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# Identification and characterization of chickenII-GnRH (*cII-GnRH*) gene of *Catla catla* (Hamilton, 1822)

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**ABSTRACT**

*Gonadotropin-releasing hormone (GnRH) is a neuropeptide hormone responsible for the release of luteinizing hormone (Lh) and follicle-stimulating hormone (Fsh) from the anterior pituitary gland . GnRH have been identified and characterized from the brain of C. catla. A cDNA fragment of 202bp was amplified and GnRH variant has been identified in the form chicken-II-type gonadotropin-releasing hormone (cII-GnRH). Sequence similarity of GnRH of Catla catla was 94% identical to C. carpio, C. auratus and 92% with C. idella and S. prenanti respectively.Phylogenetic analysis revealed that C. calta cII-GnRH is closely related to D. rerio.*

**Keywords** *:* GnRH, cDNA, Phylogenetic analysis

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## INTRODUCTION

In fish, as in all vertebrates, the regulation of reproductive action is controlled by the central nervous system. Brain is main organ which is involved in all the steps of the sexual cycle [1].Gonadotropin-releasing hormone (GnRH), a decapeptide, was originally isolated from the hypothalamus of mammals and fish. The hypothalamic-pituitary-gonadal (HPG) axis regulates puberty in fishes, primarily through the hypothalamic secretion of gonadotropin-releasing hormone (GnRH). GnRH stimulates pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone(LH) to control gametogenesis and sex steroid production.

Gonadotropin-releasing hormone (GnRH) is a neuropeptide released by hypothalamic and it acts directly on the pituitary gland [2] [3][4]. In vertebrates, nine GnRH variants have been isolated and sequenced [5] [3](LampreyI-GnRH, LampreyIII-GnRH, catfish-GnRH (cfGnRH), dogfish-GnRH (dfGnRH), salmon- GnRH (sGnRH), seabream-GnRH (sbGnRH), chickenI-GnRH (cIGnRH), chickenII-GnRH (cIIGnRH) and mammalian-GnRH (mGnRH). Recently two new forms were identified by primary structure in a protochordate: tunicateI-GnRH (tIGnRH) and tunicateII-GnRH (tIIGnRH) [6].They are all decapeptides with a highly conserved structure.In all teleost species examined, at least three GnRH variants coexist in brain, cII-GnRH being the most conspicuous one; [3] [5] sGnRH[7] [8] mGnRH[9] [10] sbGnRH[5] orcfGnRH[11] are also present .

*Catla catla* is one of the major fresh water carp fish native to Indian sub- continent [12].Considering the important role of GnRH in brain–pituitary–gonad axis regulation, in the present study, we have partial characterized *cDNA* ofGnRH, and phylogenetic analysis was performed.

## MATERIALS AND METHODS

Adult *C. catla* (500-800g) used in the experiment were collected from Powarkherda, centre of Central Institute of Fisheries Education (CIFE), Mumbai, India. The animals were brought to wet laboratory of CIFE, Mumbai and were acclimatized for 20 days before the start of experiment. The fishes were kept in FRP tanks (500L) and were daily fed twice with commercially available fish feed pellets containing 30% of protein content. For *cDNA* of GnRH characterized brainsample werecollected in RNA *later*™(Qiagen, Germany) and stored at -80°C.

**RNA extraction and Preparation of cDNA**

Total RNA was extracted from brain using Trizol™ reagent (Invitrogen, USA). *DNaseI* treatment was given to extract RNA using DNase I, RNase-free kit (Thermoscientific, USA) to remove any co-purified genomic *DNA*. Purity and quantity of total RNA was assessed by NanoDrop spectrophotometer (Thermoscientific, USA) and first stand *cDNA* was synthesized from total RNA(5µg) using RevertAid™ reverse transcriptase First Strand *cDNA*Synthesis kit (Thermoscientific, USA) as per the manufacturer's instructions.

**Molecular cloning and amplification of *cDNA*GnRH**

PCR (Takara, USA) was performed to amplify the desired DNA fragments from the template. PCR amplification was performed in a total volume of 20µL containing 2.5µL Taq buffer (10x), 0.5µL dNTP mix (10mM), 2.5µL $MgCl_2$ (25mM), 1µL each of sense and antisense primers (10pmol), 1µL of cDNA, 0.25µL of Taq DNA polymerase (5U/uL) and 12.25µL of nuclease-free water. Amplification was performed in a 96-well Takara PCR System (Takara, Japan). The PCR conditions included an initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 0.30 sec, 58°C for 0.45 sec min and 72°C for 1 min and a final extension of 10 min at 72°C.Primer used for amplifying GnRH gene was, Forward 5'- ATGGAYCCYTGYGADTGCKCYAA-3'andReverse5'-GTTTCTTCCCTGGGGTCTCAGGTAGC-3'

**Bioinformatics analysis**

BLASTn and BLASTp programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to find sequences similarity of *C.catla* cIIGnRH. A neighbour-joining tree was constructed based on the deduced amino acid sequences of other reported species using MEGA 6.0 software.

## RESULTS AND DISCUSSION

A partial *cDNA* fragment of GnRH were amplified from the brain of *C. catla* using RT-PCR. A *cDNA* fragment of 202 bp was amplified using the specific primer set **(Fig.1)**and sequence has been submitted to NCBI (Accession no.KM887435) GnRH variants have been isolated and sequenced was chicken-II-type gonadotropin-releasing hormone(cII-GnRH).Multiple sequence alignment of deduced amino acid is highly conserved across different fish**(Table 1)**

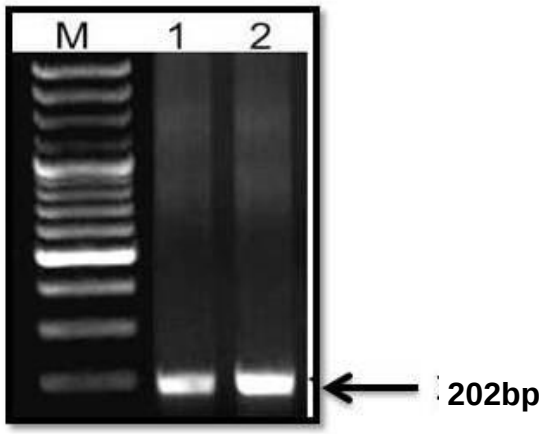


**Fig 1. PCR amplification of *GnRH cDNA* fragment of amplicon size 202bp. Lane M; 100bp plus ladder, amplified products at 60°C and 58°C annealing temperature (lane 1-2)**

**Table 1: Alignment score of *C.catla* GnRH deduced amino acid sequence with other fishes.**

| S.No | Species | Identity | Accession .no |
|---|---|---|---|
| 1 | *Cyprinus carpio* | 90% | AAO39753.1 |
| 2 | *Ctenopharyngodon idella* | 87% | ACH78253.1 |
| 3 | *Osteochilushasseltii* | 88% | AFH41000.1 |
| 4 | *Pimephales promelas* | 87% | ABS30828.1 |
| 5 | *Gobiocypris rarus* | 84% | AFJ44819.1 |

**Phylogenetic analysis**

The Neighbour joining tree constructed based on multiple sequence alignment revealed that *C. calta* GnRH is closely related to Zebra fish (*Daniorerio)* (Fig. 2)

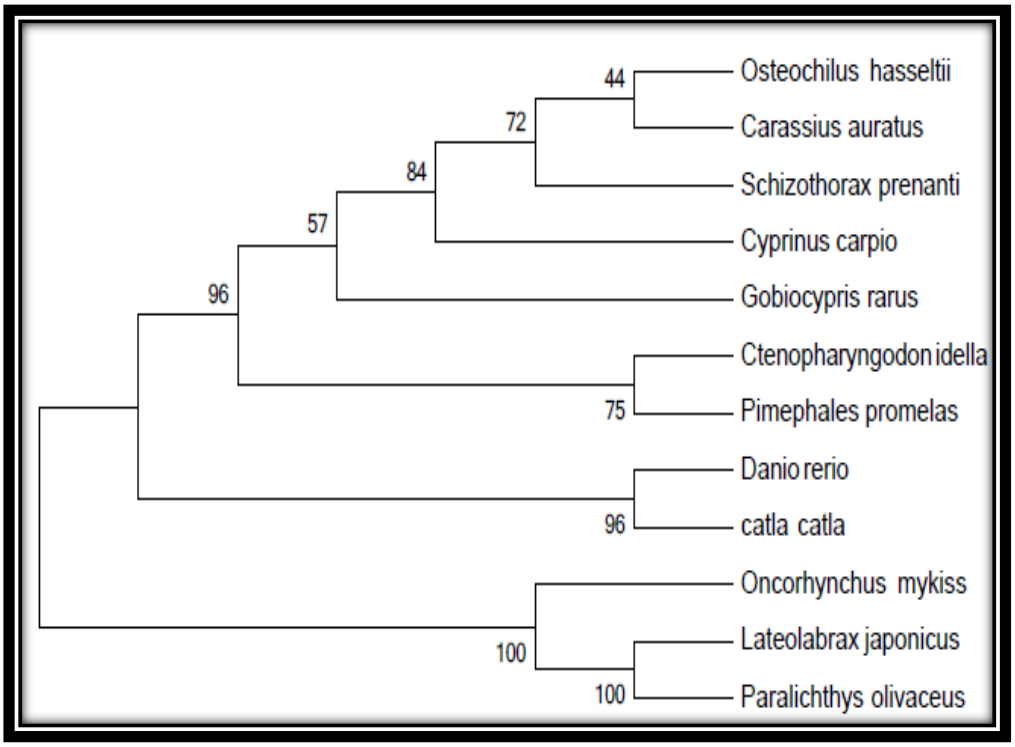


**Figure 2: Phylogenetic tree analysis of the deduced amino acid sequences of*cII-GnRH***

Gonadotropin (GTH) hormones are glycoprotein which stimulates gonadal maturation in vertebrates.GnRH is well known for its role in moderating gonadotropin release from the pituitary. Gonadotropin-releasing hormone (GnRH) is a members of family of neuropeptides that play a key role in the development and maintenance of reproductive function in vertebrates.The homology sequence of the GnRH of *C. catla* is 93 % identical to *C. carpio*and *C. auratus*.However its similarity is 92% with *C. idella* and *S. prenanti.* The Neighbour joining tree of GnRH showed that *C. catla* is closely related to *D. rerio* followed by goldfish because both are belonging same family. In the present study GnRH variants identified was chicken-II-type gonadotropin-releasing hormone. Similar type GnRH variants have been identified in *C. carpio[13]*.

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