The dopamine receptor D_5 gene shows signs of independent erosion in Toothed and Baleen whales

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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_5which displays a relatively higher affinity towards dopamine. Importantly, DRD_5knockout 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_5inactivation 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.

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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 counter-regulate and modulate the expression of the prohypertensive Angiotensin II Type 1 Receptor (AT_1R), involved in renal salt balance, blood pressure and vasoconstriction (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 analysis 124 genomes covering 16 orders, we show that independent coding debilitating mutations occurred in the ancestors of Mysticeti and Odontoceti (Cetacea). 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

Synteny analysis

To build the synteny maps for the DRD_5 gene locus in Cetacea and *H. amphibius* several annotated Cetacea genome assemblies were inspected and scrutinised using the NCBI browser, namely *O. orca* (GCF_000331955.2), *L. obliquidens* (GCF_003676395.1), *T. truncatus* (GCF_001922835.1), *D. leucas* (GCF_002288925.1), *N. a. asiaeorientalis* (GCF_003031525.1), *L. vexillifer* (GCF_000442215.1), *P. catodon* (GCF_002837175.1) and *B. a. scammoni* (GCF_000493695.1). *B. taurus* (GCF_002263795.1), a fully terrestrial relative of extant cetaceans, was used as reference. Next, (1) if DRD_5 was annotated, the following procedure was used: five flanking genes, considering only the ones characterised and tagged as protein coding, from each side of DRD_5 gene, were collected; (2) If DRD_5 gene annotations were not found, *B. taurus* DRD_5 direct flaking genes were used as reference genes to collect the corresponding flanking genes. In detail, CPEB2 was used as an anchor to search the rightwards DRD_5 flanking genes, and OTOP1 to search the leftwards genes. Regarding *H. amphibius,* 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). *B. taurus* DRD_5 flanking genes were used as reference, and using the discontiguous megablast task from blastn, the best blast hit (the hit with the highest alignment identity and query coverage) was retrieved and the coordinates of alignment in the target genome carefully inspected. *H. amphibius* 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

To perform manual gene annotation, all genomic regions of DRD_5 sequences tagged as LQ were directly collected from NCBI. Regarding cetacean species with annotated genomes but without DRD_5 gene annotations (*L. vellixifer and B. a. scammoni*), the genomic sequence ranging from the upstream to the downstream flanking genes was collected. For non-cetacean mammals, with annotated genomes but without DRD_5 annotations two procedures were followed. (1) For each species, two DRD_5 direct flanking genes were selected from a reference phylogenetic close species displaying non 'low-quality protein' DRD_5 annotation (see Supplementary Table 1) and the test species region flanked by the same reference genes was further collected. (2) If the same flanking region exhibited severe fragmentation (Ns), using the same phylogenetic close species (Supplementary Table 1) DRD_5 coding sequence as query, blastn searches were conducted against the Whole Shotgun Contigs dataset of the corresponding species via discontiguous megablast task. The genomic sequence corresponding to the blast hit with the highest alignment identity and query coverage amongst the others was retrieved.

For cetacean species with no annotated genomes (*B. bonaerensis, E. robustus, B. mysticetus, S. chinensis),* as well as *H. amphibius*, genomic sequences were retrieved through blastn searches in the corresponding genome assembly using the *B. taurus* DRD_5 coding sequence as query. The best genomic scaffold considered for each species corresponded to the blast hit with the highest query coverage and identity value amongst total hits. Due to the presence of a fragmented genomic region concerning *T. truncatus* DRD_5 gene annotation, the same blast search procedure was executed for this species, using as target the Whole Genome Shotgun contig dataset of the same species. The gene annotation concerning this species was performed in the retrieved contig corresponding to the blast hit with the highest query coverage and alignment identity value.

Collected genomic sequences were further imported into Geneious Prime 2019 (www.geneious.com) and the DRD_5 gene coding sequences were 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 premature stop codons. For Cetacea and *H. amphibius* DRD_5 *gene annotation, B.* taurus 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). The

identified mutations were next validated using the sequencing run raw data (sequencing reads) retrieved from at least two independents genomic NCBI SRA projects (when available).

Results

To examine the annotation tags and distribution of the DRD_5 gene across mammals, 119 annotated mammalian genomes available at NCBI (National Center of Biotechnology Information), 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 DRD_5 annotation tagged as 'low-quality protein' producing gene, 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), *Neophocaena asiaeorientalis asiaeorientalis* (Yangtze finless porpoise), *Delphinapterus 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), *Sousa chinensis* (Indo-Pacific humpback dolphin), or with annotated genomes lacking DRD_5 annotations: *Lipotes vexillifer* (Yangtze River dolphin) and *Balaenoptera acutorostrata scammoni* (minke whale) (Figure 1). Additionally, *Tursiops truncatus* (bottlenose dolphin), presenting a seemingly intact DRD_5 gene annotation, without the 'low-quality protein' tag, as well as *Hippopotamus amphibius* (common hippopotamus) predicted DRD_5 coding sequence (Figure 1), 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 1 presents a multiple translation alignment of the NCBI non-'*low-quality*

protein' tagged mammalian DRD_5 orthologous sequences. The alignment also includes the predicted *H. amphibius* DRD_5 sequence and excludes *T. truncatus* DRD_5 sequence, afterwards demonstrated as pseudogenized (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 (see below).

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. All analysed loci were found to be conserved, including in both *L. vexillifer* and *B. a. scammoni,* which lacked previous DRD_5 gene annotations (Figure 1). Subsequent manual annotation of all collected cetacean genomic sequences revealed DRD_5 gene erosion across all analysed species, except in *B. a. scammoni*, 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 species examined, except for *P. catodon* that presented a premature stop codon in the middle of the gene and a single nucleotide insertion near the end of the gene (Figure 2). The 2 nucleotide deletion alters the reading frame, leading to a drastic change in downstream amino acid composition. Additionally, a premature stop codon shortens the protein's expected size—31 amino acid shorter than hippopotamus—a characteristic not found in any of the examined coding DRD_5 sequences. Additionally, non-conserved mutations were found in *L. vexillifer* which presented 2 premature stop codons and *D. leucas* which displayed a single nucleotide deletion near the 5' end of the gene (Figure 2). *D. leucas* presumed DRD_5 sequence presented another noticeable feature. A massive and abrupt alignment identity decrease was observed when aligned with the *Bos taurus* (cow) reference against the genomic target region of *D. leucas*. The alignment identity drop was observed 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 (Figure 3). Regarding Odontoceti, at least one ORF-abolishing mutation was validated using available genomic Sequence Read Archives (SRAs)

194 experiments for all studied species, excluding *S. chinensis* and *L. vexillifer* for which no genomic
195 sequencing runs were available at the NCBI SRA database (Supplementary Material 1).
196 Regarding the Mysticeti suborder, a conserved single nucleotide deletion was detected in all
197 species except *B. a. scammoni* (Figure 2). A non-conserved 2 nucleotide insertion was found also
198 found in *B. bonaerensis* and an insertion of one nucleotide was detected at E. robustus DRD_5
199 sequence (Figure 2). Again, at least one ORF-abolishing mutation was validated using genomic
200 SRA experiments for all analysed species (Supplementary Material 1). Importantly, no conserved
201 mutations were detected between Odontoceti and Mysticeti clades, suggesting that DRD_5
202 pseudogenization events occurred independently after the divergence of both lineages (Figure 2).
203 To increase the robustness of our analysis, we further scrutinized the genome of the extant sister
204 clade of the Cetacea, the Hippopotamidae and were able to predict a fully functional CDS for
205 DRD_5 in *H. amphibius*, supporting the loss of *DRD_5* after Cetacea diversification.
206
207 **Other mammalian species showing DRD5 LQ tags have a coding gene**
208 Our initial analysis revealed the presence of at least one ORF-abolishing mutation in *O. aries*,
209 *P. cinereus*, *B. bison bison* and *O. princeps.* These are in some cases suggestive of gene
210 inactivation and not sequencing artefacts (Emerling et al., 2017; Lopes-Marques et al., 2017). To
211 investigate whether these DRD_5 gene sequences are eroded in these species, we performed a
212 meticulous manual annotation including SRA validation. Our results revealed sequencing reads
213 supporting the functionality of the gene, rebutting each inactivation mutation and suggesting that
214 DRD_5 is, in fact, coding in these species (Supplementary Material 2). Regarding *M. davidii*, the
215 fragmentation of the genomic region (Ns) flanked by upstream and downstream DRD_5
216 neighbouring genes impeded us to infer the DRD_5 coding status in this species, although, the
217 visible nucleotide aligning regions showed no ORF disruptive mutations, excepting for an 8
218 nucleotide deletion near the end of the gene, leading to a STOP codon, suggesting that this gene
219 possibly is lightly truncated but still functional in this species. Interestingly, regarding *O. princeps*
220 mutational SRA validation, two scenarios were observed: approximately 50% of aligned reads
221 supported the absence of a premature stop codon in the DRD_5 gene of this species, with the
222 remaining set of aligning reads supporting the presence of a premature stop codon in the same
223 species. This suggest that an allelic pseudogenization event might have occurred in *O. princeps.*
224

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 *M. murinus*, *C. cristata*, *J. jaculus* and *E. europaeus* 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 *E. edwardii* we were able to deduce a fully functional DRD_5 coding sequence. Curiously, *C. asiatica* presented a single nucleotide insertion in the 5' end of the gene (validated by genomic SRA, see Supplementary Material 3), suggesting that DRD_5 is 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 & Cañestro, 2016; Jebb & Hiller, 2018; Jeffery, 2009; Sadier et al., 2018; Sharma et al., 2018; Somorjai et al., 2018). Interestingly, gene loss mechanisms seem pervasive in lineages that endured drastic environmental adaptations in the course of evolution, such as Cetacea, entailing niche-specific physiological and morphological adaptations (Lachner et al., 2017; Lopes-Marques et al., 2019a; Lopes-Marques et al., 2018; Lopes-Marques et al., 2019b; McGowen et al., 2014; Sharma et al., 2018; Strasser et al., 2015).

By comparing 124 mammalian genomes, we document the independent erosion of a dopamine receptor, DRD_5, in both Cetacea lineages, Mysticeti and Odontoceti. 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 *C. asiatica*, 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 (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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References

Albalat R, Canestro C. 2016. Evolution by gene loss. *Nature Reviews Genetics* 17:379-391. 10.1038/nrg.2016.39

Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR. 1997. Cellular distribution of the rat D1B receptor in central nervous system using anti-receptor antisera. *Brain Research* 746:141-150. https://doi.org/10.1016/S0006-8993(96)01219-X

Beaulieu J-M, Gainetdinov RR. 2011. The Physiology, Signaling, and Pharmacology of Dopamine Receptors. *Pharmacological Reviews* 63:182. 10.1124/pr.110.002642

Bie P. 2009. Blood volume, blood pressure and total body sodium: internal signalling and output control. *Acta Physiologica* 195:187-196. 10.1111/j.1748-1716.2008.01932.x

Ciliax BJ, Nash N, Heilman C, Sunahara R, Hartney A, Tiberi M, Rye DB, Caron MG, Niznik HB, Levey AI. 2000. Dopamine D5 receptor immunolocalization in rat and monkey brain. *Synapse* 37:125-145. 10.1002/1098-2396(200008)37:2<125::AID-SYN7>3.0.CO;2-7

Dahmer MK, Senogles SE. 1996. Dopaminergic Inhibition of Catecholamine Secretion from Chromaffin Cells: Evidence that Inhibition Is Mediated by D4 and D5 Dopamine Receptors. *Journal of Neurochemistry* 66:222-232. 10.1046/j.1471-4159.1996.66010222.x

Daly G, Hawi Z, Fitzgerald M, Gill M. 1999. Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. *Molecular Psychiatry* 4:192. 10.1038/sj.mp.4000510

Emerling CA, Widjaja AD, Nguyen NN, Springer MS. 2017. Their loss is our gain: regressive evolution in vertebrates provides genomic models for uncovering human disease loci. *Journal of Medical Genetics* 54:787. 10.1136/jmedgenet-2017-104837

Giglio P, Lane JT, Barkoukis TJ, Dumitru I. 2007. Sleep Physiology. In: Barkoukis TJ, and Avidan AY, eds. *Review of Sleep Medicine*. 2nd ed. Philadelphia: Butterworth-Heinemann, 29-41.

Hollon TR, Bek MJ, Lachowicz JE, Ariano MA, Mezey E, Ramachandran R, Wersinger SR, Soares-da-Silva P, Liu ZF, Grinberg A, Drago J, Young WS, Westphal H, Jose PA, Sibley DR. 2002. Mice Lacking D5 Dopamine Receptors Have Increased Sympathetic Tone and Are Hypertensive. *The Journal of Neuroscience* 22:10801. 10.1523/JNEUROSCI.22-24-10801.2002

Hong K, Li M, Nourian Z, Meininger GA, Hill MA. 2017. Angiotensin II Type 1 Receptor Mechanoactivation Involves RGS5 (Regulator of G Protein Signaling 5) in Skeletal Muscle Arteries: Impaired Trafficking of RGS5 in Hypertension. *Hypertension* 70:1264-1272. 10.1161/HYPERTENSIONAHA.117.09757

Jebb D, Hiller M. 2018. Recurrent loss of HMGCS2 shows that ketogenesis is not essential for the evolution of large mammalian brains. *eLife* 7. 10.7554/eLife.38906

Jeffery WR. 2009. Regressive evolution in Astyanax cavefish. *Annual Review of Genetics* 43:25-47. 10.1146/annurev-genet-102108-134216

Kumar S, Dudley JT, Filipski A, Liu L. 2011. Phylomedicine: an evolutionary telescope to explore and diagnose the universe of disease mutations. *Trends in Genetics* 27:377-386. 10.1016/j.tig.2011.06.004

Lachner J, Mlitz V, Tschachler E, Eckhart L. 2017. Epidermal cornification is preceded by the expression of a keratinocyte-specific set of pyroptosis-related genes. *Scientific Reports* 7:17446. 10.1038/s41598-017-17782-4

Li H, Armando I, Yu P, Escano C, Mueller SC, Asico L, Pascua A, Lu Q, Wang X, Villar VAM, Jones JE, Wang Z, Periasamy A, Lau Y-S, Soares-da-Silva P, Creswell K, Guillemette G, Sibley DR, Eisner G, Felder RA, Jose PA. 2008. Dopamine 5 receptor mediates Ang II type 1 receptor degradation via a ubiquitin-proteasome pathway in mice and human cells. *The Journal of Clinical Investigation* 118:2180-2189. 10.1172/JCI33637

Lopes-Marques M, Machado AM, Alves QL, Fonseca MM, Barbosa S, Sinding M-HS, Rasmussen MH, Iversen MR, Bertelsen MF, Campos PF, da Fonseca R, Ruivo R, Castro LFC. 2019a. Complete inactivation of sebum-producing genes parallels the loss of sebaceous glands in Cetacea (in press). *Molecular Biology and Evolution*.

Lopes-Marques M, Machado AM, Barbosa S, Fonseca MM, Ruivo R, Castro LFC. 2018. Cetacea are natural knockouts for IL20. *Immunogenetics*. 10.1007/s00251-018-1071-5

Lopes-Marques M, Ruivo R, Alves QL, Sousa N, Machado MA, Castro FL. 2019b. The Singularity of Cetacea Behavior Parallels the Complete Inactivation of Melatonin Gene Modules. *Genes* 10. 10.3390/genes10020121

Lopes-Marques M, Ruivo R, Fonseca E, Teixeira A, Castro LFC. 2017. Unusual loss of chymosin in mammalian lineages parallels neo-natal immune transfer strategies. *Molecular Phylogenetics and Evolution* 116:78-86. https://doi.org/10.1016/j.ympev.2017.08.014

Lyamin OI, Manger PR, Ridgway SH, Mukhametov LM, Siegel JM. 2008. Cetacean sleep: An unusual form of mammalian sleep. *Neuroscience & Biobehavioral Reviews* 32:1451-1484. 10.1016/j.neubiorev.2008.05.023

McGowen MR, Gatesy J, Wildman DE. 2014. Molecular evolution tracks macroevolutionary transitions in Cetacea. *Trends in Ecology & Evolution* 29:336-346. https://doi.org/10.1016/j.tree.2014.04.001

Mezey E, Eisenhofer G, Harta G, Hansson S, Gould L, Hunyady B, Hoffman BJ. 1996. A novel nonneuronal catecholaminergic system: exocrine pancreas synthesizes and releases dopamine. *Proceedings of the National Academy of Sciences of the United States of America* 93:10377. 10.1073/pnas.93.19.10377

Miller MP, Kumar S. 2001. Understanding human disease mutations through the use of interspecific genetic variation. *Human Molecular Genetics* 10:2319-2328. 10.1093/hmg/10.21.2319

Muir WJ, Thomson ML, McKeon P, Mynett-Johnson L, Whitton C, Evans KL, Porteous DJ, Blackwood DHR. 2001. Markers close to the dopamine D5 receptor gene (DRD5) show significant association with schizophrenia but not bipolar disorder. *American Journal of Medical Genetics* 105:152-158. 10.1002/1096-8628(2001)9999:9999<::AID-AJMG1163>3.0.CO;2-2

Nery MF, Arroyo JI, Opazo JC. 2013. Accelerated Evolutionary Rate of the Myoglobin Gene in Long-Diving Whales. *Journal of Molecular Evolution* 76:380-387. 10.1007/s00239-013-9572-1

Opazo JC, Zavala K, Miranda-Rottmann S, Araya R. 2018. Evolution of dopamine receptors: phylogenetic evidence suggests a later origin of the DRD2l and DRD4rs dopamine receptor gene lineages. *PeerJ* 6:e4593. 10.7717/peerj.4593

Ott T, Nieder A. 2018. Dopamine and Cognitive Control in Prefrontal Cortex. *Trends in Cognitive Sciences*. 10.1016/j.tics.2018.12.006

Panneton WM. 2013. The mammalian diving response: an enigmatic reflex to preserve life? *Physiology* 28:284-297. 10.1152/physiol.00020.2013

Ramirez J-M, Folkow LP, Blix AS. 2007. Hypoxia Tolerance in Mammals and Birds: From the Wilderness to the Clinic. *Annual Review of Physiology* 69:113-143. 10.1146/annurev.physiol.69.031905.163111

Sadier A, Davies KTJ, Yohe LR, Yun K, Donat P, Hedrick BP, Dumont ER, Dávalos LM, Rossiter SJ, Sears KE. 2018. Multifactorial processes underlie parallel opsin loss in neotropical bats. *eLife* 7:e37412. 10.7554/eLife.37412

Sanada H, Xu J, Jose PA, Watanabe H, Felder RA. 2000. Differential expression and regulation of dopamine-1(d1) and dopamine-5(d-5) receptor function in human kidney. *American Journal of Hypertension* 13:156A-156A. 10.1016/S0895-7061(00)00649-X

Sharma V, Hecker N, Roscito JG, Foerster L, Langer BE, Hiller M. 2018. A genomics approach reveals insights into the importance of gene losses for mammalian adaptations. *Nature Communications* 9:1215. 10.1038/s41467-018-03667-1

Somorjai IML, Martí-Solans J, Diaz-Gracia M, Nishida H, Imai KS, Escrivà H, Cañestro C, Albalat R. 2018. Wnt evolution and function shuffling in liberal and conservative chordate genomes. *Genome Biology* 19:98. 10.1186/s13059-018-1468-3

Springer MS, Murphy WJ. 2007. Mammalian evolution and biomedicine: new views from phylogeny. *Biological Reviews* 82:375-392. 10.1111/j.1469-185X.2007.00016.x

Strasser B, Mlitz V, Fischer H, Tschachler E, Eckhart L. 2015. Comparative genomics reveals conservation of filaggrin and loss of caspase-14 in dolphins. *Exp Dermatol* 24:365-369. 10.1111/exd.12681

Sunahara RK, Guan H-C, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HHM, Niznik HB. 1991. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* 350:614-619. 10.1038/350614a0

Tian R, Wang Z, Niu X, Zhou K, Xu S, Yang G. 2016. Evolutionary Genetics of Hypoxia Tolerance in Cetaceans during Diving. *Genome biology and evolution* 8:827-839. 10.1093/gbe/evw037

Tiberi M, Caron MG. 1994. High agonist-independent activity is a distinguishing feature of the dopamine D1B receptor subtype. *Journal of Biological Chemistry* 269:27925-27931.

Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G, Butler J. 2009. The Sympathetic Nervous System in Heart Failure: Physiology, Pathophysiology, and Clinical Implications. *Journal of the American College of Cardiology* 54:1747-1762. https://doi.org/10.1016/j.jacc.2009.05.015

Vanyukov MM, Moss HB, Gioio AE, Hughes HB, Kaplan BB, Tarter RE. 1998. An Association Between a Microsatellite Polymorphism at the DRD5 Gene and the Liability to Substance Abuse: Pilot Study. *Behavior Genetics* 28:75-82. 10.1023/A:1021463722326

Wang X, Villar VAM, Armando I, Eisner GM, Felder RA, Jose PA. 2008. Dopamine, kidney, and hypertension: studies in dopamine receptor knockout mice. *Pediatric nephrology* 23:2131-2146. 10.1007/s00467-008-0901-3

Zeng C, Yang Z, Wang Z, Jones J, Wang X, Altea J, Mangrum Amy J, Hopfer U, Sibley David R, Eisner Gilbert M, Felder Robin A, Jose Pedro A. 2005. Interaction of Angiotensin II Type 1 and D5 Dopamine Receptors in Renal Proximal Tubule Cells. *Hypertension* 45:804-810. 10.1161/01.HYP.0000155212.33212.99

Figure 1(on next page)

Comparative synteny maps of DRD5 genomic *locus*in Cetacea and *H. amphibius*.

Blue squares indicate the presence of DRD5 in genome annotation. Red squares indicate the absence of DRD5annotation in the corresponding genome. *H. amphibius*genes found in the same genomic scaffold are represented by dark grey squares and underlined with a bar indicating the corresponding accession number. Not found genes due to the end of genomic region are represented by white squares.

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Figure 2(on next page)

Schematic representation of the DRD5gene ORF abolishing annotated mutations regarding Cetacea suborders Odontoceti (light gray) and Mysticeti(dark gray).

Thicker bars represent 2 nucleotide insertions (blue) or deletions (orange). Thinner bars represent single nucleotide insertion (blue) or deletion (orange). Premature stop codons are represented by red thick bars. Unknown regions, either resulting from genome poor assembly or coverage (Ns in *B. a. scammoni*) or due to small genomic scaffold size (*B. bonaerensis*), are represented by black regions. *H. amphibius*presents a complete functional DRD5gene. Two elucidative boxes represent the conserved mutations found in Odontoceti (2 nucleotide deletion) and in Mysticeti(1 nucleotide deletion).

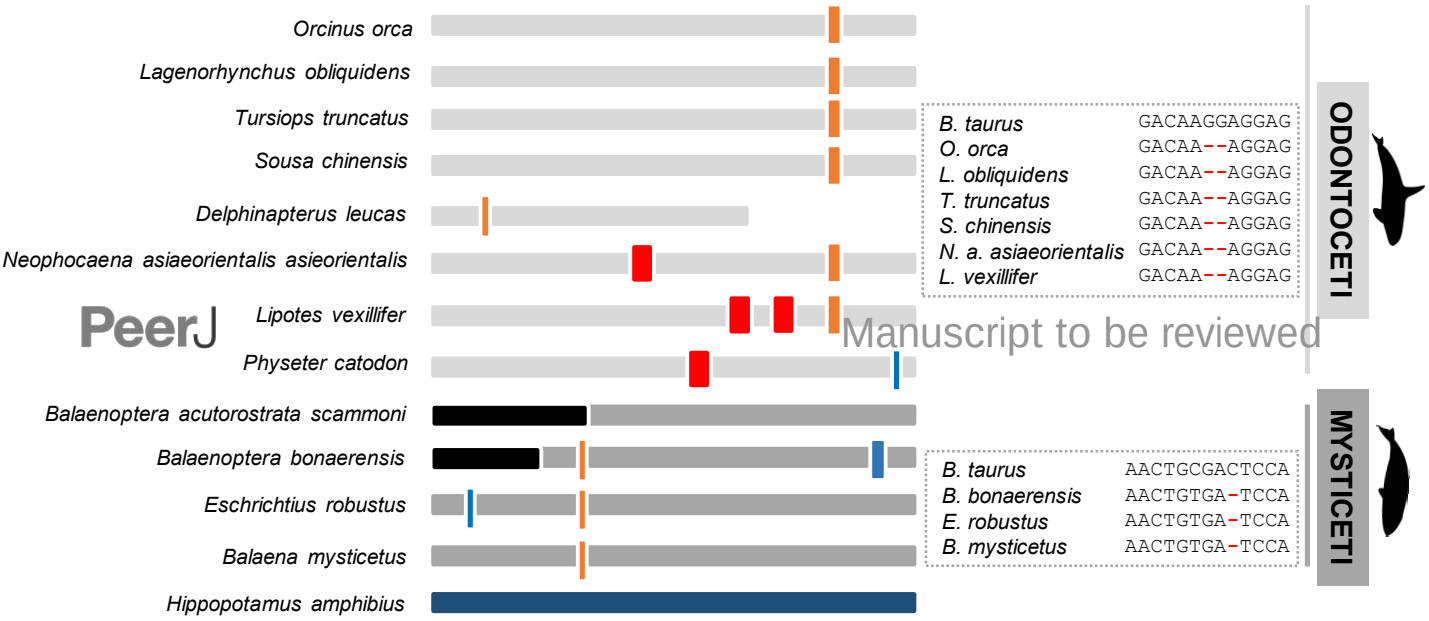

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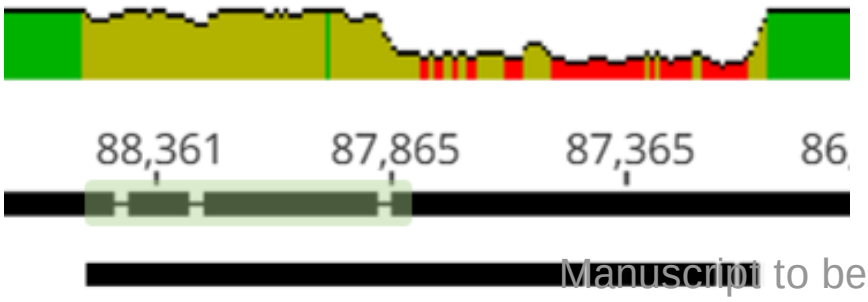
Figure 3(on next page)

*B. taurus*reference DRD5gene aligned against the genomic target region of *D. leucas*.

Lower bar represents the reference single-exon gene and the upper bar represents the genomic target region. An alignment identity graph is presented above the alignment. Green represents very high alignment identity values, followed by yellow (mid to high alignment identity values) and finally red, indicating very low alignment identity values. The hypothetical approximate DRD5 gene real size in *D. leucas*is marked by the clear abrupt alignment identity drop and is represented by a green shadowed rectangle.



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