General and Comparative Endocrinology 240 (2017) 129–137

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Research paper

Evolution of the β-adrenoreceptors in vertebrates

Kattina Zavala [a], Michael W. Vandewege [b], Federico G. Hoffmann [b,c], Juan C. Opazo [a,*]

[a] Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile
[b] Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, MS, USA
[c] Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, MS, USA

ARTICLE INFO

Article history:
Received 13 April 2016
Revised 13 October 2016
Accepted 17 October 2016
Available online 18 October 2016

Keywords:
ADRB
Genome duplication
Evolutionary medicine
Lamprey
Gene expression

ABSTRACT

The study of the evolutionary history of genes related to human disease lies at the interface of evolution and medicine. These studies provide the evolutionary context on which medical researchers should work, and are also useful in providing information to suggest further genetic experiments, especially in model species where genetic manipulations can be made. Here we studied the evolution of the β-adrenoreceptor gene family in vertebrates with the aim of adding an evolutionary framework to the already abundant physiological information. Our results show that in addition to the three already described vertebrate β-adrenoreceptor genes there is an additional group containing cyclostome sequences. We suggest that β-adrenoreceptors diversified as a product of the two whole genome duplications that occurred in the ancestor of vertebrates. Gene expression patterns are in general consistent across species, suggesting that expression dynamics were established early in the evolutionary history of vertebrates, and have been maintained since then. Finally, amino acid polymorphisms that are associated to pathological conditions in humans appear to be common in non-human mammals, suggesting that the phenotypic effects of these mutations depend on epistatic interaction with other positions. The evolutionary analysis of the β-adrenoreceptors delivers new insights about the diversity of these receptors in vertebrates, the evolution of the expression patterns and a comparative perspective regarding the polymorphisms that in humans are linked to pathological conditions.

1. Introduction

The study of the evolutionary history of genes related to human disease is a fundamental field at the interface of evolution and medicine (Gluckman et al., 2011). Molecular evolutionary studies of genes that are responsible for pathological conditions provide the genetic context on which medical researchers should work. They also are useful in providing significant information to guide genetic experiments, especially in model species where genetic manipulations can be made. Comparative studies have taken a new perspective since the discovery that some non-model species are resistant to age related diseases (Yu et al., 2011; Edrey et al., 2012; Gorbunova et al., 2012; Manov et al., 2013; Novikov and Burda, 2013; Henning et al., 2014; Faulkes et al., 2015), and by the fact that some mammals develop pathologies in a similar way to humans (Castro-Fuentes and Socas-Pérez, 2013; Tarragon et al., 2013; Braidy et al., 2015; Inestrosa et al., 2015).

From an evolutionary standpoint, several studies suggest that genes related to human disease are not a random sample from the genome, as they are likely to be of ancient origin, tend to be non-essential and are preferentially located on the periphery of gene networks (Domazet-Loso and Tautz, 2008; Dickerson and Robertson, 2012; Maxwell et al., 2014). According to Maxwell et al. (2014), a small fraction of them originated in the last 630 million of years, during the evolutionary history of vertebrates. For this group of ancient genes, the two rounds of whole genome duplication that occurred early in the history of vertebrates (Meyer and Schartl, 1999; McLysaght et al., 2002; Dehal and Boore, 2005; Putnam et al., 2008) are supposed to have played a pivotal role in shaping their evolution.

Adrenergic receptors, which are G protein-coupled molecules that mediate the action of catecholamines such as epinephrine and norepinephrine, appear to be an example of ancient genes with disease relevant phenotypes. Adrenoreceptors have been classified into two major types, α- and β-adrenoreceptors, on the basis of agonist-mediated responses. β-adrenoreceptors (ADRB) have been intensively studied as they have been linked to human pathologies. They play a critical role in the regulation of cardiovascular and pulmonary physiology, as well as other mammalian functions. They are classified into three major subgroups (ADRB1, ADRB2 and ADRB3). β_1-adrenoreceptors (ADRB1) are mostly expressed in the

* Corresponding author.
E-mail address: jopazo@gmail.com (J.C. Opazo).

http://dx.doi.org/10.1016/j.ygcen.2016.10.005
0016-6480/

heart (Bristow et al., 1986; Dorn, 2010), and they are key mediators of excitation-contraction coupling in this organ (Dorn, 2010). β_2-Adrenoreceptors (ADRB2) are mainly involved in vasodilation and hypertension, cardiac function, asthma, and preterm labor and birth (Ahles and Engelhardt, 2014). β_3-Adrenoreceptors (ADRB3) are predominantly expressed in the adipose tissue, gall bladder and portions of the colon (Krief et al., 1993; Berkowitz et al., 1995), and are involved in the activation of the nitric oxygen synthase in cardiomyocytes, relaxation of the urinary bladder, and the release of hematopoietic stem cells and progenitor cells from the bone marrow in myocardial infarction (Michel et al., 2010; Dutta et al., 2012).

The goal of this study was to investigate the evolutionary history of the β-adrenoreceptors in vertebrates, with the aim of adding an evolutionary context to the already abundant physiological information available. We assessed the diversity of β-adrenoreceptors in representative species of all major groups of vertebrates; inferred homologous relationships by examining phylogenies and synteny conservation and evaluated the role of whole genome duplications in promoting the observed gene diversity. We also examined gene expression patterns among different tissues in representative species of vertebrates. Our results show that in addition to the three already identified β-adrenoreceptor genes (i.e. *ADRB1*, *ADRB2* and *ADRB3*) in gnathostomes; there is an additional set of ADRB paralogs in cyclostomes. Our results also suggest that β-adrenoreceptors diversified as a product of the two whole genome duplications early in vertebrate evolution. Gene expression patterns are consistent across species, suggesting that expression dynamics were established deep in time during the evolutionary history of vertebrates, and have been maintained since then.

2. Materials and methods

2.1. DNA data collection and phylogenetic analyses

We annotated β-adrenoreceptor genes in representative species of all major groups of chordates. We included representative species from mammals, birds, reptiles, amphibians, lobe-finned fish, teleost fish, non-teleost ray-finned fish, cartilaginous fish, cyclostomes, urochordates and cephalochordates (Supplementary Table S1). Additionally, we also included sequences of the α-adrenoreceptors 2 (ADRA2) A, B and C, and dopamine receptors D (DRD) 1, 2, 3, 4 and 5 from humans (Supplementary Table S1). Amino acid sequences were aligned using the L-INS-i strategy from MAFFT v.6 (Katoh and Standley, 2013). Phylogenetic relationships were estimated, using gene regions that correspond to the transmembrane domains, according to maximum likelihood and Bayesian approaches. We used the propose model tool of IQ-Tree (Trifinopoulos et al., 2016) to select the best-fitting model of amino acid substitution (JTT+G). We performed a maximum likelihood analysis to obtain the best tree using the program IQ-Tree (Trifinopoulos et al., 2016), and assessed support for the nodes with 1000 bootstrap pseudoreplicates using the ultrafast routine. Bayesian searches were conducted in MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003), setting two independent runs of six simultaneous chains for 20×10^6 generations, sampling every 2500 generations, and using default priors. The run was considered to have reached convergence once the likelihood scores reached an asymptotic value and the average standard deviation of split frequencies remained <0.01. We discarded all trees that were sampled before convergence, and we evaluated support for the nodes and parameter estimates from a majority rule consensus of the last 4000 trees. Human ADRA1A, B, and D sequences were used as outgroups.

2.2. Assessments of conserved synteny

We annotated the genes found upstream and downstream of the β-adrenoreceptor genes on species representative of all main groups of vertebrates. Synteny analyses were performed in humans (*Homo sapiens*), opossum (*Monodelphis domestica*), platypus (*Ornithorhynchus anatinus*), chinese turtle (*Pelodiscus sinensis*), chicken (*Gallus gallus*), anole lizard (*Anolis carolinensis*), clawed frog (*Xenopus tropicalis*), coelacanth (*Latimeria chalumnae*), spotted gar (*Lepisosteus oculatus*) and elephant shark (*Callorhinchus milii*). Initial ortholog predictions were derived from the EnsemblCompara database (Herrero et al., 2016) and were visualized using the program Genomicus v83.01 (Muffato et al., 2010). In the case of the Japanese lamprey (http://jlampreygenome.imcb.a-star.edu.sg/) and the elephant shark (http://esharkgenome.imcb.a-star.edu.sg/) the genomic pieces containing *ADRB* genes were annotated, and predicted genes were then compared with the non-redundant protein database using Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) (Supplementary Tables S5–S9). For the genes neighboring the ADRB paralogs of vertebrates, we used the estimates of orthology and paralogy derived from the Ensembl Compara database (Herrero et al., 2016), which are obtained from an automated pipeline that considers both synteny and phylogeny to generate orthology mappings. Importantly, in many cases, protein names often do not reflect these mappings, such as in the case of CRIMP1, which is grouped with the DPYSL2, 3 and 4 paralogs, though a careful inspection of the CRIMP1 record reveals that it includes DPYSL1 as a synonym, in agreement with the Ensembl Compara assessment.

2.3. Transcript abundance assessment

ADRB transcript abundance was measured in the elephant shark, spotted gar, chicken, and human. For each species, *ADRB* expression was assessed in six different tissues (brain, heart, kidney, liver, muscle and testis). RNASeq data was gathered from a variable set of tissues available from GenBank's SRA database (Supplementary Table S2). Reference cDNA sequences of predicted genes from each genome were collected from Ensembl, for each gene only the longest cDNA sequence was included. Elephant shark cDNA sequences were collected from its own web site (http://esharkgenome.imcb.a-star.edu.sg/). Gene expression levels were estimated using RSEM v1.2.3 (Li and Dewey, 2011) which uses Bowtie v. 0.12.9 (Langmead et al., 2009) to map reads to the proper set of coding sequences. Default settings were used, and expression was measured in transcripts per million (TPM).

3. Results and discussion

3.1. Gene phylogenies and synteny analyses

Our maximum-likelihood and Bayesian phylogenies place human DRD1 and DRD5 as sister to a clade that includes all chordate ADRB receptor sequences (Fig. 1), consistent with Spielman et al. (2015) that identifies dopamine receptors D1 and D5 (*DRD1* and *DRD5*) as the genes most closely related to ADRBs. The clade that includes lancelet sequences was recovered sister to the clade that includes ADRB sequences from olfactores, the group that includes vertebrates and tunicates (Fig. 1), in agreement with the most updated organismal phylogeny (Delsuc et al., 2006). The phylogenetic position of the lancelet sequences agrees with the chimeric nature of the molecule in this group. According to Candiani et al. (2005) (Candiani et al., 2005), the receptor gene present in the genome of cephalochordates is characterized by possessing

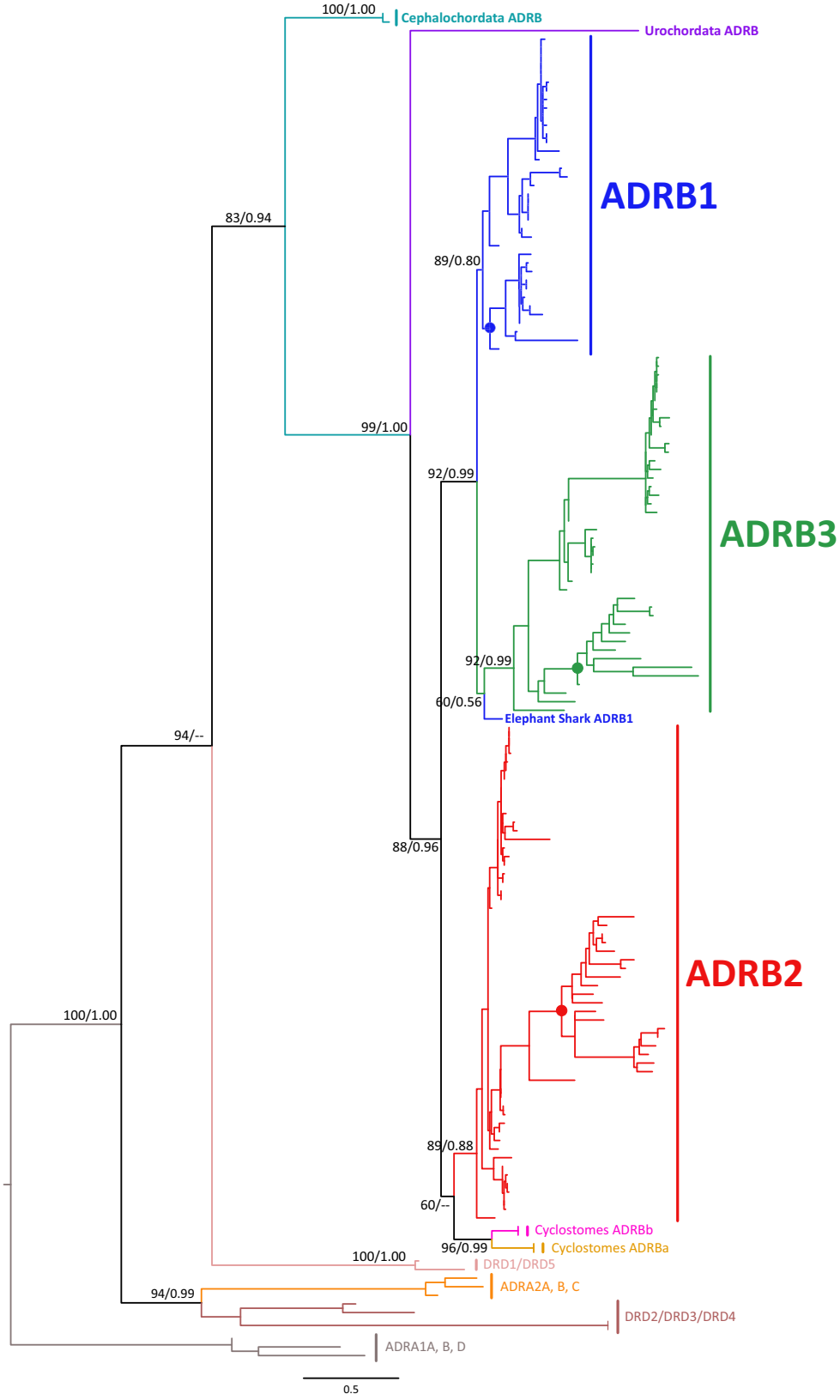

Fig. 1. Maximum likelihood phylogram depicting relationships among β-adrenoreceptors based on amino acid sequence data from transmembrane domains. Numbers on the nodes correspond to maximum likelihood ultrafast bootstrap and Bayesian posterior probabilities support values. Color dots denote the fish subtrees that are expanded in Fig. 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

high sequence similarity with both dopamine and β-adrenergic receptors.

Vertebrate ADRBs are arranged into four strongly supported clades corresponding to the three β-adrenoreceptors from

gnathostomes (*ADRB1*, *ADRB2* and *ADRB3*), plus the ADRB genes from lampreys in the fourth. Within the *ADRB1*, *ADRB2* and *ADRB3* clades the species arrangement matches the organismal phylogeny in most cases, with no evidence of additional duplications other than those derived from the teleost specific whole genome duplication, which will be discussed below. We could not find traces of *ADRB1* in the clawed frog, or of *ADRB2* and *ADRB3* in platypus, which could either reflect secondary losses or gaps in the current genome assemblies. The presence of a single gene in non-vertebrate chordates and 3 paralogs in gnathostomes suggests the expansion of the vertebrate ADRBs is probably linked to the two rounds of whole genome duplication that occurred early in vertebrate history (Meyer and Schartl, 1999; McLysaght et al., 2002; Dehal and Boore, 2005; Hoegg and Meyer, 2005).

Our synteny analyses provide additional support for the phylogeny depicted in Fig. 1. Genes found up- and downstream of the *ADRB* paralogs are in general well conserved in all main groups of gnathostomes (Fig. 2). In most surveyed species there are four upstream genes (*NHLRC2*, *DCLRE1A*, *PLEKHS1 AND CASP7*) and four downstream genes (*CCDC186*, *TDRD1*, *VWA2*, *AFAP1L2*) that are well conserved in the chromosome containing the *ADBR1* gene (Fig. 2A). For the *ADBR2* gene, synteny was mostly conserved on the downstream region of gnathostomes (Fig. 2B), where the presence of four genes (*SH3TC2*, *ABLIM3*, *AFAP1L1*, *GRPEL2*) is well conserved (Fig. 2, upper panel). In the case of the *ADRB3* gene there are upstream (GOT1L1, RAB11F1P1, BRF2, GPR124) and downstream genes (EIF4EBP1, ASH2L, STAR, LSM1) that are conserved across gnathostomes (Fig. 2C). Thus, our analyses suggest that the position of these genes has been conserved throughout the history of gnathostomes (Fig. 2).

3.2. ADRB genes in cyclostomes

In addition to the *ADRB1*, *ADRB2* and *ADRB3* genes from gnathostomes, we found four *ADBR* sequences in two lamprey species (Fig. 1), which were recovered as two distinct sister clades (Fig. 1; ADRBa and ADRBb clades). The ADRBa proteins of both cyclostome species are 96.6% similar, whereas the ADRBb proteins are 98.8%, the most feasible explanation for these high values could be a purifying selective regime since they shared a common ancestor (11–17 mya; Kuraku and Kuratani, 2006). Genes found up- and downstream of the lamprey *ADRBb* gene (*VWA2*, *SH3TC2*, *CCDC186*, *SHOC2*, *HELQ*, *DCTN1*) were conserved in both lamprey species (Fig. 3, upper panel), further defining their orthology between both

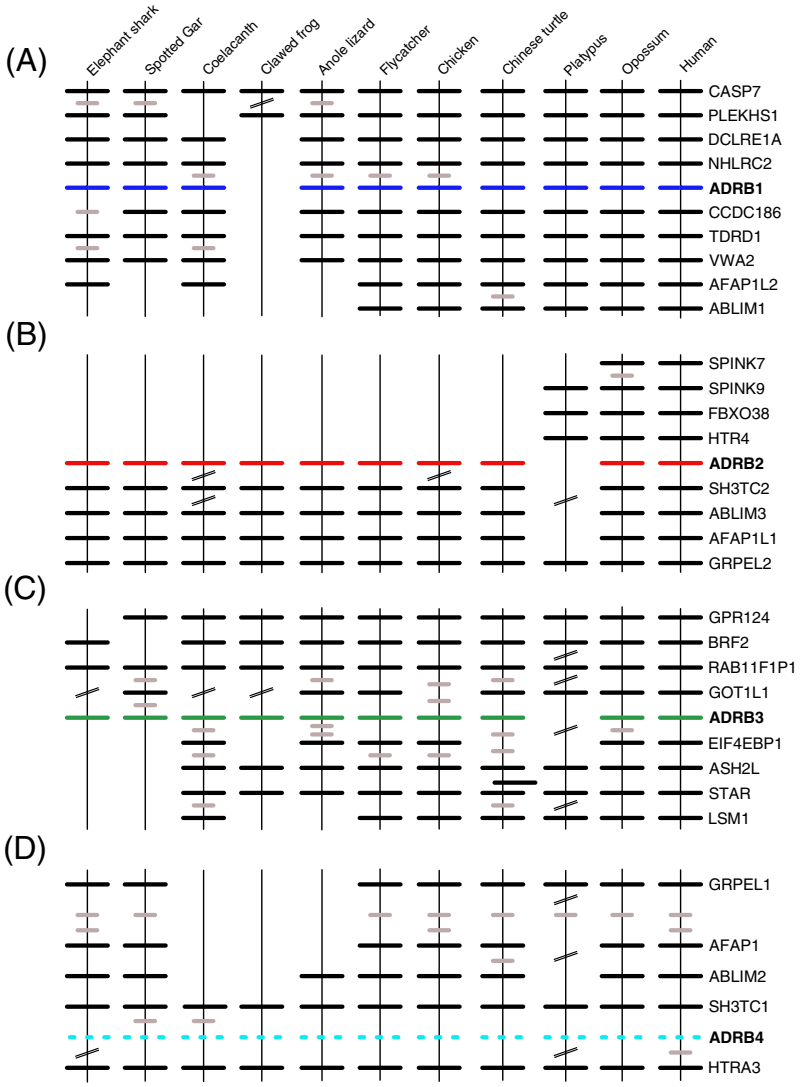

Fig. 2. Patterns of conserved synteny in the genomic regions that harbor β-adrenoreceptor genes in gnathostome vertebrates. A) genomic region that harbors ADRB1 genes. B) Genomic region that harbors the ADRB2 genes. C) Genomic region that harbors the ADRB3 genes. D) Patterns of conserved synteny in the genomic region that would be the putative location of the fourth β-adrenoreceptor gene in gnathostome vertebrates. Asterisks in the lower panel denote that the orientation of the genomic piece is from 3′to 5′.

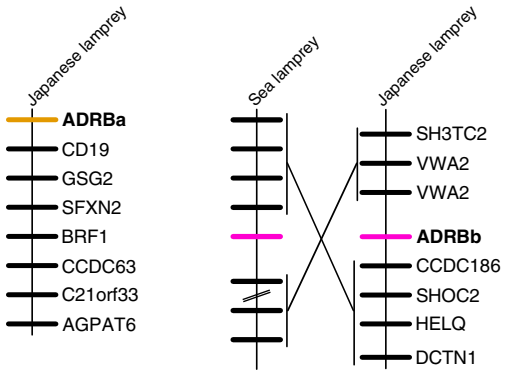

Fig. 3. Patterns of conserved synteny in the genomic regions that harbor β-adrenoreceptor genes in cyclostomes.

lamprey species. Interestingly, three out of the six annotated genes in this lamprey genomic fragment, *VWA2*, *CCDC186* and *SHOC2* (this gene is located 3 Mb upstream from ADRB1 of humans), consistently map to chromosome 10 of humans, where the *ADRB1* gene is located, suggesting that the ADRBb gene of lampreys could be orthologous to the *ADRB1* gene of gnathostomes (Fig. 1). However, this putative orthologous relationship should be taken with caution, as we also found a gene (SH3TC2) that maps to chromosome 5 of humans where the ADRB2 is located. For the lamprey ADRBa clade (Fig. 1), we were able to annotate syntenic genes only in the Japanese lamprey (Fig. 3, upper panel). Unfortunately, syntenic genes found in this genomic region did not map to any specific chromosome in humans. In fact orthologs of all annotated genes in the genomic fragment map to different human chromosomes, suggesting that this gene could be the result of a duplication event restricted to cyclostomes. Alternatively, the two lamprey *ADRB* genes might have been placed together in the phylogenetic tree due to features of the cyclostome genomes (e.g. GC bias) that differ substantially from all other vertebrate genomes. In support of this idea, genome-wide compositional analysis shows that cyclostome genomes indeed possess strong compositional bias (Qiu et al., 2011; Kuraku, 2013; Mehta et al., 2013; Smith et al., 2013) making difficult to recover the true evolutionary history of genes using phylogenetic approaches. Accordingly, other evolutionary studies working with different group of genes (e.g. KCNA, Mybs, GlobinX, Runx, p53, Reprimo) have obtained similar results like in this study i.e. cyclostome sequences tend to group together instead to their true ortholog (Qiu et al., 2011; Nah et al., 2014; Campanini et al., 2015; Opazo et al., 2015; Coffill et al., 2016; Wichmann et al., 2016)

3.3. ADRB fourth genomic region and the role of the two vertebrate specific WGDs

If the *ADRB* genes diversified as the product of the two rounds of whole genome duplications in the last common ancestor of vertebrates (Meyer and Schartl, 1999; McLysaght et al., 2002; Dehal and Boore, 2005; Hoegg and Meyer, 2005; Putnam et al., 2008), we should be able to find the genomic region that harbored the fourth *ADRB* gene copy. According to our analysis, on chromosome 4 of humans there is a group of genes that belong to the same gene family as those annotated on chromosome 5 where the human *ADRB2* gene is located (Fig. 2). According to our survey, on chromosome 4 of humans there are four genes (*SH3TC1*, *ABLIM2*, *AFAP1*, *GRPEL*) that possess ohnologs, i.e. genes that have originated by a process of whole genome duplication, that are in the same order of those found on chromosome 5. This gene arrangement was also conserved in representative species of all main groups of gnathostomes, strongly suggesting that this location could be the fourth ADRB genomic region (Fig. 2D). The fact that all vertebrate species lost the same ohnolog, the β-adrenoreceptor that was putatively located on the region corresponding to human chromosome 4, suggests this loss probably occurred shortly after the whole genome duplications. Moreover, if these four genomic locations are the product of the two vertebrate whole genome duplications we should be able to also find additional ohnolog genes in all four chromosomes (Fig. 2). In agreement with this prediction we found at least 8 ohnologs (e.g. *PDLIM*, *FGFR*, *ADAM*, *ABLIM*, *ANK*) that were present on the four genomic locations previously described (Supplementary Table S3). We also found ohnologs that were present in two (e.g., *GRPEL*, *SH3TC*, *SORCS*) or three (e.g., *DKK*, *SLIT*, *PITX*) of the chromosomes (Supplementary Table S4). Interestingly, Putnam et al. (2008) (Putnam et al., 2008) had reported that an unusually large number of genes found on human chromosomes 4, 5, 8 and 10, where the human ADRB genes are located, derive from the ancestral chordate linkage group 7 providing additional support for the role of whole genome duplications in the diversification of ADRB genes. Finally, in support of our analyses, ADRB genes are also included in the list of gene families that diversified as the product of the two rounds of whole genome duplication occurred in the last common ancestor of vertebrates (http://ohnologs.curie.fr) (Singh et al., 2015).

3.4. ADRB gene in teleost fish and the role of the teleost-specific WGD

To further assess the impact of whole genome duplications in the evolution of ADRBs, we explored the genetic complement of genes in teleost fish, a group that experienced an extra round of whole genome duplication (Meyer and Van de Peer, 2005; Kasahara, 2007; Sato and Nishida, 2010). In this analysis we included all available fish species in genome databases, as well as, individual records. We also included β-adrenoreceptor sequences from elephant shark and coelacanth. The phylogenetic prediction is to find duplicated copies of all ADRB lineages in teleost fish relative to gar, a species that did not experience the extra round of whole genome duplication. Our results revealed the presence of the three already identified ADRB genes (Fig. 4). In the case of the ADRB1 clade, our gene tree shows the presence of a single gene lineage in teleost fish (Fig. 4, upper panel) and synteny analyses provide further support as genes found up- (e.g. NHLRC2, DCLRE1A) and downstream (e.g. CCDC186, TDRD1, VWA2) the ADRB1 gene are conserved in comparison to other vertebrates. In the case of the ADRB2, the presence of two teleost clades suggests they derived from the teleost specific genome duplication (Fig. 4; lower panel). Although, the inclusion of the spotted gar sequence within one of the teleost clades (Fig. 4; lower panel) could suggest that the duplication predates the teleost radiation synteny comparisons of flanking genes (AFAP1L1A and AFAP1L1B; RNF145a and RNF145b; IL12A and IL12B) suggests they derive from the teleost specific genome duplication. The case of the ADRB3 clade is similar to the ADRB2 except that the two derived ADRB3 duplicates were retained in fewer species. One of these duplicates was retained by two species (zebrafish and cavefish) whereas the other by 8 species (Fig. 4; middle panel). Similarly, the presence of ohnolog genes (PRLHR2A and PRLHR2B; RAB11FIP1A and RAB11FIP1B) further indicates these two teleost ADRB3 paralogs are the product of the teleost specific genome duplication.

3.5. ADRB gene expression in vertebrates

In order to understand the evolutionary trends at the expression level, we characterized transcript abundance of the three ohnologs in four representative species of vertebrates. Our results indicate that the *ADRB1* gene is mostly expressed in the heart and brain in most surveyed species (Table 1). In agreement with this

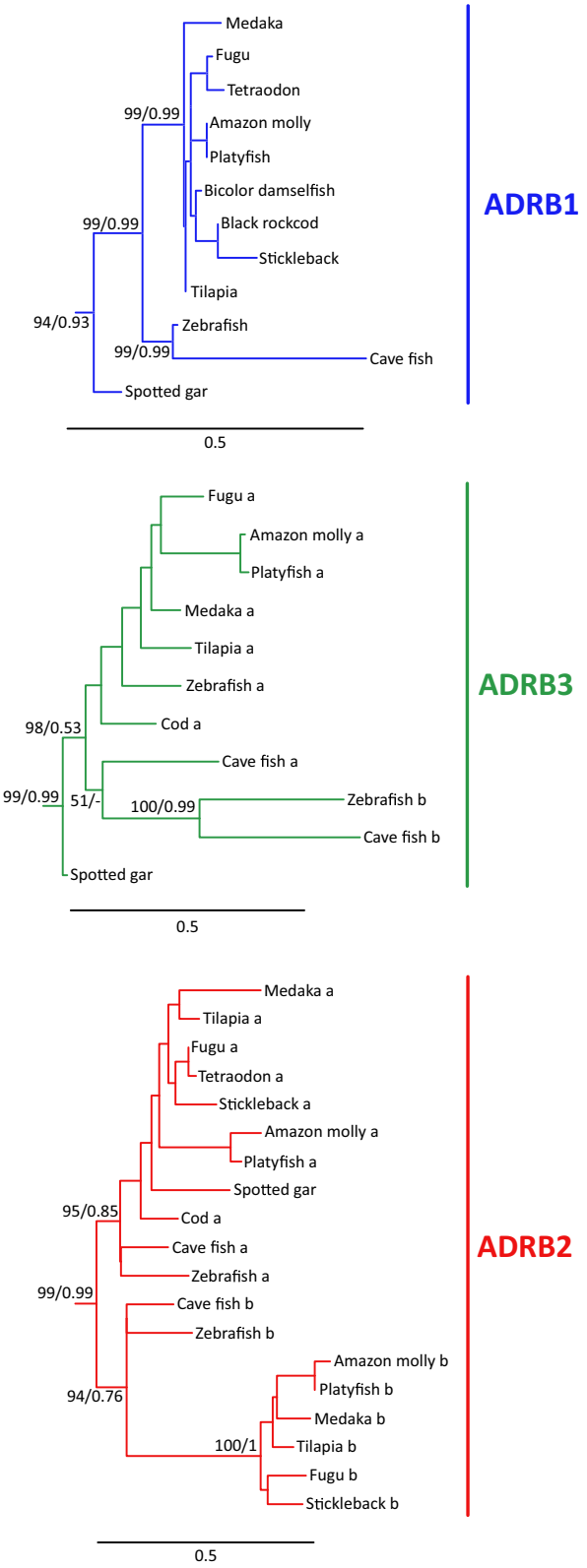

Fig. 4. Maximum likelihood phylograms depicting relationships among β-adrenoreceptors in fish. Upper panel: phylogenetic relationships among ADRB1 sequences of fish. Middle panel: phylogenetic relationships among ADRB3 sequences of fish. Lower panel: phylogenetic relationships among ADRB2 sequences of fish. Numbers on the nodes correspond to maximum likelihood ultrafast bootstrap and Bayesian posterior probabilities support values. These topologies do not represent novel phylogenetic analyses; they are the fish clades that were recovered from Fig. 1.

result, in the literature it has been described that in the heart around 77% of the isoforms correspond to the ADRB1 receptor (Bristow et al., 1986; Dorn, 2010). The exception to this pattern is the elephant shark where the *ADBR1* gene is expressed at low levels in all examined tissues (Table 1). In the chicken and spotted gar the *ADRB2* gene is most abundantly expressed in the liver and muscle, although expression is also observable in other tissues (Table 1). In the elephant shark, the expression of this gene follows a different trend, as it is highly expressed in the heart in comparison to other tissues (Table 1). In humans, we do not observe a clear pattern, as this gene is expressed at low levels in all tissues (Table 1). Finally, the ADRB3 gene is expressed at low levels in all tissues in all species (Table 1) other than chicken, where the *ADRB3* gene is mostly expressed in the heart (Table 1). The low levels of the *ADRB3* gene we quantified in most tissues in all species could be due to the fact that this gene is mostly expressed in adipocytes (Emorine et al., 1989), a tissue that was not included in our study.

3.6. ADRB polymorphisms in species related to humans

Several polymorphisms for the β-adrenoreceptors have been described in humans (Taylor and Bristow, 2004; Ahles and Engelhardt, 2014), some of which have been associated with structural, functional and clinical conditions (Taylor and Bristow, 2004; Ahles and Engelhardt, 2014). This information represents an opportunity to make a screening in non-model species related to humans, with the aim that the comparative perspective could shed light into the functional role of the β-adrenoreceptors. To do so, we compared the positions for which amino acid polymorphisms have been described in humans in a species panel that included primates, rodents and lagomorphs (Table 2). In our assessment we found that most surveyed species possess the most common alleles described for humans (Table 2). There is also a group of positions for which some species possess the most common allele, whereas others possess the alternative one (e.g. 352, 402, 404, 418; Table 2). There are some amino acid positions for which the rare allele is present in most species (Table 2). The most interesting cases are positions 49 and 27 of the *ADRB1 and ADRB2* genes, respectively, were all non-human mammals possess the alternative allele (Table 2). In the case of the position 49 of the *ADRB1* gene, which is the most important β-adrenoreceptor in modulating instant cardiac output (Taylor and Bristow, 2004; Dorn, 2010), and has been to linked to a number of cardio vascular conditions, all investigated species possess glycine, the alternative allele, given that in humans only 15% of the population possess this amino acid at this position (Maqbool et al., 1999; Börjesson et al., 2000). Although a possibility is that in non-human mammals the frequency of this polymorphism follows the human trend, and we only screened individuals belonging to the 15% of the population, the fact that all species surveyed possess the alternative allele suggest that glycine at position 49 would be common in non-human mammalian species. A similar situation is seen at position 27 of the *ADRB2* gene, which has been associated with asthma presence and severity (De Paiva et al., 2014) (Table 2). The polymorphism at position 64 of the ADRB3 is interesting, as in the literature a replacement of a tryptophan by an arginine has been associates to obesity, insulin resistance, hypertension (Ringel et al., 2000). In all these cases the fact that all/most species surveyed possess the alternative allele suggest that the specific position by itself does not produce the same physiological condition as described for humans as it effects would depend on the identity at other amino acid positions, i.e. epistatic mutations (Breen et al., 2012).

Table 1
Gene transcription profiles of the three β-adrenoreceptor genes in humans, chicken, spotted gar and elephant shark.

	Human			Chicken			Spotted gar			Elephant shark		
	ADRB1	*ADRB2*	*ADRB3*	*ADRB1*	*ADRB2*	*ADRB3*	*ADRB1*	*ADRB2*	*ADRB3*	*ADRB1*	*ADRB2*	*ADRB3*
Brain	12.9	3.19	0.37	13.19	5.88	0.04	7.1	14.51	1.82	0.12	1.97	0
Heart	6.78	2.59	0	15.65	11.32	11.13	45.65	14.74	3.75	1.09	116.58	0.08
Kidney	2.59	1.51	0.15	0.89	11.01	0	0.65	6.32	3.55	0.65	2.13	0
Liver	0.07	3.05	0	1.79	16.59	0.03	0.1	36.08	1.23	0.51	9.47	0
Muscle	0	0.78	0	0.74	93.4	0	0.13	29.83	0.19	0.44	0.89	0.53
Testis	0.92	0.49	0	0	2.25	0	0.32	1.57	0.16	0.38	0.84	0

Gene expression was measured in transcript per million (TPM).

Table 2
Summary of β-adrenoreceptor non-synonymous polymorphisms among select mammals. Amino acids matching the rare alleles in humans are in bold. Colors correspond to the three different paralogs: ADRB1 in blue, ADRB2 in red, and ADRB3 in green. Question marks denote missing information.

	ADRB1													***ADRB2***					***ADRB3***			
Position	49	59	318	324	343	352	389	399	400	402	404	418	460	16	27	34	164	220	64	251	265	268
Common Allele / Rare Allele	S/G	A/S	R/S	K/R	A/T	E/D	R/G	R/C	R/L	H/R	T/A	P/A	D/E	G/R	Q/E	V/M	T/I	S/C	W/R	T/P	T/M	L/P
Primates																						
Human	S	A	R	K	A	E	**G**	R	R	H	T	P	D	G	**E**	V	T	S	W	**P**	T	**P**
Chimpanzee	**G**	A	R	K	A	E	R	R	R	H	T	P	D	G	**E**	V	T	S	**R**	**P**	T	**P**
Gorilla	**G**	A	?	?	?	?	?	?	?	?	?	?	G	G	**E**	V	T	S	**R**	**P**	T	**P**
Orangutan	?	?	R	K	A	E	R	R	R	H	T	P	D	G	**E**	V	T	S	**R**	**P**	T	L
Gibbon	**G**	A	R	K	A	E	R	R	R	H	T	P	D	?	?	?	?	S	**R**	**P**	T	**P**
Vervet	**G**	A	R	K	A	E	R	R	R	H	**A**	P	D	G	**E**	V	T	S	**R**	S	T	**P**
Marmoset	**G**	A	R	K	A	E	R	S	R	H	**A**	P	D	G	**E**	V	T	S	**R**	**P**	T	**P**
Bushbaby	**G**	A	R	K	A	**D**	R	R	R	H	**A**	**A**	D	G	**E**	V	T	S	**R**	**P**	T	S
Glires																						
Mouse	**G**	A	R	K	A	**D**	R	**C**	R	**R**	**A**	**A**	D	G	**E**	V	T	S	**R**	**P**	T	A
Rat	**G**	A	R	K	A	**D**	R	**C**	R	**R**	**A**	**A**	D	G	**E**	V	T	S	**R**	**P**	T	A
Rabbit	**G**	A	R	K	A	**D**	R	R	G	H	N	**A**	D	G	**E**	V	T	S	C	S	P	-

4. Conclusions

We performed a comprehensive evolutionary analysis of the β-adrenoreceptor gene family in a sample of chordates including representative species of mammals, birds, reptiles, amphibians, lobe-finned fish, teleost fish, non-teleost ray-finned fish, cartilaginous fish, cyclostomes, urochordates and cephalochordates. Our analyses show the presence of three clades that correspond to the three already described β-adrenoreceptors, plus a fourth clade containing four cyclostome sequences. According to our synteny analyses, we estimated that the β-adrenoreceptor gene family diversified as a product of the two whole genome duplications in the last common ancestor of vertebrates. Additionally, chromosome 4 of humans would be the place where the fourth β-adrenoreceptor gene was located. The fact that all vertebrate species lost the fourth β-adrenoreceptor ohnolog suggests that this event occurred shortly after two vertebrate specific whole genome duplications. Gene expression patterns are generally consistent across species, suggesting that expression dynamics were established deep in time during the evolutionary history of vertebrates, and have been maintained since then. Finally, amino acid polymorphisms that have been associated to different physiological pathologies in humans appear to be common in non-human mammals, suggesting that the effect of these mutations depends more on the genomic context.

Acknowledgments

This work was funded by Fondo Nacional de Desarrollo Científico y Tecnológico – Chile (FONDECYT) grant 1160627 to JCO, and National Science Foundation – United States, EPS-0903787 to FGH.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ygcen.2016.10.005.

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