THE KISSPEPTIN SYSTEM: A NOVEL REGULATOR OF THE REPRODUCTIVE AXIS IN TELEOST FISHES

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Kisspeptin system. Discovery of elements and functions

**Discovery**

**Ligand**
- KISS1 mRNA selectively overexpressed in metastasis-suppressed melanoma

**Receptor**
- GPR54: G Protein-coupled Receptor

**System**
- Kisspeptins (Kp) are the endogenous ligand of GPR54

**Functions**

**Cancer**
- Kp is a potent inhibitor of tumor metastasis in variety of cancer types

**Reproduction**
- KISS1R mutations are linked to Hypogonadotrophic Hypogonadism in humans

**Insulin secretion**
- Important role for this system in the normal regulation of islet function

**Placentaion**
- Kp acts in the regulation of physiological trophoblast invasion in pregnancy

**Vasoconstriction**
- Kp acts as a vasoconstrictor in human, suggesting a role for KISS1R and Kp in cardiovascular system

**Ovulation**
- KP at the preovulatory period strongly suggests a role of locally produced Kp in the control of ovulation
Kisspeptin system role in Reproduction (puberty)

In 2003, three groups identified the role of the KISS1R in reproduction.

When KISS1R is mutated, mammals do not undergo puberty, their gonads are small, their sex hormones and gonadotropin levels are low, and they are sterile. Hypogonadotrophic hypogonadism.

Messager, 2004; de Roux et al, 2003
Kisspeptin system. Relevance in reproduction

ISI search (Using 5 keywords)
- METASTIN
- KISSPEPTIN
- KISS1R
- GPR54
- KISS1

Total articles = 409

In 2007, 5 of the top 10 publications in biology of reproduction

"The discovery of the role of KISSPEPTIN and its receptor, KISS1R, in PUBERTY is the most exciting finding made in the field of reproductive biology since the discovery of GnRH in the 1970s”
Regulation in the Hypothalamic-pituitary-gonadal axis (HPG)

Environmental factors
- Photoperiod
- Temperature

Physiological factors
- Nutrition
- Stress

Feedback (+)
- Anteroventral periventricular nucleus (AVPV)

Feedback (-)
- Arcuate nucleus (ARC)

Brain
- KISS1 neurons
- Hypothalamus
- KISS1R
- GnRH neurons

Anterior pituitary
- GnRH
- FSH/LH

Gonads
- Gametogenesis
- Sex steroids (Estradiol and Testosterone)
Objective of this study: “Characterization the kisspeptin system in flatfish”

Rationale

Flatfish aquaculture

Problems in reproduction of Flatfish aquaculture:

- Solea senegalensis
  - Early maturation and reduced growth rates of males

- Atlantic halibut
  - Delay of puberty and failure of reproduction in F1 males

Understanding of the control of the initiation of puberty in fish is a basic attention in the aquaculture industry

“Basic studies concerning the control of reproduction are needed”
The highly conserved nature of the kiss1r region suggests that the flanking regulatory sequences are conserved.
KISS1R promoter in teleost fishes

Two regions showed considerable similarity among species of the localization of the putative binding sites for the transcription factors MIF-1 and estrogen receptor.
Alternative splicing of KISS1R in vertebrates

Gene structure

Exon I I Exon II II Exon III III Exon IV IV Exon V

mRNA

TM1 TM2 TM3 TM4 TM5 TM6 TM7

alternative splicing mechanisms

First alternative spliced isoform detected in KISS1R in any species

Mechaly et al., 2009 Biol. Reprod. 80: 60-69

INTRON RETENTION

Senegalese sole

TM1 TM2 TM3 TM4 TM5 TM6 TM7

insertion 81bp

Sheep

TM1 TM2 TM3 TM4 TM5 TM6 TM7

insertion 105bp

In silico analysis

Non-functional isoform ?

Mouse

TM1 TM2 TM3 TM4 TM5 TM6 TM7

deletion TM5
**KISSL1R expression in brain and gonads of flatfishes**

**Senegalese sole**

**Brain**

- **Males**
  - Pubertal: **AB**, **ab**
  - Mature: **B**, **ab**
- **Females**
  - Pubertal: **B**, **b**
  - Mature: **A**

**Gonads**

- **Males**
  - Pubertal: **A**
  - Mature: **AB**
- **Females**
  - Pubertal: **A**
  - Mature: **A**

*Higher expression of Solea kiss1r_v1*

*Higher expression of Solea kiss1r_v2*

**Atlantic halibut**

**Brain**

- **Males**
  - Pubertal: **a**
  - Mature: **b**
- **Females**
  - Pubertal: **a**
  - Mature: **a**

**Gonads**

- **Males**
  - Pubertal: **a**
  - Mature: **nd**
- **Females**
  - Pubertal: **a**
  - Mature: **nd**

*Higher expression of Halibut kiss1r in the onset of puberty*

*Two orders of magnitude lower than brain*

**Differential expression patterns of the two different transcripts in brain and gonads**

Mechaly et al., 2009 Biol. Reprod. 80: 60-69

Kiss1r presented higher expression in the onset of puberty in brain and only detected in immature fish gonads

Mechaly et al., 2009 (Submitted)
Phylogenetic analysis grouped the Kiss1r sequences into three groups: *Kiss1-r1, Kiss1r-2A* and *Kiss1r-2B*, and revealed some unexpected relationships, showing that, in contrast to what had been surmised earlier, *Kiss1r-2B* is a non-mammalian form of *Kiss1r*. 

Mechaly et al., 2009 (Submitted)
### KISS1R expression in brain of vertebrates (Summary of results)

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene type</th>
<th>Pre-pubertal</th>
<th>Pubertal</th>
<th>Post-pubertal</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Inmature</td>
<td>Onset</td>
<td>Medium</td>
</tr>
<tr>
<td>Mammals</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Human</td>
<td></td>
<td>+</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td>+</td>
<td>+++</td>
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</tr>
<tr>
<td>Teleost fishes</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Nile tilapia</td>
<td></td>
<td>++</td>
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<td></td>
</tr>
<tr>
<td>Fathead minnow</td>
<td></td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Cobia</td>
<td>Kiss1r2</td>
<td>++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Grey Mullet</td>
<td>Kiss1r2</td>
<td>+++</td>
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<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Senegalese sole</td>
<td>Kiss1r_v1</td>
<td>++</td>
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<td></td>
<td>Kiss1r_v2</td>
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<tr>
<td>Atlantic halibut</td>
<td>Kiss1r-2</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Mechaly et al., 2008 Treb. Soc. Cat. Biol. 59: 95-107

- Differences in KISS1R expression between pubertal and mature and in different genes
- No consistent results
- Multiple genes or alternative splicing undetected events in other species?
Kisspeptin gene structure in vertebrates

Two different KISSPETIN detected in teleost fishes (KISS1 and KISS2)

Detection of two KISS2 isoforms in Senegalese sole produced by intron retention

Mechaly, Viñas and Piferrer. Unpublished
Specific conclusions

1- Synteny analysis showed a highly conserved nature of the *kiss1r-2* region, suggesting that flanking regulatory sequences are also likely to be conserved.

2- Bioinformatic analysis identified for the first time six conserved regions in piscine *kiss1r-2* upstream sequences, providing potential targets for *kiss1r-2* regulation in different species.

3- We found two different isoforms of *Kiss1r* generated by alternative splicing, originated by the retention of intron 3.

4- In the brain of Senegalese sole and *Atlantic halibut* *kiss1r_v1* was the most abundant isoform during the onset of puberty.

5- In the gonads, of Senegalese sole, *kiss1r_v2* was the most abundant with lower levels in females than in males. In Atlantic halibut, *kiss1r* expression levels were lower (2 order of magnitude) than those of the brain and were detected only in immature fish.

6- The *kiss1r-2B* is a non-mammalian form of *kiss1r*. *Kiss1r-1* was not found in sole and halibut, indicating that in the most derived teleosts, including the Pleuronectiformes and Tetraodontiformes, this gene may have been lost.
General conclusions

1- The several functions associated with the kisspeptin and its receptor, KISS1R, confirm that this is a pleiotropic system.

2- The different genes and isoforms, originated by duplication and alternative splicing, respectively, found in different species, add to the complexity of the kisspeptin system.

3- The relative conservation of this system across vertebrates demonstrates their importance in the regulation of essential biological processes.
Moltes gràcies