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Molecular characterization and phylogenetic analysis of Growth Hormone cDNA sequence from the *Acipenser persicus*

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Abstract: The aim of this study was to investigate the amount of variation between *Acipenseridea* family and also, study amount of phylogenetic variation between Persian sturgeon (*Acipenser persicus*) with other *Acipenseridea* regarding to the Growth Hormon (GH) gene. In this study, pre growth hormone gene of Persian sturgeon was identified for first time and applied to gene bank (JN604534.1, 2011). The total RNA was extracted from pituitary gland of Persian Sturgeon, cDNA was synthesized. The full-length cDNA sequence of Persian sturgeon contains a 645 nucleotide open reading frame, which encodes a peptide of 214 amino acids. The position of the signal peptide cleavage site was predicted to be at position 72. After cleaving of a signal peptide of 24 amino acid residues and a mature peptide of 190 aa formed. Multiple sequence alignments and Phylogenic tree were performed by using the MEGA5 program. By sequence alignment and phylogenetic analysis of amino acid residues indicated that GH widely conserved in other species were identified. The GH nucleic acid and amino acid residue sequences of Persian sturgeon had a highest similarity to these of other Acipenseridea as well as mammalian, followed by those of Anguiliformes.

Keywords: Persian Sturgeon, Growth Hormone, cDNA, Phylogenetic Analysis

1. Introduction

Growth hormone (GH) is one of the main hormones made by the pituitary gland. GH is an important hormone for somatic growth regulation and metabolism in vertebrates (3 and 5).

GH has been found in all taxonomic groups of the vertebrates, also identified in the sea lamprey. GH is the ancestral hormone with highly conserved in all known GHs. This hormone is known for its essential role in the regulation of body growth and maturation, but it can also influence on osmoregulation, reproduction and immunity. The investigation of GH in the European eel (*Anguilla anguilla*), is important as it may provide information on the conservation and variation of regulations during evolution (6). So GH is a remarkably conserved protein.

Increasing attention has been focused on the potential use of GH for fish rearing during the last decade. GH administration has been shown to accelerate growth rate in a number of animals, especially fish such as Rainbow trout, Atlantic salmon, Nile tilapia, Coho salmon, among others (4 and 8). Regarding the molecular phylogeny of the GH, many efforts have been concentrated on the characterization of GHs in fishes.

Within the Euteleostei Subdivision, the GH amino acid sequence has already been determined in species in the Ostariophysii (Cypriniformes and Siluriformes Orders), Protacanthopterygii (Salmoniformes Order), Paracanthopterygii (Gadiformes Order) and Acanthopterygii (Scorpaeniformes, Perciformes, Pleuronectiformes and Tetraodontiformes Orders) superorders (5).

The Persian sturgeon (*Acipenser persicus)* is one of most

important economically high quality meat and caviar resource of the Acipenseridea family that observed in the south of Caspian Sea and inters in the Iranian rivers, Sefidrood and Gorgan-chai (4). *Acipenser persicus* was once considered a subspecies of *Acipenser gueldenstaedtii* (Russian sturgeon). Research in 1973 found morphological, immunological, biological, and reproductive differences between the two sturgeons. Additional research to find a molecular marker to differentiate the two is ongoing (2 and 6). This specie is one of the endangered species of the sturgeon fishes and recently the capture of this species in Caspian Sea has been extremely reduced so its propagation is under governmental programming, using artificial methods (8).

The aim of this study was the characterization of Acipenser persicus GH cDNA sequence and phylogenic analysis with other available GH amino acid sequences.

2. Materials and Methods

2.1. Sampling Procedure

Persian sturgeons were captured in the southern part of the Caspian Sea and transported to the International sturgeon Research institute (Rasht, Iran). They were killed and their pituitary glands were extracted immediately. The pituitary glands were frozen and stored at -70 °C until used for pre mRNA extraction.

Total mRNA Isolation and First Strand cDNA Synthesis:

Total RNA was extracted from pituitary glands using BIOZOL reagent (CinaGen, Iran) according to the manufacturer's instruction. For the synthesis of first strand cDNA, 5 µg of the extracted total RNA was incubated with 1µg of the modified Oligo(dT) primer at 70°C, for 10 min followed by a brief centrifugation. The reaction was chilled on ice for a few minutes. 1µl RNase (CinaGen, Iran), 2 µl dNTP mixture (120 mM of each nucleotide), 4µl of 5 X enzyme buffer, 1 µl Riboluck, 5 µl DEPC water and 2 µl reverse transcriptase (CinaGen, Iran) were added with 20 µl of final volume reaction.

RT-PCR:

The specific primers used for amplification of cDNA encoding the target genes were designed from alignment of several known Acipenseridea GH nucleotide sequences and matches with the first exon region which was retrieved from the NCBI GenBank. [Acipenser sinensis (EU119864.1), *Acipenser gueldenstaedtii* (Russian Sturgeon) (AY941176.1) and *Huso huso* (AB517597.1)] The primer sequences were 5'-ATGGCATCAGGTCTGCTT (forward primer) and 5'-CTACAGAGTACAGTTGCTCTC (reverse primer).

The PCR was performed under the condition of DNA denaturation at 94ºC (5 min), followed by 35 cycles of denaturation at 94°C (30 sec), annealing at 58°C (90 sec) and extension at 72°C (30 sec), with a final extension at 72°C (25 min). Amplicons were separated by 1.5 % agarose gel electrophoresis and stained with Ethidium bromide.

DNA sequencing and data analyses:

PCR products of the appropriate size was excised from the gel, purified by gel extraction kit according to the manufacturer's protocols (Gel DNA recovery user guide, Vivantis, Iran) and sent to Takapozist Company (Iran) for sequencing. Sequence similarity analysis against GenBank database entries was performed using BLAST at the NCBI website (http://www.ncbi.nlm.nih.gov). Primer sets were generated using Primer3 program (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). Multiple sequence alignments and Phylogenic tree were performed by using the MEGA5 (Molecular evolutionary genetics analysis, Version=0.1A; http://www.megasoftware.net) program.

3. Results

GH cDNA sequence of Persian Sturgeon

Four samples of frozen Persian sturgeon pituitary glands were separately melted, homogenised with Biozol reagent and total RNA was extracted as described in material and methods. The GH cDNA were synthesized by reverse transcriptase on pre mRNA matrix and the cDNAs library was established. The cDNA was multiplied by PCR and purified by gel electrophoresis. The sequencing of the cDNA revealed an open reading frame (ORF)consists of 645 nucleotides encoding 214 amino acid residues. The order of amino acid residues of GH coded by this DNA was obtained from MEGA5. The 24 amino acid residues from the N- terminus have a high degree of homology to the signal peptide of other fish GHs. It is assumed that in the Persian sturgeon pre-GH this region probably represent the signal peptide which is cleaved upon hormone secretion. Thus, position of the signal peptide cleavage site in cDNA was predicted to be at position 72.

All investigated samples of cDNA except one (probably in result of mRNA misreading by reveres transcriptase or during sequencing of cDNA) PS GH exhibit typical GH gene feature such as length and nucleotide sequance. The amino acid sequence obtained such as four triplets for cystein residuces, a single tryptophan and stretches of other amino acids highly conserved in all known GH. Only one site of Asn-Xaa-Thr motif in GH at the C terminus region is potential site for N-linked glycosylation of the hormone. The mature GH contains 190 residues starting with a tyrosine.

The cystein residues made 2 disulfide bonds and assumed to contribute an important role in formation of tertiary structure and biological activity of the hormone (10). In contrast to Persian sturgeon and other Sturgeon species the GH of goldfish and in other Cyprinidae have 5 cystein residues (1).

Phylogenetic analysis

Several versions of sturugeon growth hormone sequences were extracted from NCBI (http://www.ncbi.nlm.nih.gov). Sequences were aligned using Clustalw X and phylogenetic analysis was performed using MEGA5 for the construction of the distance matrices, NEIGHBOR (Neighbor-Joining) for the generation of 1000 phylogenetic trees.

The alignment used for the phylogenetic analysis is presented for a sample of representative sequences in Figure 1.

The amino acid sequence of Persian sturogeon GH when

compared to reported GHs at NCBI showed highest (99%) levels of homology to the GHs of Acipenseridea and mammalian.

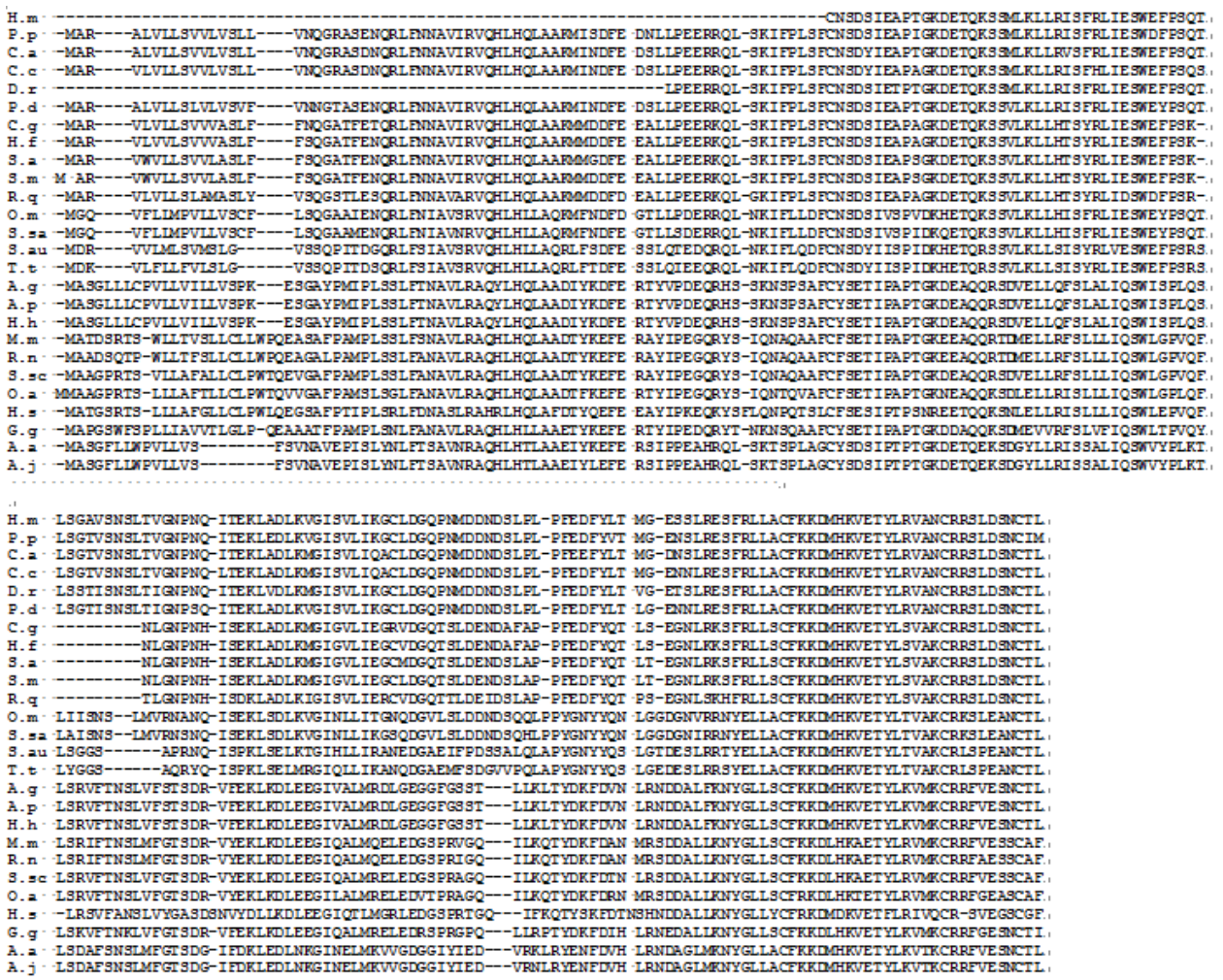

Figure 1. *The amino acid sequences of various animal GHs. The GH sequences included; Hypophthalmichthys molitrix(X60475), Pimephales promelas (AY643399), Carassius aurata(DQ350437), Cyprinus carpio(M27000), Danio rerio(AY286447), Paramisgurus dabryanus(DQ350432), Clarias gariepinus(AF416488), Heteropneustes fossili(AF147792), Silurus asotus(AY157496), Silurus meridionalis(AF530481), Rhamdia quelen(EF101341), Onchorinchus mykiss(M24683), Salmo salar (M21573), Sparus aurata(U01301), Trichogaster trichopterus(AF157633), Acipenser gueldenstaedtii(AY941176.1), Acipenser persicus(JN604534.1), Huso huso(HQ166628.1), Mus musculus(BC061157), Rattus norvegicus(V01237), Sus scrofa(JQ177096) , Ovis aries(S50877), Homo sapiens(V00520), Gallus gallus(JN675383), Anguilla anguilla(AY148493), Anguilla japonica(M24066)*

Comparison between the number of GH amino acids from Fig. 1 revealed showed great similarity and only 5 amino acids differences as Ser, Leu, Thr, Val, Arg between closely related species PS and Huso huso. Thus, there are 21 Ser, 25 Leu, 10 Thr, 11 Val and 10 Arg in PS but 22 Ser, 24 Leu, 11 Thr, 16 Val and 8 Arg in Huso huso but there are no diffrence between Ps and Russian sturgeon (Tabl.1).

Table 1. *The total amount of some amino acids in GHs from different vertebrates.*

Amino acid	*Acipenser persicus*	*Acipenser gueldenstaedtii*	*Huso huso*	*Homo sapiens*	*Rattus norvegicus*
Ser	21	21	22	20	14
Val	11	11	16	7	8
Thr	10	10	11	10	8
Leu	25	25	24	26	25
Arg	10	10	8	11	11
His	3	3	3	3	3
Cys	4	4	4	4	4
Trp	1	1	1	1	1

The phylogenic tree of vertebrates presented in Fig. 2.

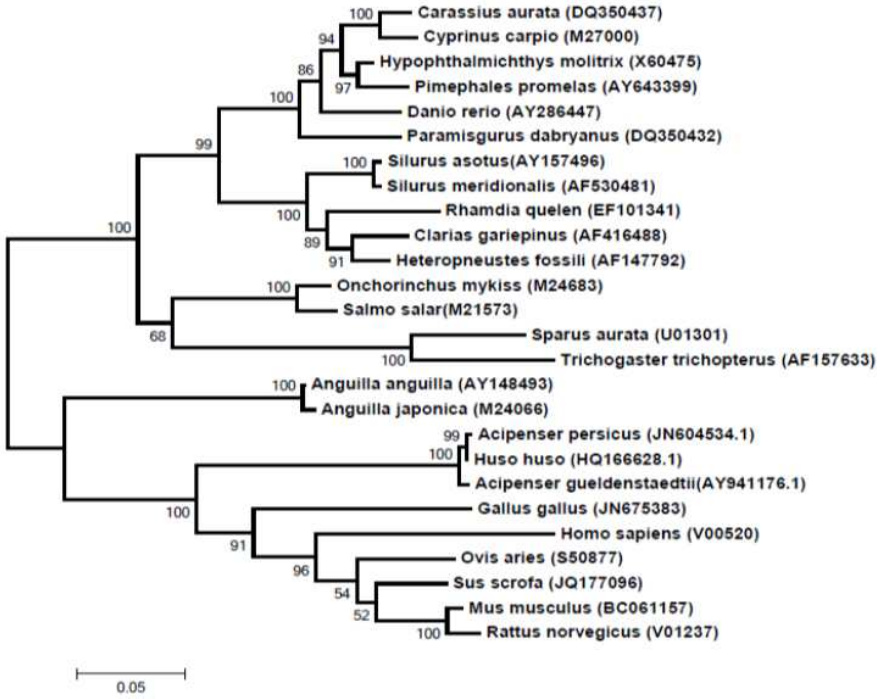

Fig 2. *The phylogenic tree of GHs some of vertebrates.*

The analysis of the GH phylogenic tree revealed that Persian sturgeon had a highest similarity to GH sequences of mammalian, followed by those of Anguiliformes whereas the amino acid residues had a highest similarity to mammalian. This result represented that the Persian sturgeon are a primitive fish and is genetically closer to mammalian than to bony fish. Moreover the aa sequences of Persian sturgeon (PS) GH have 99% similarity to beluga (Huso Huso).

4. Discussion

In this study we described sequence analysis and phylogenetic relationship of Persian Sturgeon GH to other vertebrates. Apart from a few deletions and insertions, GH is a remarkably conserved protein. GH molecule is composed of four conserved and four variable regions which are likely to be functionally important. Since the GH gene is a highly conserved protein, it provided a better resolution for more distantly related species (5, 9).

GH amino acid sequence of many teleost fish has already been determined. For example in Ostariophysii (Cypriniformes and Siluriformes Orders), Protacanthopterygii (Salmoniformes Order), Paracanthopterygii (Gadiformes Order) , Acanthopterygii (Scorpaeniformes, Perciformes, Pleuronectiformes and Tetraodontiformes Orders) (5). As a result, the aa sequences of Persian sturgeon (PS) GH have 99% similarity to beluga (Huso Huso) and highest (99%) levels of homology to the GHs of Acipenseridea and mammalian.

The comparison of aa amounts shows no difference between GH of PS and RS and only a little difference between PS and Huso huso (table. 1)

The Cystein residues, which are important for the disulfide bond formation and structural integrity of the 3-D structure of the GH protein (9) is conserved in sturgeons and located at 56, 146,187 and 192 positions.

Acknowledgements

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References

[1] Chang YS, Liu CS, Huang FL, Lo TB, 1992.The primary structures of growth hormones of three cyprinid species, bighead carp, common carp and grass carp, gen comp endocrinal. 87, 385-93

[2] IUCN (International Union for Conservation of Nature). 2010. Acipenser persicus. Available at www.iucnredlist.org/apps/redlist/details/235/0 [viewed 12/7/11].

[3] Jiang, B.C, et al, 2012. Doxycycline-regulated growth hormone gene expression system for swine. Genetics and Molecular Research 11 (3): 2946-2957

[4] Khoshkholgh M, et al, 2011.Genetic diversity in the Persian sturgeon, Acipenser percicus, from the south , Caspian J. Env. Sci. Vol. 9 No.1 pp. 17~25

[5] Luis F. Marins et al. A growth hormone-based phylogenetic analysis of euteleostean fishes including a representative species of the Atheriniformes Order, Odontesthes argentinensis. 2003. Genetics and Molecular Biology, 26, 3, 295-300

[6] Paul, K. Persian Sturgeon (Acipenser persicus) in Pond Life. 2007-2011 edition. World Wide Web electronic publication. Available at www.pondlife. me.uk/sturgeon/acipenserpersicus.php [viewed 12/8/11].

[7] Rousseau.K and Dufour. S. 2004. Phylogenetic evolution of the neuroendocrine control of growth hormone: Contribution from Teleosts. Cybium, 28(3): 181-198.

[8] Vajhi. A.R. et al; 2008 Digestive System Ultrasonography in Acipenser persicus Proceedings, The 15th Congress of FAVA. FAVA - OIE Joint Symposium on Emerging Diseases in Bangkok, Thailand on 27-30 October2008.page145-146

[9] Venugopal T, et al. 2002.Molecular cloning of growth hormone encoding cDNA of Indian major carps by a modified rapid amplification of cDNA ends strategy. J. Biosci. Indian Academy of Sciences (Vol. 27 , No. 3, (261–272)

[10] Vestling M, Murphy C, Fenselau C, Chen TT, 199.1Disulfide bonds in native and recombinant fish growth hormone. Mol Mar Biol Biotechnology, 1.73-7.