Comparative Analysis of Follicle Stimulating Hormone Beta-Subunit Gene in Mammals

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Abstract

Follicle stimulating hormone (FSH) is a glycoprotein hormone expressed by gonadotropes in the pituitary gland that regulated the development and function of gonads and is necessary for normal propagation functions in mammals. In this study, FSHβ revealed a high degree of conservation by contrasted with homologous fragment from serviceable mammals. These results suggested an insight into the characteristics of FSHβ. Using the software of RepeatMasker and Alignment, the content and distribution of SINE/tRNA-Glu and LINE/L1 in pig FSHβ subunit were detected. These insertion elements did not emerged in other artiodactylous FSHβ. It is probable positive selection during the evolutionary process of pig in artificial selection.

Keywords: mammals, evolution, FSHβ, analysis

1. Introduction

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), chorionic gonadotropin (CG) and thyroid-stimulating hormone belong to the family of glycoprotein hormones, and the chorionic gonadotropin (CG) has been found only in the placenta of equine species and primates. These hormones are heterodimers consisting of non-covalently linked common α and hormone-specific β subunits. FSH is responsible for development and survival of follicular somatic cells, and inducting of ovarian estrogen in women. In males, FSH regulates spermatogenesis by binding to the Sertoli cells (McGee & Hsueh, 2001; Plant & Marshall, 2001). Because of its necessary for unique mammalian reproduction function, FSHβ has possess evolutionarily conserved (Wallis, 2001). The FSHβ gene is characterized by excess of multiformity with allele frequencies and low genetic variation (Grigorova et al., 2007). Rearrangement sequence study identified a density of polymorphisms of 3 SNPs/1 kb in three human populations (European Estonians, Chinese Han and African Mandenkalu). Majority of these were common polymorphisms located in non-coding regions and were shared by three human populations. Although many FSH models were exist to deliberate the effects of gonadotropins on testicular functionand and testosterone as well as its role in spermatogenesis (Zheng et al., 1998). Sun et al. (2006) showed that FSH has a direct role in causing hypogonadal bone loss. Little attention has been paid to the interspecific evolution of FSH. In this study, we focused on nucleotide variation in order to demonstrate molecular evolution of the analyzed species and to provide useful data for studying the relationship of nucleotide variation in interspecific FSHβ gene.

2. Materials and Methods

In the present research, mRNA sequences of FSHβ in 20 species and DNA fragments in 15 sequences were downloaded from GenBank. All the sequences were aligned before the phyletic evolution tree was conducted. To avoid redundant sequences, the aligned nucleotide sequences were classified into different haplotypes using DNASP 4.0. The polymorphism site, the number of total mutations (M), the synonymous nucleotide diversity [p(s)], number of haplotypes (h), nonsynonymous nucleotide diversity [p(a)], the average number of nucleotide differences (Tajima, 1983) were calculated by DNASP 4.0. The Molecular Evolutionary Genetic Analysis (MEGA version 4.0) package (Tamura et al., 2007) was used to complete deletion of gaps. The Neighbor-Joining (NJ) method through bootstrap analysis (1000 pseudoreplicates) was used to construct two phylogenetic trees using the computer program MEGA. Repeats sequences were analyzed in repeat masker
3. Results and Discussion

Through analysis it was found that the full coded sequences of FSHβ ranges from 1827 (Ovis aries) to 1984 bp (Ornithorhynchus anatinus). Whereas, FSHβ identity varied from 100% for Macaca fascicularis-Macaca mulatta comparisons to 70% homology between Cervus nippon and Ornithorhynchus anatinus at the nucleotide level; this represents 100 and 65% similarity at the amino acid level, respectively. Primates had the lowest diversity compared to the average variation among all of the mammals. And the average nucleotide differences and nucleotide diversity are 0.172 and 0.043, respectively. Hence, the exogenous hormone of related species hormone or homogeneous hormones should be more conducive to captive breeding success.

![Figure 1. Analyses of amino acid sequence of FSHβ in mammals](http://repeatmasker.org/)

There was a highly conserved among the species in the deduced amino acid sequence. Through these multiple sequence alignments, some common features of FSHβ in the amino acid sequences were demonstrate. Firstly, within the mature protein region, the same positions of 12 half Cys residues of FSHβ were the same in the mammals. Six pairs of disulfide bonds of Cys residues are essential not only for the function of heterodimer FSH molecule but also for the correct folding of β subunit. Secondly, two putative N-linked glycosylation sites presented at the 25th and 42th amino acid residue in the all FSHβ subunit (Figure 1); one is located between the first and second Cys residues, while the other is located between the third and fourth Cys residue. Glycosylation of FSHβ is essential for full bioactivity and protein biosynthesis, especially in protein secretion and folding, safeguard from enzymolysis in recurrence, and the signal conduction pass by receptors-ligands binding (Ulloa-Aguirre et al., 1999). These substantial similarities suggest a common ancestral origin. It was earlier proposed that all members of the glycoprotein gene family (common-α, LHβ, FSHβ and TSHβ) evolved from a
single ancestor through gene duplications. The first duplication produced a-subunit and b-subunit and was followed by a second duplication of the ancestral β-subunit to yield the LH-β subunit gene and the ancestor of the TSHβ and FSHβ subunits which eventually gave rise to the genes for these two latter β-subunits (Li & Ford, 1998).

Figure 2. The NJ-tree of mammals based on FSHβ gene. The node number are Bootstrap values. A was inferred from DNA data and B from mRNA data

The constructed system evolution tree of FSHβ of families and species was basically conform to taxology of NCBI except for Sus scrofa, which was clade root of ungulate rather than clustered artiodactyla (Figure 2B). It provided that there was a difference evolution speed of FSHβ molecule among evolution of amniotic vertebrates (Wallis, 2001).

Using RepeatMasker and sequence alignments of FSHβ gene identified that porcine FSHβ subunit exhibits a remarkable different with those of other mammals (Figure 3). The sequence alignments of FSHβ gene among mammals indicated two microsatellite sites obviously in artiodactyla. However pig FSHβ contain (CT)n repeats, (AT)n repeats is absent. Whether this additional microsatellite sequence present in artiodactyls FSHβ is related to litter size and other reproductive traits requires further investigations.

Table 1. The analysis results of repeatmasker (http://repeatmasker.org/) of domestic pig (GenBank: D00621)

<table>
<thead>
<tr>
<th>position in query (left)</th>
<th>repeat</th>
<th>class/family</th>
<th>position in repeat (left)</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>5997 6256 (3916)</td>
<td>Pre0 SS</td>
<td>SINE/tRNA-Glu</td>
<td>1 260 (0)</td>
<td>14</td>
</tr>
<tr>
<td>7054 7092 (3080)</td>
<td>AT rich</td>
<td>Low_complexity</td>
<td>1 39 (0)</td>
<td>15</td>
</tr>
<tr>
<td>7368 7439 (2733)</td>
<td>MIR3</td>
<td>SINE/MIR</td>
<td>(55) 153 82</td>
<td>16</td>
</tr>
<tr>
<td>8055 8075 (2097)</td>
<td>AT rich</td>
<td>Low_complexity</td>
<td>1 21 (0)</td>
<td>17</td>
</tr>
<tr>
<td>8817 8990 (1182)</td>
<td>L1-2 SS</td>
<td>LINE/L1</td>
<td>(1) 6015 5843</td>
<td>18</td>
</tr>
<tr>
<td>9117 9223 (949)</td>
<td>L2b</td>
<td>LINE/L2</td>
<td>3246 3405 (21)</td>
<td>19</td>
</tr>
<tr>
<td>9885 9917 (255)</td>
<td>AT rich</td>
<td>Low_complexity</td>
<td>1 33 (0)</td>
<td>20</td>
</tr>
<tr>
<td>10031 10172 (0)</td>
<td>PRE1 SS</td>
<td>SINE/tRNA-Glu</td>
<td>1 142 (116)</td>
<td>21</td>
</tr>
</tbody>
</table>
In a computer search for repeat sequences, two retrotransposons sequences short interspersed nucleotide elements (SINE) and long interspersed nucleotide elements (LINE) showed in intron1 region and exon3 region of porcine FSHβ gene (GenBank: D00621), respectively (Table 1). Zhao et al. (1999) found an extra SINE in intron 1 of porcine FSHB gene [7]. SINEs were considered to be genomic parasites and having no benefit to the organism (Batzer & Deininger, 2002). The SINE insertion, found in introns of animal gene, may modify the gene’s transcription pattern and its presence is well known to affect the expression level (Kramerov & Vassetzky, 2005). The presence/absence of SINE resulted in allelic polymorphism of porcine FSHβ gene. The pig breeds with SINE− allele was correlated with higher litter sizes than the breeds with SINE+ allele (Shi et al., 2006). When comparing with two SINE indicated that the SINE finding by zhao was located downstream of the SINE in FSHβ gene (GenBank: D00621) and their similarity are 80%. The same high frequency SINE in pig due to probably positive selection during the evolutionary process of pig in artificial selection.

Acknowledgments

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References


