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POLYMORPHISMS OF THE CANDIDATE GENES ASSOCIATED WITH THE GROWTH TRAITS IN COMMON CARP (*Cyprinus carpio* L.)

Deyana Gencheva[1*], Stefka Stoyanova[2]

Trakia University – Stara Zagora, 6000
Faculty of Agriculture
[1]Department of Genetics, breeding and reproduction
[2]Department of Biology and Aquaculture

***Email: dhristova@uni-sz.bg**

Abstract

The implementation of modern molecular genetic technologies for the improvement of genetic resources enables the precise and efficient selection for economically relevant traits in fish farming and aquaculture – agricultural sectors with the most intensive development on a global scale. With regard of this the identification of "major" or "candidate" genes associated with economically important traits using DNA-based marker techniques is a necessary prerequisite for conducting a breeding programme in the long term.

The genetic studies of many researchers in the field of aquaculture are primarily focused on the identification of nucleotide variations in polymorphic sites of genes associated with the growth performance in Teleosts. The somatotropic axis genes, among which those coding for the growth hormone (GH), the growth hormone receptor (GHR), insulin-like growth factor I (IGF-I) and myostatin (MSTN) are of particular interest. The results obtained in different fish species of economic importance, including common carp (*Cyprinus carpio*), demonstrated clearly that the genetic variation in these loci could be associated with the growth performance of the species' representatives.

The purpose of this overview was to present the achievement of scientists and specialists in the field of fish farming and aquaculture related to genes responsible for the growth performance of a number of fish species in order to reveal the possibilities and accomplishments of functional genomics in an improvement of marker-assisted selection techniques in *Cyprinus carpio*.

Keywords: common carp **(***Cyprinus carpio* L.), growth hormone gene (GH), growth hormone receptor gene (GHR), insulin-like growth factor I gene (IGF-I), myostatin gene (MSTN).

INTRODUCTION

Fish farming and aquaculture are among the most rapidly developing agricultural sectors worldwide (Zaykov, 2008). FAO data affirm that the production of fish and fish products follows a constantly ascending trend and about 2050 it is expected to attain 80 000 000 t (FAO, 2012). For cyprinids, in particular, the production up to 2013 was 4 080 045 t (FAO, 2015).

The main reasons for the expansion of aquaculture in many countries, as affirmed by Staykov (1997) are the necessity for increased production of animal protein, the utilisation of low-productive and non-productive lands, the demands of the international market for marine and freshwater aquaculture species and related dietetic products, and last but not least, opening new vacancies to reduce unemployment in poor regions of the world.

A typical member of Teleosts, the common carp *(Cyprinus carpio)* is a freshwater species from the *Cyprinidae* family, with the body length of up to 120 cm and body weight of up to 35 kg (Nelson, 2016; Imsiridou et al., 2009). In European climatic conditions, this size could be attained within 3–4 years.

Data provided from the Ministry of Agriculture and Forests demonstrate that the carp is the most popular and preferred species for consumption in Bulgaria, EUROSTAT data affirm that in the EC, carp production has increased over 15 000 t by 2009 and that Poland and the Czech Republic are the largest producers.

These data agree with the fact that carp is a traditional dish for Central European countries as well as for Bulgaria (Fish farming and aquaculture in Europe, 2012).

The application of modern molecular genetic technologies in livestock husbandry, including fish farming, is a prerequisite of precise and efficient selection for economically important traits using DNA markers, also known as marker-assisted selection (MAS) (Lo Presti et al., 2009, Hristova et al., 2012).

Hence the identification of major, candidate genes associated with economic traits is the initial important step in a selection programme (Ruane and Colleau, 1996; Dekkers, 2004).

IDENTIFICATION OF POTENTIAL CANDIDATE GENES FOR GROWTH

Growth traits are economically important traits under the genetic control of numerous genes from the species' genome and are significantly influenced by environmental factors.

It is important to note that during the identification of a potential candidate gene for growth, it should be established how allelic variation in the respective polymorphic locus influences gene regulation provided that a large part of genes comprise three major regions – one or several coding exons, a gene promoter responsible for DNA transcription into mRNA and one or several non-coding sequences (introns) (De-Santis and Jerry (2007).

Somatogenesis is a polygenic trait resulting from numerous physiological pathways that regulate the metabolism and muscle growth in vertebrate animals, including Teleosts (De-Santis and Jerry, 2007).

In this sense, somatotropic axis genes and genes from the transforming growth factor family have raised interest as potential candidate genes in livestock selection programmes, and fish farming in particular**.**

The somatotropic axis plays a key role in systemic metabolism and physiology and comprises several primary coding genes – for growth hormone (GH), growth hormone releasing hormone (GHRH), growth hormone inhibiting hormone (GHIH), insulin-like growth factors I and II (IGF-I; IGF-II) and associated receptors and transporter proteins.

The somatotropic axis functions through the reception of signals from the environment via the brain epiphysis and their translation through the hypothalamus into regulatory hormones e.g. GHRH and GHIH (Moriyama et al., 2000).

In aquaculture, the research efforts in this field are mainly focused on genes coding for growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factor I (IGF-I), as well as myostatin (MSTN) – another essential factor of growth, more precisely of myogenesis.

GROWTH HORMONE (GH) GENE

From the viewpoint of its physiological function in the body, the GH is the main regulator of postnatal development through stimulation of anabolism, cell division, protein synthesis and skeletal growth. The GH gene is further involved in lipolytic activity through fat oxidation, inhibition of glucose transport to peripheral tissues (diabetogenic activity) and regulation of ribosome translational activity, with a subsequent effect on protein synthesis (Goodman, 1993).

The results of numerous studies on single nucleotide polymorphisms (SNP) in polymorphic sites of GH in animal species of economic significant as cattle (Tambasco et al., 2003; Pozzi Pereira et al., 2005) pigs (Cheng et al., 2005), poultry (Nie et al., 2005) etc. have clearly demonstrated that even minimum amounts of the genetic variance in growth performance could be associated with an additive effect of alleles from the growth hormone locus. Similar studies have been carried out in aquaculture species as well.

Regarding the structural organisation of the GH gene in fish, it was established that similarly to mammalian GH gene, it was presented by 5 exons and 4 introns in carps (Hong and Schartl, 1993), while in several other fish species, an additional exon was present (Ohkubo et al., 1996).

An interesting detail from the early evolution of Teleosts is the mutation associated with duplication of the GH gene resulting in identification of a double copy (GH-I and GH-II) in salmonids' genome (*Oncorhynchus mykiss*, *Oncorhynchus* etc.), as well as in the common carp (*Cyprinus carpio*) and the tilapia (*Oreochromus niloticus*) (Tao and Boulding, 2003). The genetic variation in the growth hormone was due mainly to mutations from the type of SNPs and single sequence repeats (SSRs), mainly in intronic regions and less frequently in exonic sequences of the gene (Gross and Nilsson, 1999; Yue and Orban, 2002; Almuly et al., 2005).

GROWTH HORMONE RECEPTOR GENE

The growth hormone receptor (GHR) gene codes for a transmembrane protein from the class 1 receptors within the cytokine family. Being a receptor, GHR mediates the communication between the GH gene and target cells transmitting stimulation signals through the cell membrane resulting in initiation of transcription of multiple

genes, including the gene coding for insulin-like growth factor I (IGF-I) (Kobayashi et al., 1999).

The GHR gene has been cloned and characterized in a number of fish species as rainbow trout (*Oncorhynchus mykiss*) (Very et al., 2005), Atlantic salmon (*Salmo salar*) (Benedet et al., 2005), goldfish (*Carassius auratus*) (Lee et al., 2001) etc. The GHR gene is single-copy with 9 exons in mammals and an additional exon in the double copy of the gene in fish (GHR-I and GHR-II) (Ozaki et al., 2006). The GHR-I and GHR-II genes are transcribed together while their expression is not the same with tissue-specific features in the liver and adipose tissue (Saera-Vila et al., 2005). Although at present the genetic variation in this locus in fish is still unknown, it could be a possible subject of future research in fish genome, including carps, due to the evidence for strong association of GHR polymorphisms and growth performance traits in some vertebrates as birds (Huang et al., 1993), cattle (Curi et al., 2005) and so on.

INSULIN-LIKE GROWTH FACTOR I GENE

In Teleosts, the IGF-I gene codes for insulin-like growth factor I, a peptide made up of 70 amino acids. Except for salmonids, the gene is single-copy (Stahlbom et al., 1999).

The genetic structure of the gene is known only in some fish species including carps (Vong et al., 2003) and thus, available publications emphasizing its potential application as a candidate gene for growth performance are rather limited. One of the first studies has identified SNPs in some genes of the somatotropic axis, including IFG-I in Arctic charr using PCR-RFLP (Tao and Boulding, 2003). The association of the different genotypes with some growth traits in this species was established, although it was not relevant.

MYOSTATIN GENE

Having in mind that the gene coding for myostatin (MSTN) is one of the components of control at muscle tissue level, it is of particular interest in investigations of genetic variation in loci associated to the growth in fish.

Its importance is due to the direct interaction of MSTN with GH in muscle fibers resulting in regulation of the expression of myogenic regulatory factors and consequently, stimulation or inhibition of the differentiation of myoblasts in muscle fibers (Liu et al., 2003).

MSTN, also known as growth/differentiation factor-8 (GDF-8) is a protein in terrestrial vertebrates (MacPheron et al., 1997), whose importance for growth at the phenotype level was initially demonstrated in mice.

The results showed that mutations in MSTN gene lead to 30% increase in growth rate resulting from muscle fibers' hyperplasia and hypertrophy. In fish, the MSTN gene includes 3 exons and 2 introns that are rather similar in Teleosts – Atlantic salmon (Østbye et al., 2001), rainbow trout (Rescan et al., 2001) etc. It is a double-copy gene (MSTN-1; MSTN-II), and each copy is expressed in various tissues as skeletal muscle in terrestrial vertebrates and respectively gills, kidney and sexual glands in fish (Biga et al., 2005).

Polymorphic variations of the gene in fish are mainly in exon I in species as American channel catfish (*Ictalurus punctatus*) (Kocabas et al., 2002). The data associating fish growth performance with MSTN genotypes are yet limited.

ASSOCIATION ANALYSES OF GROWTH PERFORMANCE GENES IN CARPS

The investigation of associations between some SNPs in candidate genes and their phenotypic expression is a suitable and rapid approach for identification of genetic markers of growth as a quantitative, polygenic determined trait (Tao and Boulding, 2003).

The presence of such a relationship is a proof that the associated gene is directly responsible for the genetic control of the respective trait from one hand and from the other, in case when functional polymorphisms is situated very close to the studied marker, it follows that both loci are in the so-called *linkage disequilibrium* (*LD*) (Lynch and Walsh, 1998).

In this context, several actual global research studies on the detection of polymorphisms in specific loci of genes coding for GH, IGF-I and MSTN in carps and attempts for their association with phenotypic growth traits in this freshwater aquaculture species should be mentioned. Liu et al. (2017) reported a significant association of polymorphisms in the GH gene with some growth traits in carps.

A total of 306 carps were genotyped via PCR amplification of 6 polymorphic sites of the gene with subsequent single-strand conformation polymorphism (SSCP) analysis and separation of fragments via non-denaturing polyacrylamide gel electrophoresis (PAGE). Feng et al. (2014) reported 3 SNPs in intron 2 (g.3759T>G, g.7627T>A and g.7722T>C) and one coding (nonsynonymous) SNP mutation (g.7892C>T) leading to the substitution of various amino acid residues in exon 3 of the carp IFG-1 gene.

The genotyping was performed in 289 carps using PCR-RFLP analysis and direct sequencing of SNPs in the GH gene.

The authors demonstrated a significant association of g.7627T>A mutation with body weight and length in the studied carp population with a tendency for higher body weight by 5.9% in fish from the homozygous AA genotype vs the homozygous TT genotype.

This study affirmed that the information provided by individual SNPs for association analyses was often limited with regard to phenotypic traits. That is why Feng et al. (2014) recommended grouping of individuals in haplogroups combining several SNPs at a time, which would substantially increase the reliability of such analyses. The linkage disequilibrium test showed that SNPs in intron 2 and exon 3 were unlinked (r = 0.011–0.254). High frequency of allele C (0.799), compared to allele T (0.201) was identified with regard to the g.7892C>T mutation in the IGF-I gene. In their analysis of genetic variation in the MSTN gene via sequencing, Sun et al. (2012) identified 2 SNPs (c.371+749A>G, c.371+781T> C) in intron 2 as well as 2 SNPs (c.42A>G, c.72C> T) in exon 3, in a sample of 162 carps.

High frequencies (between 50.31 and 92.28) of alleles in studied polymorphic loci were observed. The authors established a strong association of fish from the homozygous GG genotype with body weight for c.42A>G.

The relationship between the heterozygous CT genotype and higher body weight was demonstrated also for c.72C>T. On the contrary, no association of other identified SNPs with studied growth traits was found out by Sun et al. (2012).

The authors attributed this tendency to the specific nature of studied polymorphisms and concluded that probably, there were silent or synonymous mutations without a change in amino acid composition in the structure of the coded protein and ultimately, without phenotypic expression.

CONCLUSIONS

1. The presented overview demonstrates the application of molecular genetic studies on marker-assisted selection in a global rapidly expanding livestock production branch as aquaculture. Furthermore, the information on the genetic diversity on the DNA loci level in fish species farmed in Bulgaria is very scarce or absent.

2. That is why the results of investigations on the genetic variation in specific genes associated with growth performance in fish, including carps, would be a valuable basis for decision-making with regard to improvement of current breeding programmes in national fish farming practice.

3. The information from genetic analysis of nucleotide variation at some growth loci would assist in the determination of genetic diversity in *Cyprinus carpio* population for sustainable conservation of genetic resources of this traditional for the country species.

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