Molecular cloning of growth hormone receptor (GHR) from common carp (*Cyprinus carpio* L.) and identification of its two forms of mRNA transcripts*

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Abstract The cDNA of growth hormone receptor (GHR) was cloned from the liver of 2-year common carp (*Cyprinus carpio* L.) by reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA end (RACE). Its open reading frame (ORF) of 1806 nucleotides is translated into a putative peptide of 602 amino acids, including an extracellular ligand-binding domain of 244 amino acids (aa), a single transmembrane domain of 24 aa and an intracellular signal-transduction domain of 334 aa. Sequence analysis indicated that common carp GHR is highly homologous to goldfish (*Carassius auratus*) GHR at both gene and protein levels. Using a pair of gene-specific primers, a GHR fragment was amplified from the cDNA of 2-year common carp, a 224 bp product was identified in liver and a 321 bp product in other tissues. The sequencing of the products and the partial genomic DNA indicated that the difference in product size was the result of a 97 bp intron that alternatively spliced. In addition, the 321 bp fragment could be amplified from all the tissues of 4-month common carp including liver, demonstrating the occurrence of the alternative splicing of this intron during the development of common carp. Moreover, a semi-quantitative RT-PCR was performed to analyze the expression level of GHR in tissues of 2-year common carp and 4-month common carp. The result revealed that in the tissues of gill, thymus and brain, the expression level of GHR in 2-year common carp was significantly lower than that of 4-month common carp.

Keywords: growth hormone receptor (GHR), molecular cloning, transcript, expression analysis, common carp.

Growth hormone receptor (GHR) belongs to the hematopoietic receptor superfamily[1]. The action of growth hormone (GH) in regulating growth[2], reproduction[3] and immunity[4] has been elucidated. The binding of GH to the GHR on target tissues triggers a cascade of tyrosine and protein phosphorylation events, which culminates in the biological action of GH[5,6]. Up to date GHR cDNAs have been cloned from many species[7—9], including various kinds of mammalian animals; avian of chicken and domestic pigeon; reptilian of soft-shelled turtle (*Pelodiscus sinensis japonicus*) and amphibian of African clawed frog (*Xenopus laevis*). In the year of 2001, Lee et al. cloned the full length cDNA of GHR from goldfish (*Carassius auratus*)[10]. This is the first report about the cloning of GHR in teleost fish. Subsequently Tse successfully cloned the GHR cDNA from black seabream (*Acanthopagrus schlegelii*), and found two kinds of GHR cDNAs in all the tissues, which resulted from a 93 bp intron alternatively spliced[11]. The diversity of GHR has been proved in mammals. For example, 8 GHR mRNAs (V1-V8) have been identified in human[12], 10 different GHR cDNAs were cloned from a cow endometrium cDNA library, and the expression of the 5′ UTR of GHR in rat liver has shown sexual dimorphism. Besides black seabream, two GHR cDNAs have been successfully cloned from rainbow trout (*Oncorhynchus mykiss*) recently[13].

In this study, we cloned GHR from common carp (*Cyprinus carpio* L.) and identified its two forms of transcripts, which will provide more valuable information on the gene structure, function and expression of freshwater fish.

1 Material and methods

1.1 Fish and sampling

The 2-year and 4-month common carp (*Cyprinus carpio* L.) were cultured in the Guanqiao Experi-

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mental Statition in Wuhan. When the fishes were transferred to the laboratory, the liver, spleen, kidney, headkidney, thymus, gill and brain were carefully removed and immediately stored in the liqulid nitrogen.

1.2 RT-PCR and RACE

About 50 mg of each tissue was used for extraction of total RNAs, which was performed with Trizol (Invitrogen, Japan) following the user's manual.

A primer GP1 (5′-CCATGGGTGGAGTTCATC-3′) was designed according to GHR Box2 region which is highly conservative. The oligo (dT) adaptor primer (AP) used for the first strain cDNA synthesis was 5′-GTTTTCCCAGTCACGAC(T)n-3′, so the specific adaptor primer AP was 5′-GTTTTCCCAGTCACGAC-3′. The first strain cDNA synthesis and the PCR amplification of 3′ RACE were performed with TaKaRa RNA PCR kit (AMV) Ver 3.0, referring to the protocols. The template was from the liver of controlled carp. Then PCR was performed with the primer of GP1and AP, under the condition of 94 ℃ denaturation for 5 min, running 30 cycles of 94 ℃ 30 sec; 60 ℃ 30 sec; 72 ℃ 1.5 min, and 72 ℃ elongation for 5 min.

To obtain the GHR cDNA with a complete coding region, the sense primer 5′ UTR (5′-GAAACGATGTTCGGGTGATT-3′) was designed according to the 5′ UTR of goldfish and grass carp (*Ctenopharyngoden idella*). At the same time the reverse gene specific primer GP2 (5′-CTCTGCAGGGTCATCAAGGT-3′) was designed according to the partial cDNA sequence of common carp obtained by 3′ RACE. Two primers were used to amplify the coding region and part of non-coding region at 5′ end.

1.3 Cloning and sequencing

PCR products were separated by agarose gel electrophoresis, and the amplified products were purified from the gels using the Glass Milk Extraction Kit (Fermentas). The extracted products were ligated into PMD18-T vector (TaKaRa) and used to transform competent *E. coli* DH5α cells. Positive colonies were screened by the method of PCR. The recombinant plasmids were sequenced by the dideoxy chain termination method with M13 universal primers. The data were automatically collected on the ABI PRISM 3730 Genetic Analyzer.

1.4 Tissue expression of GHR

Of the 1 μg total RNA isolated from liver, spleen, kidney, headkidney, thymus, gill and brain of 2-year common carp was used to synthesize the first strain cDNA. The first strain cDNA was used as PCR amplification template with the primers of Gf: 5′-GTGCGTGAGAACATAACC-3′ and Gr: 5′-CAGTGGGAGTTGTTTCTG-3′ which could specifically amplify a part of GHR cDNA. The negative control contained no template. The expected amplified fragment size was 224 bp. Amplification of β-actin was as the internal reference in PCR. The primers for β-actin cDNA amplification were actinF 5′-CAGATCATGTTTGAGACC-3′ and actinR 5′-ATTGCCAATGGTGATGAC-3′ which covered an intron in genome, and the expected amplified fragment from cDNA was 460 bp. The reaction was performed with an initial denaturation of 5 min at 94 ℃, followed by 30 cycles of 45 sec at 94 ℃; 30 sec at 60 ℃; 45 sec at 72 ℃. The final step was 10 min at 72 ℃. The PCR products were fractionated on a 1.5% agarose gel.

The same procedures were performed using the total RNAs isolated from liver, spleen, kidney, headkidney, thymus, gill and brain of 4-month common carp.

1.5 Genomic DNA amplification

Genomic DNA was prepared from the liver of 2-year common carp[14]. The PCR was performed to trap the intron with the template of the genomic DNA and a pair of primers of Gf and Gr, under the condition of 94 ℃ denaturation for 10 min, running 30 cycles of 94 ℃ 45 sec; 60 ℃ 30 sec; 72 ℃ 1.5 min, and 72 ℃ elongation for 10 min.

1.6 Computer-aided sequence analysis of cloned DNA

The sequences were analyzed for similarity with other known sequences by BLAST program. The signal peptide prediction was performed by SignalP program. The protein family signature was identified by InterPro[15] program. The phylogenetic tree was constructed based on the full length amino acid sequences of partial known GHRs using neighbor-joining algorithm within MEGA version 3.0[16].

2 Results

2.1 Cloning of GHR cDNA from the liver of 2-year common carp

A partial sequence of 1102 bp was obtained by 3′ RACE, and a product with the size of 1196 bp obtained using the primers of 5′ UTR and GP2. The BLAST searches on the NCBI database indicated that the two sequences resemble to GHR cDNA of other species (http://www.ncbi.nlm.nih.gov/BLAST/). Putting the two fragments together and omitting the identical nucleotide sequence of the overlapping, the GHR cDNA including a complete coding region of 2252 bp was obtained (GenBank accession number: AY741100) (Fig. 1).

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1                                                        GAAACGATGTT
12   CGGGTGATTTTTGAGGTTGATCTGACCACGTTTTTGCATCGTTTAAAAGGGGGAGAATAC
72   CGAGAGACCCACAACACGCAAGTCTGTTGATCCGGATGAACGAACTGTTGAGAAAGTAAA
132  AACTCGCAACAGATTTTTCTCGCGGACAACTTCTCTGGAGCTGAGGAGACAGCAGAAGCT
192  ATGGCTTACTCTCTCTCGCTCGGTCTGCTCTACCTGGGCTTGCTGTGTGGAAACGGACTG
       M  A  Y  S  L  S  L  G  L  L  Y  L  G  L  L  C  G  N  G  L
252  GTGTCTGCAAGATCCGAGCTGTTCACTCCAGATCCAAGCAGAGGACCTCATTTTACAGGC
       V  S  A  R  S  E  L  F  T  P  D  P  S  R  G  P  H  F  T  G
312  TGCCGCTCCAGAGAGCAGGAGACCTTCCGTTGCTGGTGGAGCGCTGGGATCTTCCAGAAC
       C  R  S  R  E  Q  E  T  F  R  C  W  W  S  A  G  I  F  Q  N
372  CTCACCGAGCCTGGAGCTCTCAGGGTCTTCTACCAGACAAAAAATTTCCTCTCTGAGTGG
       L  T  E  P  G  A  L  R  V  F  Y  Q  T  K  N  F  L  S  E  W
432  CAGGAGTGTCCAGACTACACACGTACTGTGAAAAATGAGTGCTACTTCAACAAAACCTTC
       Q  E  C  P  D  Y  T  R  T  V  K  N  E  C  Y  F  N  K  T  F
492  ACACAGATCTGGACCTCGTACTGCATTCAGCTGCGCTCAGTGCGTGAGAACATAACCTAT
       T  Q  I  W  T  S  Y  C  I  Q  L  R  S  V  R  E  N  I  T  Y
552  GACGAGGCCTGCTTTACAGTAGAGAACATAGTGCATCCTGACCCACCAATTGGGCTGAAC
       D  E  A  C  F  T  V  E  N  I  V  H  P  D  P  P  I  G  L  N
612  TGGACTCTATTAAATGTGAGTCGCTCGGGGTTGCACTTTGACGTCCTTGTGCGCTGGGCT
       W  T  L  L  N  V  S  R  S  G  L  H  F  D  V  L  V  R  W  A
672  CCCCCTCCGTCAGCAGATGTGCAGATGGGCTGGATGAGCCTGGTGTACCAGGTTCAGTAC
       P  P  P  S  A  D  V  Q  M  G  W  M  S  L  V  Y  Q  V  Q  Y
732  CGGGTCAGAAACAACTCCCACTGGGAAATGCTGGACCTGGAGAGTGGCACACAGCAGTCC
       R  V  R  N  N  S  H  W  E  M  L  D  L  E  S  G  T  Q  Q  S
792  ATCTACGGTTTACATACTGACAAAGAGTATGAAGTCCGGGTGCGCTGCAAGATGTCAGCC
       I  Y  G  L  H  T  D  K  E  Y  E  V  R  V  R  C  K  M  S  A
852  TTTGACAACTTTGGCGAATTCAGTGACAGCATCATTGTGCATGTGGCACAGATACCAAGC
       F  D  N  F  G  E  F  S  D  S  I  I  V  H  V  A  Q  I  P  S
912  AAAGAATCAACGTTCCCGACGACGTTGGTGTTGATTTTTGGAGTGATTGGAGTGGTGATT
       K  E  S  T  F  P  T  T  L  V  L  I  F  G  V  I  G  V  V  I
972  CTTCTTGTCCTCCTCATCTTCTCTCAACAACAGAGGTTGATGGTAATATTTTTACCACCT
       L  L  V  L  L  I  F  S  Q  Q  Q  R  L  M  V  I  F  L  P  P
1032 ATTCCTGCACCTAAAATAAAAGGCATCGACCCAGAGCTGCTGAAGAATGGAAAGCTTGAC
       I  P  A  P  K  I  K  G  I  D  P  E  L  L  K  N  G  K  L  D
1092 CAGCTCAATTCTTTGCTGAGCAGTCAGGATATGTACAAGCCGGACTTCTACCATGAGGAT
       Q  L  N  S  L  L  S  S  Q  D  M  Y  K  P  D  F  Y  H  E  D
1152 CCATGGGTGGAGTTCATCCAGCTGGACCTTGATGACCCTGCAGAGAAGAATGAGAGTTCT
       P  W  V  E  F  I  Q  L  D  L  D  D  P  A  E  K  N  E  S  S
1212 GATACACAACATCTGCTGGGCTTGTCTCGCTCAGGCTCTTCTCACTTCCTTAATTTCAAA
       D  T  Q  H  L  L  G  L  S  R  S  G  S  S  H  F  L  N  F  K
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(To be continued on the next page)

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1272  AGTGACAACGATTCGGGTCGTGCTAGCTGCTACGACCCAGAAATCCCAAATCCCAAGGAC
       S  D  N  D  S  G  R  A  S  C  Y  D  P  E  I  P  N  P  K  D
1332  TTGGCTTCTTTTCTGCCTGGCCATTCAGGACGAGGAGATAACCACCCTCTGGTTTCCAGA
       L  A  S  F  L  P  G  H  S  G  R  G  D  N  H  P  L  V  S  R
1392  AGCAGCTCATCCATCCCTGATCTTGGTTTCCAGCAGACATCAGAAGTGGAGGAGACTCCC
       S  S  S  S  I  P  D  L  G  F  Q  Q  T  S  E  V  E  E  T  P
1452  ATTCAAACGCAACCAGCTGTGCCCAGCTGGGTTAACATGGACTTTTATGCCCAAGTAAGT
       I  Q  T  Q  P  A  V  P  S  W  V  N  M  D  F  Y  A  Q  V  S
1512  GATTTCACACCAGCAGGAGGTGTCGTGCTTTCACCTGGACAACTGAACAGCTCTCCAGTG
       D  F  T  P  A  G  G  V  V  L  S  P  G  Q  L  N  S  S  P  V
1572  AAAAAGAAGGGAGAAGGGAATGAGAAGAAGATACAATTCCAGTTGCTTTCCGATGGAGCC
       K  K  K  G  E  G  N  E  K  K  I  Q  F  Q  L  L  S  D  G  A
1632  TACACCTCAGAGAACACAGCCAGGCTGCTTTCTGCCGATGTGCCACCCAGCCCTGGTCCT
       Y  T  S  E  N  T  A  R  L  L  S  A  D  V  P  P  S  P  G  P
1692  GAGCAGGGGTACCAAGCATTCCCAACCCAAGCCGTTGAGGGGAACCTCTGGAATGGTGAG
       E  Q  G  Y  Q  A  F  P  T  Q  A  V  E  G  N  L  W  N  G  E
1752  TACCTGGTGTCCGCCAATGATTCCCAGACGCCGTGCCTGGTTCCTGAAGCTCCTCCAGCC
       Y  L  V  S  A  N  D  S  Q  T  P  C  L  V  P  E  A  P  P  A
1812  CCCATACTGCCACCAGTATCAGACTATACTGTAGTGCAGGAAGTGGATGCCCAGCACAGC
       P  I  L  P  P  V  S  D  Y  T  V  V  Q  E  V  D  A  Q  H  S
1872  CTCCTCCTGAATCCTCCTTCCTCACAGCCTGCGATATGCCCTCACAGCCCAAACAAACAT
       L  L  L  N  P  P  S  S  Q  P  A  I  C  P  H  S  P  N  K  H
1932  CTCCCTGTAATCCCAACCATGCCCATGGGGTACCTCACCCCAGACCTTCTGGGAAACCTG
       L  P  V  I  P  T  M  P  M  G  Y  L  T  P  D  L  L  G  N  L
1992  AACCCATGAAGGGACTAAAAAGCATAAAGTTCATGGTCTTGTTGTACATTTTCACTGCTG
       N  P  *
2052  GAAAGTTGCATGAATGGCGTGACACGTGACGGAAATTCAGCACATGAATTTTCTCTGCTG
2112  TACGCAACAAAATGGAAGAAGCTGAAAACGTTTCTTTTCTGTCAGCTGTTGCAGTAGTGA
2172  TACGCTAGCTCACCGAGAGATTTTAAATGTGCTTTCGATTTACAAACAGAAACAGCTGGT
2232  TACATGCAAAAAAAAAAAAAA
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Fig. 1. Nucleotide and deduced amino acid sequences of common carp GHR. Signal peptide predicted by SignalP is marked by a black line. Conserved cysteine residues in the extracellular domain are marked by pink letters. Potential *N*-glycosylation sites are marked by blue letters. Transmembrane domain is shadowed. Box1 and Box2 regions are double underlined.

2.2 Expression of two transcripts in 2-year and 4-month common carp revealed an alternative splicing

RT-PCR was performed using the primers of Gf and Gr, and the result showed that the amplification product in the liver of 2-year common carp was 224 bp in size, which differs from the transcripts in other tissues where a 321 bp fragment was identified. In all the tissues of 4-month common carp including liver, only the 321 bp fragment was identified (Fig. 2), which implies that a 97 bp sequence was deleted in the transcript of liver. The sequencing result confirmed this implication. However, the expression level in gill, thymus and brain of 2-year common carp was significantly lower when compared with that in 4-month common carp (Figs. 2 and 3).

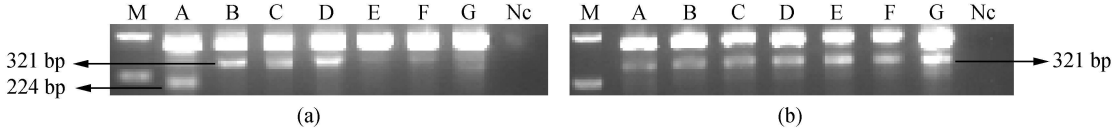

Fig. 2. Expression of two GHR genes in different tissues. (a) Expression of GHR in 2-year common carp. (b) Expression of GHR in 4-month common carp. M, molecular weight marker (DL2000); A, liver; B, spleen; C, kidney; D, headkidney; E, thymus; F, gill; G, brain; Nc, negative control without the template.

4-mon: gtgcgtgagaacataacctatgacgaggcctgcttacagtggagaacatagggtgagttaatagctgtgaatctgctgaatgaagg

2-year: gtgcgtgagaacataacctatgacgaggcctgcttacagtggagaacata----------
4-mon: tttacacagttacagaatgatctgtgttggaggtctgacttcaaatgttttgtgacttccagtgcatcctgacccaccaattgggctga

2-year: ---------- gtgcatcctgacccaccaattgggctga
4-mon: actggactctattaaatgtgagtcgctcggggttgcacttgacgtccttgtgcgctgggctccccctccgtcagcagatgtgcaga

2-year: actggactctattaaatgtgagtcgctcgggggttgcactttgacgtccttgtgcgctgggctccccctccgtcagcagatgtgcaga
4-mon: tgggctggatgagcctggtgtaccaggttcagtaccgggtcagaaacaactcccactg
2-year: tgggctggatgagcctggtgtaccaggttcagtaccgggtcagaaacaactcccactg

Fig. 3. Comparison of the amplified products from the liver of 2-year and 4-month common carp using the primers of Gf and Gr. '-' means deleted nucleotides.

Through the genomic DNA analysis by PCR and sequencing, we obtained the same result as indicated above. From the result, we considered that the alternative splicing of the intron might be correlated with the development of the common carp.

2.3 Homology and phylogenetic analysis

Amino acids sequence of GHR in common carp shared 92.2% identity to GHR of goldfish, 90% to grass carp, 36.4% to human, and 36.2% to rat. Morever, the extracellular domain is more conserved than the intracellular domain (Table 1).

Table 1. Amino acid identities between common carp GHR and GHRs of other species (%)

Species	Whole receptor	Extracellular domain	Intracellular domain
Common carp	100	100	100
Grass carp	90.4	92.3	89.0
Goldfish	92.2	95.9	89.2
Cherry salmon	51.1	59.7	44.1
Gilthead seabream	45.9	49.6	43.5
African clawed frog	35.1	40.2	32.5
Soft-shelled turtle	36.4	41.9	32.6
Domestic pigeon	36.3	41.2	32.3
Human	36.4	40.4	33.2
Norway rat	36.2	38.6	33.0
Sheep	35.8	37.9	33.2
Monkey	34.7	40.4	30.2
Pig	36.9	40.4	32.9
Dog	37.1	42.1	32.1

A phylogenetic tree constructed based on the full length amino acid sequences of common carp and other known GHR showed the relationship of common carp with other species (Fig. 4).

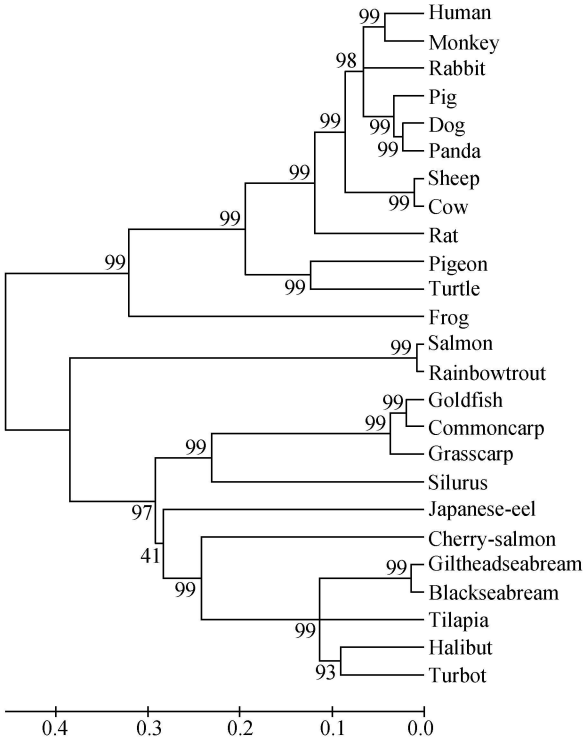

Fig. 4. Phylogenetic tree showing the relationship of common carp with other species analyzed by GHR amino acids comparison. The sequences were aligned by CLUSTAL W program and the phylogenetic tree was constructed by neighbor-joining methods using MEGA version3.0. The species included: human (Human, AAA52555), monkey (Monkey, AAK62288), rabbit (Rabbit, 1401239A), pig (Pig, AAZ15732), dog (Dog, NP_001003123), giant panda (Panda, AAK72050), sheep (Sheep, NP_001009323), cow (Cow, O46600), Norway rat (Rat, NP_058790), domestic pigeon (Pigeon, BAC43750), soft-shelled turtle (Turtle, AAG43525), African clawed frog (Frog, AAF05775), coho salmon (Salmon, AAK95625), rainbow trout (Rainbowtrout, AAW27914), goldfish (Goldfish, AAK60495), common carp (Commoncarp, AY741100), grass carp (Grasscarp, AAP37033), Silurus meridionalis (Silurus, AAP97011), Japanese eel (Japanese-eel, BAD20706), cherry salmon (Cherry-salmon, BAD51998), gilthead seabream (Giltheadseabream, AAM00431), black seabream (Blackseabream, AAN77286), Mozambique tilapia (Tilapia, BAD83668), bastard halibut (Halibut, BAC76398), turbot (Turbot, AAK72952).

3 Discussion

The GHR cDNA cloned from the liver of 2-year common carp was translated into a transmembrane glycoprotein of 602 aa (Fig. 1), including an extracellular ligand-binding domain of 244 amino acids (aa), a single transmembrane domain of 24 aa and an intracellular signal-transduction domain of 334 aa. The characteristic landmark of GHR is the YGEFS motif found within common carp GHR in the position of aa224—228. In common carp, the first aa of this motif is a phenylalanine instead of tryosine as goldfish GHR[10]. So the motif turns to a FGEFS motif accordingly. The result of CLUSTAL showed that in mammalian GHRs the aa in the situation is tryosine without exception. However, in avian, reptilian, amphibian and all the teleost the aa turns to phenylalanine. Though the significance of a phenylanine instead of a tyrosine in the motif is unknown yet, it is considered a conservative change for both aa residues containing an aromatic side chain[8].

In the extracellular domain of common carp GHR, the 6 conserved cysteine residues are believed to play significant roles. These cysteine residues are probably engaged in forming disulfide bonds between C41 and C51, between C83 and C94, and between C108 and C126 according to their homologous positions when compared with human GHR[17]. The aa216 in the extracellular domain is an unpaired cysteine found in all GHRs identified so far. Interestingly, in mammalian, avian and reptilian GHRs the unpaired cysteine occurs after the FGEFS motif in a position proximal to the transmembrane domain. In amphibian and all the teleost GHRs, the unpaired cysteine is located upstream the FGEFS motif in a position about 30 aa away from the transmembrane domain. It appears, therefore, that the FGEFS motif and the occurrence of the unpaired cysteine upstream the FGEFS motif are the characteristic of lower vertebrate GHRs.

There are 6 potential *N*-glycosylation sites in the extracellular domain of commn carp GHR. The first one is located at aa60. A homologous site for this is found in avian, reptilian, amphibian and all the teleost, but not in mammalian. The second one is located at aa97. A homologous site for this is found in mammalian, avian, reptilian and some of the teleost. The third one is located at aa117. A homologous site for this is found in goldfish, grass carp and *Silurus meridionalis* only. The fourth one is located at aa140. A homologous site for this is found in all species except for cherry salmon. The fifth one is located at aa145. A homologous site for this is found in all species GHRs except for Japanese eel. The sixth one is located at aa184. A homologous site for this is found in all species. There are two additional potential *N*-glycosylation sites in common carp GHR compared with mammalian GHR. The physiological significance of these additional potential *N*-glycosylation sites remains to be investigated, particularly in view of the possible involvement of glycosylation in ligand binding[18, 19].

Two highly conserved regions named Box1 and Box2 are found within the intracellular domain. Box1 is a site for JAK2 binding[20]. The PPVPVP sequence conserved in mammalian, avian, reptilian and amphibian is changed to PPIPAP in common carp, and changed to PPVPAP in some other teleost. However, this is a relatively conservative change as valine, isoleucine and alanine all carry non-polar aliphatic side chains. The Box2 region is believed to be involved in the proliferative response of the receptor[21]. In fact, the sequence around the critical phenylalanine residue (WVEFI) is identical in all GHRs except that in Mozambique tilapia (*Oreochronus mossambicus*) GHR. The conserved Box1 and Box2 regions in fish GHRs suggest a post-receptor signaling mechanism in fish akin to mammalian GHRs.

Out of the 8 conserved intracellular tyrosine residues found in most GHRs, seven are found in common carp, namely Y312, Y317, Y371, Y436, Y481, Y549 and Y591. Conservation of these tyrosine residues varies to some extent among species. Y436 and Y591 are found in all GHRs, indicating their essential role in mediating some common biological functions across species. Y312 is found in all species except for guinea pig, rainbow trout and coho salmon. Y317 is found in all species except for human and monkey. Y371 is less conservative and changed into cysteine in avian, reptilian and amphibian. Y481 is found only in all fish species. Y549 is found in all species except for cherry salmon.

The phylogenetic tree (Fig. 4) based on GHR amino acid sequences puts together all fish tested, and displays the same clustering as the present hierarchy of vertebrate species. The evolution of mammalian, avian, reptilian, amphibian and fishes accords with the current evolution law from lower to higher. In

view of the phylogenetic diversity of fish, the elucidation of the GHR sequences of other fish species would provide a molecular means for fish classification and taxonomy.

As can be seen in Table 1, the extracellular domain, which defines the ligand binding site, is more conserved than the intracellular domain which defines the signaling events. The conservation of extracellular domain partly explains why fish GHRs can recognize GHs of other species[10,11].

Because of intron alternatively splicing, sexual dimorphism and so on, GHR has variants. This is because GHR accommodates the diversity of GH. Two different amplified fragments were found in common carp. The sequencing results of the fragments and partial genomic DNA indicated that the two fragments are generated by alternative splicing of the GHR gene in which an intron of 97 bp is either removed or retained during mRNA processing. There is a stop codon in the intron included in the larger one of PCR amplified fragments, making the translation terminate and forming the truncated GHR. The truncated GHR corresponds to the partial sequence of extracellular domain of common GHR (Fig. 3), while the smaller one of PCR amplication fragments, because of the intron spliced, corresponds to the whole GHR. Besides GHR, another protein termed growth hormone binding protein (GHBP), which can form complexes with circulating GH, was also found in serum and cytoplasm of most species. GHBP is either identical, or highly homologous, to the extracellular domain of the GHR. In mouse and rat two different mRNAs encoding GHR and GHBP respectively were found. They both formed through alternative splicing of the primary GHR transcript[22]. It is believed that GHBP can enhance the growth-promoting effects of GH *in vivo*, probably by increasing the half-life of GH in the circulation. Though the truncated GHRs were found in many higher animals[22] and turbot (*Scophthalmus maximus*)[23—25], most of the truncated GHRs including a section of hydrophobic amino acids are membrane-anchored proteins, except the GHBP. So they are still a membrane-anchored protein. A truncated GHR was also found in monkey[22]: the absence of a hydrophobic transmembrane domain suggests that the protein would not function as membrane-bound receptor, while the absence of the amino acids critical for GH binding suggests that it would not function as circulating GHBP. The exact biological role of the truncated protein is unclear. A 0.7 kb mRNA transcript was also found in chicken[22]. Only the first 95 amino acids of the 221 amino acids hormone-binding domain of the receptor are encoded. These 95 amino acids are considered important for GH binding by mutagenesis and crystallography studies[26]. In the 3′UTR of the 0.7 kb transcript, there is a domain of AAUAAA and a GU-rich region. The 0.7 kb transcript should be produced by RNA cleavage between the domain of AAUAAA and a GU-rich region.

Expression analysis revealed that in the tissues of gill, thymus and brain, the expression level of GHR in 2-year common carp was significantly lower than that of 4-month common carp, which corresponds to the intensity of thymus's function in different development stage. Thymus is developing in 4-month common carp, and the immune function of thymus in this stage is increasing. However, thymus is in a degenerated stage when the common carp grows to an age of 2-year. Thymus is one of the most important immune organs. But the expression of GHR in fish thymus has not been reported. The expression experiment provides us a direct proof that GHR does exist in thymus. RT-PCR performed with the primers of Gf and Gr showed that the amplified products from all the tissues of 4-month common carp are in the same size of 321 bp. But the amplified products from 2-year common carp showed different size, a 224 bp product from liver and a 321 bp product from other tissues. Though the exact mechanism of the phenomenon is unclear, we consider that it is associated with development: the membrane-anchored GHR occurs in liver due to the intron alternatively spliced mechanism when common carp develops in some degree, or more possibly the membrane-anchored GHR exists from the very beginning, but in the early stage of development this form of GHR is not dominant so it cannot be detected by the common PCR method. This is the first report about the variety of GHR in freshwater fish. The biological significance and action mechanism of this truncated GHR need further investigation.

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