Classification of Dopamine Receptor Genes in Vertebrates: Nine Subtypes in Osteichthyes

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Key Words

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Abstract

Dopamine neurotransmission regulates various brain functions, and its regulatory roles are mediated by two families of G protein-coupled receptors: the D₁ and D₂ receptor families. In mammals, the D₁ family comprises two receptor subtypes (D₁ and D₅), while the D₂ family comprises three receptor subtypes (D₂, D₃ and D₄). Phylogenetic analyses of dopamine receptor genes strongly suggest that the common ancestor of Osteichthyes (bony jawed vertebrates) possessed four subtypes in the D₁ family and five subtypes in the D₂ family. Mammals have secondarily lost almost half of the ancestral dopamine receptor genes, whereas nonmammalian species kept many of them. Although the mammalian situation is an exception among Osteichthyes, the current classification and characterization of dopamine receptors are based on mammalian features, which have led to confusion in the identification of dopamine receptor subtypes in nonmammalian species. Here we begin by reviewing the history of the discovery of dopamine receptors in vertebrates.

The recent genome sequencing of coelacanth, gar and elephant shark led to the proposal of a refined scenario of evolution of dopamine receptor genes. We also discuss a current problem of nomenclature of dopamine receptors. Following the official nomenclature of mammalian dopamine receptors from D₁ to D₅, we propose to name newly identified receptor subtypes from D₆ to D₉ in order to facilitate the use of an identical name for orthologous genes among different species. To promote a nomenclature change which allows distinguishing the two dopamine receptor families, a nomenclature consortium is needed. This comparative perspective is crucial to correctly interpret data obtained in animal studies on dopamine-related brain disorders, and more fundamentally, to understand the characteristics of dopamine neurotransmission in vertebrates.

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Introduction

Dopamine is one of the most intensively studied modulatory neurotransmitters due to its implication in many brain functions such as neuroendocrine regulation, motor programming, motivational behaviors, learning and memory [Hornykiewicz, 1966; Vogt, 1973; Tuomisto and

Mannisto, 1985; Gerfen et al., 1987; Schultz, 1998; Goldman-Rakic, 1999; Matsumoto et al., 1999; Kehagia et al., 2010]. Dopamine exerts its actions on target cells by activating specific membrane receptors. In mammals, five dopamine receptor genes have been isolated, and they have been characterized extensively over the last 30 years.

Dopamine receptors are more numerous in other groups of vertebrates. Thus, several types of dopamine receptors found in nonmammalian species do not have a one-to-one homologous counterpart with mammalian dopamine receptors. The data obtained in different vertebrate species are therefore difficult to interpret and compare. In addition, the 'official' classification and nomenclature of dopamine receptors are based only on the set of receptors found in placental mammals. This hampers meaningful and coherent comparison of the effects of dopamine receptor agonists/antagonists between mammalian and nonmammalian species. To alleviate this problem, defining relations of homology among dopamine receptor genes in vertebrates is essential.

Here we review the history of the discovery of dopamine receptors in mammals and other vertebrate groups, and then summarize the classification of dopamine receptors based on evolutionary analyses. We conclude by reviewing the problem posed by the mammalian-centered nomenclature of dopamine receptors, and provide a broader view of dopamine receptor evolution in vertebrates.

Two Families of Dopamine Receptors in Mammals

Before the molecular era, two categories of dopamine receptors, named D_1 and D_2 , were identified based on pharmacological, biochemical and physiological criteria. The D_1 receptor is defined by specific agonists that increase cAMP synthesis and by antagonists that block this effect. By contrast, agonists of the D_2 receptor inhibit cAMP synthesis or modulate other signaling pathways, and these effects are blocked by a series of D_2 receptor antagonists, including antipsychotic drugs [Kebabian and Calne, 1979; Beaulieu and Gainetdinov, 2011].

Molecular cloning techniques led to the isolation of DNA sequences from rat and human, confirming that D_1 and D_2 receptors are encoded by different genes [Bunzow et al., 1988; Dearry et al., 1990; Monsma et al., 1990; Sunahara et al., 1991]. These DNA sequences also led to the discovery that dopamine receptors are membrane proteins with seven hydrophobic segments, a structural signature of the G protein-coupled receptor. D_1 and D_2 re-

Fig. 1. Two different classes of dopamine receptors. **a** Schema of the structure of D_1 and D_2 families of dopamine receptors. The two families possess different characteristics in terms of genomic structure, G protein coupling and physiological effects. **b** A simplified phylogenetic tree showing the relationship of D_1 and D_2 families with other monoamine receptors in vertebrates [modified from Yamamoto et al., 2013]. Monoamine receptors sharing the same natural agonist are not necessarily close to each other.

ceptors both belong to group A (rhodopsin-like) of the G protein-coupled receptor superfamily, but have different structural features (fig. 1a). The D_1 receptor gene is intronless, and the deduced structure of the D_1 receptor protein exhibits a short third cytoplasmic loop and a long C-terminal tail. When this sequence is expressed in heterologous cells, the receptor is coupled to the Gs family of G proteins, and D_1 receptor agonists promote adenylyl cyclase activity in those cells. In contrast to the D_1 receptor, the D_2 dopamine receptor gene contains several in-

trons and encodes a long third cytoplasmic loop and a short C-terminal tail. In heterologous cells, the D_2 receptor is coupled to the Gi/Go family of G proteins, and its agonists inhibit adenylyl cyclase activity. Although functions of the D_1 and D_2 receptors are often described by their effects on cAMP production, dopamine receptors also regulate a number of other signal transduction pathways, including calcium and potassium channels and arachidonic acid synthesis, thus providing versatile signal modulations to a single cell [reviewed in Jaber et al., 1996; Missale et al., 1998; Sidhu and Niznik, 2000; Vallone et al., 2000].

After the first two D_1 and D_2 receptors were isolated, other dopamine receptor-related sequences were discovered. Three of them were initially identified in rats: two different D_1 -like receptor genes which encode proteins that are able to activate adenylyl cyclase, termed D_{1A} and D_{1B} [Monsma et al., 1990; Tiberi et al., 1991], and a novel D_2 -related receptor [Sokoloff et al., 1990]. Despite its high sequence similarity to the D_2 receptor gene, its pharmacological profiles and tissue distribution were distinct from those of D_1 or D_2 receptors. Thus, it was identified as a 'third' subtype and accordingly named the D_3 dopamine receptor. Finally, D_4 and D_5 receptor genes were identified from human genomic DNA. Although the sequence of the D_4 receptor gene was similar to D_2 and D_3 receptor genes, it displayed higher affinity to clozapine than the other subtypes of dopamine receptors [Van Tol et al., 1991]. The D_5 receptor showed the highest sequence similarity with the known D_1 receptor and the biochemical properties similar to D_1 stimulating adenylyl cyclase, but displayed higher affinity for dopamine than the D_1 receptor [Sunahara et al., 1991].

Later studies revealed that the human D_5 and the rat D_{1B} receptor sequences are in fact orthologous [Jarvie et al., 1993]. Genes are referred to as 'orthologous' when homologous sequences present in different species originate from a gene already present in their common ancestor. In contrast, genes are 'paralogous' when homologous sequences are generated by a gene duplication event. Due to this history of receptor identification and terminology, the two receptors of the D_1 family are called D_1 and D_5 in humans, but are often called D_{1A} and D_{1B} in nonhumans.

Interestingly, D_1 and D_2 dopamine receptors are not phylogenetically more related to each other than to other monoamine receptors [Callier et al., 2003; Le Crom et al., 2003; Yamamoto et al., 2013] (fig. 1b). This suggests that the ability to bind dopamine, shared by the two receptors, was acquired independently by convergence during evolution.

To summarize, five receptor genes have been isolated from mammalian species, and they were assigned either to the D_1 or to D_2 families. D_1 and D_5 , also referred to as D_{1A} and D_{1B} in nonhuman vertebrates, belong to the D_1 receptor family while D_2 , D_3 and D_4 belong to the D_2 receptor family. Note that the two dopamine receptor 'families' are also called 'classes'. These terms are interchangeable and the former is often used in the field of molecular biology, referring to gene families, while the latter is often used in the field of pharmacology. Here we will use the term 'family' throughout this review.

Additional D_1 Family Dopamine Receptor Genes in Nonmammalian Species

When additional dopamine receptor subtypes were found in nonmammalian species, the classification and nomenclature of dopamine receptors became further complicated. A third subtype in the D_1 family, named D_{1C} , was initially characterized in *Xenopus laevis* [Sugamori et al., 1994] and then in some teleosts such as tilapia, eel and carp [Lamers et al., 1996; Cardinaud et al., 1997; Hirano et al., 1998]. Similar to the other D_1 family members, it is encoded by an intronless gene and is able to enhance adenylyl cyclase activity in transfected cells, but its sequence, pharmacological profile and resistance to desensitization make it significantly different from other D_1 family receptors [Sugamori et al., 1994; Cardinaud et al., 1997; Le Crom et al., 2004]. The D_{1C} receptor was named following the rat nomenclature; therefore, all subsequently identified receptor genes of the D_1 family were named in the alphabetical order. The D_{1D} receptor, isolated from the chicken genome [Demchyshyn et al., 1995], displays different pharmacological profiles from the *Xenopus* and teleost D_{1C} receptor, with relatively low protein sequence identity, and is thus considered to be a separate subtype. A D_{1D} receptor gene was then also found in zebra finch [Kubikova et al., 2010], which suggested that the D_{1D} receptor subtype may be specific to birds. An additional D_1 receptor subtype was found in the carp retina. Although its deduced protein structure is similar to that of the D_1 family, the sequence of its third cytoplasmic loop contains a unique amino acid stretch 'RTCPLQHRAI', which is not present in any other dopamine receptor of the D_1 family. Without any pharmacological/physiological characterization, it was named D_{1X} , as an 'unidentified' subtype of the D_1 family [Hirano et al., 1998].

The availability of large sets of genome sequences from numerous vertebrate species enabled undertaking exten-

Fig. 2. Four subtypes of D_1 family dopamine receptors in Osteichthyes. Here is presented a phylogenetic tree of protein sequences of D_1 family dopamine receptors obtained from the maximum likelihood method using PhyML 3.0 [Guindon and Gascuel, 2003; Dereeper et al., 2008; Guindon et al., 2010]. D_1 -like sequences from coelacanth, gar (<http://www.ensembl.org/index.html>) and elephant shark (<http://esharkgenome.imcb.a-star.edu.sg/blast/>) were added to the set of protein sequences used in Yamamoto et al. [2013]. The tree was rooted on the amphioxus D_1 -like dopamine receptor (AmphiAmR1). Bootstrap values are indicated in red (color in online version only), and the length of the horizontal lines is proportional to sequence divergence (scale bar: 2.0 arbitrary units). All D_1 family receptors found in gnathostomes (jawed vertebrates) belong to the clade of either D_{1A} , D_{1B} , D_{1C} or D_{1E} , identified in Yamamoto et al. [2013], suggesting that the four subtypes preexisted in the gnathostome ancestor (a simplified tree is shown at the top left). Teleosts have additional copies in each subtype, except in D_{1E} . The nomenclature of the dopamine receptor subtypes follows as proposed in Yamamoto et al. [2013].

sive phylogenetic analysis of D_1 family dopamine receptor sequences, spurring us to propose a renewed classification of the D_1 family [Yamamoto et al., 2013] (fig. 2). Firstly, we demonstrated that anamniote D_{1C} and avian D_{1D} receptor genes are in fact orthologous, not paralogous as originally proposed. Therefore, the avian D_{1D} was renamed to D_{1C} to make the nomenclature compliant with the phylogenetic classification. We also found that the carp D_{1X} receptor sequence is one of the D_{1B} paralogous genes, probably as a result of teleost-specific genome duplication [Jaillon et al., 2004]. To account for this finding, the D_{1B} sequence was renamed to D_{1Ba} , and D_{1X} was

renamed to D_{1Bb} . Finally, we found a previously unidentified D_1 receptor gene in the genomes of zebrafish and several sauropsid species, which we named the D_{1E} receptor to avoid any confusion with the previous avian D_{1D} receptor (currently reclassified as the amniote D_{1C} receptor).

Thus, based on phylogenetic analysis, it appears that ancestral Osteichthyes possessed four paralogous genes of the D_1 family dopamine receptors: D_{1A} , $D_{1B(X)}$, D_{1C} (previously characterized as D_{1D} in birds) and D_{1E} receptor genes. As a consequence of gene duplications and gene losses, the repertoire of the D_1 family receptor genes

differs between vertebrate lineages. For instance, the D_{1C} receptor gene has been secondarily lost in the mammalian lineage, and the D_{1E} receptor gene has been lost independently in several lineages of modern vertebrates. As a consequence, mammals have kept only two of the ancestral subtypes, while some species such as chicken, turkey, coelacanth, and zebrafish have kept all four subtypes. In addition, zebrafish possess seven D_1 family receptor genes resulting from four ancestral subtypes, i.e. they have two paralogous genes for each subtype of the D_1 family except for the D_{1E} receptor.

The recent genome sequencing of elephant shark (*Callorhynchus milii*), a Chondrichthyes (cartilaginous fish) [Venkatesh et al., 2014], allowed us to further specify the evolution of D_1 family receptor genes in jawed vertebrates. Three D_1 -like sequences were found in the elephant shark genome, and they belong to the D_{1A} , D_{1B} and D_{1C} subtypes (fig. 2). This suggests that the four D_1 family receptor subtypes found in Osteichthyes probably existed before the split of Osteichthyes from Chondrichthyes, and thus were present in the gnathostome ancestor.

The pharmacological characterization of D_1 family dopamine receptors does not necessarily fit with the phylogenetic relationships. As described above, the avian D_{1C} was misidentified as ' D_{1D} ' due to its pharmacological and physiological properties which are significantly different from ' D_{1C} ' in amphibians and teleosts [Demchyshyn et al., 1995]. Similarly, the amphioxus ' $\text{AmphiD1}/\beta$ ' receptor has pharmacological properties similar to the vertebrate D_1 family, but it is phylogenetically closer to the vertebrate β -adrenergic receptors [Candiani et al., 2005; Burman et al., 2007, 2009]. A more recently identified ' AmphiAmR1 ' is the amphioxus gene phylogenetically closest to the vertebrate D_1 , and is prob-

ably a D_1 ortholog [Burman et al., 2007; Yamamoto et al., 2013]. Altogether, these analyses suggest that functional properties of monoamine receptors are quite plastic during evolution.

Additional D_2 Family Dopamine Receptor Genes in Nonmammalian Species

Relationships of the different D_2 family receptor sequences found in nonmammalian species are not clear, and there is no recent classification that takes into account a wide range of vertebrate species.

It has been shown that zebrafish possess three D_2 -like receptor genes and three D_4 -like receptor genes. Initially, they were named D_{2a} , D_{2b} and D_{2c} [Boehmler et al., 2004] and D_{4a} , D_{4b} and D_{4c} respectively [Boehmler et al., 2007]. Later, the nomenclature of the three D_2 and D_4 receptor genes was changed in zebrafish databases such as Ensembl (http://www.ensembl.org/Danio_rerio/Info/Index) and ZFIN (<http://zfin.org/>). The D_{2b} sequence was renamed as D_{2like} (D_{2l}) to account for the significant difference of this receptor sequence from the other two paralogous genes, and the D_{2c} sequence was renamed as D_{2b} . Similarly, the D_{4b} sequence was renamed as $D_{4-related}$ (D_{4-rs}), and the former D_{4c} was renamed as D_{4b} .

In order to clarify the classification of the dopamine receptors of the D_2 family in vertebrates, we carried out a phylogenetic analysis using the maximum likelihood method [Dereeper et al., 2008] on the protein sequences of this family from a wide range of vertebrate groups. In addition to the well-defined subtypes of the D_2 , D_3 and D_4 receptors, two additional clades were identified: one con-

Fig. 3. Five subtypes of D_2 family dopamine receptors in Osteichthyes. Here is presented a phylogenetic tree of protein sequences of D_2 family dopamine receptors obtained from the maximum likelihood method using PhyML 3.0 [Guindon and Gascuel, 2003; Dereeper et al., 2008; Guindon et al., 2010]. The protein sequences from various vertebrate species were obtained from the Ensembl genome browser (<http://www.ensembl.org/index.html>), GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) and Elephant Shark Genome Project (<http://esharkgenome.imcb.a-star.edu.sg/>), and they were aligned using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). The tree was rooted on *Drosophila* D_2 -like dopamine receptor (DopR2). Bootstrap values are indicated in red (color in online version only), and the length of the horizontal lines is proportional to sequence divergence (scale bar: 2.0 arbitrary units). In addition to the conventional D_2 , D_3 and D_4 , additional clades of D_{2l} and D_{4-rs} are formed (a simplified tree is shown at the top left).

The right panel shows comparative gene synteny of the chromosome loci bearing D_2 , D_{2b} , D_3 , D_4 and D_{4-rs} receptor genes in representative vertebrate species. Gene synteny also supports the non-orthologous relationship between D_2 and D_{2b} , as well as between D_4 and D_{4-rs} . Orthologs of the neighbor genes of D_{2l} (*ETHE1* and *XRCC1*) are found on the human chromosome 19, but the D_{2l} locus is replaced by another gene (*ZNF575*). The presence of D_2 and D_{2l} genes in genomes of coelacanth and gar, in addition to those of teleosts, strongly suggests that they preexisted before the Sarcopterygii-Actinopterygii split, instead of being duplicated as a consequence of the teleost-specific genome duplication. The absence of D_{2l} and D_{4-rs} in tetrapods suggests that they were secondarily lost before the divergence of tetrapods. The nomenclature of the dopamine receptor subtypes follows rules proposed in ZFIN (<http://zfin.org/>).

(For figure see next page.)

taining the zebrafish D_{2l} receptor, and the other containing the zebrafish D_{4-rs} receptor (fig. 3). Importantly, genomes of coelacanth (the order of Coelacanthiformes which is a nontetrapod Sarcopterygii) and of gar (the or-

der of Lepisosteiformes which is a nonteleost Actinopterygii) have sequences that are orthologous to the zebrafish D_{2l} and D_{4-rs} receptors, thus strongly suggesting that the D_{2l} and D_{4-rs} receptor genes are not specific to the te-

Fig. 4. Exon-intron organization of dopamine receptor genes of the D_2 family. For each gene, boxes correspond to coding exons (E) and the dashed lines correspond to intron or noncoding regions of the genes. The size of the boxes and lines is arbitrary. The number of amino acids encoded by each exon is indicated within the box, except when the coding sequence is not complete. Three amino acids at the exon-intron boundary are also shown, even when the splicing site does not respect the coding phase of the sequence. For the D_2 , D_3 and D_4 dopamine receptor subtypes, human and zebrafish (Danio) sequences are shown as representative examples of gnathostome genes. For the D_{2l} and D_{4rs} receptor subtypes, the coelacanth (Coel) has been chosen as a descendant of early diverging gnathostomes. Although incomplete, the sequences of the D_{2l} -like and D_{4l} -like receptors of lamprey are also shown, as representative of agnathans. The genomic sequences of the $D_2/D_{2l}/D_3$ receptors encompass seven exons separated by introns branching at

exactly the same place for the three paralogous sequences in different species, from teleost to mammals. The amino acids at the exon-intron boundary are also generally highly conserved. The D_4/D_{4rs} receptors are translated from four exons only, with intron sequences located again at the same place in the genomic sequences available from different species. In these subtypes, the two first exons and the last one are conserved with those of the $D_2/D_{2l}/D_3$ receptor genes, and the third exon corresponds to a fusion of exons 3, 4, 5 and 6 of the $D_2/D_{2l}/D_3$ receptor genes. This gene organization is also found for the lamprey genes, even if their sequence is only partial. Taken together, the organization of the gene sequences of the D_2 family clearly underlines the existence of two groups originating from two ancestral paralogous genes, the first one comprising the $D_2/D_{2l}/D_3$ receptor genes and the second one comprising the D_4/D_{4rs} receptor genes.

leost lineage, but preexisted in the common ancestor of Osteichthyes.

Gene synteny describes the physical colocalization of two or more genes at a given locus on the same chromosome, and the presence of shared synteny between species is a good indication that the genomic region is conserved from a common ancestor. We also performed gene synteny analysis and found that, consistent with the published data [Boehmler et al., 2004], syntenic genes of the zebrafish D_{2a} (chromosome 15) and D_{2b} receptor

genes (previously named D_{2c} ; chromosome 5) are found on the chromosomes bearing the D_2 gene in several tetrapod species (human chromosome 11, mouse chromosome 9, turkey chromosome 26). By contrast, tetrapod genomes did not show conserved synteny with the D_{2l} (previously named D_{2b} in zebrafish) locus. This supports the notion that the teleost D_{2l} receptor gene is not orthologous to the tetrapod D_2 . The conserved gene synteny of the D_4 loci was less obvious. Nonetheless, we also found some conservation between the D_{4a} locus of sev-

eral teleost species and the D_4 locus in tetrapods, coelacanth and gar. A conserved synteny of the D_{4-rs} (previously named D_{4b} in zebrafish [Boehmler et al., 2007]) locus was found on the chromosomes of coelacanth and gar, but not of tetrapods.

Altogether, it is likely that the ancestors of Osteichthyes possessed five D_2 family dopamine receptor genes: D_2 , D_3 , D_4 , D_{2l} and D_{4-rs} receptor genes. Since none of the tetrapod species possess D_{2l} or D_{4-rs} receptor genes, they have probably been secondarily lost before the divergence of tetrapods.

The exon-intron organization of D_2 family receptor genes displays high similarity among the D_2 , D_{2l} and D_3 receptor genes on the one hand, and D_4 and D_{4-rs} receptor genes on the other (fig. 4). The genomic sequences of $D_2/D_{2l}/D_3$ receptors encompass seven exons. In contrast, D_4/D_{4-rs} receptors encompass only four exons, and the third exon corresponds to a fusion of exons 3, 4, 5 and 6, which are present in the $D_2/D_{2l}/D_3$ receptor genes. Interestingly enough, the lamprey genome exhibits two genes corresponding to the D_2 family: one displaying the exon-intron organization similar to the $D_2/D_{2l}/D_3$ receptor genes, and the other to the D_4/D_{4-rs} receptor genes. Thus, the analysis of the exon-intron organization gives credence to the two clades displayed by the phylogenetic tree.

Evolutionary Scenario of Dopamine Receptor Genes in Line with the 2R Hypothesis

Many genes are present as single copies in genomes of amphioxus and ascidians, whereas mammals contain three or more orthologs. Such observations led to the 2R hypothesis which speculates that gnathostomes (jawed vertebrates) experienced two rounds of whole-genome duplication [Lundin, 1993; Holland et al., 1994; Pébusque et al., 1998; Skrabanek and Wolfe, 1998; Panopoulou and Poustka, 2005]. Although this hypothesis is still debated, it is tempting to draw a scenario of the evolution of dopamine receptor genes in this context (fig. 5).

For the D_1 family dopamine receptor genes, four paralogous genes exist in Osteichthyes, in contrast to a single D_1 family receptor gene (AmphiAmR1) in the amphioxus genome [Burman et al., 2007; Yamamoto et al., 2013]. This observation fits well with the 2R hypothesis. Based on sequence similarity and gene synteny, the first duplication of the D_1 -like gene of stem chordates would have given rise to a D_{1A}/D_{1B} -like gene and a D_{1C}/D_{1E} -like gene, and the second duplication would have given rise to the four D_{1A} , D_{1B} , D_{1C} and D_{1E} receptor genes. The presence

of the D_{1A} , D_{1B} and D_{1C} receptor genes in elephant shark indicates that the second round of duplications probably occurred before the separation of the Osteichthyes and Chondrichthyes lineages. If one of the duplications occurred before the gnathostome-cyclostome split [Kuraku et al., 2009], two paralogs of the D_1 family receptor genes would be expected to be present in cyclostomes. However, up to now only one D_1 family receptor gene, which is slightly more related to the D_{1A}/D_{1B} receptor gene, has been found in lamprey. Since the genomic information is still incomplete in cyclostomes, it is difficult to bring support to the duplication timing in the stem vertebrates.

In the case of the D_2 family receptor genes, both molecular phylogeny and intron-exon organization strongly support that the D_2 family of dopamine receptors is divided into two clades, one containing $D_2/D_{2l}/D_3$ sequences and another containing D_4/D_{4-rs} sequences. The presence of one lamprey gene of the D_2 receptor family in each clade (D_2 -like and D_4 -like receptors) suggests that the first round of duplication occurred before the gnathostome-cyclostome split.

The dopamine receptor genes of the D_2 family found in elephant shark are classified within the clades of the D_2 , D_3 and D_4 receptor sequences. This suggests that the second round of genome duplications generated the D_2 , D_3 , D_4 and D_{4-rs} paralogs, and that elephant shark lost the D_{4-rs} receptor gene. The D_{2l} receptor gene may have been generated by a gene duplication event that occurred after the Osteichthyes-Chondrichthyes split, and has been secondarily lost in the stem tetrapods.

Since no D_2 family receptor gene has been found in the genomes of nonvertebrate chordates (tunicates and amphioxus), it is difficult to infer the situation of stem chordates. Outside of chordates, genes assigned to the ' D_2 dopamine receptor family' are found in the sea urchin (a nonchordate deuterostome) as well as in several species of protostomes, based on molecular phylogenies. Considering that tunicate and cephalochordate genomes have lost numerous genes, it is possible that these two groups of chordates have independently lost genes encoding D_2 family receptors.

The Problem of Nomenclature of the Dopamine Receptor Genes

The nomenclature of the dopamine receptor genes is quite confusing and unsatisfactory for two main reasons: (1) it does not seriously take into account the existence of additional dopamine receptor subtypes in nonmammalian species, and (2) the nomenclature of the two families

Fig. 5. Hypothetical evolutionary scenario of dopamine receptor genes in vertebrates. **a** Evolutionary scenario of gene duplications of dopamine receptor genes drawn in line with the 2R hypothesis. The arrows indicate hypothetical genome duplications, and the arrowhead indicates a gene duplication. A single D_1 -like and D_2 -like gene in the stem chordates were probably duplicated twice, before and after the gnathostome-cyclostome split, raising four subtypes in stem gnathostomes. The D_2 and D_{2l} genes were further duplicated in the stem Osteichthyes, resulting in five D_2 family dopamine receptor genes in Osteichthyes. **b** Evolutionary scenario within the group of Osteichthyes based on the presence of dopamine receptor genes in modern species. Zebrafish possess extra copies of many subtypes, presumably due to the teleost-specific genome duplication. The tetrapod lineage has secondarily lost the D_{2l} and D_{4rs} receptor genes, and mammals have further lost D_{1C} and D_{1E} receptor genes.

of dopamine receptors is not consistent, as alphabetical suffixes are applied for subtypes of the D_1 family (D_{1A} , D_{1B}), whereas numeric suffixes are applied for subtypes of the D_2 family (D_2 , D_3 , D_4).

The IUPHAR Committee on Receptor Nomenclature and Drug Classification (<http://www.guidetopharmacology.org/>) considers only receptor genes which are present in the human genome. Thus, the receptor subtypes which have been lost in mammals were named independently from the IUPHAR Committee by using alphabetical suf-

fixes, i.e. D_{1C} [Sugamori et al., 1994], D_{1D} [Demchyshyn et al., 1995] and D_{1X} [Hirano et al., 1998] (fig. 6a). Accordingly, the modified nomenclature proposed by Yamamoto et al. [2013], which accounts for the correct sequence relationship of the different subtypes within the D_1 family, also used the alphabetical suffix from D_{1A} to D_{1E} (fig. 6b). As a result, the successive findings of D_1 family receptor genes in nonmammalian species resulted in a nomenclature that is inconsistent between the D_1 family (alphabetical suffix) and the D_2 family (numeric suffix).

Fig. 6. Inconsistent nomenclature of dopamine receptor genes. The nomenclature of dopamine receptor genes is quite confusing because of successive findings of new subtypes in nonmammalian species and the misidentification of homologous relationships. Here we clarified the evolutionary relationships of dopamine receptor genes of both the D_1 and D_2 families, which is shown as simplified phylogenetic trees. The same phylogenetic position represents the same gene. Nomenclatures for teleost-specific paralogs are indicated in parentheses. **a** Old nomenclatures used in previous publications. **b** Nomenclatures applied in this article. Our publication on D_1 family receptor genes [Yamamoto et al., 2013] fol-

lowed the alphabetical nomenclature used in earlier studies. The newly identified gene was named as D_{1E} because the ' D_{1D} ' was already used for the avian D_{1C} [Demchyshyn et al., 1995]. The nomenclature for the D_2 family receptor genes followed that of Ensembl and ZFIN. **c** A proposition of nomenclature which is coherent with the evolutionary scenario of dopamine receptor genes. **d** A proposition of nomenclature in which subtypes within each D_1 and D_2 family are represented by letters (D_{1A} , D_{1B} , D_{1C} , etc./ D_{2A} , D_{2B} , D_{2C} , etc.). **e** A proposition of nomenclature which reflects only chronologic order of gene discovery, and requires minimal changes from the currently accepted nomenclature.

Ideally, a nomenclature of receptors should be coherent with the phylogenetic classification. When we take into account the evolution of the two different classes of dopamine receptor genes in Bilateria, each receptor subtype could be classified according to duplication events. For example, the first duplication in the D_1 family would give rise to D_{1A} and D_{1B} , and the second duplication would give rise to D_{1A1} , D_{1A2} , D_{1B1} , D_{1B2} , etc. (fig. 6c). The problem of this system is that a nomenclature cannot be fixed without performing a rigorous phylogenetic analysis. In addition, it encounters the necessity of renaming when the evolutionary classification is modified.

An alternative possibility is to distinguish the two families as D_1 and D_2 , and arbitrarily assign an alphabetical suffix for subtypes within each family (fig. 6d). This is the case for the nonmammalian D_1 family subtypes, and it follows the rule used for many other monoamine recep-

tors, such as serotonin receptor subtypes ($5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, and $5HT_{2A}$, $5HT_{2B}$, $5HT_{2C}$, etc.). In this system, the alphabetical order would not necessarily reflect the evolutionary relationship, but it would keep the distinction between the D_1 and D_2 families, which are distantly related to each other. This nomenclature change would require the renaming of D_2 to D_{2A} , D_3 to D_{2C} and D_4 to D_{4D} . The acceptance of such changes would not be easy since the numeric nomenclature ' D_2 , D_3 and D_4 ' for the D_2 family is so extensively utilized.

A third possibility is to rename all the subtypes in numeric order following the current nomenclature proposed by IUPHAR. The official database of the IUPHAR and gene databases use numeric suffixes such as D_1 and D_5 , and the terms D_{1A} and D_{1B} are considered as 'previous or unofficial names'. Looking at the use of terminology in other genome databases, the current tendency of using

numeric suffixes seems to become a rule. In the Ensembl and ZFIN databases, the zebrafish D_{1C} receptor gene has been recently renamed D_6 , and the D_{1E} receptor has been renamed D_7 . In this line, the additional D_2 family receptor subtypes, ' D_{2I} ' and ' D_{4-rs} ', should be renamed D_8 and D_9 , respectively (fig. 6e).

The problem of this last option is that it does not differentiate between the two different dopamine receptor families. However, its main advantage is that it requires a minimal change of already accepted nomenclature. To facilitate everyone using the same terminology which reflects correct orthologous relationships, naming all the dopamine receptor genes using consecutive numbering may be a good compromise. In order to achieve a nomenclature change that permits distinguishing between two different receptor families (alphabetic suffix; fig. 6d), a nomenclature consortium needs to be organized to promote an official change of the well-accepted terminology such as ' D_2 ', ' D_3 ' and ' D_4 '.

Conclusion

The dopamine receptors have been highly diversified as a consequence of gene duplications during vertebrate evolution. Overall, nine dopamine receptor genes exist in

the group of Osteichthyes: four in the D_1 family (D_1 , D_5 , D_6 and D_7) and five in the D_2 family (D_2 , D_3 , D_4 , D_8 and D_9). Mammals have lost many dopamine receptor genes as compared to other vertebrate groups, keeping only five of the ancestral dopamine receptor genes (from D_1 to D_5).

Examining the functional significance of such receptor multiplicity is essential to gain a better understanding of the nature of dopamine neurotransmission in vertebrates. Since the duplicated paralogous genes are kept in many species, it is unlikely that all these genes are functionally redundant. Cautious evolutionary and comparative approaches will contribute to deciphering the physiological significance of the vast diversity of dopamine systems, and will enable proper interpretations of information obtained from animal models of human pathologies involving dysfunction of dopamine systems.

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