Supplementary material

Tri-m-cresyl phosphate and PPAR/LXR interactions in seabream hepatocytes: revealed by computational modeling (docking) and transcriptional regulation of signaling pathways

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**Sparus aurata** fatty acid binding protein (FABP) sequence analysis

An analysis of amino acid sequences was performed using the NCBI’s BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) for characterizing the *Sparus auratus* FABP (Accession no. HQ228170). The deduced amino acid sequence consists of 132 amino acids and shows the highest similarity to the sequence of FABP subtype 7. To establish the degree of identity of FABP7 among fish species, the sequence of *Sa*FABP7 was aligned with other FABP7 fish sequences using CLUSTAL OMEGA, multiple sequence alignment program (http://www.ebi.ac.uk/Tools/msa/clustalo/). FABP7 multiple amino acid sequence alignment is presented in Figure S1. The extent of identity between FABP7 sequence in seabream and in other fish species ranges from 85.61% to 91.67% (Table S1).

D. rerio  
MVDAFCATWKLDSQNFDDYMKALGVGFATRQVNVTQPVTAISKDGKVKKVKTSTFRN

O. niloticus  
MVEAFCATWKLDSQNFDDYMKALGVGFATRQVNGVTKPTVTAISKDGKVKKVKTSTFRN

O. latipes  
MVDSFCATWKLDSQNFDDYMKALGVGFATRQVNVTQPVTAISKDGKVKKVKTSTFRN

T. rubripes  
MVDAFCATWKLDSQNFDDYMKALGVGFATRQVNVTQPVTAISKDGKVKKVKTSTFRN

S. aurata  
MVEAFCATWKLDSQNFDDYMKALGVGFATRQVNVTQPVTAISKDGKVKKVKTSTFRN

S. partitus  
MVDAFCATWKLDSQNFDDYMKALGVGFATRQVNVTQPVTAISKDGKVKKVKTSTFRN

L. crocea  
MVFAFCATWKLDSQNFDDYMKALGVGFATRQVNVTQPVTAISKDGKVKKVKTSTFRN

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D. rerio  
TEISFKLGEFDETTADDRHVKSTSVLEGDNLRQVRQWQGKRKEIHDGKMVMTLTF

O. niloticus  
TEISFKLGEFDETTADDRHVKSTSVLEGDNLRQVRQWQGKRKEIHDGKMVMTLTF

O. latipes  
TEISFKLGEFDETTADDRHVKSTSVLEGDNLRQVRQWQGKRKEIHDGKMVMTLTF

T. rubripes  
TEISFKLGEFDETTADDRHVKSTSVLEGDNLRQVRQWQGKRKEIHDGKMVMTLTF

S. aurata  
TEISFKLGEFDETTADDRHVKSTSVLEGDNLRQVRQWQGKRKEIHDGKMVMTLTF

S. partitus  
TEISFKLGEFDETTADDRHVKSTSVLEGDNLRQVRQWQGKRKEIHDGKMVMTLTF

L. crocea  
TEISFKLGEFDETTADDRHVKSTSVLEGDNLRQVRQWQGKRKEIHDGKMVMTLTF

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D. rerio  
EGVQAVRTYEKA

O. niloticus  
EGVQAVRTYEKA

O. latipes  
EGVQAVRTYEKA

T. rubripes  
EGVQAVRTYEKA

S. aurata  
EGVQAVRTYEKA

S. partitus  
EGVQAVRTYEKA

L. crocea  
EGVQAVRTYEKA

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Figure S1. FABP7 multiple amino acid sequence alignment from different fish species. GenBank accession numbers for the different sequences shown in the alignment are: *Sparus aurata*, AEN83586.1; *Danio rerio*, NP_571680; *Oreochromis niloticus*, XP_003442977; *Oryzias latipes*, XP_004082256; *Takifugu rubripes*, XP_003962578; *Stegastes partitus*, XP_008275136; *Larimichthys crocea*, XP_010744948. Positions that are identical in all sequences are indicated by an asterisk (*), fully conserved ‘strong groups’ with double dots (:) and fully conserved ‘weak groups’ by a single dot (.).
Table S1. Identity (in %) in amino acid sequences of FABP7

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<td>S.a.</td>
<td>85.61</td>
<td>90.15</td>
<td>85.61</td>
<td>89.39</td>
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(S.a.) Sparus aurata, AEN83586; (D.r.) Danio rerio, NP_571680; (O.n.) Oreochromis niloticus, XP_003442977; (O.l.) Oryzias latipes, XP_004082256; (T.r.) Takifugu rubripes, XP_003962578; (S.p.) Stegastes partitus, XP_008275136; (L.c.) Larimichthys crocea, XP_010744948.

To date, FABP7 was identified as FABP brain-like according to its highest tissue expression pattern. However, nomenclature of FABPs has become increasingly confusing as some tissues contain more than one FABP subtype. In particular, zebrafish FABP7 transcripts are detected in brain, spinal cord, retina, testis, liver, intestine, and muscle. Similarly, the tertiary structure of FABP subtypes is highly conserved and, despite extensive studies on the structure of FABPs, their precise physiological role remains unclear. Several recent studies demonstrated the important role of hepatic FABP7 in controlling fatty acids or fibrates binding and lipid homeostasis. In this context, Bijland et al. demonstrated that fenofibrate increased transcription of genes involved in fatty acids binding (i.e. FABP1, FABP2, FABP4, and FABP7) in the liver of cholesteryl ester transfer protein (CETP) transgenic mice. Furthermore, 0.5% clofibrate activates PPARs which directly induce the transcription peroxisome proliferator response element (PPRE)-mediated of FABP7 in the liver of zebrafish. Karanth et al. suggested that FABP7 is an important carrier of FA ligands to nuclear receptors, such as PPARs. Once activated, these nuclear receptors form heterodimers with retinoid X receptors (RXR) which in turn bind to PPRE in FABP7 gene and stimulate the transcription. Again, a recent study highlighted that lycopene inhibits hepatic steatosis via miR-21-induced downregulation of its downstream target gene, FABP7, in mice fed a high-fat diet. Finally, hepatic FABP7 plays important role during the thermoregulation in Sparus aurata. In fact, low water temperature for 21 days increased transcription of FABP7 gene, leading to lipid metabolism dysfunction and potentially to cell death.

References