The relation between growth hormone (GH) gene and Cytochrome b gene in three salmon types

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ABSTRACT

In the present study, growth hormone (GH) gene and Cytochrome b gene in Salmo trutta caspius and Salmo trutta fario were discussed, the rate of relationships between salmonids were analyzed by GH and Cytochrome b gene. The GH gene is a genetics marker in nuclear DNA that expressed paternal traits in salmons, furthermore, Cytochrome b gene also is genetics marker that expressed maternal DNA in mitochondrial genomics. With two genes we documented that there were high homology between sequences of GH gene and Cytochrome b gene, hence the salmonids types, specially salmo trutta caspius, salmo salar and salmo trutta fario probably had similar ancient in bony fishes.

Keywords: Salmons, mitochondrial, cytochrome b, growth hormone gene, genetics

INTRODUCTION

The salmo types including, salmo trutta caspius (S. t. caspius), salmo salar (S. Salar) and Salmo trutta fario (S. t. fario), is important for economic aquaculture industry. They live in the part of rivers and exhibit homing behavior. Therefore the difference of population related to migration of them (sometimes connected to the sea). There are studies related to physiological and genetic on the salmons, confirmed marker genetic differentiation (Fergusen, 1989, Guyomard, 1989).

The research on the marker of genetics most concentrated on the paternal and maternal traits in fishes. There are some genes for paternal traits specially growth hormone (GH) gene, that associated with various quantitative traits (reviewed in Peter and Marchant, 1995). However, GH gene is an important trait in other animals, like cattle that regulate somatic growth in muscle and the skeletal body (Harvey et al., 1995; Rocha et al., 1991; Hoj et al., 1993; Pilla et al., 1994; Schlee et al., 1994a, b; Lagziel et al., 1996), and pigs (Casas-Carrillo et al., 1994; Nielsen et al., 1995).

Salmonid fishes have two GH genes (GH1 and GH2) resulting from their polyploid ancestry (Agellon et al., 1988; Rentier-Delrue et al., 1989; Forbes et al., 1994). The polymorphism of GH genes has been detected and compared between solmonids(Gross and Nilsson, 1995).

The full length of GH gene in salmonids sequenced and deposited in Genebank. In salmo trutta caspius was found 2048 bp. (Accession number, JN241634.1), containing, five exons and six introns in the length. (Rezaei and Akhshabi, 2011 a and b; Rezaei and Akhshabi, 2012). The result of sequence in Salmo Salar (Johansen et al., 1989), however there were not report regards sequence of GH gene in S. t. fario, but also we can conclude the variation is low.
between *S. t. fario*, *S. Salar* and *S. t. Caspius*, because the result of mitochondrial DNA that related to maternal traits had been high homology between salmonids. The mitochondrial genomic in salmonids including 13 protein coding genes, 22 transfer tRNA genes, and 2 ribosomal RNAs genes corresponding to the 12S and 16S transcripts, that was followed between the species of salmonids including, the *O. mykiss* (Zardoya et al., 1995), *S. salar* (Hurst et al., 1999), and other salmonids such as *S. alpinus* (Doiron et al., 1999), *C. Lavaretus* (Miya and Nishida, 2000) and *S. fontinalis* (Doiron et al., 2002).

Recently, in *S. t. Caspius* for the full length of cytochrome b gene in mitochondrial DNA was found and deposited by (Jamshidi and Kalbasi, 2009) in Genebank (Accession number, JN995186), this gene has one exon in full length, the result of alignment with another sequence of cytochrome b in *S. t. Capius* and *S. Salar*, there were high homology between sequences. In this study we have two aims, first, is their relationship between someone's or salmon types has been common ancestor? Second, how much relationship between genetic markers engaged in paternal and maternal traits?

**MATERIALS AND METHODS**

Samples and DNA isolation: The samples are included, *S. t. caspius* and *S. t. fario* were obtained from the rivers of Tonekabon - Iran, the fishes both male and female had three years age old.

DNA isolation: Total genomic DNA from *S.t.caspius* and *S.fario* was isolated from powdered tissue has taken from muscle body following described by Sambrook et al., (1998). Briefly, the samples was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (24:25: 1). Vortexed 10x is then centrifuged at 3000 x g for 5 min at room temperature. DNA was precipitated overnight at 4°C with then washed with 2 vol. 100% ethanol. Then the DNA genomics amplified cytochrome b gene was separated by 1.5 % agarose gel electrophoresis. After electrophoresis, the DNA full length was visualized ethidium bromide and then was taken photos by gel DOC Bio RAD Company.

Designing of primers and PCR amplification: There were not any report regards GH gene in *S.t. Caspius* in Genebank, hence we used other sequences specially *S. Salar* and *S. t. fario* for designing primers, so were designed three pair of primers from first to end of the GH gene, we used a DNAMAN program (USA) and also the BLAST NCBI Network system for designing primers (Table 1). The primers could amplify three different sizes of fragments including, 910, 312 and 819 bp. The primers has also able cross amplified, means a forward primer of first fragment can match with a reverse primer of the second or third fragment, hence, we could amplify that a full length of GH gene in *S. t. Caspius*.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd Primer(SsGH1):</td>
<td>ACATACTCAACCGCAACCCGCACCTTCAAG</td>
<td>910 bp.</td>
</tr>
<tr>
<td>Rev Primer (SsGH2):</td>
<td>GTGACAGGTCCACTCCTGCTATTCA</td>
<td></td>
</tr>
<tr>
<td>Fwd Primer (SsGH3):</td>
<td>GATAATAGGGAATCTCAAAGCTGT</td>
<td>312 bp.</td>
</tr>
<tr>
<td>Rev Primer (SsGH4):</td>
<td>CTCAAATACCTCTAGTAACTGGA</td>
<td></td>
</tr>
<tr>
<td>Fwd Primer (SsGH5):</td>
<td>CATCACAATATTTGACTATATCAG</td>
<td>819bp.</td>
</tr>
<tr>
<td>Rev Primer (SsGH6):</td>
<td>CAGATTAGGCCTTGGCCCTGACCTGA</td>
<td></td>
</tr>
<tr>
<td>Sequence Primer:</td>
<td>ATCTGGTAGAGCCTGACTCCA</td>
<td></td>
</tr>
</tbody>
</table>
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Designing primers of GH gene for S.t. fario:

Were designed primers of GH gene in S. t. fario, according to reports of sequences of GH gene in salmonids in Genebank, by DNAMAN program (USA) and the BLAST NCBI Network system, because we thought probably there is a high homology between S.t. fario and other salmonids. The primers could amplify three fragments, including, 1495, 1500 and 1493 bp. These primers also could amplify by crossly, means, first forward primer with third reverse primer could amplify a fragment, also these results confirmed regards other forward and reverse primer in GH gene (Table 2).

Table 2: Primers were designed for amplified of GH gene in S. t. fario.

<table>
<thead>
<tr>
<th>Product Size (bp)</th>
<th>Primer Set I-Forward</th>
<th>Primer Set I-Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1495</td>
<td>AATCATCTTGCAATTAAGAG</td>
<td>CCTTAGTTGAAGGCAGTGGGT</td>
</tr>
<tr>
<td>1500</td>
<td>GCATGTTATGCCCCCTTAACC</td>
<td>CAGTCCTGTGGCCCTAAAGGT</td>
</tr>
<tr>
<td>1493</td>
<td>TGAACCTAAAGTGCAATGAAGTCA</td>
<td>AACCTGGAGACAGGCTCTT</td>
</tr>
</tbody>
</table>

Primers of cytochrome b in S. t. fario:

Primers designed to specifically amplify the cytochrome b gene based on conserving sequences from regions identified by the alignment of all the available sequence data from several salmonid species. These primers can amplify from first to end of cytochrome b gene including 1191 bp. These primers including:

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Forward Primer 5’</th>
<th>Reverse Primer 5’</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>GACTTGAAAAACCACCGTTG</td>
<td>CTCCGGATCTCCGGATTACAAGAC</td>
</tr>
<tr>
<td>II</td>
<td>GCATGTTATGCCCCCTTAACC</td>
<td>CAGTCCTGTGGCCCTAAAGGT</td>
</tr>
<tr>
<td>III</td>
<td>TGAACCTAAAGTGCAATGAAGTCA</td>
<td>AACCTGGAGACAGGCTCTT</td>
</tr>
</tbody>
</table>

The PCR programs:

The PCR reaction used 10 microgram PCR reactions contained: 1 µl template DNA, 2 µl forward primer (100 ng/µl), 2 µl reverse primer (100 ng/µl), 2 µl dNTP mix (2.5mM each), 5 µl 10X ChromTaq Assay buffer, 0.5 µl ChromTaq enzyme (3U/µl), Water 37.5 µl, in a total volume, 50 µl. 94°C of 5 min, 35 cycles of 94°C 30 Sec., 55°C 30 Sec., and 72°C 1 min. Two to ten µl of each PCR reaction were run on 1.5% agarose gels in TAE buffer containing ethidium bromide. One µl 500bp, DNA ladder (Gibco-BRL) was used as a size standard. Then the PCR products after purification by the Chromous kit purification were sent to the Chromous Geni Company-India for doing sequence.

Sequencing of the GH gene in S.t.caspius, and cytochrome b gene in salmo trutta fario: For sequencing of GH gene and cytochrome b gene we designed one set of primer for per gene, that process of the sequencing including:

PCR Purification: Amplified PCR product was purified using QIA quick PCR Purification Kit Protocol:

A. Added 5 volumes of Buffer PB to 1 volume of the PCR sample and mixed. B. Placed a QIAquick spin column in a provided 2 ml collection tube. C. Centrifuged at 8000 rpm for 30–60 s. D. Discarded flow-through. Placed the QIA quick column back into the same tube. E. Washed with 0.75 ml Buffer PE to the QIA quick column and centrifuged for 30–60 s. F. Discarded the flow-through and placed the QIA quick column back in the same tube. G. Centrifuged the column for an additional 1 min at maximum speed. H. Placed QIA quick column in a clean 1.5 ml microcentrifuge tube. K. To elute PCR product, added 40 µl of B H2O to the center of the
QIAquick membrane and centrifuged the column for 1 min.

**Sequencing of Amplified GH gene and Cytochrome b gene:** Sequencing was performed along with the Forward and reverse primers in ABI 3730XL high throughput sequencer machine. Forward and reverse sequences were assembled and edited.

**RESULTS**

Study variations at the DNA level contribute to the genetic characterization of *Salmons*. We used GH of gene and cytochrome b gene. According to the annotation GH genes, these are genes linked to economic traits and polymorphism genetics which are governed by many genes. Following to the sequences of the *salmon* GH gene and cytochrome b were published in the BLASTn on the National Centre for biotechnology information (NCBI) network service, was designed a fragment of almost 3kb. for *S.t.caspius* and also *S.t.fario* (Figure 1). Regarding cytochrome b gene a full length (1191 bp.) were amplified and are shown in Figure 2.

![Fig. 1: PCR amplification of high quality *salmo t. caspius* using the primers of designed Samples were separated by electrophoresis in 1.5% gel electrophoresis. M. Size marker 500 bp.](image1.png)

![Fig. 2: PCR amplification of high quality *s. t. fario* using the primers of designed Samples were separated by electrophoresis in 1.5% gel electrophoresis. M. Size marker 1000bp.](image2.png)

**Comparison of the GH gene and cytochrome b in the *s. t. caspius* and *S. t. fario* with other *salmons*:** There are high homology between bony fishes, such as *s. t. fario*, *s. salar*, *s. t. caspius* and *rainbow trout* regarding marker genetics as mitochondrial genetics (cytochrome b) (Rezaei and Akhshabi, 2012) , In related to, GH gene were compared between *s. t. caspius* was recorded in Genebank (JN241634.1) with *s.salar* was recorded in Genebank (AY614010), the result showed there were high homology between sequences, in figure 3, however the number of exons of GH in *s.salar* more than *s. t. caspius* (6 exon in *s. salar* and 5 exons in *s. t. caspius*) but was similar almost the position of exons in *s.salar* and *s. t. caspius*. Moreover the number of introns in *s. salar* and *s. t. caspius* was 5 numbers that was same between
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sequences, also the position of introns were similar between together of sequences.

comparisons between the cytochrome b gene in the S. t. fario and other Salmons, including S. Salar, S. t. fario and S. t. caspius populations by the DNA gene program. Therefore the results are shown; there is a high homology between sequences. In figure 4. A 99% DNA sequence similarity was observed, but there were five nucleotides differences from the first of the two sequences and end of two sequences between a sequence of S. t. fario and S. t. caspius. But regarding S. salar there was 93 percent homology between sequences. However there are some different the shape of salmons, S. t. caspius and S. Salar with s. t. fario but the analysis are shown homology was 93 percent, especially with s. salar. Moreover these results innovated that S. t. fario in Iran is originated from S. Salar that the ancestor of S. t. fario had migrated from the Atlantic Ocean to White Sea and then to Caspian Sea, because we collected samples has been connected from Rivers to Caspian Sea.

Fig. 3: Comparison of sequences of the Cytochrome b gene between S.t. caspius, s.salar and s. t. fario. The maximum identity is shown, there is a high homology between sequences.

Fig. 4: Comparison of sequences of the GH gene between S.t. caspius and s.salar. The maximum identity is shown, there is a high homology between sequences.

DISCUSSION

In the present study the phylogenetic analysis of Salmons showed that there were a high homology between salmons such as S.t. caspius, s. t. fario and s. salar according to some genes that had cited marker genes in salmons, the genes such as a cluster gene from mitochondrial genomic, as Cytochrome b, Cytochrome C oxidase (I, II, III), (reviewed by Bihington & Hebert, 1991; Beckenbach, 1991; Carr and Marshall, 1991; McVeigh and Davidson, 1991; Bernatchez et al., 1992; Whitmore et al., 1992; Ovenden et al., 1993). GH genes (GH1 and GH2), in Atlantic salmon (Johansen et al., 1989; Male et al., 1992), rainbow trout (Agellon et al., 1988a;
Rentier-Deirue et al., 1989), and common carp (Chiou et al., 1990). Mini-satellite DNA (Fields et al., 1989; Taggart and Ferguson, 1990, 1991; Turner et al., 1991; Bentzen et al., 1993; Stevens et al., 1993), random amplified polymorphic DNA (RAPD) markers amplified with single primers of arbitrary nucleotide sequence (Elo & Vuorinen, 1993).

However, the nuclear DNA will express paternal traits and mitochondrial DNA will express maternal DNA in salmons but also together can have been similar aims for studies of phylogenetics analysis. In this study, we sequenced both Cytochrome b and GH genes in s. t. fario and s. t. caspius, the results were compared between sequences of reported in Genebank – NCBI Network system, there were high homology between sequences of Cytochrome b and GH genes, however there is some variation in the shape of s. t. fario with s. t. caspius, and S. salar. In similarity the Atlantic populations there are four black stripes and variable number of small irregular black spots white halos on the body sides. In S. t. fario, in hatchery trout has a bluish gray body color and no black strips, however, they are larger than Atlantic salmon, s. t. caspius but more regular in shape, and less intensively pigmented, moreover there are red spots are always observed in populations of s. t. fario. Nevertheless, our studies showed the among of variation between salmons is very less (around 2-3 %) regarding Cytochrome b, GH gene, however, we have to do more studies on mini and microsatellites, RFLP, RAPD and other genetics marker for getting better results. But the more reports indicated that GH gene is influence on the growth of the body and genetics marker that is part of nuclear DNA, expressed paternal traits. Furthermore, Cytochrome b and C oxidase expressed from mitochondrial DNA genomic that penetrated on the maternal traits, that's important for studies of phylogenetics and evolution of salmons.

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**REFERENCES**


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