympathetic tone, promoting vasoconstriction, thus yielding a hypertensive phenotype (Hollon et al., 2002; Li et al., 2008).

The observed gene loss distribution, and predicted phenotypic outcome, is consistent with the peripheral vasoconstriction mechanism described in Cetacea, suggested to counterbalance

deep-diving induced hypoxia (Ramirez et al., 2007; Tian et al., 2016). In fact, Cetacea have developed a number of physiological adaptations to offset hypoxia: notably, increased blood volume and oxygen-transport protein levels (hemoglobin, neuroglobin and myoglobin), allowing oxygen stores in blood, muscle tissues, and brain, reduced heart rate or bradycardia, apnea and peripheral vasoconstriction (Nery et al., 2013; Panneton, 2013; Ramirez et al., 2007; Tian et al., 2016). Peripheral vasoconstriction allows the regional compartmentalization of blood supplies, reducing blood flow in more hypoxia-tolerant tissues, such as skin, muscle, spleen or kidney, while maintaining arterial blood flow to the central nervous system and heart (Panneton, 2013). Moreover, the deviation of muscle blood supply reduces lactate accumulation (Panneton, 2013). Thus, in Cetacea, DRD_5 loss could contribute for the peripheral vasoconstriction requirements of diving. Also, by shifting renal salt balance, DRD_5 could also play a role in the maintenance of an adequate blood volume and pressure (Bie, 2009; Hong et al., 2017; Li et al., 2008; Zeng et al., 2005).

The predicted vasoconstriction phenotype is in agreement with a previous work reporting episodes of adaptive evolution (positive selection) in genes related with hypoxia tolerance in Cetacea, including genes involved in oxygen transport and regulation of vasoconstriction (Tian et al., 2016). In addition, by expanding their analysis to other non-aquatic hypoxic environments, such as underground tunnels, they uncovered convergent evolution scenarios in species adapted to diving and burrowing (Tian et al., 2016). A similar convergence is observed in the present work. In fact, in the mole *Chrysochloris asiatica* (Cape golden mole), DRD_5 was also predicted non-functional. Although this was the single DRD_5 loss example found outside Cetacea, one cannot discard the putative contribution of alternative molecular events (i.e. post-translational mechanisms) towards trait loss (Sadier et al., 2018), or event distinct physiological adaptations to overcome oxygen deprivation.

Besides diving physiology, DRD_5-dependent sympathetic tone alterations could also contribute to the idiosyncratic sleep behavior observed in Cetacea (Huelsmann et al., 2019; Lopes-Marques et al., 2019b; Lyamin et al., 2008). Several physiological adjustments occur during sleep, encompassing thermoregulation, as well as endocrine, immune, pulmonary and cardiovascular functions (Giglio et al., 2007). In most mammals, sleep states lead to a decrease of the sympathetic tone, inducing vasodilation and decreasing blood pressure (Giglio et al.,

2007). Thus, DRD_5 loss could prevent sympathetic tone decrease in resting states paralleling the unihemispheric sleeping behavior and long-term vigilance observed in Cetacea.

Conclusions

Overall, our findings provide evidence for natural occurring KO for DRD_5. Besides highlighting a molecular signature for vasoconstriction and blood pressure regulation in Cetacea, naturally occurring DRD_5 KO could also provide useful frameworks to gain insight into hypertension and heart failure-induced peripheral vasoconstriction responses in humans (Triposkiadis et al., 2009; Wang et al., 2008).

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519

Figure 1

Schematic representation of the DRD5 gene ORF abolishing annotated mutations regarding Cetacea parvorders Odontoceti and Mysticeti.

Phylogenetic relationships derived from a Maximum Likelihood (ML) tree with the DRD5 sequences from Mysticeti and Odontoceti species, as well as from *Hippopotamus amphibius*(hippopotamus), *Bos taurus*(cattle) and *Homo sapiens*(human). DRD_5 ORF-abolishing mutations are mapped in the corresponding branch. Vertical thick bars represent 2-nucleotide insertions (yellow) or deletions (orange). Vertical thin bars represent single nucleotide insertion (yellow) or deletion (orange). In-frame premature stop codons are represented by red triangles. Fragmented or incomplete genomic regions are represented by black regions. *Delphinapterus leucas*(beluga whale) predicted DRD_5 shortness is represented by a smaller bar. Functional branches are represented by green bars, nonfunctional branches are represented by grey and dark grey bars (Odontoceti and Mysticeti branches, respectively). Example of DRD_5 open reading frame (ORF) inactivating mutations concerning Mysticeti clade (A) and Odontoceti clade (excluding *Physeter catodon*, the sperm whale) (B). Numbers above characters represent the alignment position index.

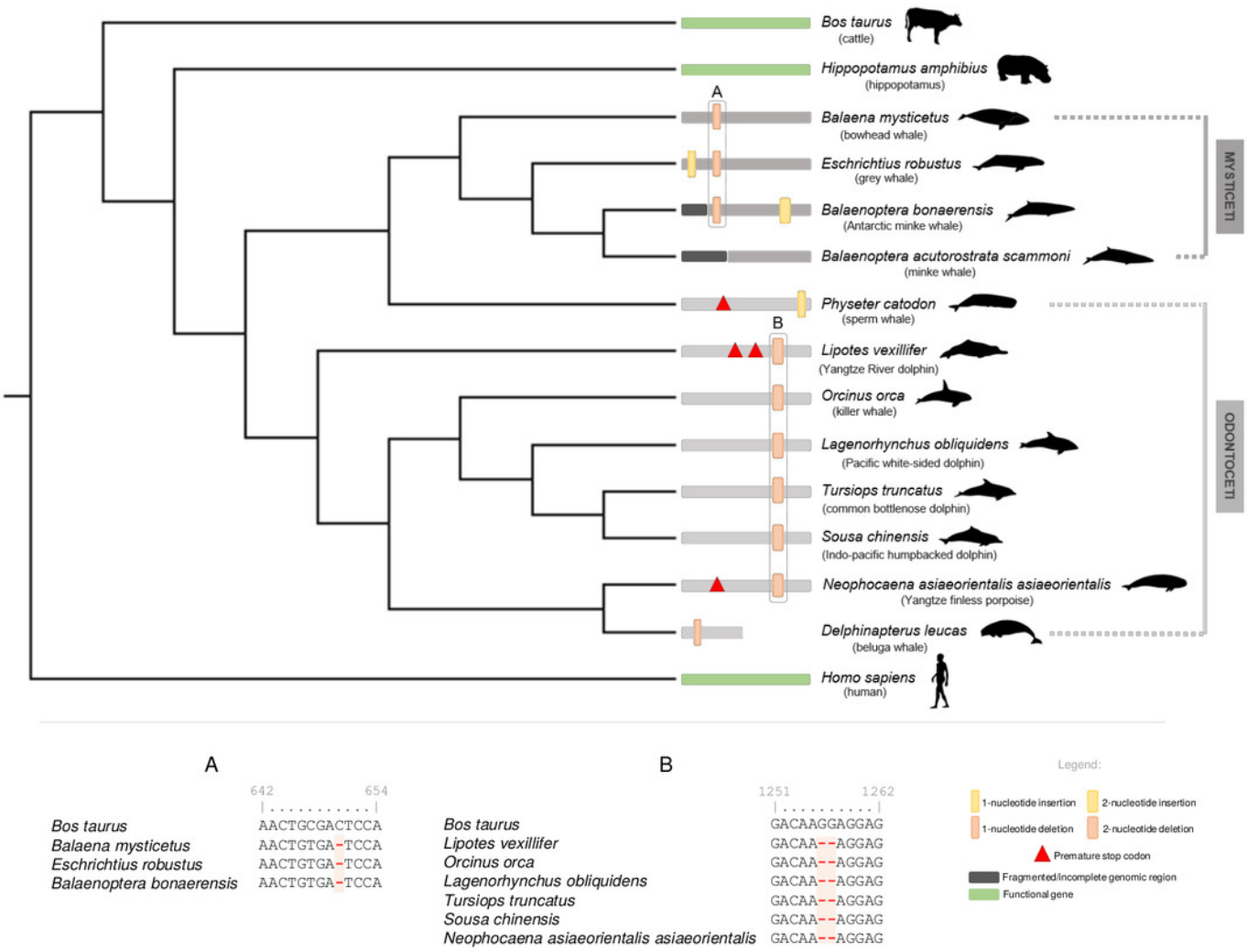
Bos taurus
(cattle)
Hippopotamus amphibius
(hippopotamus)
A
Balaena mysticetus
(bowhead whale)
Eschrichtius robustus
(grey whale)
Balaenoptera bonaerensis
(Antarctic minke whale)
Balaenoptera acutorostrata scammoni
(minke whale)
MYSTICETI
Physeter catodon
(sperm whale)
B
Lipotes vexillifer
(Yangtze River dolphin)
Orcinus orca
(killer whale)
Lagenorhynchus obliquidens
(Pacific white-sided dolphin)
Tursiops truncatus
(common bottlenose dolphin)
Sousa chinensis
(Indo-pacific humpbacked dolphin)
Neophocaena asiaeorientalis asiaeorientalis
(Yangtze finless porpoise)
Delphinapterus leucas
(beluga whale)
ODONTOCETI
Homo sapiens
(human)
A
642
654
Bos taurus AACTGCGACTCCA
Balaena mysticetus AACTGTGA-TCCA
Eschrichtius robustus AACTGTGA-TCCA
Balaenoptera bonaerensis AACTGTGA-TCCA
B
1251
1262
Bos taurus GACAAGGAGGAG
Lipotes vexillifer GACAA--AGGAG
Orcinus orca GACAA--AGGAG
Lagenorhynchus obliquidens GACAA--AGGAG
Tursiops truncatus GACAA--AGGAG
Sousa chinensis GACAA--AGGAG
Neophocaena asiaeorientalis asiaeorientalis GACAA--AGGAG
Legend:
1-nucleotide insertion
2-nucleotide insertion
1-nucleotide deletion
2-nucleotide deletion
Premature stop codon
Fragmented/incomplete genomic region
Functional gene

Table 1(on next page)

Selection (dN/dS) analyses with CODEML from PAML for the seven different branch categories.

Likelihood ratio tests (LRT) corresponding p -value concerning each branch category is also presented.

Table 1: Results of selection (dN/dS) analyses with CODEML from PAML for the seven different branch categories. Likelihood ratio tests (LRT) corresponding p -value concerning each branch category is also presented.

Branch category	dN/dS **(ω)**
Functional branches (*Homo sapiens, Bos taurus, Hipoppotamus amphibius*)	Statistically significantly ≠ from 1 (p = 0.0300)
Mysticeti branches (*Balaena mysticetus, Eschrichtius robustus, Balaenoptera bonaerensis, Balaenoptera acutorostrata scammoni*)	Not statistically significantly ≠ from 1 (p = 0.291)
Odontoceti branches (*Physeter catodon*, *Lipotes vexillifer, Orcinus orca, Lagenorhynchus obliquidens, Tursiops truncatus, Sousa chinensis, Neophocanea asiaeorientalis asiaeorientalis*)	Not statistically significantly ≠ from 1 (p = 0.692)
Common cetacean branch	0,135 (p = 0.001, statistically significantly ≠ from 1)
Stem Mysticeti branch	1.174 (p = 0.905, not statistically significantly ≠ from 1)
Stem Odontoceti (excluding *Physeter catodon*, sperm whale) branch	0.213 (p = 0.045, statistically significantly ≠ from 1)
***Physeter catodon* (sperm whale) ancestor branch**	0.320, (p = 0.460, not statistically significantly ≠ from 1)