Search for cryptorchidism candidate genes: a comparative genetic approach

T. Kunej*, J. Ogorevc *, P. Dovc*

Introduction

Cryptorchidism is defined as incomplete descent of one (unilateral) or both (bilateral) testes and associated structures from the abdomen through the inguinal canal into the scrotum. It is common in humans, pigs, horses and companion animals (2-12 %) but rare in cattle, sheep and goat (≤ 1 %) (Amann and Veeramachaneni, 2007). The complete descent of testis occurs in most species prenatally with the exception of the dog, where it occurs postnatally. Defects in testis descent cause several problems ranging from impaired spermatogenesis and reduced fertility to increased rate of testicular neoplasia and testicular torsion. It is widely accepted that cryptorchidism has a significant genetic component, for instance mutations in INSL3 gene have been recognized as a cause of cryptorchidism (Foresta et al., 2008). For this reason breeding from affected individuals is not recommended.

We assembled cryptorchidism candidate loci database. In order to support involvement of different candidate loci in cryptorchidism we combined different study approaches, each supporting the evidence for involvement of identified genomic regions to cryptorchidism. However, information extracted from methodologically focused studies is often fragmented. To integrate data from different study approaches we used holistic, map driven approach. The map based review reveals positional overlaps between candidate loci and allows complementation of different pieces of evidence.

Material and methods

We reviewed the literature published up to 12/2009 searching for the relevant publications through PubMed and WoS using key phrases: genetics, gene candidates, cryptorchidism, testicular descent, male infertility, QTL, microarray, association, miRNA, epigenetic, reproduction, and assisted reproduction. Human clinical syndromes that may cause or feature cryptorchidism were retrieved from Online Mendelian Inheritance in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim) and Disease database (http://www.diseasesdatabase.com/). The data for cryptorchidism-related animal experiments were retrieved from the Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org/), AnimalQTL database (http://www.animalgenome.org/QTLdb/) and Online Mendelian Inheritance in Animals - OMIA database (http://omia.angis.org.au/). Putative miRNA target sites in candidate genes were obtained using Sanger's mirBase Targets - Version 5 (http://microrna.sanger.ac.uk/). Ensembl transcript identifiers for candidate genes were obtained from Ensembl database - Release 50 (http://www.ensembl.org/) and cross-sectioned to the list of identifiers with putative miRNA target sites.

*University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3, 1230 Domžale, Slovenia
Additional information on miRNA and miRNA target sequences was extracted from the Patrocles database (http://www.patrocles.org/). The functional relationship between candidate genes has been established using Ingenuity Pathway Analysis (http://www.ingenuity.com).

Results and discussion

In our study we have reviewed 144 candidate loci (genomic regions and genes) involved in cryptorchidism in humans and animals. Candidate loci are mapped on human gene map together with syntenic regions identified in different species using comparative genetic approach (Figure 1a). The candidate loci for cryptorchidism were identified considering six different research approaches in eight different species (human, cattle, pig, horse, sheep, dog, rat, and mouse). Cryptorchidism associated loci were located on 18 human chromosomes, whereas on chromosomes 4 and 11 an overlap between the genomic regions involved in chromosome mutations with the position of candidate gene has been identified. Alternatively, the candidate loci were mapped on the bovine gene map using the bovine-human synteny map.

1. Clinical syndromes in humans

The list of clinical syndromes with known genetic mutations that feature cryptorchidism published by Barthold (2008) was expanded for additional clinical syndromes extracted from the OMIM and Disease databases.

2. Transgenics and knock-outs

From the MGI database and from literature we retrieved 33 mice and one rat KO and transgenic experiments that resulted in phenotypes associated with cryptorchidism.

3. Association studies/mutation screening

Six genes (LHCGR, ESR1, NR5A1 (SF-1), RXFP2 (LGR8/GREAT), INSL3, AR) showed association between sequence variation/mutation screening and cryptorchidism in human and INSL3 in sheep and dog. Ferlin et al. (2005) found no difference between the numbers of CAG and GGC repeats, resulting in variable lengths of PolyGln/PolyGly in the androgen receptor (AR) gene and cryptorchidism; however it has been proposed that a particular combination of the PolyGln/PolyGly sequence may be linked to cryptorchidism. Studies of insulin-like 3 (INSL3) and estrogen receptor 1 (ESR1) showed opposing results: Galan et al. (2007) found no association, while Yoshida et al. (2005) and Wang et al. (2008) reported association between ESR1 sequence polymorphisms and cryptorchidism.

4. Chromosomal abnormalities

In our study we included results from nine different studies reporting 28 different chromosomal abnormalities associated with cryptorchidism.

5. Expression patterns

Our survey revealed 43 genes reported to be differentially expressed between cryptorchydism and normal group in human, rat and horse using microarray (Barthold et al., 2008) and immunohistochemistry approaches (Hejmej et al., 2005).

6. Proteomic level

Hutson et al. (1998) investigated the effect of exogenous calcitonin gene-related peptide (CGRP) in neonatal pigs. They found that exogenous CGRP (CALCB) stimulated migration of inguinal testes that had been arrested in the line of descent while ectopic testes did not
respond. The results support the assumption that CGRP plays a role in testicular descent and make CALCB a promising candidate gene for cryptorchidism.

By using six different approaches we collected data for cryptorchidism from eight different species. Comparative genomics approach allowed us to use information from various species. Positions of genes for individual species were identified by searching for ortholog genes in databases or by using synteny maps. For example, MGI database contains information about mammalian ortholog genes for different species (e.g. cattle, pig, sheep, and rabbit), locations for genes not available in databases were estimated using synteny maps.

### Table 1: The summary of candidate loci for cryptorchidism.

<table>
<thead>
<tr>
<th>Study approach</th>
<th>Number of loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical syndromes</td>
<td>35 (30)</td>
</tr>
<tr>
<td>Knock-out and transgenic experiments</td>
<td>34</td>
</tr>
<tr>
<td>Chromosomal abnormalities</td>
<td>28</td>
</tr>
<tr>
<td>Association studies</td>
<td>6</td>
</tr>
<tr>
<td>Expression studies</td>
<td>53</td>
</tr>
<tr>
<td>Proteomic studies</td>
<td>1</td>
</tr>
<tr>
<td>Studies reporting individual locus more than once by different study approaches</td>
<td>11</td>
</tr>
</tbody>
</table>

We found 11 genes (LHCGR, RXFP2, INSL3, MSX1, CYP19A1, ESR1, AR, WTI, HRAS, TNNI2, and TNNT3) associated with cryptorchidism identified by at least two different approaches.

Animal models are of great importance for the functional analysis of genes. As reported by Mortell et al., (2005) different cryptorchidism animal models exist; INSL3 and HOXA11 knockout mice, flutamide rat, surgical rat, and rabbit models. However, extrapolating the gained knowledge from one species to another is often difficult, mainly due to the different anatomical and physiological characteristics. We identified overlaps between genomic regions involved in the chromosome abnormalities and candidate genes. Duplication on position 4p16 overlaps with MYOG gene location and translocation breakpoint on 11p15.5 overlaps with HRAS, TNNI2, and TNNT3 genes. However, to our knowledge to date there are no small non-coding RNAs and epigenetic factors associated to cryptorchidism.

The functional relationships among most promising candidate loci were established using Ingenuity Pathway Analysis (Fig.1 b). The obtained results can be further applied for identification of novel candidate genes which have before not been tested for association with cryptorchidism. Better understanding of signaling pathways involved in the descent of testes and associated structures from the abdomen into the scrotum will enable more efficient identification of candidate loci in multiple species. At this stage the Ingenuity Pathway Analysis can significantly improve the incomplete physiological and developmental knowledge. However, the quality of the IPA absolutely depends on the quality of gene ontology and trait ontology databases which are critical for good quality of the IPA analysis.
Figure 1: a: part of the cryptorchidism gene map showing candidate genes positioned on human chromosomes 1-7. b: Ingenuity map integrating top candidate genes for cryptorchidism.

Conclusion
Due to the relatively high frequency of cryptorchidism in different mammalian species numerous studies have been performed to elucidate the genetic background of this defect. We exploited the comparative genetic approach and extracted cryptorchidism related data from publications and different public databases in order to identify candidate loci associated with cryptorchidism in multiple species. Our approach revealed 11 loci (LHCGR, RXFP2, INSL3, MSX1, CYP19A1, ESR1, AR, WT1, HRAS, TNNI2, and TNNT3) for which the association with chryptorchidism was found using at least two different approaches.

Acknowledgements
We thank Slovenian Research Agency (ARRS) for co-financing this study (P4-0220).

References