



# Neuropeptide Y family receptors Y1 and Y2 from sea lamprey, *Petromyzon marinus*



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## ABSTRACT

The vertebrate gene family for neuropeptide Y (NPY) receptors expanded by duplication of the chromosome carrying the ancestral Y1–Y2–Y5 gene triplet. After loss of some duplicates, the ancestral jawed vertebrate had seven receptor subtypes forming the Y1 (including Y1, Y4, Y6, Y8), Y2 (including Y2, Y7) and Y5 (only Y5) subfamilies. Lampreys are considered to have experienced the same chromosome duplications as gnathostomes and should also be expected to have multiple receptor genes. However, previously only a Y4-like and a Y5 receptor have been cloned and characterized. Here we report the cloning and characterization of two additional receptors from the sea lamprey *Petromyzon marinus*. Sequence phylogeny alone could not with certainty assign their identity, but based on synteny comparisons of *P. marinus* and the Arctic lamprey, *Lethenteron camtschaticum*, with jawed vertebrates, the two receptors most likely are Y1 and Y2. Both receptors were expressed in human HEK293 cells and inositol phosphate assays were performed to determine the response to the three native lamprey peptides NPY, PYY and PMY. The three peptides have similar potencies in the nanomolar range for Y1. No obvious response to the three peptides was detected for Y2. Synteny analysis supports identification of the previously cloned receptor as Y4. No additional NPY receptor genes could be identified in the presently available lamprey genome assemblies. Thus, four NPY-family receptors have been identified in lampreys, orthologs of the same subtypes as in humans (Y1, Y2, Y4 and Y5), whereas many other vertebrate lineages have retained additional ancestral subtypes.

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## 1. Introduction

The neuropeptide Y family of peptides has the largest family of G protein-coupled receptors for peptides in the vertebrates with seven members that arose in early vertebrate evolution (Larhammar and Salaneck, 2004; Larsson et al., 2008, 2009). The ancestor of the vertebrates had three primordial receptors that probably arose through local duplications, namely the predecessors of the Y1, Y2 and Y5 subfamilies (Fig. 1). Before the radiation of the gnathostomes (jawed vertebrates), two tetraploidizations, or genome doublings, took place that quadrupled the chromosome number (Nakatani et al., 2007; Putnam et al., 2008) approximately 500 million years ago. These two events are often referred to as 1R and 2R for first and second round of tetraploidization. After the loss of some of the duplicates, seven NPY-family receptors remained (Fig. 1) when the classes of gnathostomes began to diverge from

one another, and all seven subtypes have been found in representatives for cartilaginous fishes (Larsson et al., 2009), lobe-finned fishes (Larhammar and Bergqvist, 2013) and ray-finned fishes (unpublished). These seven receptor subtypes can be grouped into three subfamilies based on their ancestry and sequence similarity: the Y1 subfamily (with members Y1, Y4, Y6, Y8), the Y2 subfamily (Y2, Y7) and the Y5 (alone) subfamily. The Y3 receptor was proposed from pharmacological experiments but its existence as a separate entity and gene has never been verified. The gene designations for the receptor subtypes are NPY1R, NPY2R, NPY4R, etc.

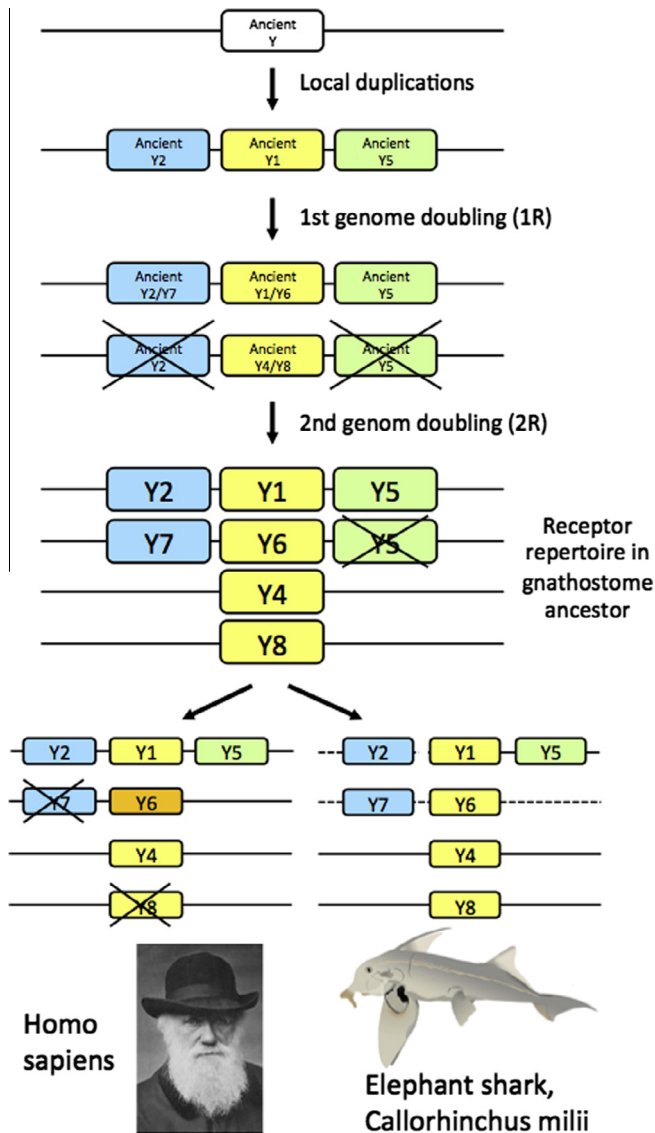
Subsequently, gene losses and a few additional gene duplications have taken place in some of the lineages: mammals and birds have lost Y8 independently, mammals have lost Y7, and a functional Y6 receptor has been lost independently in some mammals, lizards, frogs and teleost fishes, whereas teleost fish have acquired a duplicate of Y2 named Y2-2 and a duplicate of Y8 named Y8b (Starbäck et al., 2000; Larsson et al., 2008, 2009; Fallmar et al., 2011; Sundstrom et al., 2012).

The tetraploidizations also duplicated the ancestral NPY gene to generate PYY (peptide YY) (Soderberg et al., 2000; Sundstrom et al.,

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**Fig. 1.** Deduced gene duplication scheme for the vertebrate NPY receptor family based on analyses in several basal vertebrate lineages (Larsson et al., 2009; Larhammar and Bergqvist, 2013). Human Y6 is a pseudogene as indicated by orange color. The dashed lines for the elephant shark indicate that synteny has not yet been confirmed. Note that Y1 and Y5 are indeed located close to each other in the elephant shark as reported in the present study (see Fig. 4), like in the coelacanth *Latimeria chalumnae* (Larhammar and Bergqvist, 2013). Y1, Y2 and Y5 are syntenic in chicken as are Y6 and Y7, supporting the scenario shown (Bromee et al., 2006).

2008). Later PP (pancreatic polypeptide) appeared in the early lobe-finned lineage as a copy of PYY (Larhammar and Bergqvist, 2013). Duplicates of both NPY and PYY arose in the teleost fish ancestor as a result of another tetraploidization named 3R (Jaillon et al., 2004) and these genes are therefore named NPYa, NPYb, PYYa and PYYb (Sundstrom et al., 2008).

This multiplicity of receptors and peptides probably explains why this system has been found to influence a wide range of physiological parameters. One of the most powerful effects is the stimulation of appetite (Zhang et al., 2011), but the list of effects also includes increased blood pressure, reduction of pain and anxiety, as well as effects on tumor growth, bone density and gastrointestinal functions, and correlations with depression, schizophrenia and alcohol intake (Cox, 2007; Lee and Herzog, 2009; Morales-Medina et al., 2010; Zhang et al., 2011). Especially intriguing from both a

functional and evolutionary point of view are the opposing effects on food intake where NPY stimulates via Y1 and Y5, PYY inhibits via Y2 and PP has been reported to inhibit food intake via Y4, see (Simpson and Bloom, 2010; Zhang et al., 2011) for reviews. This implies that at some point during evolution after the duplication some of the peptide and receptor copies must have switched to the opposite function.

The best way to address the evolution of functional changes is to investigate the roles of the various peptides and receptors in species representing distantly related vertebrate lineages. The first step is to identify all components of the peptide-receptor system in these species. We have previously identified the full repertoire of NPY-family peptides and receptors in a broad range of species and have performed studies of peptide-receptor preferences especially in mammals, chicken, the western clawed frog *Xenopus (Silurana) tropicalis*, and zebrafish (Bromee et al., 2006; Fallmar et al., 2011; Holmberg et al., 2002; Larhammar et al., 2001; Lundell et al., 2002; Salaneck et al., 2000; Sundstrom et al., 2013). Each lineage displays some unique partnership features for peptides and receptors. For instance, in mammals PP and Y4 have a clear preference for each other, and this selectivity is greater in rat (Lundell et al., 1996) than in human, while the chicken Y4 receptor binds to the three native ligands with almost equal affinities (Lundell et al., 2002). In the zebrafish, which has the three peptides NPYa, PYYa and PYYb and the seven receptors Y1, Y2, Y2-2, Y4, Y7, Y8a and Y8b, one of the major differences is that PYYa has lower affinity than the other two peptides for Y2 (Sundstrom et al., 2013) whose closest relative, the teleost-specific Y2-2 receptor, has very similar affinities for the three peptides (Fallmar et al., 2011). Another difference between the subtypes in zebrafish is that all three peptides have higher affinity for Y4 than for the other receptors (Sundstrom et al., 2013). In the western clawed frog, only three of the six receptors were possible to express in cell culture (Sundstrom et al., 2012). Among these Y8 had higher affinity for all three native peptides (NPY, PYY and PP) than either Y5 or Y7. Probably Y8 is the only one of these three for which PP has a biologically relevant affinity in the frog.

An important vertebrate lineage for studies of the evolution of the NPY system is the lampreys because they probably diverged from the jawed vertebrates soon after the two tetraploidizations as concluded from genome studies of the sea lamprey *Petromyzon marinus* (Smith et al., 2013) and the Arctic lamprey *Lethenteron camtschaticum* (also called *Lethenteron japonicum*) (Mehta et al., 2013) and supported by detailed studies of the visual opsin gene family (Lagman et al., 2013). Three peptides have been described, namely NPY, PYY and a third peptide named PMY which is probably unique to lampreys (Conlon et al., 1991; Montpetit et al., 2005; Soderberg et al., 1994; Wang et al., 1999). Regarding NPY-family receptors, we have previously reported the cloning and functional characterization of a Y1-subfamily receptor from the river lamprey *Lampetra fluviatilis* (Salaneck et al., 2001) that we proposed is most likely an ortholog of mammalian Y4. Recently we reported the Y5 receptor in *P. marinus* (Xu et al., 2013). Previously, we also identified a Y2-like sequence in *L. fluviatilis* (Larsson et al., 2008; Xu et al., 2013) although it was uncertain whether it was an ortholog of gnathostome Y2 or Y7. Two of these receptors have also been characterized in detail with regard to mRNA distribution in *P. marinus* brain using *in situ* hybridization, namely Y4 and Y5, both of which were found to have broad distribution with only minor differences (Perez-Fernandez et al., 2013, 2014).

Here we present a fourth NPY receptor in *P. marinus* and *L. camtschaticum*. Due to the uncertainty inherent in sequence-based phylogenetic analyses between lampreys and gnathostomes, we also compared synteny. Because the new lamprey gene is located close to Y5, like Y1 is in gnathostomes, we conclude that

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