

RESEARCH ARTICLE

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The evolution of vertebrate somatostatin receptors and their gene regions involves extensive chromosomal rearrangements

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Abstract

Background: Somatostatin and its related neuroendocrine peptides have a wide variety of physiological functions that are mediated by five somatostatin receptors with gene names *SSTR1-5* in mammals. To resolve their evolution in vertebrates we have investigated the *SSTR* genes and a large number of adjacent gene families by phylogeny and conserved synteny analyses in a broad range of vertebrate species.

Results: We find that the *SSTRs* form two families that belong to distinct paralogs. We observe not only chromosomal similarities reflecting the paralogy relationships between the *SSTR*-bearing chromosome regions, but also extensive rearrangements between these regions in teleost fish genomes, including fusions and translocations followed by reshuffling through intrachromosomal rearrangements. These events obscure the paralogy relationships but are still tractable thanks to the many genomes now available. We have identified a previously unrecognized *SSTR* subtype, *SSTR6*, previously misidentified as either *SSTR1* or *SSTR4*.

Conclusions: Two ancestral *SSTR*-bearing chromosome regions were duplicated in the two basal vertebrate tetraploidizations (2R). One of these ancestral *SSTR* genes generated *SSTR2*, -3 and -5, the other gave rise to *SSTR1*, -4 and -6. Subsequently *SSTR6* was lost in tetrapods and *SSTR4* in teleosts. Our study shows that extensive chromosomal rearrangements have taken place between related chromosome regions in teleosts, but that these events can be resolved by investigating several distantly related species.

Keywords: Somatostatin receptors, Whole genome duplications, Chromosome rearrangements

Background

The availability of a large variety of annotated and assembled vertebrate genome sequences has made it possible to address specific evolutionary questions on a genome-wide scale. This includes both large-scale analyses of genome evolution [1-5] and targeted comparative evolutionary studies of specific gene families. The Ensembl genome database (www.ensembl.org) includes genomes for representatives of most vertebrate classes, as well as suitable out-groups for the study of vertebrate evolution [6]. The recent addition of the genomes of the Comoran coelacanth *Latimeria chalumnae* and the spotted gar *Lepisosteus oculatus* complements the previous set of species with pivotal out-groups for

tetrapods and teleost fishes, respectively. They are especially important for studies of genomic events that have taken place in either teleost or tetrapod evolution, as is the case for the chromosomal regions described in the present study.

The basal vertebrate whole genome duplications (2R) [1,3,4] and subsequently the teleost-specific genome duplication (3R) [2,7] have expanded numerous endocrine and neuronal gene families, see for example references [8-15]. Here we have subjected the chromosomal regions harboring the somatostatin receptor family genes to a detailed analysis by collecting sequences from a broad range of vertebrate genomes, including several teleost fishes as well as the spotted gar and the coelacanth.

Somatostatin, the short peptide responsible for inhibition of growth hormone release, was sequenced from sheep hypothalamus in 1973 [16] and its discovery was one of the achievements highlighted by the 1977 Nobel

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Prize in physiology or medicine. Subsequently this 14-amino-acid peptide was sequenced in numerous other vertebrate species and was found to be highly conserved during evolution. Somatostatin is widely distributed and serves both as a neuroendocrine peptide regulating the pituitary, a neuropeptide acting on other neurons, and as an endocrine peptide. In accordance with this, somatostatin has been reported to have many physiological effects [17]. A somatostatin-related peptide was discovered in mouse and human and was named cortistatin or somatostatin-2 [18]. It is now known to be present throughout the tetrapods. In teleost fishes additional somatostatin-like peptides exist named somatostatin 3–6, each encoded by a separate gene [19]. All of these duplicates may have arisen through chromosome duplications in 2R and 3R [19,20].

After the first identification of binding sites for somatostatin, evidence began to accumulate for more than one receptor subtype. The cloning era of G-protein-coupled receptors led to the discovery of five somatostatin receptor subtypes in mammals, named *SSTR1* through 5 [21]. The conserved structure of somatostatin receptor genes consists of a single exon encoding protein products of approximately 360 to 420 amino acids. The somatostatin receptors have been classified into two subfamilies based upon their degree of sequence identity: The human *SSTR1* and *SSTR4* amino acid sequences share 70% sequence identity in the region spanning TM1 to TM7 (including the loops), while *SSTR2*, -3 and -5 share 56–66% amino acid sequence identity to each other. All five receptor subtypes inhibit adenyl cyclases [22] and they can also trigger other second messenger pathways to various extents.

Homologs of the mammalian somatostatin receptors have been described in several teleost fishes, see *Nelson & Sheridan (2005)* [23] for review. However, no *SSTR4* subtype has yet been described in a teleost fish. The known SSTR repertoire in chicken is the same as in mammals and several of the receptors have been studied functionally [24,25]. It was proposed several years ago that the SSTR family expanded in 2R [21] although it was not clear how the appearance of the five members correlated with the two genome doublings. A more recent phylogenetic analysis [26] presented a tree that was unresolved both with respect to species taxonomy and somatostatin receptor subtypes. Other investigators have proposed that the SSTRs arose from a series of duplications throughout vertebrate evolution [27,28].

Our analyses allow us to conclude that the chromosome duplications in early vertebrate evolution (2R), and in the teleost tetraploidization (3R), can explain the known repertoire of vertebrate somatostatin receptors. Furthermore, we have discovered that one of the teleost receptors represents a sixth ancestral vertebrate subtype

that we have called *SSTR6*, which is still present in some teleost fishes, the spotted gar and the coelacanth, but has been lost in tetrapods. Thus, the somatostatin receptor system obtained its present complexity already in the early stages of vertebrate evolution. By centering our analyses around the SSTR genes we could also disentangle complex rearrangements in the SSTR-bearing chromosome regions in teleost fish genomes. This has implications for analyses of conserved synteny and the assignment of orthology for genes located in these regions.

Results

Phylogenetic analysis of the SSTR gene family; identification of a sixth SSTR subtype

Somatostatin receptor amino acid sequences were collected from genome databases for several species representing most of the vertebrate classes: In addition to tetrapod and teleost fish genomes, the genomes of the Comoran coelacanth (*Latimeria chalumnae*) and the spotted gar (*Lepisosteus oculatus*) were investigated in order to provide relative dating points earlier in the evolution of lobe-finned fishes (Sarcopterygii) and ray-finned fishes (Actinopterygii), respectively. The identified amino acid sequences include predictions from several previously unknown SSTR sequences. These results are summarized in Table 1, and detailed descriptions of the identified sequences are included as Supplemental note 1 (see Additional file 1).

The SSTR amino acid sequences identified in the genome databases were used to create an alignment for phylogenetic analyses in order to determine the identity of previously unknown SSTR sequences and study the evolution of this gene family. Using the human kisspeptin-1 receptor as out-group, the resulting phylogenetic maximum likelihood (PhyML) tree in Figure 1 shows that the vertebrate SSTR family consists of six subtype clusters representing the five known SSTR subtypes *SSTR1* through *SSTR5*, as well as a previously unrecognized sixth subtype. We have named these sequences *SSTR6* in our studies. In agreement with previous analyses of fewer sequences [21,23,27], the tree has two well-defined ancestral branches; one including *SSTR2*, -3 and -5, and one containing the *SSTR1* and -4 as well as the *SSTR6* subtype. Both branches are well-supported, and the separate SSTR subtypes form well-supported clusters within each branch, using both bootstrapping and SH-like approximate likelihood ratio statistics (see Additional file 2, Figures S1 and S2). Some subtypes are missing from some species' genome databases (see Additional file 1, Supplemental note 1). Notably, sequences of the sixth subtype, *SSTR6*, could not be identified in any of the investigated tetrapod sequences, and *SSTR4* sequences could not be identified

Table 1 Summary of the identified somatostatin receptor sequences analyzed in this study

	Genus and species (genome assembly version)	Assigned sequence names	Chromosome/linkage group / genomic scaffold locations
Mammals	<i>Homo sapiens</i> (GRCh37)	Human <i>SSTR1</i>	14: 38.68 Mb
		Human <i>SSTR2</i>	17: 71.16 Mb
		Human <i>SSTR3</i>	22: 37.60 Mb
		Human <i>SSTR4</i>	20: 23.02 Mb
		Human <i>SSTR5</i>	16: 1.12 Mb
	<i>Mus musculus</i> (NCBIM37)	Mouse <i>SSTR1</i>	12: 59.31 Mb
		Mouse <i>SSTR2</i>	11: 113.48 Mb
		Mouse <i>SSTR3</i>	15: 78.37 Mb
		Mouse <i>SSTR4</i>	2: 148.22 Mb
		Mouse <i>SSTR5</i>	17: 25.63 Mb
	<i>Canis familiaris</i> (BROAD2)	Dog <i>SSTR1</i>	8: 19.58 Mb
		Dog <i>SSTR2</i>	9: 10.00 Mb
		Dog <i>SSTR3</i>	10: 30.40 Mb
		Dog <i>SSTR5</i>	6: 42.65 Mb
	<i>Monodelphis domestica</i> (BROAD05)	Opossum <i>SSTR1</i>	1: 278.65 Mb
Opossum <i>SSTR2</i>		2: 217.49 Mb	
Opossum <i>SSTR3</i>		8: 91.98 Mb	
Opossum <i>SSTR4</i>		1: 598.18 Mb	
Opossum <i>SSTR5</i>		6: 153.47 Kb	
Birds	<i>Gallus gallus</i> (WASHUC2)	Chicken <i>SSTR1</i>	5: 39.75 Mb
		Chicken <i>SSTR2</i>	18: 9.00 Mb
		Chicken <i>SSTR3</i>	1: 53.39 Mb
		Chicken <i>SSTR4</i>	3: 3.27 Mb
		Chicken <i>SSTR5</i>	14: 5.64 Mb
Reptiles	<i>Anolis carolinensis</i> (AnoCar2.0)	Anole lizard <i>SSTR1</i>	a
		Anole lizard <i>SSTR2</i>	2: 96.75 Mb
		Anole lizard <i>SSTR3</i>	5: 22.84 Mb
		Anole lizard <i>SSTR5</i>	GL343263.1: 1.76 Mb
Amphibians	<i>Xenopus tropicalis</i> (JGI_4.2)	Frog <i>SSTR1</i>	GL172781.1: 1.07 Mb
		Frog <i>SSTR2</i>	GL172812.1: 1.79 Mb
		Frog <i>SSTR3</i>	GL172724.1: 1.41 Mb
		Frog <i>SSTR4</i>	GL172884.1: 512.43 Kb
		Frog <i>SSTR5</i>	GL172659.1: 446.17 Kb
Coelacanth	<i>Latimeria chalumnae</i> (LatCha1)	Coelacanth <i>SSTR1</i>	JH126598.1: 0.53 Mb
		Coelacanth <i>SSTR2</i>	JH126581.1: 3.45 Mb
		Coelacanth <i>SSTR3</i>	JH129649.1: 0.21 Mb
		Coelacanth <i>SSTR4</i>	JH126648.1: 2.61 Mb
		Coelacanth <i>SSTR5</i>	JH129247.1: 0.21 Mb
		Coelacanth <i>SSTR6</i>	JH127490.1: 0.26 Mb
		Coelacanth <i>SSTRX</i>	JH126581.1: 3.47 Mb
Spotted gar	<i>Lepisosteus oculatus</i> (LepOcu1)	Spotted gar <i>SSTR1</i>	LG7: 4.44 Mb
		Spotted gar <i>SSTR2</i>	LG10: 34.84 Mb
		Spotted gar <i>SSTR3</i>	LG12: 34.19 Mb
		Spotted gar <i>SSTR5</i>	LG13: 4.69 Mb
		Spotted gar <i>SSTR6</i>	LG28: 1.08 Mb

Table 1 Summary of the identified somatostatin receptor sequences analyzed in this study (Continued)

Teleost fish	<i>Danio rerio</i> (Zv9)	Zebrafish <i>SSTR1</i>	17: 10.35 Mb
		Zebrafish <i>SSTR2a</i>	3: 63.08 Mb
		Zebrafish <i>SSTR2b</i>	12: 1.73 Mb
		Zebrafish <i>SSTR3a</i>	3: 29.75 Mb
		Zebrafish <i>SSTR3b</i>	Scaffold Zv9_NA631: 3.42 Kb
		Zebrafish <i>SSTR5a</i>	24: 16.78 Mb
		Zebrafish <i>SSTR5b</i>	1: 55.01 Mb
		Zebrafish <i>SSTR6</i>	7: 19.63 Mb
<i>Gasterosteus aculeatus</i> (BROADS1)	Stickleback <i>SSTR2a</i>	groupXI: 9.50 Mb	
	Stickleback <i>SSTR2b</i>	groupV: 6.81 Mb	
	Stickleback <i>SSTR3a</i>	groupXI: 15.59 Mb	
	Stickleback <i>SSTR5a</i>	groupXI: 11.72 Mb	
	Stickleback <i>SSTR5b</i>	groupIX: 14.95 Mb	
	Stickleback <i>SSTR6</i>	scaffold_47: 436.21 Kb	
<i>Oryzias latipes</i> (MEDAKA1)	Medaka <i>SSTR2a</i>	8: 10.93 Mb	
	Medaka <i>SSTR2b</i>	scaffold5841: 160 bp	
	Medaka <i>SSTR3a</i>	8: 2.80 Mb	
	Medaka <i>SSTR3b</i>	1: 29.10 Mb	
	Medaka <i>SSTR5a</i>	8: 13.75 Mb	
<i>Tetraodon nigroviridis</i> (TETRAODON8)	Green puffer <i>SSTR2a</i>	3: 10.44 Mb	
	Green puffer <i>SSTR2b</i>	2: 4.83 Mb	
	Green puffer <i>SSTR3a</i>	3: 15.06 Mb	
	Green puffer <i>SSTR3b</i>	18: 10.39 Mb	
	Green puffer <i>SSTR3c</i>	Un_random: 59.49 Mb	
	Green puffer <i>SSTR5b</i>	18: 2.40 Mb	
<i>Takifugu rubripes</i> (FUGU4)	Fugu <i>SSTR2a</i>	scaffold_115: 411.36 Kb	
	Fugu <i>SSTR2b</i>	scaffold_3: 3.77 Kb	
	Fugu <i>SSTR3a</i>	scaffold_359: 200.56 Kb	
	Fugu <i>SSTR3b</i>	scaffold_407: 33.36 Kb	
	Fugu <i>SSTR5b</i>	scaffold_189: 267.65 Kb	
	Fugu <i>SSTR6</i>	scaffold_164: 38.74 Kb	
Invertebrates	<i>Drosophila melanogaster</i> (BDGP5)	Fruit fly <i>Drostar1</i>	3L: 18.55 Mb
		Fruit fly <i>Drostar2</i>	3L: 18.48 Mb

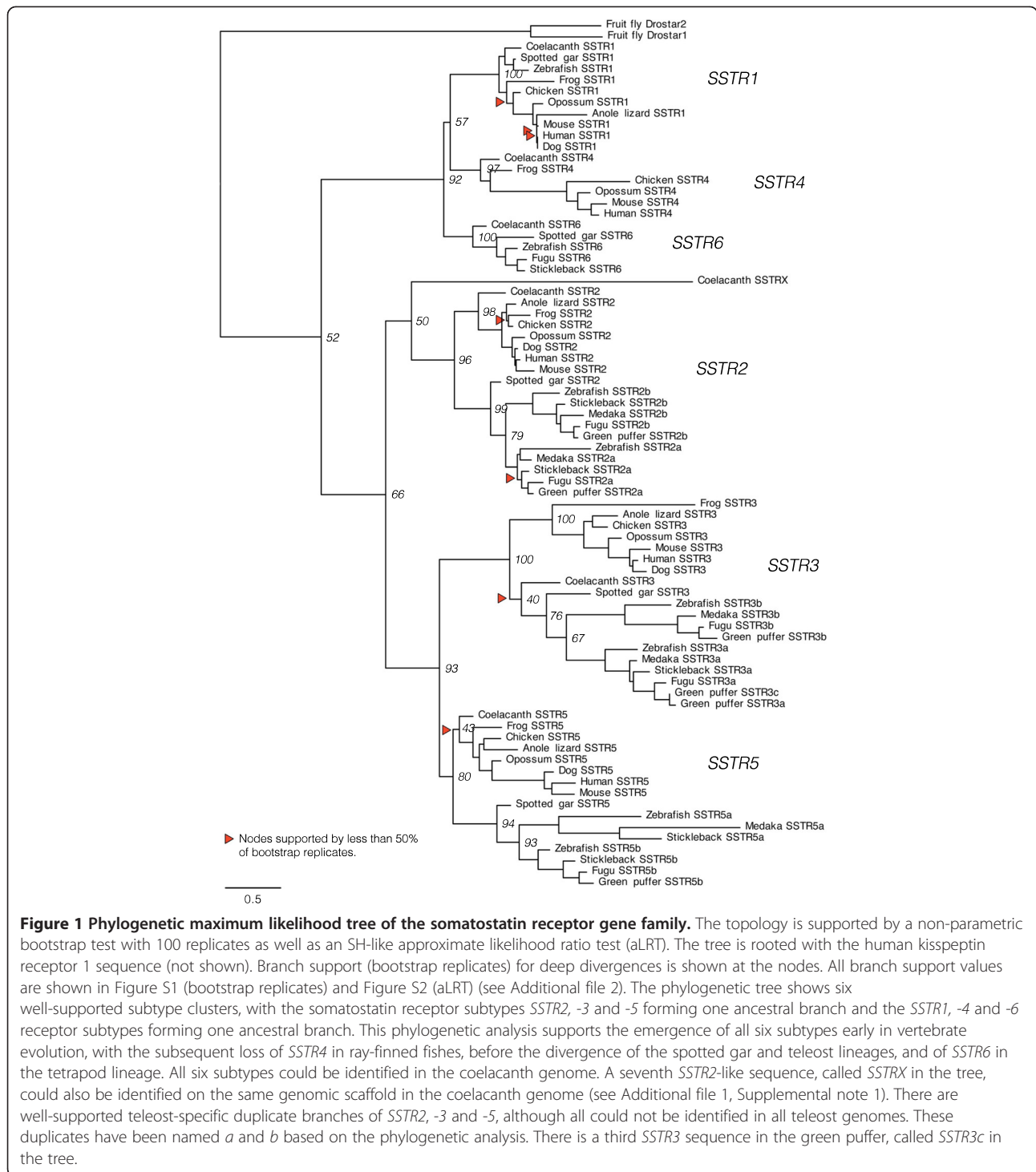
^a The Anole lizard *SSTR1* sequence could not be identified in the most updated assembly (AnoCar2.0), however it is located on genomic scaffold_0 at 284.26 Kb in the previous assembly (AnoCar1.0, Ensembl database version 60).

in teleost fishes or in the spotted gar. All six SSTR subtypes are represented in the coelacanth, demonstrating that the absence of *SSTR4* genes in the spotted gar and teleost fishes, and of *SSTR6* genes in tetrapods likely resulted from secondary gene losses. In teleost fishes, an *SSTR1* sequence could only be identified in the zebrafish genome.

There are teleost specific duplicates of *SSTR2*, -3 and -5 forming well-supported *a*- and *b*-clusters within their respective subtypes. In the spotted gar genome only single copies of the *SSTR2*, -3 and -5 sequences were found, and these branch basal to the respective teleost-specific *a*- and

b-duplicate clusters, which strongly supports the duplication of *SSTR2*, -3 and -5 early in the teleost lineage. Taken together this means that some teleost species may have up to eight different SSTR family members, including an SSTR subtype that has not been previously described. In our analyses, the zebrafish genome has this repertoire of receptors: *SSTR1*, -2*a*, -2*b*, -3*a*, -3*b*, -5*a*, -5*b* and -6.

The known *Drosophila* allatostatin C receptor 1 and 2 sequences called *Drostar1* and *Drostar2* were included in the phylogenetic analyses due to their close sequence and functional similarity with the mammalian somatostatin receptor [29]. These sequences cluster together



basal to the vertebrate SSTR sequences and most probably represent an independent duplication event. It was not possible to identify true SSTR orthologs in the tunicates *Ciona intestinalis* and *Ciona savignyi*, or in the Florida lancelet (amphioxus) *Branchiostoma floridae*.

Syntenic gene families

In addition to making a phylogenetic tree of somatostatin receptors in vertebrates, our aim was to determine whether the SSTR genes were duplicated in the chromosome doublings in 2R. To test this hypothesis, syntenic (neighboring) gene families in the SSTR gene-bearing

chromosome regions were analyzed with respect to their phylogenies, using both neighbor joining (NJ) and PhyML methods, and the chromosomal locations of the member genes (see Methods below). In total, 47 syntenic gene families were analyzed. Our results of the conserved synteny analyses are presented as tables comparing the chromosomal locations of all the identified syntenic family member genes in the genomes of human, chicken, zebrafish, stickleback and medaka. Due to size restrictions, the tables have been included as additional data files (see Additional files 3 and 4). The phylogenetic trees of all the neighboring gene families have also been included as additional files (see Additional files 5 and 6). These tables and phylogenetic trees are the bases for our description of the results below.

Conserved synteny analysis of the *SSTR1*, -4 and -6 chromosome regions

The chromosomal locations of the *SSTR* genes as well as the early divergence of two ancestral *SSTR* branches in the phylogenetic tree suggested that the *SSTR1*, -4 and -6 genes derive from one ancestral *SSTR* gene, and the *SSTR2*, -3 and -5 genes from a separate ancestral *SSTR* gene, and that these two ancestral genes were located in distinct paralogs (related chromosome groups). Therefore two separate analyses of conserved synteny were done. To investigate whether the *SSTR1*, -4 and -6 genes arose by duplications of a single ancestral gene in 2R we have carried out phylogenetic analyses of 17 syntenic gene families and the chromosomal locations of all neighboring family members were noted and compared between species (see Additional file 3). In summary, all but two of the 17 identified syntenic gene families in the *SSTR1*, -4 and -6 chromosome blocks (Table 2) have phylogenetic trees that either support or are consistent with duplications early in vertebrate evolution (see Additional file 5). These gene families have tree topologies with subtype clusters diverging in the same time window as 2R, i.e., after the divergence of invertebrate chordates and vertebrates but before the divergence of lobe-finned fishes (including tetrapods) and ray-finned fishes (including teleosts). The PhyML topologies of the RIN and PYG gene families are shown as examples in Figure 2. Several gene families also have teleost-specific duplicate clusters, supporting subsequent duplications in 3R, see for example *PYGM* and *RIN2* clusters in Figure 2 as well as the teleost *FLRT1* orthologs (see Additional file 2, Figure S4). Some of the neighboring families have inconsistencies between the NJ and PhyML trees (see Additional file 1, Supplemental note 2), however they were considered supportive if they showed the topology described above for at least one of the methods.

Table 2 Neighboring gene families analyzed for conserved synteny in the *SSTR1*, -4 and -6-bearing chromosome blocks

Symbol ^a	Description ^a	Root (if other than <i>D. melanogaster</i>)
ABHD12	<i>Abhydrolase domain containing 12</i>	
CFL	<i>Cofilin and destrin (actin depolymerizing factor)</i>	
FLRT	<i>Fibronectin leucine rich transmembrane protein</i>	<i>C. savignyi</i>
FOXA	<i>Forkhead box A</i>	
ISM	<i>Isthmin homolog</i>	<i>C. intestinalis</i>
JAG	<i>Jagged</i>	
NIN	<i>Ninein (GSK3B interacting protein)</i>	
NKX2	<i>NK2 homeobox 1 and 4</i>	
PAX	<i>Paired box 1 and 9</i>	
PYG	<i>Glycogen phosphorylase; brain, liver and muscle variants</i>	
RALGAPA	<i>Ral GTPase activating protein, alpha subunit</i>	
RIN	<i>Ras and Rab interactor</i>	
SEC23	<i>Sec23 homologs A and B</i>	
SLC24A	<i>Solute carrier family 24 members 3 and 4</i>	<i>B. floridae</i>
SNX	<i>Sorting nexin 5, 6 and 32</i>	
SPTLC	<i>Serine palmitoyltransferase, long chain base subunit 2 and 3</i>	
VSX	<i>Visual system homeobox</i>	<i>C. elegans</i>

^a Gene family names and descriptions are based on approved HUGO Gene Nomenclature Committee (HGNC) gene symbols and descriptions, or known aliases from the NCBI Entrez Gene database. Where not all known protein subtypes/isoforms are part of the gene family, the included subtypes are specified.

The positional and phylogenetic data combined demonstrate the conserved synteny between the chromosome blocks containing *SSTR1*, -4 and -6 in the analyzed genomes. In the human genome, these correspond to well-defined regions on chromosomes 14 and 20, and to a lesser degree 11 and 19. Although no *SSTR6*-bearing chromosomal region was used in the selection of syntenic gene families, the ISM (Figure S8), PYG (Figure S13), RIN (Figure S15) and SLC24A (Figure S17) families have members neighboring the *SSTR6* genes in one or several of the teleost genomes (see Additional file 3). This dataset also shows that rearrangements between the homologous chromosome regions have been common in the teleost lineage. For example, genes located on both chromosomes 14 and 20 in the human genome have orthologs that are distributed primarily between chromosomes 13, 17 and 20 in the zebrafish genome in a way that suggests the substantive exchange of paralogs between these regions. This can be seen for the teleost

Table 3 Neighboring gene families analyzed for conserved synteny in the *SSTR2*, -3 and -5-bearing chromosome blocks

Symbol ^a	Description ^a	Root (if other than <i>D. melanogaster</i>)
ADAP	ArfGAP with dual PH domains	
ATP2A	ATPase, Ca ⁺⁺ transporting, cardiac muscle, fast twitch	
C1QTNF	C1q and tumor necrosis factor related protein	<i>B. floridae</i>
CABP	Calcium binding protein 1, 3, 4 and 5	
CACNA1	Calcium channel, voltage dependent, T type alpha subunit	
CREBBP	CREB binding protein	
CYTH	Cytohesin	
FAM20	Family with sequence similarity 20	
FNG	Fringe homolog ^b	
FSCN	Fascin homolog 1 and 2, actin-bundling protein	
GGA	Golgi-associated, gamma adapting ear containing, ARF-binding protein	
GLPR	Glucagon, glucagon-like and gastric inhibitory polypeptide receptors	<i>C. intestinalis</i>
GRIN2	Glutamate receptor, ionotropic, N-methyl D-aspartate 2	
KCNJ	Potassium inwardly-rectifying channel, subfamily J member 2, 4, 12 and 14	<i>C. intestinalis</i>
KCTD	Potassium channel tetramerisation domain containing 2, 5 and 17	
METRN	Meteorin, glial cell differentiation regulator	<i>B. floridae</i>
NDE	nudE nuclear distribution gene E homolog	
RAB11FIP	RAB11 family interacting protein 3 and 4 (class II)	
RADIL	Ras association and DIL domains/Ras interacting protein	<i>B. floridae</i>
RHBDF	Rhomboïd 5 homolog	
RHOT	Ras homolog gene family, member T1 and T2	
RPH3A	Rabphilin 3A homolog/double C2-like domains, alpha	
SDK	Sidekick cell adhesion molecule	<i>C. elegans</i>
SOX	Sex-determining region Y-box 8, 9 and 10	
TEX2	Testis expressed 2	
TNRC6	Trinucleotide repeat containing 6	
TOM1	Target of myb1	
TTYH	Tweety homolog	
USP	Ubiquitin specific peptidase 31 and 43	
WFIKKN	WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing	<i>B. floridae</i>

^a Gene family names and descriptions follow the same system as Table 2.

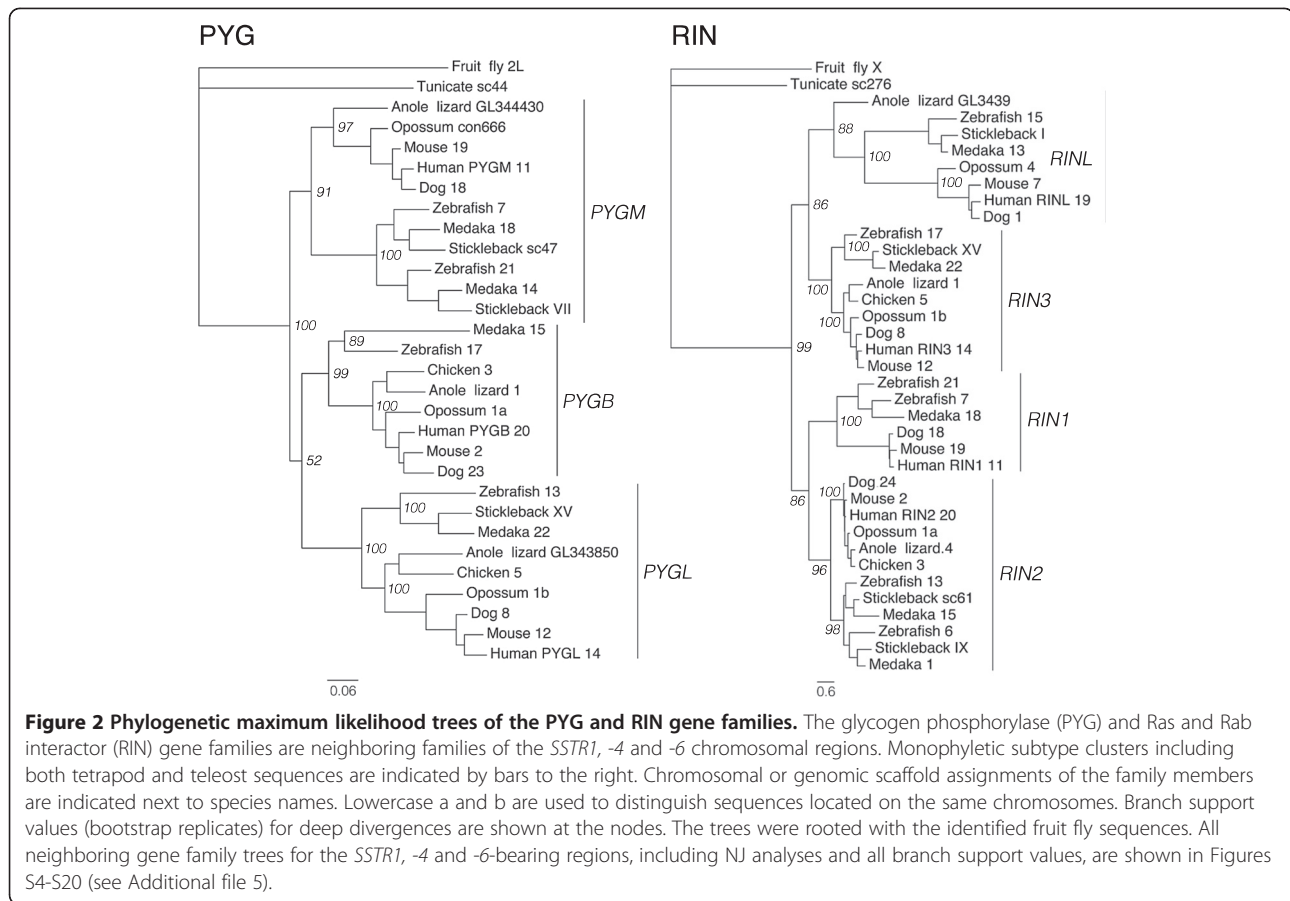
^b Complete description: Lunatic, manic and radical fringe homolog. O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase.

orthologs of the *PYGL* and *PYGB* genes compared with the *RIN3* and *RIN2* orthologs (Figure 2). In the stickleback and medaka genomes there seems to have been fewer rearrangements: orthologs of genes located on human chromosome 20 are located on stickleback linkage group XV between approximately 3.75 and 4 Mb, and on medaka chromosome 22 between approximately 16 and 16.34 Mb, which suggests translocation of small chromosomal blocks (see Additional file 3).

Conserved synteny analysis of the *SSTR2*, -3 and -5 chromosome regions

For the investigation of paralogy relationships between the chromosomal regions that harbor the *SSTR2*, -3 and -5 genes, 30 syntenic gene families were analyzed as described above for the *SSTR1*, -4 and -6-bearing chromosome regions. To identify these gene families, the *SSTR2*, -3 and -5-bearing chromosome regions in the chicken and stickleback genomes were analyzed for conserved synteny (see Methods below). Two separate starting points (chicken and stickleback) were used because the chromosomal locations of the *SSTR* genes in the teleost genomes, with *SSTR2*, -3 and -5 homologs located on the same chromosome, suggest a different expansion scenario than the tetrapod genomes, including chicken (Table 1). In this way we could collect a dataset of neighboring gene families without favoring one scenario over the other. In summary, 23 of the 30 syntenic gene families in the *SSTR2*, -3 and -5 chromosome blocks have tree topologies that support an expansion from one ancestral vertebrate gene through 2R (see Additional file 6, Figure S21–S50). Four are consistent with 2R, but show some inconsistencies between phylogenetic methods (see Additional file 1, Supplemental note 3) - ADAP (Figure S21), FAM20 (Figure S28), RPH3A and TOM1 (Figure S47) - while only two are considered inconclusive - CABP (Figure S24) and GGA (Figure S31) (see Additional file 6). Many of the analyzed families also show topologies that support the duplication of family members in 3R. The PhyML topologies of the GRIN2 family (Figure 3) and of the FNG and FSCN families (Figure 4) are shown as examples.

As described previously, the chromosomal locations of all neighboring family members were compared between species (see Additional file 4) and the phylogenetic tree topologies of the neighboring gene families were used to infer the paralogy and orthology relationships. The identified conserved synteny blocks correspond to regions of human chromosomes 7, 16, 17, 19 and 22 in the human genome. This dataset shows that there have been extensive chromosome rearrangements between the paralogous chromosome regions in the teleost genomes, and to some extent in the human genome. For example, many gene families with members on chicken chromosome 14 have orthologs distributed between human



chromosomes 16 and 7, and stickleback linkage groups V, IX and XI. In the zebrafish genome these same gene families have orthologs spread over more chromosomes: most are on chromosomes 3, 12, 1 and 24, but there are individual orthologs of two families on chromosome 22 and an unmapped genomic scaffold (see Additional file 4). Several of these rearrangements can be seen for members of the *GRIN2* (Figure 3), *FNG* and *FSCN* (Figure 4) families, with teleost-specific duplicates in different subtype clusters co-located on the same chromosomes. The human *GRIN2B* sequence also seems to have translocated to chromosome 12. The *GRIN2A*, -2*B* and -2*C* clusters show well-supported teleost duplicate branches, supporting a duplication in 3R (Figure 3). In these branches we observe teleost genes located on the same chromosomes (for instance zebrafish *GRIN2A*, -2*B* and -2*C* orthologs, all on chromosome 3), likely due to the chromosomal rearrangements. The *FSCN* gene family has several teleost sequences located on the same chromosomes, for instance on zebrafish chromosome 3, medaka chromosome 8 and stickleback linkage group XI, and the *FNG* family has teleost duplicates in the *LFNG* cluster (Figure 4). However, the topology is not clear for the *LFNG* teleost duplicates. These rearrangements in the teleost lineage likely explain why the *SSTR2a*, -3*a* and

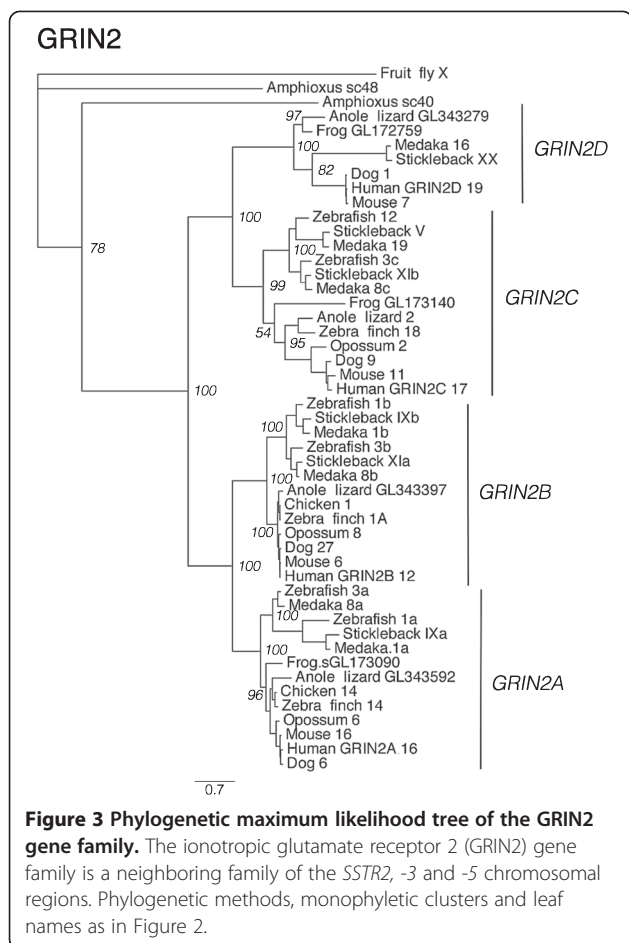
-5*a* genes also are located in the same chromosomal regions in teleost genomes (Table 1), as will be discussed below.

A few gene families identified in the analysis of conserved synteny, namely *ATP2A* (Figure S22), *CABP* (Figure S24), *GLPR* (Figure S32) and *RPH3A* (Figure S42) (see Additional file 6), have individual paralogs on different chromosomes or genomic scaffolds, as described in Supplemental note 3 (see Additional file 1).

Discussion

Evolution of the *SSTR* family

Our phylogenetic analyses of the *SSTR* gene family provide strong support for expansion and diversification in both the 2R and 3R events, giving rise to six different *SSTR* subtype genes early in vertebrate evolution and subsequently expanding the *SSTR2*, -3 and -5 branch in the teleost lineage. Our evolutionary scheme of the *SSTR* gene family expansion is presented in Figure 5. The sixth subtype, which we have called *SSTR6*, was previously unrecognized. We have identified it in the ray-finned fishes, including the spotted gar and the teleosts, as well as in the coelacanth, a member of the lobe-finned fish lineage. Thus, it was clearly present before the divergence of lobe-finned and ray-finned fishes. Its

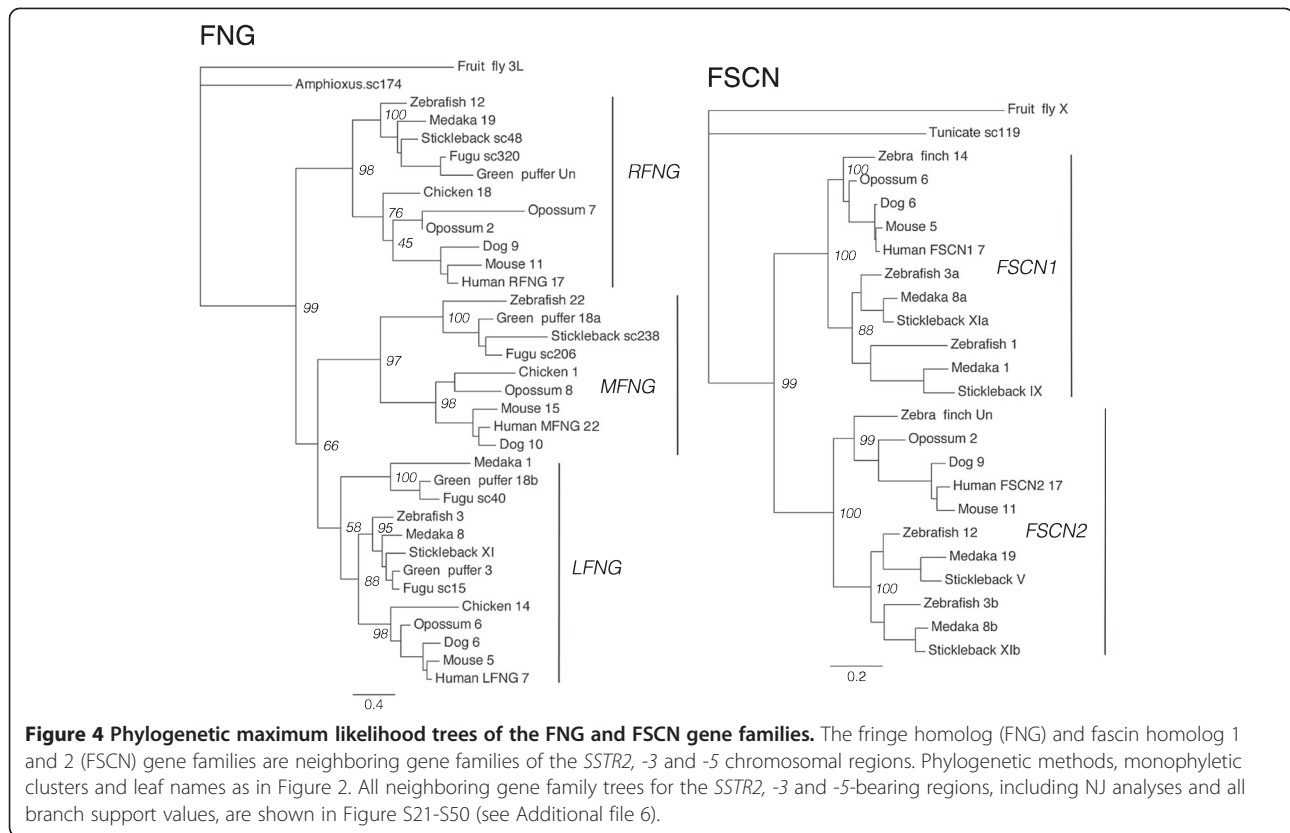


chromosomal position in the teleosts supports origin in 2R (*see below*). Conversely, the *SSTR4* gene was only identified in the lobe-finned fishes, including tetrapods and the coelacanth. These losses are likely the result of secondary and independent events: *SSTR6* from the lineage leading to tetrapods some time after the divergence of the coelacanth lineage, and *SSTR4* from the ray-finned fish before the divergence of the spotted gar and the lineage leading to teleosts (Figure 5). The topology of the *SSTR1*, -4 and -6 branch supports this scenario (Figure 1). All six *SSTR* subtypes that emerged early in vertebrate evolution are represented in the genome of the coelacanth. The additional seventh coelacanth sequence that we have called *SSTRX* is located on the same genomic scaffold with the same orientation as the *SSTR2* sequence (Table 1) and it clusters in the most basal position in the *SSTR2* cluster. This, together with its branch length in the tree, indicates that it is a lineage-specific duplicate of *SSTR2* with a higher evolutionary rate.

The somatostatin system has been reported to have arisen prior to the divergence of insects and vertebrates, i.e., before the protostome-deuterostome split.

Drosophila melanogaster and other insects have a somatostatin-like 15-amino-acid peptide that has been named ASTC for allatostatin C [30]. Two ASTC receptors were identified in *D. melanogaster*, with closest relationship to human somatostatin and opioid receptors [29]. The receptors were named *Drostar1* and -2 and seem to have arisen through a lineage-specific duplication in insects. We propose that two ancient *SSTR* genes were present before the emergence of vertebrates based on our comparative analyses. However, we were unable to identify any unambiguous *SSTR* family members in the genome databases of the amphioxus *Branchiostoma floridae*, and the tunicates *Ciona intestinalis* and *Ciona savignyi*. The latter are members of the urochordate lineage which constitutes the closest extant relatives of vertebrates [31,32]. A previous analysis of G-protein coupled receptor sequences in the Florida lancelet (*Branchiostoma floridae*) genome identified several lancelet-specific expansions of somatostatin-, galanin- and opioid receptor-like sequences, totaling 90 distinct sequences in this cluster [33]. Among these sequences, 18 cluster together with the human *SSTR* sequences, although the resolution of this phylogenetic analysis is very low. In any case, these lineage-specific duplications preclude the identification of true orthologs to the vertebrate somatostatin receptors, although there are several candidates. We have identified three putative somatostatin receptor sequences in the genome of the sea lamprey *Petromyzon marinus*, and their database identifiers are noted in Table S1 (see Additional file 7). However, due to the incomplete status of this genome assembly, and thus the lack of synteny data, we refrain from speculating about their orthology relationships.

Somatostatin receptors have been described for several teleost fish species in addition to the ones that we have studied. In each species usually one or a few sequences have been reported, except for goldfish, *Carassius auratus*, where eight sequences have been published [34-37]. Our additional tree presented in Figure S3 (see Additional file 2) confirms previous suggestions [23,28] that two of these correspond to *SSTR1* as a result of the goldfish-specific fourth tetraploidization (4R) that took place some 12–15 MYA [38,39]. Other goldfish sequences correspond to subtypes *SSTR2*, *SSTR3a* and *SSTR3b*. The three *SSTR5*-like sequences in goldfish were initially named 5a, 5b, and 5c. The one named 5c is orthologous to 5b in our comparisons and the ones named 5a and 5b appear to be 4R duplicates of 5a (see Additional file 2, Figure S3). The latter have accumulated as many as 66 amino acid differences in this short time period (resulting in 83% sequence identity), whereas the *SSTR1* 4R-generated duplicates differ at only 5 positions. In the orange-spotted grouper, *Epinephelus*

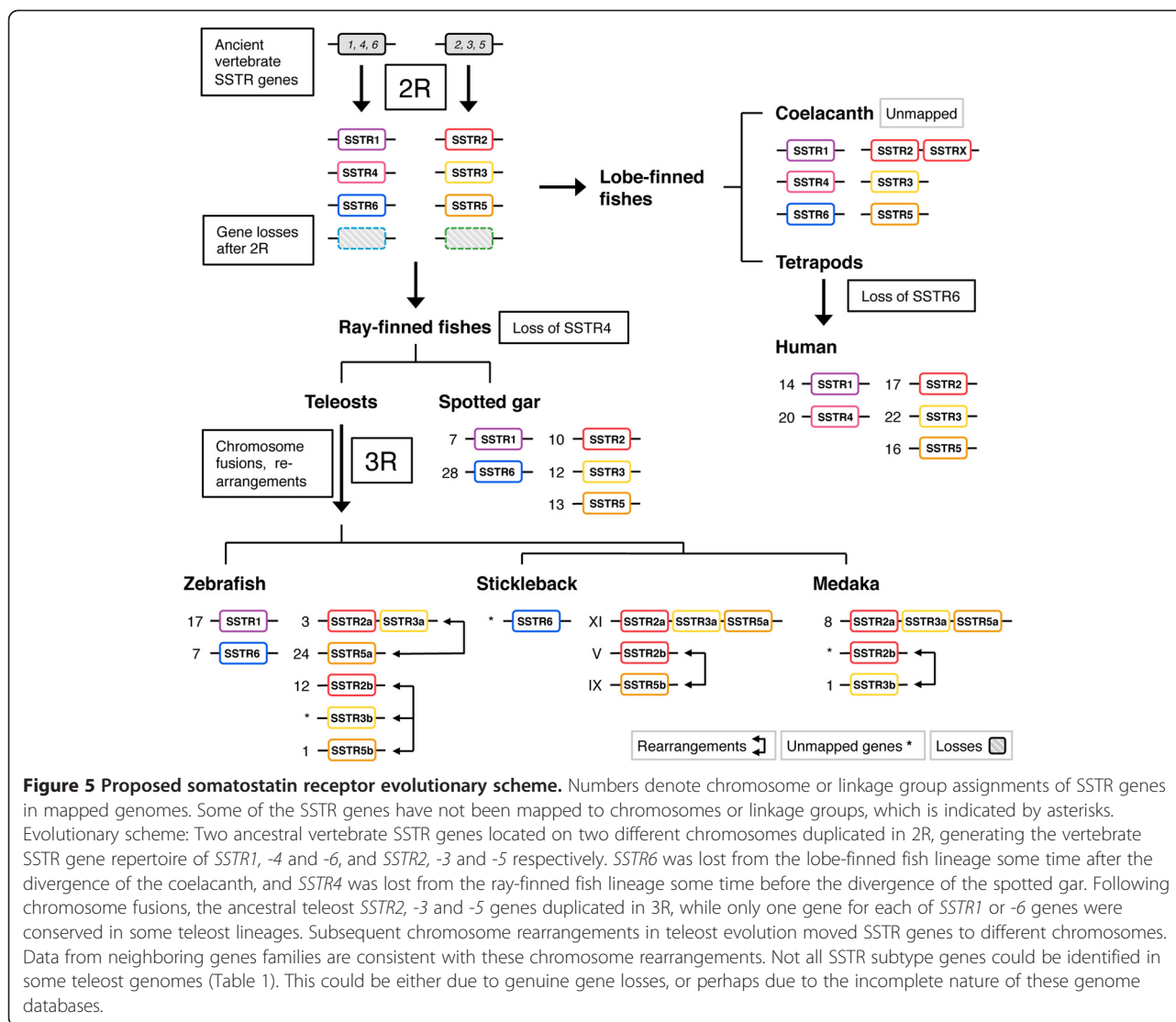


coioides, four sequences have been reported [27] that we can now identify as *SSTR1*, *SSTR2b*, *SSTR3a*, and *SSTR5a* (see Additional file 2, Figure S3). The *SSTR3* sequence determined in the black ghost knifefish *Apteronotus albifrons* [40], an electric fish, is *SSTR3b*, and the two sequences from the cichlid *Astatotilapia burtoni* [41] are *SSTR2a* and *SSTR3a*. In the rainbow trout, *Oncorhynchus mykiss*, three sequences have been reported [42,43]. In our additional analysis the two sequences identified as *SSTR1a* and *-1b* [43,44] can be correctly identified as two copies of *SSTR6* likely resulting from the salmonid fourth tetraploidization (see Additional file 2, Figure S3).

SSTR-bearing chromosome regions were duplicated in vertebrate whole genome duplications

Two separate analyses of conserved synteny were carried out in order to test the hypothesis that each of the *SSTR1*, -4, -6 and *SSTR2*, -3, -5-branches of the *SSTR* gene family was multiplied as a result of duplications of two distinct chromosome regions. In total we have compared the chromosomal locations of genes in 47 gene families located in *SSTR*-bearing chromosome regions. These positional data were combined with phylogenetic analyses of the gene families to infer the likely orthology and paralogy relationships within each family, as well as to determine the time

window of the duplications and chromosome rearrangements. As a whole, these analyses show that the *SSTR1*, -4 and -6-bearing chromosome regions on the one hand, and the *SSTR2*, -3 and -5-regions on the other, belong to distinct paralogons that were formed by chromosome duplications during the same time period in early vertebrate evolution. Using relative dating in the phylogenetic analyses, as well as the species distribution of the genes, we can place the duplication events to the period after the divergence of invertebrate chordates and vertebrates, but before the divergence of lobe-finned fishes (including tetrapods) and ray-finned fishes (including teleosts). This means that the identified regions of paralogy likely resulted from duplications of ancestral chromosome regions in the same time window as the early vertebrate tetraploidizations. Thus, our analysis provides further support for 2R. Our analyses also indicate that these two paralogy regions duplicated further in the time-window of the teleost-specific whole genome duplication 3R, although for the *SSTR* gene family only duplicates of *SSTR2*, -3 and -5 were retained (Table 1). Based on the phylogenetic analyses of the *SSTR* family (Figure 1, Additional file 2), these duplicates have been named adding the letters *a* and *b* to the gene symbols. Our proposed evolutionary scenario for the evolution of the *SSTR*-bearing chromosome regions is presented in Figure 6.

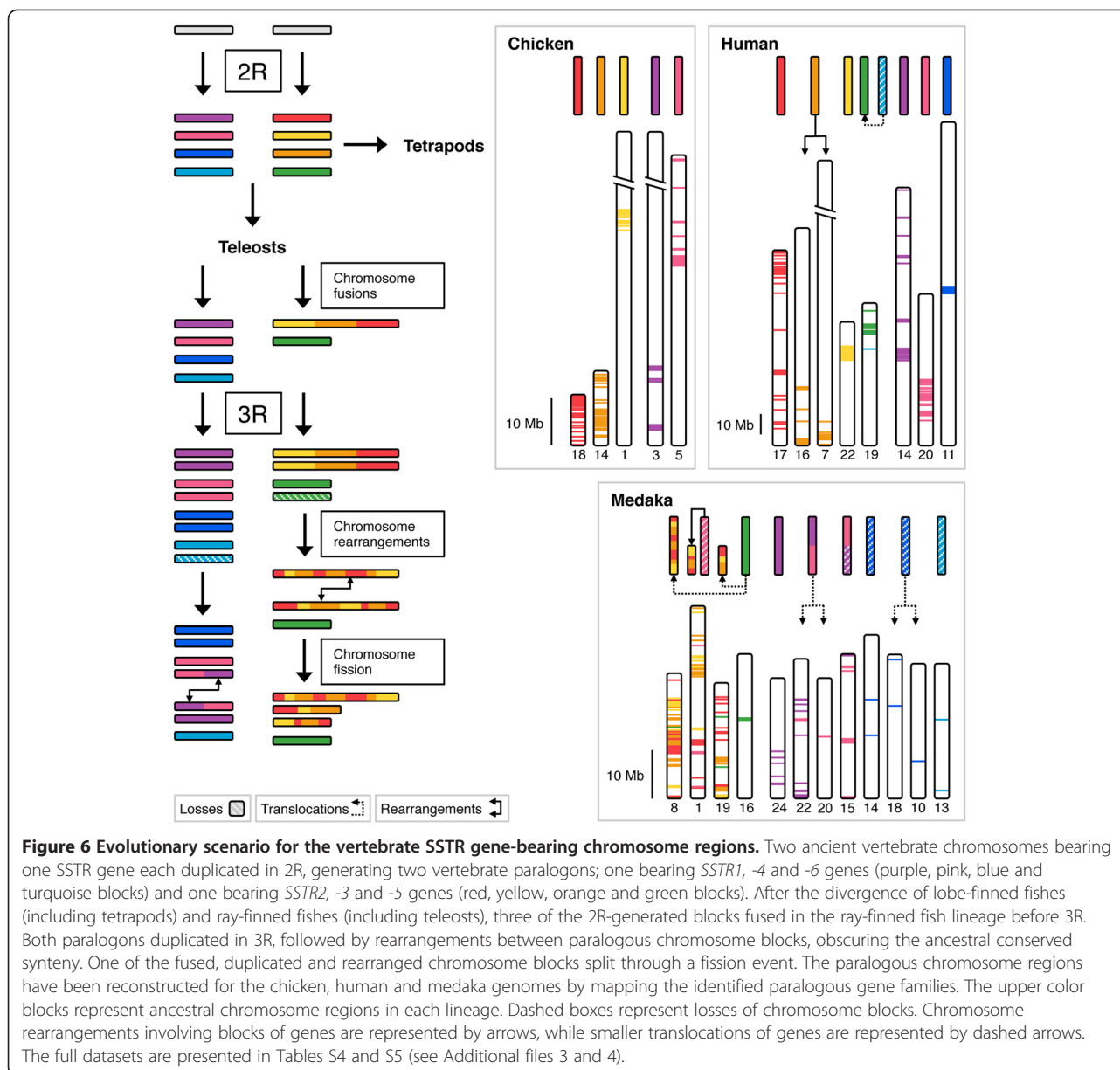


The paralogous regions we have identified bearing *SSTR1*, -4, and -6-genes, and *SSTR2*, -3 and -5-genes, and the time window for their origin, are consistent with previous large-scale genomic analyses. In the analysis of paralogous chromosome regions in the human genome compared to the *Branchiostoma floridae* genome [3] these regions (Figure 6) correspond to ancestral chordate linkage groups numbered 11 and 15 respectively, indicating an origin in 2R. A separate reconstruction of the vertebrate ancestral genome [4] also inferred that these regions originated from two separate ancestral chromosomes that quadrupled in 2R. In the latter analysis the *SSTR1*, -4 and -6-bearing regions correspond to the ancestral linkage group called G and the *SSTR2*, -3 and -5-bearing regions to ancestral linkage group called I. The analysis of the first medaka draft genome [5], as well as the aforementioned reconstruction of the ancestral vertebrate genome, support the conclusion that both

paralogous regions duplicated further in 3R, but that there have been several major rearrangements that obscure the paralogy relationships. The medaka genome is an appropriate starting point for the discussion of chromosomal rearrangements in the teleost lineage since it seems to have preserved more of the ancestral teleost genome organization [5].

Chromosomal rearrangements in teleost genomes

Initially, the locations of the *SSTR2a*, -3a and -5a duplicates in teleost genomes suggested that the expansion of the somatostatin receptor family might have partially occurred through other mechanisms than 2R. In the medaka and stickleback genomes all three paralogs are located within regions of approximately 11 Mb on chromosome 8 and 9 Mb on linkage group XI, respectively. In the zebrafish, *SSTR2a* and -3a are located approximately 33 Mb apart on chromosome 3 while



SSTR5a is located on chromosome 24. In the green puffer *SSTR2a* and *-3a* are located approximately 5 Mb apart on chromosome 8 and additionally the *SSTR3b* and *-5b* genes are co-localized on chromosome 18 approximately 8 Mb apart (see Table 1 for locations). These arrangements would suggest that ancestral segmental duplications were involved. However, in all non-teleost genomes, notably that of the spotted gar, the *SSTR2*, -3 and -5 paralogs are located on different chromosomes or linkage groups (Table 1). To make sure that our analysis of conserved synteny did not favor the 2R scenario over the ancestral tandem duplication scenario, both the chicken and the stickleback genomes were used as starting points for the identification of neighboring

families in the *SSTR2*, -3 and -5 paralogon. For the *SSTR1*, -4 and -6 paralogon we parted from the human and chicken genomes, since the locations of the SSTR genes did not indicate different expansion scenarios in tetrapods and teleosts. Based on the combined positional and phylogenetic data using tetrapods as well as teleosts we conclude that both of the SSTR-bearing paralogs have undergone a series of inter- and intra-chromosomal rearrangements in the teleost lineage that obscure the ancestral organization. To deduce these rearrangements we have compared lists of neighboring gene family members in the identified paralogous chromosome regions between the human, chicken, zebrafish, stickleback and medaka genomes. The results of

this analysis are presented in Additional files 3 and 4 and our suggested scenario is summarized in Figure 6.

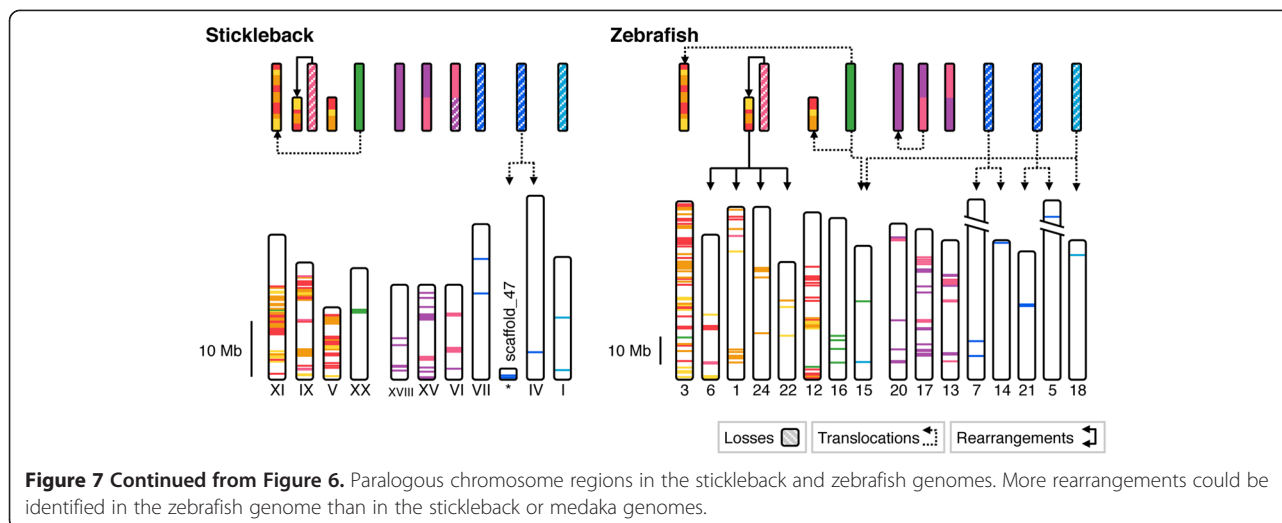
The analysis of conserved synteny for the *SSTR2*, -3 and -5-paralogy regions shows that many of the gene families, not only *SSTR*, display the same paralog translocations between the homologous chromosome regions generated in 2R. Notable examples are the *CYTH* (Figure S27), *FSCN* (Figure S30), *GGA* (Figure S31), *GRIN2* (Figure S33), *KCNJ* (Figure S34), *KCTD* (Figure S35), *SOX* (Figure S44) and *TNRC* (Figure S46) families (see Additional file 6): In all the analyzed genomes these families have two or three 2R-generated subtype genes located on the same chromosome regions with 3R-generated duplicates on other chromosomes (see Additional file 4). The *GRIN2* PhyML tree is shown as an example in Figure 3 and the *FSCN* PhyML tree can be seen in Figure 4.

This situation allows us to infer the scenario presented in Figure 6: Three of the four 2R-generated paralogous chromosome blocks were fused into the same chromosome in the ray-finned fish lineage sometime after the spotted gar had branched off approximately 350 MYA and before 3R in the teleost lineage (for time point estimates see *Amores et al. (2012) [45]*). After the 3R event and before the last common ancestor of the studied species, the now duplicated fused chromosome blocks exchanged paralogs and subsequently one of them was split by fission events. In all the analyzed teleost genomes we observe these fused and rearranged regions on at least three chromosomes (Figures 6 and 7). There seem to have been more fissions and rearrangements in the zebrafish lineage (Figure 7). It is likely that many of the rearrangements occurred as part of larger blocks and subsequently local rearrangements have jumbled the ancestral order. This scenario is corroborated by the orthology relationships inferred from the phylogenetic analyses

of the neighboring families (see Additional file 6, Figures S21-S50). The fact that it is 2R-generated duplicates that have been co-located by the chromosome fusions, and not primarily 3R-generated duplicates, shows that the fusions occurred before 3R.

We could see similar chromosomal rearrangements in the paralogous regions bearing *SSTR1*, -4 and -6 genes, although not to the same extent. Due to the lower degree of *SSTR* gene retention after 2R and 3R in this paralogon, fewer neighboring families could be identified as belonging to the paralogy block. Nonetheless some gene families seem to have translocated duplicates between homologous chromosomes after 3R (Figure 6). The highest degree of such translocations can be seen in the zebrafish where the *ABDH* (Figure S4), *FOXA* (Figure S7), *JAG* (Figure S9), *NIN* (Figure S10), *NKX2* (Figure S11), *PAX* (Figure S12), *PYG* (Figure S13), *RALGAPA* (Figure S14) and *VSX* (Figure S20) families have duplicates of 2R-generated subtypes located on the same chromosome (see Additional file 3).

There have been some indications of these translocations in previously published large-scale genomic analyses. For instance, in the analysis of the published medaka genome [5] the rearrangements after 3R between the *SSTR2*, -3 and -5-paralogous regions on chromosomes 1, 8 and 19 are apparent. Our analyses allow us to resolve the events in greater detail: We conclude that these rearrangements in the teleost lineage were preceded by fusions of 2R-generated chromosome blocks before 3R, with subsequent paralog translocations and chromosome fissions after 3R (Figure 6). This fusion scenario is supported by a large-scale reconstruction of the ancestral vertebrate genome [4], where these reorganizations were concluded from comparative genomic analyses including the medaka. However, these analyses suggested that the fusions occurred before the



divergence of lobe-finned and ray-finned fishes. Our conserved synteny analysis on the other hand shows that the tetrapod genomes have no signs of ancestral fusions in this paralogon (Figure 6, see Additional file 4). Together with the locations of the *SSTR2*, -3 and -5 genes on different linkage groups in the spotted gar genome (Table 1), our data instead point towards a time frame for the chromosomal fusions after the divergence of the gar lineage and before 3R in the teleost lineage. Both these large-scale analyses also support our scenario for the rearrangements between *SSTR1*, -4 and -6-paralogous regions in the teleost lineage after 3R.

The recent mapping of the spotted gar genome [45] concluded that its genome organization is more similar to that of the human genome than to teleost genomes. We were able to predict sequences for all SSTR genes except *SSTR4* in the genome of the spotted gar, and located them to five different genomic linkage groups (Table 1). The cited analyses of conserved synteny between the spotted gar genome and the human, zebrafish and stickleback genomes are concurrent with our own, and demonstrate that the linkage groups we have identified as SSTR-bearing in the spotted gar share conserved synteny with SSTR-bearing chromosome regions in the other genomes (see supporting information in *Amores et al. (2011)* [45]).

It is to be expected that duplicated chromosomes, as well as duplicated chromosomal regions that display similarity, can undergo rearrangements such as translocation to the same chromosome. We were surprised to find that regions that arose as separate chromosomes in 2R, perhaps 500 MYA, have been fused in the ray-finned fish lineage and subsequently exchanged 2R-generated paralogs after the 3R event approximately 300 MYA. Any such rearrangements require extensive analyses in order to be disentangled. We had completed our comprehensive analyses arriving at the scenario shown in Figure 6 when the spotted gar genome became available and confirmed our suggested scenario. The teleost rearrangements described here may severely hamper efforts to use conservation of synteny for identification of orthologs between teleosts and other vertebrates. Fortunately, the spotted gar constitutes a very important out-group for comparison with chromosomal events involving or surrounding 3R and it will greatly facilitate such analyses [45].

Implications for synteny analyses and orthology assignment

Our studies of the two vertebrate paralogons bearing SSTR genes have potentially far-reaching implications for comparative genomic studies such as analyses of conserved synteny. The identification of conserved synteny is essential for the correct assignment of orthology

and paralogy relationships between genes, and therefore for the evolutionary studies of gene families [46].

We describe here how 2R-generated duplicated chromosome blocks fused in the ray-finned fish lineage, and how subsequently these fused blocks duplicated in 3R and exchanged paralogs between each other, likely in blocks, blurring much of the conserved synteny patterns generated by the whole genome duplications. There have also been intra-chromosomal rearrangements within these chromosomal blocks, as well as a fission event splitting one of the 3R-duplicated blocks. At this point it is worth noting that the scenarios describing the evolution of the *SSTR2*, -3 and -5-bearing chromosome regions and the *SSTR1*, -4 and -6-bearing regions differ, with the latter showing no sign of fusions after 2R and inter-chromosomal exchange of paralogs only after 3R (Figures 6 and 7).

These types of rearrangements of the genomic structure make it exceedingly complicated to sort out the evolution of genomic regions and to infer orthology and paralogy relationships within gene families. We show that it is possible to resolve these events if one considers both the positional data between several different genomes as well as the phylogenies of the gene families shared between the chromosome regions. These analyses also demonstrate the importance of having the appropriate out-groups to determine the (relative) time points of the events. We could confirm the likely ancestral paralogy relationships between the chromosome regions by comparing our findings against the genomes of the spotted gar and the coelacanth, which were released during the final stages of our analyses. The spotted gar genome, which has been assembled to linkage groups, proved essential to confirm the ancestral location of the SSTR genes on different chromosomes, and therefore to support both the duplication of the chromosome regions in 2R and the time window of the chromosome block fusions.

The chromosomal rearrangements that we have described here undoubtedly complicate the assignment of orthology based on synteny analyses. For the SSTR family we found that the assignment of SSTR genes to specific subtypes needs to take into consideration firstly that there is a sixth previously undescribed ancestral vertebrate subtype, *SSTR6*, which is more closely related to *SSTR1* and *SSTR4*; secondly, that teleost fishes may have additional paralogs resulting from the shared third whole genome duplication (3R); and thirdly that additional duplicates have been generated by independent fourth genome duplications in some lineages. The teleost *SSTR6* sequences that we have identified in this study were annotated as *SSTR1a* in the zebrafish genome database and *SSTR4* in the stickleback and fugu genome databases.

Conclusions

By combining analyses of conserved synteny with phylogenetic data we can conclude that two vertebrate ancestral SSTR genes on different chromosomes diversified in the basal vertebrate whole genome duplications, 2R, one giving rise to *SSTR1*, -4 and -6 subtype genes, and one giving rise to *SSTR2*, -3 and -5 subtype genes. The *SSTR6* subtype was previously unrecognized, and could be identified in all teleost fish genomes, the spotted gar genome as well as the genome of the Comoran coelacanth. Conversely, *SSTR4* subtype genes could only be identified in the analyzed tetrapod genes as well as the coelacanth. Taken together these results indicate that six SSTR subtype genes were ancestral to both lobe-finned and ray-finned fishes, but that reciprocal losses have occurred. Subsequently *SSTR2*, -3 and -5 conserved duplicates from the teleost-specific whole genome duplication, 3R. Although there have been losses of SSTR subtype genes, the paralogous genome regions could be identified in both tetrapod and teleost genomes. The positional and phylogenetic data from the analysis of conserved synteny indicate that there have been significant rearrangements between paralogous chromosome regions in the teleost genomes, especially between *SSTR2*, -3 and -5-bearing chromosome regions. These rearrangements would explain the co-localization of *SSTR2*, -3 and -5 genes in several teleost genomes. That these rearrangements occurred in the teleost lineage is corroborated by comparison with the spotted gar genome, representing a lineage that diverged before teleost evolution.

Methods

Identification of SSTR sequences in Ensembl genome databases

Amino acid sequences of SSTR family members were identified in the Ensembl genome browser (<http://www.ensembl.org>) using the automatic protein family prediction feature. All SSTR sequences and their locations have been verified against Ensembl release 67 (May 2012). In most analyzed genomes the identified "somatostatin receptor type" protein family included somatostatin receptors of *SSTR1*, -2, -3, -4 and -5-type as well as neuropeptide B/W receptors of *NPBWR1* and -2-type. The NPBWRs share sequence similarity to both SSTRs and opioid receptors, however phylogenetic analysis as well as their chromosomal locations indicate that they constitute a separate family of GPCRs [9]. Hence only the SSTR sequences were considered in our analyses.

The SSTR sequences from the following Ensembl genome databases were collected and their database identifiers and locations noted: *Homo sapiens* (human), *Mus musculus* (mouse), *Canis familiaris* (dog), *Monodelphis domestica*

(grey short-tailed opossum), *Gallus gallus* (chicken), *Anolis carolinensis* (Carolina anole lizard), *Silurana (Xenopus) tropicalis* (Western clawed frog), *Latimeria chalumnae* (Comoran coelacanth), *Danio rerio* (zebrafish), *Oryzias latipes* (medaka), *Gasterosteus aculeatus* (three-spined stickleback), *Tetraodon nigroviridis* (green spotted pufferfish), *Takifugu rubripes* (fugu), *Ciona intestinalis* (vase tunicate) and *Drosophila melanogaster* (fruit fly). Database identifiers, location data and annotation notes of all SSTR sequences, as well as genome assembly versions for each species, are listed in Additional file 7.

To account for possible failures in the automatic identification of SSTR protein family members TBLASTN searches were also carried out in the Ensembl databases as well as in the National Center for Biotechnology Information (NCBI) Reference Sequence and trace archive databases using the known human SSTR sequences as queries. *Branchiostoma floridae* (Florida lancelet, amphioxus) genomic scaffolds were sought by TBLASTN searches in the NCBI Reference Sequence database using the known human family member sequences as queries. Additionally, complementary searches for teleost fish sequences were performed in the NCBI reference sequence database using the identified zebrafish *SSTR1* and *SSTR6* sequences.

Identification of SSTR sequences in the *Lepisosteus oculatus* genome

SSTR sequences were sought in the *Lepisosteus oculatus* (spotted gar) genome assembly *LepOcu1* (GenBank ID: GCA_000242695.1) available from NCBI (<http://www.ncbi.nlm.nih.gov/genome/assembly/327908/>). The sequences of the assembled linkage groups as well as unplaced scaffolds were downloaded and a local search database was set up. TBLASTN searches were carried out in this local database applying the BLAST+ 2.2.26 executable application available from <ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/> with the known human SSTR sequences as well as the identified coelacanth sequences as search queries. The near full-length BLAST hits were evaluated by reciprocal tblastn searches in the NCBI reference sequence database and those that matched identified SSTR sequences were included in preliminary neighbor joining (NJ) trees (see "Phylogenetic analyses" below) to assert their identities. The positions within the linkage groups of those BLAST hits that clustered confidently within the SSTR NJ tree were noted and the corresponding genomic sequences were inspected in order to predict the full length of the SSTR genes in the spotted gar (see "Sequence alignments and editing of gene and protein sequences" below).

Identification and analysis of neighboring gene families/ Conserved synteny analysis

Lists of gene predictions corresponding to the different SSTR-bearing chromosome blocks were downloaded using the BioMart function in the Ensembl database version 56. The chromosome blocks were defined as 15 Mb in each direction of the SSTR gene in question, although in many cases this definition encompassed the entire chromosome. These blocks were compared with each other in order to identify those gene families, as defined by Ensembl's automatic protein family prediction, that are represented on several of the blocks across different species.

For the analysis of the *SSTR1*, -4 and -6-bearing regions, the human and chicken chromosome blocks carrying the *SSTR1* and *SSTR4* genes were compared with each other. The gene families that were represented on both chromosomes in the human genome were selected for the analysis of conserved synteny and this list was complemented with those gene families that were represented on both chicken chromosomes as well as at least one of the human chromosomes. In this way we could account for any possible lineage-specific rearrangements in any of these genomes. The chromosome blocks in the human genome (assembly GRCh36) were between map positions 23 Mb and 53 Mb on chromosome 14 and between 8 Mb and 38 Mb on chromosome 20. The chromosome blocks in the chicken genome (assembly WASHUC2) were between map positions 24 Mb and 54 Mb on chromosome 5 and between 1 bp and 18 Mb on chromosome 3. These blocks represent the chromosome regions bearing *SSTR1* and *SSTR4* genes respectively in each species. Teleost genomes were not considered in this selection of neighboring gene families since there seems to have been a lineage-specific loss of *SSTR4* early in ray-finned fish evolution. Our preliminary phylogenetic analysis indicated that teleosts, spotted gar and coelacanth had another distinct SSTR gene instead, *SSTR6*, which we could take advantage of in the analysis of conserved synteny. This gene has not been assigned to a chromosome location except for in the zebrafish genome. We attempted including the chromosome regions of zebrafish *SSTR1* and -6 in the selection of neighboring gene families, but this provided no additional ones.

For the analysis of the *SSTR2*, -3 and -5-bearing regions, the chicken and stickleback chromosome blocks were both used. The gene families that were represented on all three chromosomes in each of these genomes were chosen for the analysis of conserved synteny. The chromosome blocks in the chicken genome (assembly WASHUC2) were between map positions 38 Mb and 69 Mb on chromosome 1, as well as the whole of chromosomes 14 (approximately 15.8 Mb)

and 18 (approximately 10.9 Mb). The blocks in the stickleback genome (assembly BROADS1) correspond to the full linkage groups V (approximately 12.25 Mb), IX (approximately 20.24 Mb) and XI (approximately 16.20 Mb). Linkage groups V and IX carry the *SSTR2b* and *SSTR5b* genes respectively, and linkage group XI carries three SSTR genes: *SSTR2a*, *SSTR5a* and *SSTR3*. The stickleback genome was favored over other teleost genomes as all the SSTR genes predicted in this genome assembly have been mapped.

The predicted amino acid sequences of all the identified protein family members were downloaded for subsequent alignment and phylogenetic analysis, and the locations of the corresponding predicted genes were noted (see Additional files 8 and 9). Locations have been verified against Ensembl version 67 (May 2012) to ensure the information is up to date. To a large extent the same species were included in the phylogenetic analyses of the neighboring gene families as in the SSTR tree, with the following exceptions: coelacanth and spotted gar sequences were not considered and green spotted pufferfish and/or fugu sequences were only included when the preliminary phylogenetic analyses showed inconclusive teleost fish topologies. Additionally, sequences from the *Macropus eugenii* (tammar wallaby, assembly 1.0), *Taeniopygia guttata* (zebra finch, assembly 3.2.4), *Meleagris gallopavo* (turkey, assembly 2.01), *Ciona savignyii* (transparent tunicate, assembly 2.0) and *Branchiostoma floridae* (Florida lancelet, amphioxus, assembly 2.0) genome databases were used to complement missing gene predictions in the genome databases for grey short-tailed opossum, chicken and vase tunicate for some gene families. For those few gene families where no fruit fly, amphioxus or tunicate sequences could be identified, the *Caenorhabditis elegans* predicted protein family members were collected from the Ensembl database (assembly WBcel215). Database identifiers, location data and annotation notes of all neighboring family sequences are included in supplemental tables (see Additional files 8 and 9).

Sequence alignments and editing of gene and protein sequences

The identified amino acid sequences were aligned using the ClustalWS sequence alignment program with standard settings (Gonnet weight matrix, gap opening penalty 10.0 and gap extension penalty 0.20) through the JABAWS 2 tool in Jalview 2.7 [47]. The alignments were manually inspected and edited in Jalview 2.7 in order to curate wrongly predicted sequences and adjust poorly aligned sequence stretches. Short, incomplete or highly diverging amino acid sequence predictions were curated manually by analyzing the corresponding genomic

sequence (including full intron sequences and flanking regions) with respect to consensus for splice donor and acceptor sites and sequence homology to other family members. In this way erroneous automatic exon predictions and exons that had not been predicted could be ratified.

Phylogenetic analyses

Phylogenetic trees were made using the Phylogenetic Maximum Likelihood (PhyML) method [48] supported by a non-parametric bootstrap analysis of 100 replicates and assuming the LG matrix of amino acid substitution by Le and Gascuel [49]. This method was applied using the web-application of the PhyML 3.0 algorithm available at <http://www.atgc-montpellier.fr/phyml/> or the executable PhyML-aBayes (3.0.1 beta) program with the following settings: amino acid frequencies (equilibrium frequencies), proportion of invariable sites (with optimised *p-invar*) and gamma-shape parameters were estimated from the datasets; the number of substitution rate categories was set to 8; BIONJ was chosen to create the starting tree and the nearest neighbor interchange (NNI) tree improvement method was used to estimate the best topology; both tree topology and branch length optimization were chosen.

Initially, phylogenetic trees were made using the neighbor joining (NJ) method applied through ClustalX 2.0 [50] with standard settings and a non-parametric bootstrap analysis with 1000 replicates. These trees have been included for the neighboring gene families in Additional files 5 and 6 in order to complement the PhyML tree topologies and provide a reference for discussion in the cases where tree topologies were inconclusive (see Results). For both NJ and PhyML tree topologies, bootstrap values higher than 50% were considered supportive.

For the SSTR-family tree (Figure 1) more careful measures were taken in order to account for the larger amount of protein subtypes and animal taxa in this tree compared to the neighboring gene families. The Phylogenetic Maximum Likelihood analysis was repeated using both a non-parametric bootstrap analysis of 100 replicates, and an SH-like approximate likelihood ratio test (aLRT) [51], in both cases selecting both NNI and subtree pruning and regrafting (SPR) tree improvement methods rather than only NNI. Additionally, the amino acid substitution model for the phylogenetic analysis was chosen using ProtTest 3.0 [52] with the following settings: Likelihood scores were computed selecting the JTT, LG, DCMut, Dayhoff, WAG, Blosom62 and VT substitution model matrices, with no add-ons and a Fixed BioNJ JTT base tree. Based on this analysis the JTT model of amino acid substitution was chosen.

In most cases the identified fruit fly sequences were used as out-groups to root the trees, and where such a sequence could not be found the identified amphioxus or tunicate sequences were used as the out-group instead. The inclusion of amphioxus and/or tunicate in the phylogenetic analyses provides the relative dating for the time window of the 2R events. For two gene families *C. elegans* sequences had to be identified due to the lack of fruit fly sequences. For the SSTR-family tree (Figure 1) the human kisspeptin receptor (*KISS1R* or *GPR54*) sequence was chosen as an out-group in order to accurately show the branching point of the identified fruit fly SSTR-family genes. Kisspeptin receptors are GPCRs closely related to the somatostatin receptors [53] (see also Additional file 5; Figure S15 in *Nordström et al. (2008)* [33]), diverging before the protostome-deuterostome split, therefore providing a reasonable out-group for our phylogenetic analysis of the SSTR family.

Description of additional files

The following additional data files are available with the online version of this paper. The spreadsheets in Additional files 7, 8 and 9 include comprehensive information about all sequences analyzed in this study, such as database identifiers, location data and annotation notes. Figures of all phylogenetic analyses used in the study are included in Additional files 2,5,6. The positional data underlying our evolutionary scenario is presented in Additional files 3 and 4. All final curated sequence alignments made for the phylogenetic analyses, as well as the original rooted phylogenetic tree files, have been provided as citable file sets with persistent identifiers - see references [54,55]. Detailed notes on the identification of SSTR sequences in the genome databases, as well as detailed descriptions of the neighboring family tree topologies, are included in Additional file 1.

Additional files

Additional file 1: Supplemental notes 1–3. Detailed descriptions of the results, including the identification of SSTR sequences in genome databases as well as the phylogenetic analyses of neighboring gene families.

Additional file 2: Figures S1–S3. All phylogenetic analyses of the SSTR gene family.

Additional file 3: Table S4. Positional data for the *SSTR1*, -4 and -6-bearing chromosome regions. The members of the identified neighboring gene families in these chromosome regions are charted by species and chromosome/genomic scaffold. These charts show the identified paralogous chromosome regions in the human, chicken, medaka, stickleback and zebrafish genomes. Each species is included in a separate tab in the spreadsheet.

Additional file 4: Table S5. Positional data for the *SSTR2*, -3 and -5-bearing chromosome regions. The members of the identified neighboring gene families in these chromosome regions are charted by species and chromosome/genomic scaffold. These charts show the identified paralogous chromosome regions in the human, chicken,

medaka, stickleback and zebrafish genomes. Each species is included in a separate tab in the spreadsheet.

Additional file 5: Figures S4–S20. Phylogenetic trees of the *SSTR1*, -4 and -6-neighboring gene families. Figures are numbered S4-S20 and include both neighbor joining and phylogenetic maximum likelihood trees of the gene families described in Table 2.

Additional file 6: Figures S21–S50. Phylogenetic trees of the *SSTR2*, -3 and -5-neighboring gene families. Figures are numbered S21-S50 and include both neighbor joining and phylogenetic maximum likelihood trees of the gene families described in Table 3.

Additional file 7: Table S1. Database identifiers, location data and annotation notes of all SSTR sequences identified and included in this study.

Additional file 8: Table S2. Database identifiers, location data and annotation notes of *SSTR1*, -4 and -6-neighboring gene family sequences, including information for those gene families that were discarded from the analysis. Each gene family is included in a separate tab in the spreadsheet.

Additional file 9: Table S3. Database identifiers, location data and annotation notes of *SSTR2*, -3 and -5-neighboring gene family sequences, including information for those gene families that were discarded from the analysis. Each gene family is included in a separate tab in the spreadsheet.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DOD and GS participated in the study design, performed phylogenetic and chromosome analyses and co-wrote the article. CAB performed genome database searches, sequence analyses and alignments as well as phylogenetic and chromosome analyses. DL conceived and co-designed the study, participated in all analyses and co-wrote the article. All authors participated in the draft of the manuscript and have read and approved the final version.

Acknowledgements

The authors would like to thank Lars G. Lundin for valuable discussions. This work was supported by grants from the Swedish Research Foundation and Carl Trygger's Foundation. The funding bodies had no role in the conception and design of this study; in the collection, analysis, and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

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Received: 3 September 2012 Accepted: 21 November 2012

Published: 29 November 2012

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doi:10.1186/1471-2148-12-231

Cite this article as: Ocampo Daza et al.: The evolution of vertebrate somatostatin receptors and their gene regions involves extensive chromosomal rearrangements. *BMC Evolutionary Biology* 2012 **12**:231.

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