Gene expression profiles defining host resistance to Infectious Pancreatic Necrosis in Atlantic salmon fry

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• Infectious diseases = serious problem for salmon farming
• Host resistance is universally heritable

Selective breeding can be applied to improve disease resistance

• Aquaculture species potentially useful models to understand genetic basis of host resistance to pathogens
IPN Resistance

- IPN – endemic birnavirus causes mortality at two windows
- **Stage 1: QTL Mapping Resistance Loci** - Single locus explains most genetic variation in resistance in fresh & sea water

- Marked contrast in mortality level between RR and SS homozygotes

![Chart showing genetic variation explained by QTL on chromosome 26](chart.png)

• **Stage 2: Goals:**
  - SNP markers to accurately predict QTL genotype @ population level
  - Understanding of underlying biological mechanisms

• **Advances in genomic tools and technology:**
  - Reference genome sequences for A. salmon and rainbow trout
  - Microarrays and RNA Sequencing
  - Tools for high density SNP genotypes
    i. RAD genotyping by sequencing & SNP array
    ii. Whole genome sequencing

<table>
<thead>
<tr>
<th>Population</th>
<th>Mortality Rate (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAD01 SNP Genotype</td>
</tr>
<tr>
<td></td>
<td>RR</td>
</tr>
<tr>
<td>TEST</td>
<td>0.10 (0.03)</td>
</tr>
<tr>
<td>VALIDATION</td>
<td>0.11 (0.01)</td>
</tr>
</tbody>
</table>

→ **Commercial application:**
SNP panel used as genetic test to select resistant broodstock

*Houston et al. 2012, BMC Genomics*
(iii) Whole genome sequencing pools of RR and SS fish

Yeargroup 1: 22 x SS = Pool 1
Yeargroup 2: 24 x SS = Pool 2
Yeargroup 1 22 x RR = Pool 3
Yeargroup 2 24 x RR = Pool 4

Illumina PCR-free
WGS 30X per pool
HiSeq 2500

Align reads to Atlantic salmon reference genome (99 % mapped)

Compare pools within and across yeargroups for QTL-concordant SNPs and other polymorphisms
Markers (nearly) concordant with QTL type;

- QTL SS fixed for susceptibility SNP allele
- QTL RR almost fixed for resistance SNP allele
IPN Transcriptomics

- Gene expression contrast between resistant and susceptible fry
  - Families of fry given bath challenge of IPNV

- Replicated challenge & control tanks for each TP
- Whole fry homogenate (pools of 6 biological replicates per condition); RNA extracted and hybridised to Agilent 44K array

Houston et al. 2010, Heredity

Test 1: Comparison of R and S families
• Microarray comparison of host response to infection in resistant and susceptible families

Gene expression susceptible families

Gene expression resistant families

Mechanisms of resistance likely to be early in infection process

Interferons and other cytokines

1 day

7 days

20 days

log2 (Fold-Change)

-log10 (p-value)
• RNA-Seq focus on early timepoints (prior to infection and 1 day post infection)
  – Offspring genotyped to determine underlying QTL status (RR and SS)
  – RNA extracted from whole fry and sequenced on Illumina HiSeq 2000

Test 2: Comparison of RR and SS genotypes within segregating families

- 3 segregating families
- 2 timepoints (0 and 1 DPI)
- 2 genotypes (RR & SS)
- 6 fry per condition
  ➢ 72 fry transcriptomes
IPN Transcriptomics

• Atlantic salmon fry transcriptome assembled
  ➢ 1.6 bn reads → aligned to ICSASG_v1 genome assembly
  ➢ ~113K transcripts from ~80K putative genes
  ➢ ~63% annotated, covers 88% of publicly-available ESTs

• Differential expression RR vs SS
  i. Genome-wide comparison of gene expression
  ii. Identification of human homologues → pathway enrichment
  iii. QTL region differential expression
Pre-infection (Timepoint 0) comparison of RR vs SS

- 628 significantly differentially expressed transcripts
- KEGG pathway enrichment (Kobas 2.0)
- Functional connection network (top 30)

<table>
<thead>
<tr>
<th>KEGG Pathway</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein processing in endoplasmic reticulum</td>
<td>0.001254</td>
</tr>
<tr>
<td>Fatty acid metabolism</td>
<td>0.001257</td>
</tr>
<tr>
<td>Influenza A</td>
<td>0.003696</td>
</tr>
<tr>
<td>Fatty acid biosynthesis</td>
<td>0.004733</td>
</tr>
<tr>
<td>PPAR signaling pathway</td>
<td>0.007884</td>
</tr>
<tr>
<td>Fructose and mannose metabolism</td>
<td>0.010228</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>0.011012</td>
</tr>
<tr>
<td>AMPK signaling pathway</td>
<td>0.011752</td>
</tr>
<tr>
<td>Biosynthesis of unsaturated fatty acids</td>
<td>0.017246</td>
</tr>
</tbody>
</table>

Possible link between IPN resistance and fatty acid metabolism (Morais et al. 2012)
**One day post-infection comparison of RR vs SS**

- 316 significantly differentially expressed
- KEGG pathway enrichment (Kobas 2.0)
- Functional connection network (top 30)

<table>
<thead>
<tr>
<th>KEGG Pathway</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tight junction</td>
<td>1.51E-05</td>
</tr>
<tr>
<td>Ribosome</td>
<td>3.45E-05</td>
</tr>
<tr>
<td>Protein digestion and absorption</td>
<td>0.000350864</td>
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<tr>
<td>Pancreatic secretion</td>
<td>0.003120276</td>
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<tr>
<td>Alanine, aspartate and glutamate metabolism</td>
<td>0.006104826</td>
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<tr>
<td>Biosynthesis of amino acids</td>
<td>0.007011666</td>
</tr>
<tr>
<td>Mineral absorption</td>
<td>0.013506223</td>
</tr>
<tr>
<td>Complement and coagulation cascades</td>
<td>0.031222676</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy (HCM)</td>
<td>0.048987447</td>
</tr>
</tbody>
</table>

May reflect responses to virus seen in susceptible fish only
IPN Transcriptomics

- Resistant and susceptible genotypes differ in their gene expression in pathways related to:
  - Cellular protein turnover
  - Tight junction regulation

- How to distinguish cause and effect?

Gamil et al. 2015, Viruses
Suzuki 2013, Cell Mol Life Science

IPNV modulates intestinal epithelial barrier for entry (Sundh et al. 2011)
• Differential expression of genes in QTL region (RR vs SS)
  ➢ Positional and functional candidates
Differential expression of genes in QTL region (RR vs SS)

Pre-infection

1 day post infection

Position on contig tcf1000000010_0-0_ssa26_0_0

Same gene diff expressed: RNASEq & microarray
Conclusions

i. 30 X WGS of homozygous resistant and susceptible pools → detailed polymorphism map of narrow QTL region
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iv. One transcript with constitutive differential expression in QTL region → potential cis-acting effect
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v. Cell protein turnover & regulation of tight junction have roles in host resistance to IPNV infection
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