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Molecular evolution of growth hormone gene family in old world monkeys and hominoids

Chun Ye[a,1], Ying Li[a,b,c,1], Peng Shi[a], Ya-ping Zhang[a,b,*]

[a]*Laboratory of Cellular and Molecular Evolution, and Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China*
[b]*Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming 650091, China*
[c]*The Graduate School, Chinese Academy of Sciences, Beijing 100039, China*

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Abstract

Growth hormone is a classic molecule in the study of the molecular clock hypothesis as it exhibits a relatively constant rate of evolution in most mammalian orders except primates and artiodactyls, where dramatically enhanced rate of evolution (25–50-fold) has been reported. The rapid evolution of primate growth hormone occurred after the divergence of tarsiers and simians, but before the separation of old world monkeys (OWM) from new world monkeys (NWM). Interestingly, this event of rapid sequence evolution coincided with multiple duplications of the growth hormone gene, suggesting gene duplication as a possible cause of the accelerated sequence evolution. Here we determined 21 different GH-like sequences from four species of OWM and hominoids. Combining with published sequences from OWM and hominoids, our analysis demonstrates that multiple gene duplications and several gene conversion events both occurred in the evolutionary history of this gene family in OWM/hominoids. The episode of recent duplications of CSH-like genes in gibbon is accompanied with rapid sequence evolution likely resulting from relaxation of purifying selection. GHN genes in both hominoids and OWM are under strong purifying selection. In contrast, CSH genes in both lineages are probably not. GHV genes in OWM and hominoids evolved at different evolutionary rates and underwent different selective constraints. Our results disclosed the complex history of the primate growth hormone gene family and raised intriguing questions on the consequences of these evolutionary events.

Keywords: Growth hormone; Primates; Gene duplication; Positive selection

1. Introduction

The mammalian pituitary growth hormone (GH) stimulates the growth and metabolism of muscle, bone, and cartilage cells and plays an important role in development. Most mammals only possess one GH gene in their genomes, while humans have five GH-related genes all located in chromosome 17q22–24 (Harper et al., 1982). These genes are the pituitary growth hormone gene (hGHN) and four placentally expressed genes, growth hormone variant (hGHV), chorionic somatomammotropin hormone A and B (hCSHA and hCSHB), and an hCSH-like gene (hCSHL). These five genes collectively make up the human growth hormone (hGH)/chorionic somatomammotropin hormone (hCSH) gene family. Functional members of the hGH/hCSH family have 217 amino acid residues (including signal peptide sequences) encoded by 5 exons, and these sequence

Abbreviations: GH, growth hormone; CSH, chronic somatommamotropin hormone; NWM, new world monkey; OWM, old world monkey; Mya, million years ago.

* Corresponding author. Laboratory of Cellular and Molecular Evolution, and Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China. Tel.: +86 871 5190761; fax: +86 871 5195430.
E-mail addresses: zhangyp1@263.net.cn, zhangyp@public.km.yn.cn (Y. Zhang).

[1] Those authors contributed equally to this work.

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similarity among the members displays a greater than 90% at DNA level (Chen et al., 1989). Members of the hGH/hCSH family work together to maintain the nutritional balance between the mother and fetus during pregnancy. As a research model of the endocrinology of human pregnancy, the rhesus macaque GH/CSH gene family has also been characterized, which contains five genes, mGHN, mGHV, mCSH1, mCSH2, and mCSH3, but how these genes are organized at the genomic level is not clear yet (Golos et al., 1993). Furthermore, the marmoset, a new world monkey, has been identified to have eight GH-like genes (Wallis et al., 2001; Wallis and Wallis, 2002). While in the bushbaby and slow loris (prosimians), a single copied GH gene was observed (Adkins et al., 2001; Wallis et al., 2001), thus the multiple gene duplications giving rise to a cluster of GH-like genes in higher primate start before the divergence of NWM from OWM/hominoids lineage and after the split of prosimians and simians.

GH sequence is quite conserved in most mammals with only a few amino acid substitutions. In primates, however, the human and rhesus macaque GHs differ from the inferred GH sequence of the common ancestor of eutherian mammals by about 60 amino acids, demonstrating a burst of rapid substitutions during primate evolution (Wallis, 1994). Recent studies further defined the date of the rapid evolution to a time after the divergence of simians and the tarsier but before the separation of new world monkeys (NWM) from old world monkeys (OWM) and hominoids (Adkins et al., 2001; Liu et al., 2001; Wallis et al., 2001). The temporal proximity of gene duplication events and rapid sequence changes has led to the hypothesis that the rapid evolution was due to relaxation of functional constraints or positive selection after gene duplication (Liu et al., 2001). Wallis (1996), however, believed that the rapid evolution predated gene duplication because the majority of primate-specific substitutions are shared among all the loci of higher primates. On the contrary, Liu et al. (2001) argued that the sequence similarity in duplicated genes could be the result of gene conversion.

To study the complex history of the GH/CSH gene family in OWM/hominoids in detail, we sequenced GH/CSH gene family from four species in Catarrhini. Our phylogenetic analysis suggested that the evolutionary mechanism of GH/CSH gene family in OWM/hominoids is consistent with the so-called birth-and-death process. Further more, our results show that different members of this gene family evolved differently, even homologous genes in different lineages evolved at remarkable different rates and underwent different selective constraints.

2. Materials and methods

Genomic DNA was prepared from four species of primates, including three OWMs, the Sub-nosed Golden Monkey (*Rhinopithecus roxellana*), the Douc Langur (*Pygathrix nemaeus*), and the Assamese Macaque (*Macaca assamensis*) and one ape, the White-cheeked Gibbon (*Hylobates leucogenys*). Members of the GH/CSH gene family in these four species were amplified using the following primers designed by Wallis et al. (2001): GH sense: 5′-TGGCTATCCTGACATCCTTTCCCGC-3′ and GH antisense: 5′-CCACCCCATAATATTAGAGAAGGACAC-3′. The pair of primers spans the full-length GH gene (including 5 exons and 4 introns) and some regulatory elements. In order to reduce the errors due to PCR recombination and mutation, we performed three times of independent PCR with one time using *pfu* polymerase (Takara, China) and the other two using taq polymerase (Takara, China). The PCR products obtained by using *taq* polymerase were cloned into PMD18-T vector (Takara, China) and transformed into an ultracompetent *E. coli* cell (Takara China). Those performed by using *pfu* polymerase were cloned into PCR-Script Amp Cloning Kit, according to the manufacturer's protocol (Stratagene, La Jolla, CA.), and transformed into the ultracompetent *E. coli* cell (Takara). Plasmids carrying PCR fragment were extracted and sequenced in both directions. For each PCR product, 15–40 clones were sequenced using ABI 3700 DNA sequencer (PE Biosystems, USA). Those sequences identified by at least two times of independent PCR were taken into consideration. Moreover, any single nucleotide variant that occurred uniquely in single clone was assumed to be PCR error and changed to match the consensus nucleotide found in other clones at that site. Thus, most nucleotide variants and recombination due to PCR error were removed from consideration.

Sequences were aligned using CLUSTAL X program (Jeanmougin et al., 1998) followed by manual adjustments. Other OWM and hominoid GH/CSH gene sequences retrieved from GenBank were also used in the analysis (Table 1). Kimura's (K80) (Kimura, 1980) model was used to infer neighbor-joining (NJ) (Saitou and Nei, 1987) tree implemented in the program MEGA 2.1 (Kumar et al., 2001). Then 1000 replications of bootstrap analyses were done. Statistical tests for gene conversion were performed using the program GENECONV 1.81 (Sawyer, 2000) implementing Sawyer's (1989) methods, with the full-length sequences as input data. Analyses were repeated where mismatches were either not allowed, or allowed but given a relative penalty of 1, 2, or 3. Ancestor sequences of all interior nodes of the GH gene tree were inferred by the distance-based Bayesian methods (Zhang and Nei, 1997). Numbers of synonymous substitutions (s), numbers of nonsynonymous substitutions (n), potential numbers of synonymous sites (S), potential numbers of nonsynonymous sites (N), pairwise nonsynonymous substitutions per nonsynonymous site (d_N), and synonymous substitutions per synonymous site (d_S) are all calculated by modified N-G method (Zhang et al., 1998) implemented in MEGA 2.1(Kumar et al., 2001).