Fast evolution of growth hormone receptor in primates and ruminants

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Abstract Pituitary growth hormone (GH) evolves very slowly in most of mammals, but the evolutionary rates appear to have increased markedly on two occasions during the evolution of primates and ruminants. To investigate the evolutionary pattern of growth hormone receptor (GHR), we sequenced the extracellular domain of GHR genes from four primate species. Our results suggested that GHR in mammal also shows an episodic evolutionary pattern, which is consistent with that observed in pituitary growth hormone. Further analysis suggested that this pattern of rapid evolution observed in primates and ruminants is likely the result of coevolution between pituitary growth hormone and its receptor.

Keywords: pituitary growth hormone, pituitary growth hormone receptor, coevolution.

The mammalian pituitary growth hormone (GH) stimulates the growth and development of cells. GH is secreted by anterior pituitary and transported to many tissues by blood circulation, where it binds to growth hormone receptor (GHR) on the surface of target cells to carry out its function[1]. GHR belongs to the hematopoietic cytokine receptor family, whose members all share similar structural and functional features. GHR is a single transmembrane receptor and can be divided into three domains the extracellular domain (ECD), the transmembrane domain (TMD) and the intracellular domain (ICD). Human GHRs include 638 amino acids encoded by nine exons (from exon2 to exon10): most of exon2 encodes for signal peptide; the last eight nucleotides of the exon2, exon 3 7, and the first nine nucleotides of exon8 encode the extracellular domain of the mature protein (738 nucleotides altogether); most of exon8, exon9 and exon10 encode the transmembrane domain and intracellular domain (1122 nucleotides altogether)[2,3]. The ECD domain encoded by exon 3 7 is the structural and functional center of GHR interacting with GH[2].

Species specificity[4,5] is one of the characters of GHR.

For example, human GH is able to bind GHRs from other mammals with high affinity, while GHs from other mammals cannot bind human GHR. Although GH and GHR gene sequences vary greatly among different species, the species specificity in primates is mostly due to substitution of one pair of binding sites, which are His/Asp at position 171 of GH and Leu/Arg at position 43 of GHR. Previous study showed that the substitution of position 43 of GHR is prior to that of 171 of GH[6], suggesting the substitution of GH may result from adaptability to the changes of GHR.

In most mammals, GH evolves at a rather low rate of $(0.22\pm0.03)\times10^{-9}$ substitutions/amino acid site/year, which appears to have increased by 25 50 times on two occasions, during the evolution of primates and ruminants[7]. This is the so-called "episodic evolution". Because of the interaction between GH and ECD of GHR, one may expect that ECD of GHR also shows similar episodic evolutionary pattern with GH[4,8].

To test this hypothesis, we amplified and sequenced ECD sequences of GHR from four primate species. Combining them with other mammalian GH and GHR gene sequences available from GenBank, we analyzed the evolutionary pattern of GHR together with its ligand.

1 Materials and methods

1.1 Materials

GHR gene sequences are available in GenBank only from several primate species; they are human (*Homo sapiens*), macaque (*Macaca macaca*), olive baboon (*Papio anubis*) and squirrel monkey (*Saimiri boliviensis*). To get GHR gene from more species, we sequenced ECD of GHR from four primate species the snub-nosed golden monkey (*Rhinopithecus roxellana*), the Douc langur (*Pygathrix nemaeus*), the red howler (*Alouatta Seniculus*) and the white-faced saki (*Pithecia pithecia*). PCR reagents and purification kit were bought from TaKaRa China Biomedical Inc. Sequencing reaction mixtures were BigDye[TM] Terminator Kit V2.0 from PE Inc., and primers were made by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd.

1.2 Primers

We designed eight pairs of primers (Table 1) based on the GHR sequences from human and mouse to amplify exon 3 7 of primate GHR.

1.3 Amplifying and sequencing of the ECD of GHR

PCR amplification was carried out in a 50 μL volume containing 40 mmol dNTPs, 10 pmol primer, 5 μL 10× PCR reaction buffer, 0.25 U *Taq* polymerase, 20 mmol BSA, 20 ng total genomic DNA as template and about

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Table 1   Primers

Names[a]	Sense primers	Anti-sense primers
Exon3w	5′-GTATATCCAACCTGCCTTC-3′	5′-ACATCTCCTCCCAGTCTC-3′
Exon3n	5′-ACAGGGATGACTAATGATTTTC-3′	5′-TCAGTCACTCTTCCAGTTAC-3′
Exon4n	5′-TAGCTCTGGTTTCTTAAACAGG-3′	5′-CATTGATTGCATAAATCACA-3′
Exon5w	5′-TAGGCAGAAGTACCAAACGG-3′	5′-TACAACATGATTTTTGGAAC-3′
Exon5n	5′-TACAACATGATTTTTGGAAC-3′	5′-GCTTCCCCATTTATTTAGTC-3′
Exon6w	5′-AAGTTACTCTCTTATAAAGT-3′	5′-GTTAAACTGGACAGCAAG-3′
Exon6n	5′-ACTAATGCTCTGTTGAAT-3′	5′-GTGTAAGGTGTAGCAACATC-3′
Exon7n	5′-AGTGTTCATTGGCATTGAGT-3′	5′-TGGACAACACACTACCAGTG-3′

a) w represents outside primer far from object, and n represents inside primer nearer to object. e3   7 of white-faced saki, e4 and e7 of Douc langur, e3, e4, e6, e7 of snub-nosed golden monkey and e3, e4, e5, e7 of red howler, can be obtained with only primer n, while the e3, e5, e6 of Douc langur, e5 of Sub-nosed golden monkey and e6 of Red howler can only be obtained by both primer "n" and primer "w" using nested PCR.

36 μL water. Reaction conditions were: 1 cycle of 95   for 3 min and 35 cycles of 94   for 1min, 55   60   for 1 min, 72   for 1 min and 1 cycle of 72   for 10 min. PCR products were purified using PCR purification kit. Sequencing reaction mixtures contained 2 μL of Terminator Ready Reaction mix, 5 μL of template, 1 μL (2 pmol/L) of primer in a total volume of 8 μL. Cycle sequencing was carried out for 25 cycles at 94   for 10 s, 50   for 5 s, 60   for 4 min. Then we collected and sequenced the products.

1.4   Assembling ECD sequence of GHR

The same sequences from two directions were assembled using software SeqMan[9] to get a correct sequence, and then sequences of the same exon were aligned individually using ClustalW[10]. In BioEdit[11], we deleted the intron sequences, and got the sequences from exon3 to exon7. At last, we obtained four integrated sequences of ECD of GHR by orderly assembling all the exons of each species. Because in humans the third exon begins at the 71st nucleotide and the seventh exon ends at the 784th nucleotide, we deleted the first two and the last one nucleotide of the ECD of GHR sequences in order not to change the open reading frame of GHR.

1.5   Amino acid sequences alignment of ECD, TMD and ICD of GHR gene and evolutionary analyses

We downloaded all available amino acid sequences of GHR from eutherian mammals and also GHR of opossum, pigeon and chicken as outgroups. Using ClustalW[10], we aligned them with sequences obtained in our study separately for ECD and TMD/ICD domains.

Wallis used a star phylogeny when calculating the evolutionary rates for GH gene in different mammalian lineages[7], assuming that the main orders of eutherian mammals diverged at a single point, about 75 million years BP[12]. In order to make comparison with his results, we used the same assumption. To obtain the star phylogeny trees, we reedited the trees of ECD and TMD/ICD constructed by using Mega2.1[13] with the amino acid of GHR gene in software TreeView. Amino acid p-distance for each branch of GHR was estimated by codeml in PAML[14]. According to the divergence times and the P-distances we can easily calculate the amino acid substitution rate ($\times 10^{-9}$ substitutions/amino acid site/year) for each branch. Pairwise AA distances correlations between GH and ECD, GH and TMD/ICD, ECD and TMD/ICD of GHR were calculated using Pearson's correlation coefficient[15] implemented by SPSS 10.0.1[16].

1.6   Divergence times

In order to compare evolutionary rates of GHR in mammals with those of GH, we used the same divergence times (Fig. 1) as those used by Wallis[7]. They are: new world monkey and old world monkey, 40 Ma[17]; human
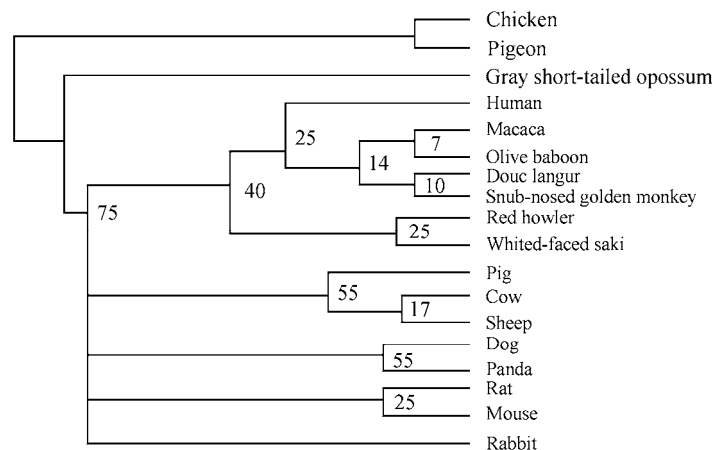

Fig. 1.   Divergence time (Million years BP).

and old world monkey, 25 Ma[17] ; Colobini and Cercopithecini, 14 Ma[17]; macaque and Papio/Olive baboon, 7 Ma[17]; rhinopithecus/snub-nosed golden monkey and Presbytis/Douc langur, 10 Ma[17]; Red howler and white-faced saki, 25 Ma[17]; cow and sheep, 17 Ma[18]; pig and ruminants, 55 Ma[18]; mouse and rat, 25 Ma[19]; dog and panda, 55Ma[20].

2 Results

All GHR amino acid sequences alignment for ECD and TMD/ICD are shown in Figs. 2 and 3.

We calculated the amino acid substitution rates of

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               5      15      25      35      45      55      65      75      85      95 100
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
human   FPWLLIIIFGIFGLTVMLFVFLFSKQQRIKMLILPPVPVPKIKGIDPDLLKEGKLEEVNTILAIHDSYKPEFHSDDSWVEFIELDIDE--PDEKTEESDT
macaca  .............................................N...........A...............................--...N.G...
cow     ...F.......L..A.T.YLLI.........................N..H..YN...........D--......G...
pig     ...F............I..LLI.........................N..H..Y.............D--......G...
rat     ...F.........VA....VI..........................G...N...D.YN...........D--A.....
mouse   ...F.........VA.....VI.........................G...N...D.YN...........ADV.....G...
rabbit  ...F................I.........................Q.......YN...........D--......G...
sheep   ...F.......L...T..LLI.........................N..H..YN...........D--......G...
dog     ...F.........MI..L.I...........................N.......YN...........D--L.....G...
panda   ...F.........MI..L.I...........S.............S.....N.......YN...........D--......G...
olive   .......Q...............Y.......N............-.............K.........A..S-.........-.....
opossum .H.F.......L....V....IL.......L...............K...........S......Q.YN...........D--....A.....
chicken ...F.VVV..VC..A.TAILI.L...P.L...F...............K..D...S...S..N..TQLYN..L..........D--S...NRV...
pigeon  ...F.VV....AC..A.TVILI.L...S.L....F..............K...D...S...S..N..TQLYN..L..........ED--....NRV...

               105     115     125     135     145     155     165     175     185     195 200
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
human   DRLLSSDHEKSHSNLGVKDGDSGRTSCCEPDILETDFNANDIHEGTSEVAQPQRL-KGEADLLCLDQKNQNNSPYHDACPATQQP-SVIQAEKN-KPQPL
macaca  ........Q.............Y...........N...................-..............K.............-.....
cow     .....N.....LNIF.A..D......Y......A..HVS.MCD...........-.....IS...........SN..A..S...-...LV.E.-..R..
pig     ....NN.....LTI..A.ED.......Y.........VCD..A...........-.............SN..A.....-...L..E.-..R..
rat     .....D.Q...AGI..A..D......YD....D...HTS.MCD....F...K.-.A...........LK...-.Y..SLGSLH.-.ITLTMED-.....
mouse   .....N.....AGI..A..D......YD....D...HTS.MCD..LKF..S.K.-NM...........LK.L.-Y..SLGSLH.-.IT.TVEEN..
rabbit  .....NS.Q..L.V.AA..D......Y.......N....S.GCD.N........-..................VS..A...-E.VL..ED-..R..
sheep   .....N.....L.IF.A..D......Y.......HVS.MCD...........-.....I...........SN..A..S...-...LV.E.-..R..
dog     .....N.....LNI..A..D......Y.........S.VCD...........-...V...............ST.TT.T....-.I.L.KE.-..R..
panda   .....N.....LNI..A..D......Y.........S.VCD...........-...I...........S...ST.TA.N....-G..L.KE.-..R..
olive   ........Q.............Y...........N...................-..............K.........A..S-.........-.....
opossum ......ERQ..LNY..A..D.......LS.PASCNS..DI.HS...-P.T...F..EP.-------E.SpS.TP.PNGGLLSEGGQ.R..
chicken .....D..L....C..A..D....A..Y....P....S.S.TCDAI.DID.FKKVTEK.E.....HR.DDVEALQSL.NTD....HTST.S.SR-ESW.P
pigeon  .....E..L....C..A..D....A.......P....S.S.TCDAI.DID.FKKVTEK.E.....GR.DNDE.LPSL.NTD....RMSTRP.NS-Q.W.P

               205     215     225     235     245     255     265     275     285     295 300
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
human   PTEGAESTHQAAHIQLSNPSSLSNIDFYAQVSDITPAGSVVLSPGQKNKAGMSQCDMHPEMVSLCQENFLMDNAYFCEADAKKCIPVAPHIKVESHIQPS
macaca  ..D....................A.....................................D.I...........................E..
cow     LIG.T......V.T.......A...................N.........T.NP...T...V.TP..A..IV.......V....Y.AL..VEA....E..
pig     IIS.TD....T..T.......A...................I.........L.V..P.PA..I.........AM...VE...RLA..
rat     LGSET.....LPSTPM.S.V..A................G.........I...LA.GNTQL.VAAP.....YS.NS.....S......AA...MEATTCVK..
mouse   LSSET.A...L.STPM...T..A................G.........I...IA.GNTQR.VATP.....YS.NS.....S......A...RMEAT.C.K..
rabbit  L.GEI...L...PS.....N..A...................N...A..V.....T..I.........A...VD...RVE..
sheep   .IG.T......V.T.......A...................N.........T.NP...T...V.TP5.AD.IV.S.....V....Y.AL..DVEA....E..
dog     LIS.T...Q....T.......A.............L...........I.P...P..VA...A..I................T....VEA..RVE..
panda   LIS.T.....P.......A...................IAP...P..V....A..I................T....VEA..RGE..
olive   ..D....................A...................L........D.I...........................E..
opossum LPN.M..SSL.V.AH...Q...A......................S...I...G.R..A.P.RG..IL..T.........VS....AEP.PRTE.R
chicken FADSTD.ANPSVQT....QN..T.T..................S.V.RA..ESCT------EQ..T.........V....A.ISQEED.PRV.EQ
pigeon  FADSIDAASPS..N....QN..R.T..................S.VARAR.EFCT------EQ..TL.........V....A.IS.EED.PRV.AQ

               305     315     325     335     345     355     365     375 381
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
human   LNQEDIYITTESLTTAAGRPGT-GEHVPGSEMPVPDYTSIHIVQSPQGLILNATALPLPDKE-FLSSCGYVSTDQLNKIMP
macaca  F..............T.......-T..I...........................G...-................
cow     F..............T...S..-A....S..I.....................V.........-................
pig     F..............T...SA.-A.CA.S.....................V...........-................
rat     F..............T.RMSE.-ADTA.DA.-.......TV.T.K..R..............KK................Q
mouse   F..............T.QMSE.-ADIA.DA..S......TV.T...R...............KN.P.............Q
rabbit  F..............T.E.S..-A.DA...........L.....V...AT...........-...............L.
sheep   F..............T...S..-A.N..S..I.....................V.........-................
dog     F..............T..QS..-T.RAVS.............I...R..V............-................
panda   F..............V..Q..-A.RA.S..I...........R..V...........-................
olive   F..............T.......-T..I...........................G...-................
opossum FSP..V.........T..SS.I-AGGPS.A...................V.....V...-..T.............Q
chicken SCN..T.F.......TGINL.ASMAET.SM...........H.....V.E..-.NM...............
pigeon  ICN..T.F.......TGISL.ASTAET.SP.V..........H.....V.....V....-.NM...............
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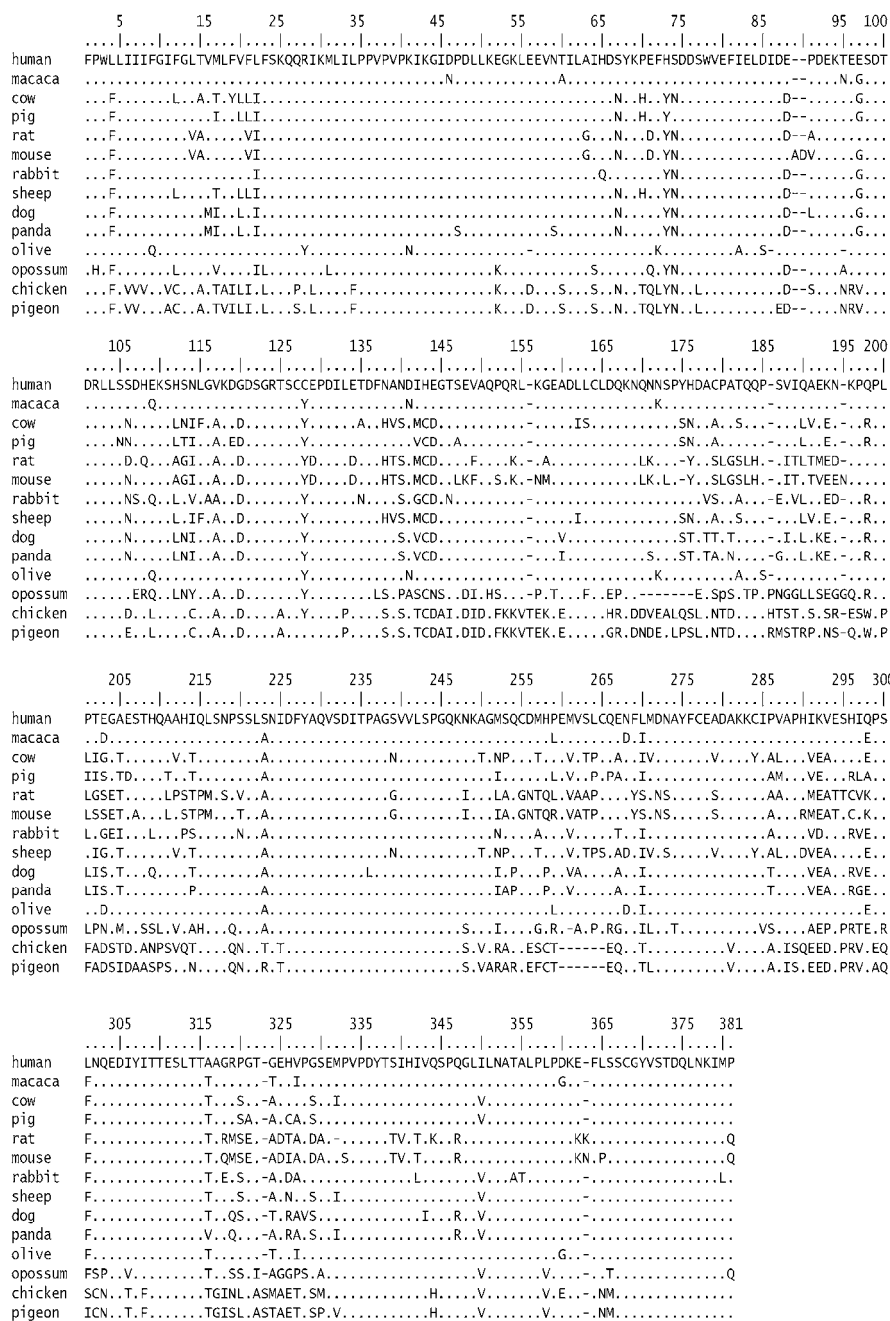
Fig. 2. Alignment of amino acid sequences of EMD of GHR genes in mammals and birds. Note: The GenBank accession numbers of GHR are: human GHR, NM_000163; macaque GHR, AF209081; cow GHR, NM_176608; pig GHR, AF238492; rat GHR, NM_017094; mouse GHR, NM_008117; rabbit GHR, AF015252; sheep GHR, M82912; dog GHR, NM_001003123; panda GHR, AF395535; Olive (Olive baboon) GHR, AF150751; opossum (Gray short-tailed opossum) GHR, AF238491; chicken GHR, NM_001001293; pigeon GHR, U20353. The GenBank accession numbers of sequence data obtained by this study are: Douc (Douc langur) GHR e3 e7: AY958705, AY958709, AY958713, AY958717, AY958721; Snub (Snub-nosed golden monkey) GHR e3 e7: AY958706, AY958710, AY958714, AY958718, AY958722; Howler (Red howler) GHR e3 e7: AY958707, AY958711, AY958715, AY958719, AY958723; Saki (White-faced saki) GHR e3 e7: AY958708, AY958712, AY958716, AY958720, AY958724.
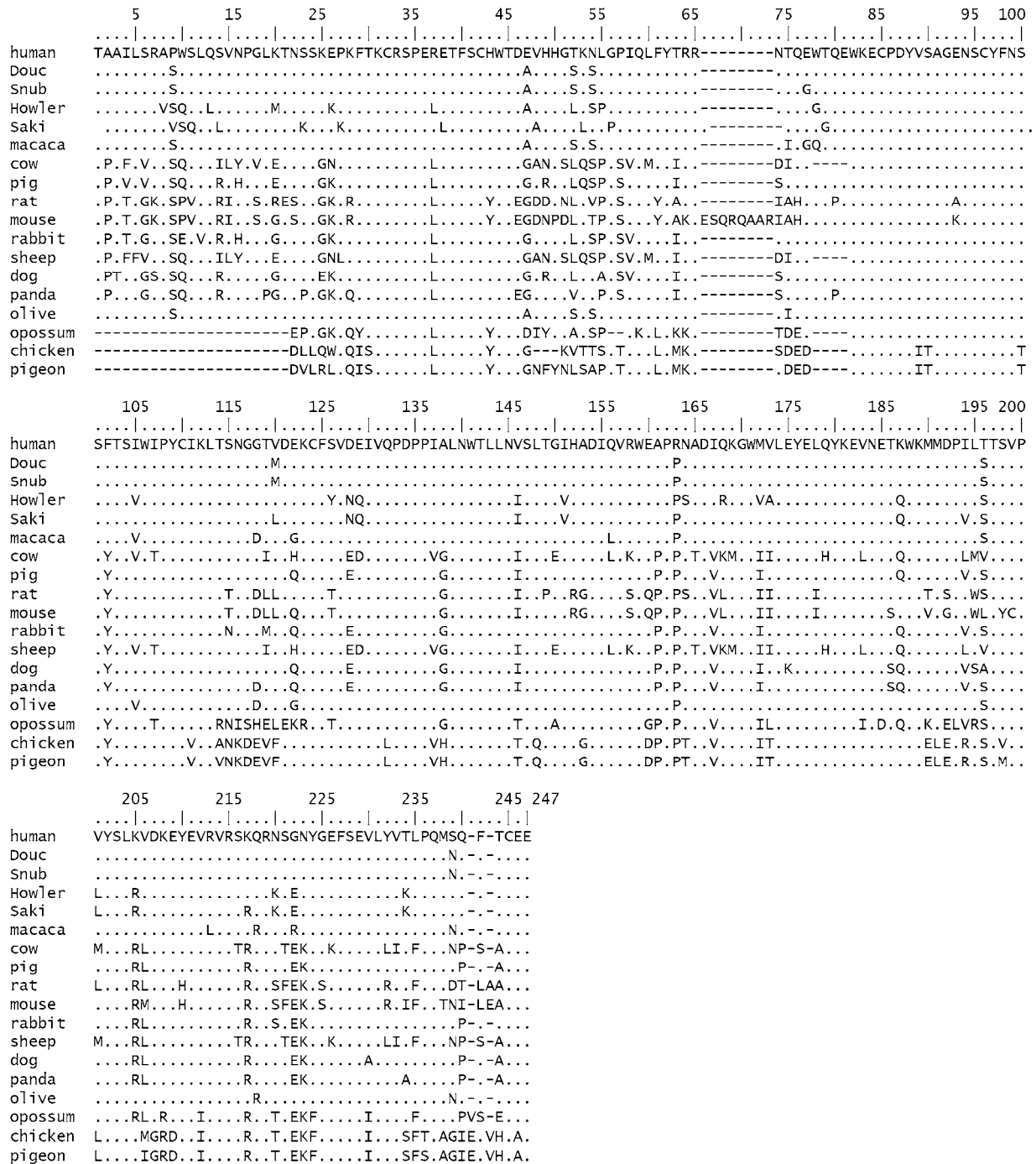
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             5       15      25      35      45      55      65      75      85      95 100
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
human    TAAILSRAPWSLQSVNPGLKTNSSKEPKFTKCRSPERETFSCHWTDEVHHGTKNLGPIQLFYTRR--------NTQEWTQEWKECPDYVSAGENSCYFNS
Douc     ........S.........................................A....S.S...........-------.......................
Snub     ........S.........................................A....S.S..........--------...G....................
Howler   .......VSQ..L.......M.....K...........L..........A....L.SP.........--------....G....................
Saki     .......VSQ..L........K...K...........L........A....L..P..........--------....G......................
macaca   ........S.........................................A....S.S...........--------.I.GQ..................
cow      .P.F.V..SQ...ILY.V.E....GN...........L...........GAN.SLQSP.SV.M..I..--------DI..----...............
pig      .P.V.V..SQ...R.H...E....GK...........L...........G.R..LQSP.S....I..--------S.......................
rat      .P.T.GK.SPV..RI..S.RES..GK.R.........L.....Y..EGDD.NL.VP.S...Y.A..--------IAH...P............A......
mouse    .P.T.GK.SPV..RI..S.G.S..GK.R.........L.....Y..EGDNPDL.TP.S...Y.AK.ESQRQAARIAH.................K......
rabbit   .P.T.G..SE.V.R.H...G....GK...........L...........G....L.SP.SV....I..--------.......................
sheep    .P.FFV..SQ...ILY...E....GNL..........L...........GAN.SLQSP.SV.M..I..--------DI..----...............
dog      .PT..GS.SQ...R....G....EK............L...........G.R..L..A.SV....I..--------S......................
panda    .P...G..SQ...R....PG..P.GK.Q.........L...........EG....V..P.S....I..--------S.....P.................
olive    ........S.........................................A....S.S...........--------.I...................
opossum  ------------------EP.GK.QY.......L.....Y...DIY..A.SP--.K.L.KK..--------TDE.----...................
chicken  --------------------DLLQW.QIS......L.....Y...G---KVTTS.T...L.MK.--------SDED----.......IT.........T
pigeon   --------------------DVLRL.QIS......L.....Y...GNFYNLSAP.T...L.MK.--------.DED----.......IT.........T

             105     115     125     135     145     155     165     175     185     195 200
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
human    SFTSIWIPYCIKLTSNGGTVDEKCFSVDEIVQPDPPIALNWTLLNVSLTGIHADIQVRWEAPRNADIQKGWMVLEYELQYKEVNETKWKMMDPILTTSVP
Douc     ..................M..................................P............................................S....
Snub     ..................M..................................P............................................S....
Howler   ....V..................Y.NQ................I....V.........PS...R...VA.............Q........S....
Saki     ..................L.......NQ................I....V.........P...................Q......V.S....
macaca   ....V...........D...G..................L....P.....................................S....
cow      .Y..V.T..........I..H....ED.......VG.......I...E.....L.K..P.P.T.VKM..II.....H...L...Q......LMV....
pig      .Y.................Q....E..........G.......I.........P.P..V...I.............Q......V.S....
rat      .Y...........T..DLL.....T..........G.......I..P..RG....S.QP.PS..VL...II....I...........T.S..WS....
mouse    .Y...........T..DLL.Q...T..........G.......I.....RG....S.QP.P...VL...II....I.......S...V.G..WL.YC.
rabbit   .Y..........N...M..Q....E..........G.......I.........P.P..V...I.............Q......V.S....
sheep    .Y..V.T..........I..H....ED.......VG.......I...E.....L.K..P.P.T.VKM..II.....H...L...Q......L.V....
dog      .Y.................Q....E..........G.......I.........P.P..V...I..K...........SQ......VSA....
panda    .Y...............D...Q....E..........G.......I.........P.P..V...I.............SQ......V.S....
olive    ....V...........D...G..................................P..................................S....
opossum  .Y....T......RNISHELEKR..T..........G.......T...A........GP.P...V...IL.........I.D.Q..K.ELVRS....
chicken  .Y........V..ANKDEVF...........L....VH.......T.Q....G......DP.PT..V....IT.................ELE.R.S.V..
pigeon   .Y........V..VNKDEVF...........L....VH.......T.Q....G......DP.PT..V....IT.................ELE.R.S.M..

             205     215     225     235     245 247
         ....|....|....|....|....|....|....|....|....|.|
human    VYSLKVDKEYEVRVRSKQRNSGNYGEFSEVLYVTLPQMSQ-F-TCEE
Douc     ......................................N.-.-.-...
Snub     ......................................N.-.-.-...
Howler   L...R..............K.E...........K......-.-...
Saki     L...R..........R..K.E...........K......-.-...
macaca   ...........L...R...R.................N.-.-.-...
cow      M...RL.........TR...TEK..K.....LI.F...NP-S-A...
pig      ....RL..........R....EK.............P-.-A...
rat      L...RL...H......R..SFEK.S......R..F...DT-LAA...
mouse    ....RM...H......R..SFEK.S......R.IF..TNI-LEA...
rabbit   ....RL..........R..S.EK.............P-.-...
sheep    M...RL.........TR...TEK..K.....LI.F...NP-S-A...
dog      ....RL..........R....EK......A.........P-.-A...
panda    ....RL..........R....EK..........A.....P-.-A...
olive    ...................R.................N.-.-.-...
opossum  ...RL.R...I....R..T.EKF.....I....F....PVS-E...
chicken  L....MGRD..I....R..T.EKF.....I...SFT.AGIE.VH.A.
pigeon   L....IGRD..I....R..T.EKF.....I...SFS.AGIE.VH.A.
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Fig. 3.   Alignment of amino acid sequences of ICD/TMD of GHR genes in mammals and birds. Note: The same as in Fig. 2.

mammalian GHR separately for ECD and TMD/ICD domain (Figs.4 and 5). We observed that in most of mammals, GHR evolved at rather low rates (from 0 to 1×10^{-9} substitutions/amino acid site/year), but during the evolution of primates (the common ancestral branch of old world monkeys and new world monkeys), ruminants(the common ancestral branch of cow and sheep) and rodents(the common ancestral branch of rat and mouse), the rates showed substantial increase, which are 2   5 times of those of other lineages. This result is consistent with that observed in GH, indicating that the episodic evolutionary pattern observed in ECD of the GHR in mammals proba-

bly results from coevolution of GHR with its ligand, although the episodic evolutionary rate of GHR is much lower than that of GH (about 25 50 times of those of other lineages)[7].

From Figs. 4 and 5, we found that in most mammals the rates of ECD of GHR are close to those of TMD/ICD of GHR (for the ECD region, the rates range from 0 to 1×10^{-9} substitutions/amino acid site/year; for the TMD/ICD region, the rates range from 0.5×10^{-9} to 1.0×10^{-9} substitutions/amino acid site/year). The branches C/c, D/d and E/e also evolve at high rate, and there are no difference in rate between ECD and TMD/ICD of GHR (For the ECD region, the rates were 4.16×10^{-9}, 1.36×10^{-9} and 2.60×10^{-9} substitutions/amino acid site/year respectively; for the TMD/ICD region, the rates were 4.12×10^{-9}, 1.58×10^{-9} and 1.89×10^{-9} substitutions/amino acid site/year respectively). But on the branches A/a and B/b, rates of ECD were 2 3 times higher than that of TMD/ICD. This result showed that the ECD, which was considered more conservative[21], evolved at much higher rate than TMD/ICD, indicating that the correlation of GHR and GH was specific to the ligand-binding domain. This is further in support of the viewpoint of coevolution of GH and GHR.

Pairwise amino acid distance correlations between GH and GHR were calculated using Pearson's correlation coefficient value r. We calculated the r values for GH vs. ECD, GH vs. TMD/ICD and ECD vs. TMD/ICD. The results are shown in Table 2. The corresponding correlation coefficient r values are 0.498 ($p<0.01$), 0.369 ($p<0.01$) and 0.886 ($p<0.01$) for GH vs. ECD, GH vs. TMD/ICD and ECD vs. TMD/ICD, respectively. Although the r values between GH and ECD and between GH and TMD/ICD were not very high, controls whose distance of one

gene is randomly assigned to be not corresponding to its comparing partner were all lower than 0.147 ($p<0.01$), indicating highly correlated coevolution between GH and the ECD of GHR[22].

3 Discussion

GH has to bind to GHR to fulfill its function. Accordingly, genes encoding GH and the binding domain of GHR should be subjected to a mutual selection pressure to maintain or enhance the affinity to each other. If this were the case, we would observe that the evolution pattern of GHR, especially the binding domain of GHR was consistent with that of GH. Our results certainly confirmed this conjecture. At the same time, the results of Pearson's correlation analysis further proved the coevolution of GH and GHR. All of these made us believe that the rapid evolution of GHR in primates and ruminants probably resulted from coevolution of GHR and GH.

On branches F/f and G/g of GHR in primates (Figs. 4 and 5), although the rates of GHR of these two branches also increased (2 3 times that of TMD/ICD), we cannot figure out its coevolution with GH because the corresponding GH sequences are not available. As for branches H, I, J and K (Fig. 4), not only the corresponding GH sequences but also the sequence of TMD/ICD of GHR are unavailable, we thus cannot test the coevolution between the receptors and their ligands.

Generally two possible explanations could account for the rapid evolution seen in primates and ruminants: one is positive selection, and the other is relaxation of "purifying selection"[23,24]. From Figs. 4 and 5, we noted that for branches leading to primates and ruminants the evolutionary rates increased at a short timescale, but then slowed down again. Relaxation of "purifying selection" seems
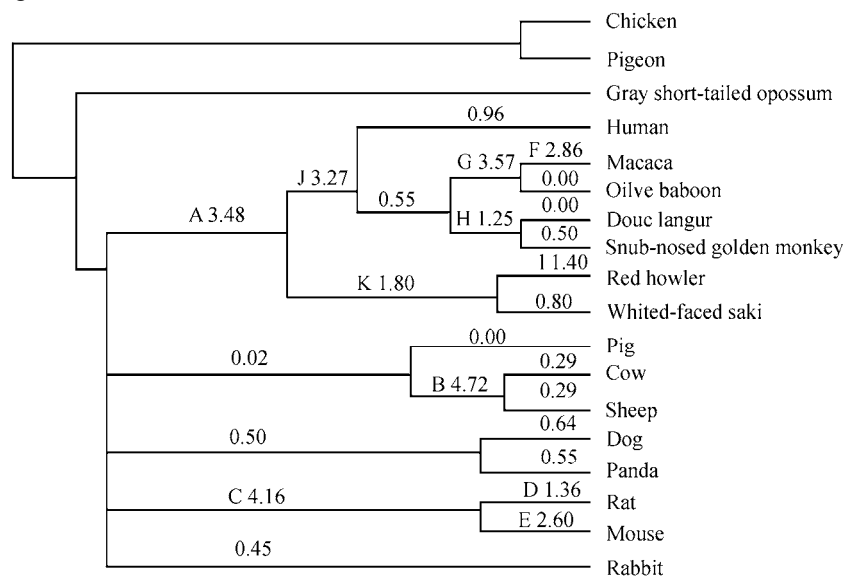
Fig. 4.   Amino acid substitution rates for ECD of GHR in mammals and birds ($\times10^{-9}$ substitutions / amino acid site / year).
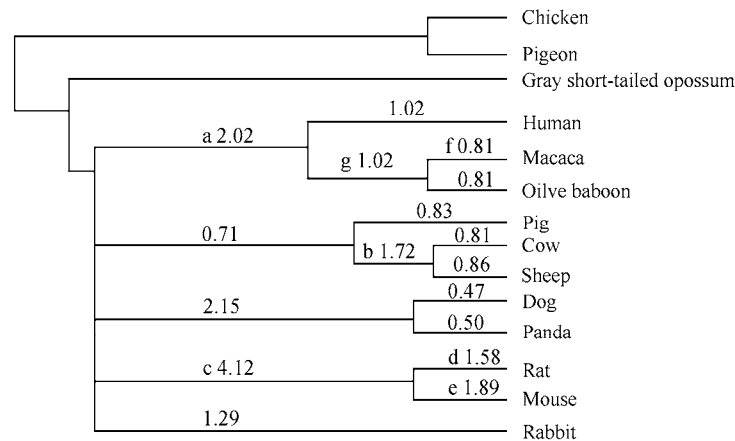
Fig. 5.   Amino acid substitution rates of ICD/TMD of GHR in mammals and birds ($\times 10^{-9}$ substitutions / amino acid site / year).

Table 2   Pearson's correlation coefficient[a]

Pairs and controls	r	p
GH and ECD of GHR	0.498	0.000
Control	0.001	0.994
GH and TMD/ICD of GHR	0.369	0.006
Control	0.121	0.380
ECD and TMD/ICD of GHR	0.886	0.000
Control	0.147	0.284

a) *r* represents values of Person's correlation coefficient. *p* represents the significance of this value; controls are chosen at random for each of the comparison pairs and it is believed that there are no interactions between receptors and ligands. Because the GH sequences from Olive baboon, gray short-tailed opossum and pigeon are unavailable, Person's correlation coefficient values were only calculated with the sequences except for these three. GenBank accession numbers of GH used in the calculation are: human GH, BC075012; macaque GH, L16556; cow GH, V00111; pig GH, M22761; rat GH, U62779; mouse GH, X02891; rabbit GH, Z38127; sheep GH, S50877; dog GH, AF069071; panda GH, AF540936; chicken GH, M35609. GenBank accession numbers of corresponding GHR are shown in Fig. 2.

cannot explain this phenomenon[7], while, if the fast evolution is due to positive selection, in view of the interaction of GH and GHR and the action of the internal or external environment, the evolutionary rate of GHR would be expected to slow down after a period of rapid evolution. So positive selection seems more reasonable and should be the main cause of the rapid evolution seen in GHR gene of primates and ruminants.

According to our results, in primates the rapid evolution in GHR occurred after the primates split from other mammals but before new world monkeys split from old world monkeys. The rapid evolution of GH is dated to the period after the divergence of new world monkeys from prosimians, but before the separation of old world monkeys and new world monkeys[25]. Without GHR sequences from prosimians, we could not detect whether the period of rapid evolution of GHR also happened after the divergence of new world monkeys from prosimians. Meanwhile, the amino acid substitution rate for episode of the

fast evolution in primate is probably underestimated because the fast evolutionary time is overestimated.

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