

Using Transcriptomics to Develop Predictive Models for Identifying Pigs with Superior Immune Response and Improved Food Safety

Christopher K. Tuggle, Iowa State University

EADGENE European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety

Genomics for Animal Health: Outlook for the Future

13- 14th October 2009, Muséum National d'Histoire Naturelle, Paris, France

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EADGENE Finding genetic markers and RNA classifiers for improving pork safety

Collect Sal shedding phenotypes (n = 1,000+)

Collect sequence data from RNAs and predict SNPs in genes

Collect expression data on response to Salmonella infection

Create DNA Bank for community use

Select SNPs in interesting genes, genotype and test association

Test RNA classifiers for predicting shedding

Field validation of markers

Field validation of putative RNA classifiers

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EADGENE Challenge Study to Measure Phenotypic Variation in Immune Response

- 40 pigs infected with *S. Typhimurium* on day 0
- Fecal *Salmonella* shedding levels measured from day 2-20
- Shedding levels was used to select extreme phenotypic classes
 - Four animals showed little to no shedding after day 7 → "low shedders" LS
 - Six animals shed continuously up to day 20 → "persistent shedders" PS
- Whole blood RNA from all 10 animals at day 0 and day 2 was analyzed using Affymetrix chips

Why whole blood?
 1. Serial collection possible without killing subsets of pigs
 2. Practical sample collection on farm for many pigs

Utthe et al. 2009; Huang, Utthe et al. 2009
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EADGENE Phenotypic Variation in Immune Transcriptional Response

Number of *annotated* probesets with significant differences in blood expression

| | q shed (t0+t2) | q shed (at t0) | q shed (at t2) | q shed by infection |
|----------|----------------|----------------|----------------|---------------------|
| q < 0.05 | 0 | 0 | 243 | 1,442 |
| q < 0.10 | 0 | 0 | 1,313 | 3,308 |

Transcriptional response to infection depends on shedding class!
What are the pathways these genes represent?

Utthe et al. 2009; Huang, Utthe et al. 2009
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EADGENE NIH DAVID analysis of genes showing large response to infection (|FC| > 1.5; q < 0.1)

| Persistent shedders (PS) | Low shedders (LS) |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Generally: Intracellular response genes | Generally: Extracellular response genes |
| <ul style="list-style-type: none"> Response to biotic stimulus, immune resp. Proteasome, endopeptidase activity protein catabolism Protein kinase cascade, reg. of NFκB cascade programmed cell death, apoptosis immunoglobulin domain Vacuole/lysosome/lytic vacuole TOLL receptor signaling, NFκB/IL1R Sig. M. SCLEROSIS/DIABETES/ARTHRITIS SH2 domain Pleckstrin ASTHMA/LUPUS- Genetic Assoc CHOLERA-Genetic Assoc signal peptide intrinsic to plasma membrane signal transducer, receptor activity Extracellular matrix Morphogenesis, organ morphogenesis Cell-cell signaling Fibrinogen alpha/beta/gamma Ion channel activity | <ul style="list-style-type: none"> signal peptide, cell commun., receptor Response to biotic stimulus, immune resp. Integral to plasma membrane extracellular matrix cell-cell signaling, structural component carbohydrate/heparin binding angiogenesis tissue/organ development/remodeling Fibronectin type III organ morphogenesis cell cycle, M phase nucleosome, nucleus/nuclear protein nucleic acid binding, reg. of biol. process microtubule organizing center RNA localization, RNA splicing Response to DNA damage stimulus chromosome segregation Cellular processes- phys., metabolic Zinc finger, ion binding |

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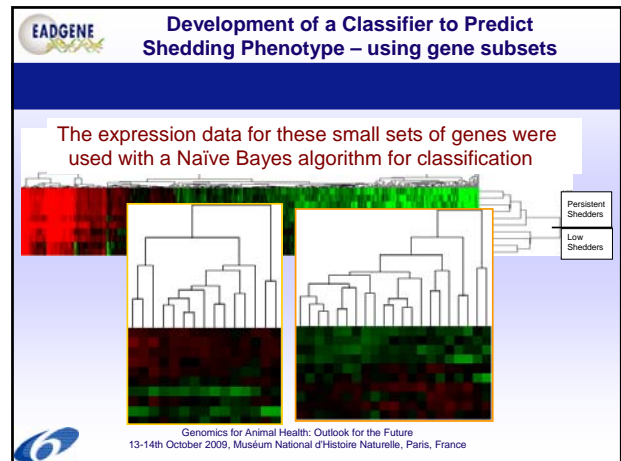
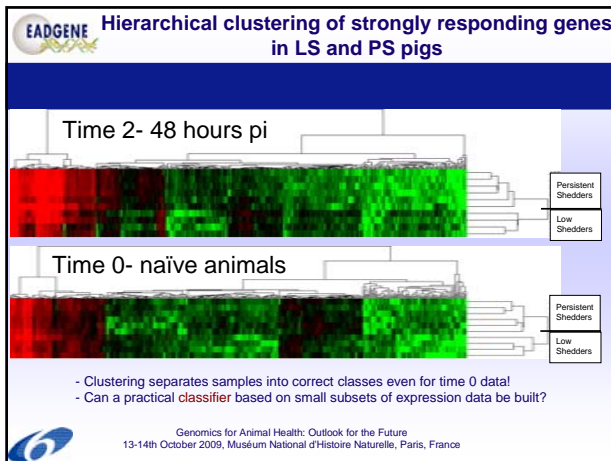
EADGENE Shed by infection interaction genes: Strong overlap of up-regulated genes in LS with down-regulated genes in PS

- 300 shed by infection genes with **strongly opposite** expression pattern in two classes!
- Low Shedder response is not just a "non-response"

q < 0.1 for shed by infection interaction; FC > |1.5| t2/t0

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Development of a Classifier to Predict Shedding Phenotype – using gene subsets

- Time 2 data correctly predicted the shedder class (Low or Persistent) 70-100% of the time.
- Similar results (80 % accuracy) were also achieved for three small classifiers built with time 0 data
- Thus there are sets of 18-25 genes whose pattern of expression-- *prior to infection*--can be used to distinguish these animals even before they express their shedding phenotype!

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Conclusions

- Differentially expressed gene lists identified very different biological pathways in LS and PS pigs
- Expression patterns of small sets of shed x infection genes could predict shedding phenotype
- New populations will need to be created to test this result- new grant funded in 2009:
 - ◆ Extend *in vivo* blood response RNA analysis to >100 animals
 - ◆ Integrate with *in vitro* blood response to LPS of same animals
 - ◆ Extend to field population of 500 animals with shedding data

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Questions?

Or time to party?

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