Detection of a quantitative trait locus associated with resistance to whipworm infections in pigs

Per Skallerup¹,², Peter Nejsum¹,², Claus B Jørgensen¹, Harald H Göring³, Alan Archibald⁴, Merete Fredholm¹, and Stig M Thamsborg²

1) Department of Veterinary Clinical and Animal Sciences, UCPH, Denmark
2) Department of Veterinary Disease Biology, UCPH, Denmark
3) Texas Biomedical Research Institute, San Antonio, TX, USA
4) The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK
Why this project?

- Whipworms (*Trichuris* spp.) infect a variety of hosts, including production animals and humans.
- High infection levels may cause growth retardation, anaemia and haemorrhagic diarrhoea.
- Limited knowledge of how *Trichuris* spp. infections are regulated by the host’s immune system.

Bethony et al. 2006
Trichuris suis

- Trichuris suis has a global distribution with highest prevalence in outdoor production systems.
- The pig-T. suis system may serve as a unique model for T. trichiura in humans.

http://para-tech.dk/the-product/
Elucidating the host genetic component in *Trichuris* infections

Breed and strain differences in resistance to gastrointestinal nematodes due to genetic variation
(Miller 1908; Ackert et al. 1935; Wakelin 1975)

Nematode infections have moderate to high heritabilities
(Stear et al. 1997; Davies et al. 2006; Kaufmann et al. 2011)

- *Trichuris suis* FEC in pigs: $h^2=0.31$-$0.73$ (Nejsum et al. 2009)
- *T. trichiura* FEC in humans: $h^2=0.28$ (Williams-Blangero et al. 2002)

A considerable part of the phenotypic variation can be explained by the host’s genetic make-up

Find genetic marker/genes
Objectives of the study

1. Conduct a genome-wide scan to detect quantitative trait loci (QTLs) associated with resistance to *Trichuris suis* (discovery study)

2. Validate the results in unrelated pigs (validation studies)
Design of discovery study

19 sows (Landrace/Yorkshire) x 13 boars (Duroc)

195 DLY piglets (F1)

*T. suis* + *A. suum* trickle infection (10 wks-24 wks)

Phenotype:
✓ FEC (week 8 p.i.)

SNP genotyping (Illumina 7K SNP chip)

Data cleaning
Statistical analysis
A putative quantitative trait locus (QTL) on porcine chromosome 13

SSC13: 26,203,876 – 30,067,019 bp

Plot of P-values for the association between *T. suis* FEC (week 8 p.i.) and SNPs located on chromosome 13; n=195
Validation studies

Three of the lead SNPs were selected for replication:

- stSG1354613_233 (‘ST’)
- IL00001116 (‘IL’)
- 0_DOCK3_DS076720.1_45 (‘DOC’)
Design of validation studies

Validation Study 1
82 piglets
Natural infection (T. suis + A. suum)
Trait: FEC (mean week 12 and 14)

Validation Study 2
31 piglets, 8 weeks old
Single infection (5,000 T. suis eggs)
Trait: FEC (mean day 47 and 51)

Validation Study 3
178 piglets
Trickle infection (weekly)
Duration: 6 wks
Trait: Worm counts
## SNP ST: Association with faecal egg counts

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position on SSC13 (bp)</th>
<th>Study</th>
<th>Faecal egg counts (FEC)</th>
<th>Genotypic means ± SD (median)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>stG1354613_233 (ST)</td>
<td>26,595,058</td>
<td>Discovery (n=195)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>531 ± 516 (520)</td>
<td>225 ± 407 (20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stG1354613_233 (ST)</td>
<td>26,595,058</td>
<td>VS1+VS2 (n=113)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>621 ± 1064 (75)</td>
<td>330 ± 1027 (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## SNP IL: Association with faecal egg counts

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position on SSC13 (bp)</th>
<th>Study</th>
<th>Faecal egg counts (FEC)</th>
<th>Genotypic means ± SD (median)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL0001116</td>
<td>26,667,538</td>
<td>Discovery (n=195)</td>
<td>AA</td>
<td>533 ± 505 (530)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AB</td>
<td>223 ± 399 (20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BB</td>
<td>51 ± 153 (0)</td>
<td></td>
</tr>
<tr>
<td>VS1+VS2</td>
<td>704 ± 1117 (160)</td>
<td>VS1+VS2 (n=113)</td>
<td>AA</td>
<td>704 ± 1117 (160)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AB</td>
<td>314 ± 1002 (50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BB</td>
<td>93 ± 282 (30)</td>
<td></td>
</tr>
</tbody>
</table>
No association with worm counts

<table>
<thead>
<tr>
<th>SNP</th>
<th>Study</th>
<th>Worm burden</th>
<th>Genotypic means ± SD (median)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>ST</td>
<td>VS3 (n=178)</td>
<td>162 ± 119 (150)</td>
<td>139 ± 145 (90)</td>
<td>63 ± 71 (50)</td>
</tr>
<tr>
<td>IL</td>
<td></td>
<td>163 ± 120 (150)</td>
<td>144 ± 145 (90)</td>
<td>63 ± 71 (50)</td>
</tr>
<tr>
<td>DOC</td>
<td></td>
<td>137 ± 131 (95)</td>
<td>145 ± 135 (120)</td>
<td>170 ± 127 (150)</td>
</tr>
</tbody>
</table>
Inspection of candidate region (SSC13)

Human homologue HSA 3p21.33-22.1: The QTL encompasses 31 genes

Four candidate genes were identified:

- **CX3CR1** - chemokine (C-X3-C motif) receptor 1
- **CCR8** - chemokine (C-C motif) receptor 8
- **VIPR1** - vasoactive intestinal peptide receptor 1
- **ACKR2** - atypical chemokine receptor 2
What we have delivered in this project

- Whole-genome scan of a resource population revealed a putative QTL on SSC13.
- We validated the QTL in unrelated populations.
- We have identified four candidate genes (chemokine receptors).
- We encourage further studies of the ST and IL markers and the candidate genes.
Acknowledgements

University of Copenhagen
Lise-Lotte Christiansen
Helena Mejer