This little piggy went to market:
applications of genomics for sustainable pig health

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Iowa State University
Why is animal health so important?

- Disease causes loss of up to 15% of potential profit
  - Mortality
  - Product condemnation
  - Treatment costs (prevention and treatment)
  - Reduced production efficiency
  - Lower genetic gain
  - Reduced consumer acceptance
Comprehensive disease control program

- Biosecurity
- Sanitation
- Eradication
- Vaccination
- Antibiotics
- Genetic resistance
Why take a genetics approach to improve animal health?

- Improves animal welfare
- Environmentally sound approach
- Sustainable improvements
- Addresses consumer concerns about antibiotics
- Animal health affects human health
  - Zoonotic diseases
  - Food poisoning
Evidence for Host Resistance: Porcine Respiratory and Reproductive Syndrome (PRRSV)

- PRRS is a 700 million dollar issue
- Variation in severity among infected pigs (exp. challenges)
  - Antibody response and lung lesions:
  - Meishan < Duroc (Halbur et al., 1998)
- Evidence of genetic resistance relative to reproductive failure (Lewis et al., 2009)
  - matings per conception (PRRSV neg.)
    - $h^2 = 0.04$
  - matings per conception (PRRSV pos.)
    - $h^2 = 0.46$
The PRRS Host Genetics Consortium (PHGC)

Joan Lunney. USDA BARC         Bob Rowland, KSU

- Understanding the role of host genetics in resistance to PRRSV infection, and the effects of PRRS on pig health and related growth.
- Uses a nursery pig model to assess pig resistance/ susceptibility to primary PRRSV infection.
- After acclimation, pigs infected with PRRSV and followed for 42 days post infection (dpi).
- Blood samples collected at 0, 4, 7, 10, 14, 21, 28, 35 and 42 dpi, and weekly weights recorded.
Results of Genomic Analyses
JAS 90: 1733

Viral Load
- Chr 4
  - 1 Mb region explains 15% of genetic variance
  - Includes important candidate genes

Weight Gain
- Chr 4
  - 11% of genetic variance
Effects of SSC4 SNP WUR10000125 in PHGC (NVSL) and Genome Canada (KS06) trials

**VIRAL LOAD**

- **KS06**: n=683, -2.7±0.7, p=0.0002
- **NVSL**: n=1023, -4.5±0.6, p<0.0001

**WEIGHT GAIN**

- **KS06**: n=682, 1.0±0.3, p<0.0031
- **NVSL**: n=983, 2.0±0.3, p<0.0001
A SNP introduces a new acceptor splice site, which causes five nucleotides to be added to the transcript (GBP5).

The 5bp transcript causes a frame-shift in the protein that introduced an early stop codon.

The susceptible genotype is missing the C-terminus of the protein.  

Koltes et al. BMC Genomics 16:412.
Porcine Circovirus 2

- Circular single stranded DNA virus
- Infect monocytes, macrophages and dendritic cells
- Two main clusters of strains exist: PCV2a and PCV2b
- PCV2 is environmental stable and resistant to common disinfectants
- Pigs become seropositive between 6 - 18 weeks of age
- Vaccines available (2008)
Previous evidence suggested that host genetics influences PCVAD

- Zhou et al., 2006 - Sero-prevalence in 46 farms in Zhejian (China) was higher in Landrace than in Yorkshire and Duroc sows
- Opriessnig et al., 2006 - Landrace developed more severe lymphoid lesions than Duroc and Large White in experimental challenges
- Opriessnig et al., 2009 - Landrace had significantly more severe PCV2-associated lesions than Pietrain in experimental challenges
- Bates et al., 2009 - Variation in PCV2 immune response has an important host genetic contribution
 Genome Canada project: Application of genomics to improving swine health and welfare

N ~ 1,000
Genetic lines (n = 14)
Genetic programs (n = 7)
PCV2b experimental challenge – 28 days (n ~ 1,000)

LW: Large White
LR: Landrace

LWxLR

Average daily gain
PCV2 IgM and IgG (ELISA)
Viremia (Real Time qPCR)
Tempus blood collection (RNA)
Tail clips (Genotyping, 60K SNPs)

0 7 14 21 28 dpi

Ciobanu et al.
Indicators of PCVAD susceptibility (n=974)

PCV2 antibody response following PCV2b challenge

PCV2b viremia following experimental challenge

Average daily gain during PCV2b challenge
SSC7 and SSC12 explained ~ 15% of genetic variance

* Kachman, 2014
Alleles from 2 major SNPs influence viremia, immune response and growth during PCV2b infection.

No effects on fat or muscle growth, age at puberty, litter size traits, weight loss during lactation etc., in non-challenged pigs.
Frequency of favorable allele for the SNP located on SSC12 provides opportunities for Marker Assisted Selection.
Genomic Differences Between Pre-weaning Survival and Mortality of Piglets Following PEDV Outbreaks

Francesca Bertolini, PhD, Department of Animal Science, Iowa State University

Graham Plastow

John Harding

Benny Mote

Max Rothschild
Coronavirus

Infects the cells lining the small intestine
- Watery Diarrhea
- Vomit
- Dehydration

The incubation period is approximately 2 days and diarrhea lasts for 7 to 14 days

Rapid spread across all breeding and growing pigs with almost 100% morbidity (pigs affected) within 5 to 10 days

Porcine Epidemic Diarrhea (PED) virus

Song and Park, 2012
First reported in Europe in the 1970s (England and Belgium) and later in Asia, 1980s

First diagnosed in the United States in mid-May 2013

Reported in Canada in January 2014

Source: USDA
MORTALITY/RESILIENCE

AGE-DEPENDENT PEDV RESILIENCE
(Shibata et al. 2000)

Mortality

100%

PRE-WEANED PIGS

0%

POST-WEANED PIGS

ADULT PIGS
Alanyl (membrane) aminopeptidase

Antibodies against pAPN block the infection (Sik et al. 2003; Li et al. 2007)

**Immunofluorescence assays** (Li et al. 2007)

Other coronaviruses such as TGEV, HCoV-229E and FeCoV all use APN as their cellular receptor (Delmas et al., 1992; Yeager et al., 1992; Tresnan et al., 1996; Tresnan and Holmes, 1998; Kolb et al., 1998)
AIM

INVESTIGATE THE GENETIC DIFFERENCES BETWEEN SURVIVING AND DEAD PRE-WEANING PIGLETs THAT CAN BE LINKED TO PEDV RESILIENCE
**MATERIALS AND METHODS**

**Animals**

Dead and surviving neonatal pigs during the acute phase of a PED outbreak before the development of maternal antibody

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<th>ALIVE</th>
<th>TOT</th>
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<td><strong>106</strong></td>
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DATA ANALYSES

Genotyping with the 80K SNPchip

Retaining SNPs in autosomes and call rate > 0.90

1Mb windows

Mean Fst of each window

Normalization of the mean Fst value

GOrilla and Enrichr
RESULTS

Samples distribution
Results

Normalized Fst Plot

162 ANNOTATED GENES

APN=0.2
# GENE ENRICHMENT

**GOrilla**

<table>
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<tr>
<th>COMPONENT</th>
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<td>cell projection part</td>
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<td>Golgi membrane</td>
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## GENE ENRICHMENT

**GORILLA**

<table>
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<td>ATPase activity</td>
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<tr>
<td>substrate-specific transporter activity</td>
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<tr>
<td>ATPase activity, coupled to movement of substances</td>
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<tr>
<td>ATPase activity, coupled</td>
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Results

GENE ENRICHMENT

**Virus Perturbations from GEO up**
Click the bars to sort. Now sorted by **combined score**.

- SARS-dORF6_36Hour_GSE47962
- A-Vietnam-1203-2004(H5N1)_18Hour_GSE28166
- icSARA deltaORF6_3Hour_GSE33267
- SARS-CoV 24Hour_GSE47962
- icSARA deltaORF6_24Hour_GSE33267
- A-CA-04-2009(H1N1)_0Hour_GSE47960
- SARS-CoV MA15_Day4_PFU-10^4_GSE33266
- SARS-BatSRBD_24Hour_GSE47962
- hMPV_6Hour_GSE8961
- A-CA-04-2009(H1N1)MA_Day1_GSE36328

**Virus Perturbations from GEO down**
Click the bars to sort. Now sorted by **combined score**.

- HHV-8_72Hour_GSE6489
- icSARA deltaORF6_12Hour_GSE33267
- A-Vietnam-1203_CIP048_RG4-2004(H5N1)NS1trunc_24Hour_GSE43204
- SARS-CoV_24Hour_GSE17400
- A-Netherlands-602-2009(H1N1)_36Hour_GSE40844
- A-CA-04-2009(H1N1)MA_Day3_GSE36328
- icSARS CoV_36Hour_GSE33267
- SARS-BatSRBD_12Hour_GSE47960
- icSARS-CoV_60Hour_GSE33267
- icSARA deltaORF6_36Hour_GSE33267

**GENES** under or over expressed in several virus infections, including **Coronaviruses**.
CONCLUSIONS

Found evidence of several genes that can be linked to pig resilience

- Genes linked to Golgi apparatus
- Genes involved in the regulation of ER
- Genes involved in ion transports

Further studies to confirm and investigate the role of each gene in pre-weaning pig resilience
PEDV Study 2

viral inoculum - ~50% survival rate  Case and Controls

❖ 3 farms
❖ VD = found dead with diarrhea
❖ VM = terminated pig for welfare reasons before it died naturally
❖ VS = survived for 7 days
❖ We considered both VM and VD as DEAD

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<td>VS</td>
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DEAD=143
SURVIVED=61


mFst analysis with 500kb overlapping windows

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9: 680000000-690000000: common in the first analysis
mFst analysis with 500kb overlapping windows combining the 2 PEDV data sets

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Regions partially overlapping
Regions totally overlapping in both
New regions
DETAILS FOR CHROMOSOME 7 AND ANPEP GENE

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PEDV1+PEDV2 1Mb 500Kb overlapping

ANPEP gene Window

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A new approach:

Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus


Nature Biotechnology 34, 20–22 (2016)
doi:10.1038/nbt.3434
Published online 07 December 2015

http://www.nature.com/nbt/journal/v34/n1/full/nbt.3434.html
UK: African Swine Fever-resistant pig created

A disease-resistant piglet has been produced using new genetic engineering. It is the first animal created from ‘gene-editing’.

The piglet was born four months ago at Edinburgh’s Roslin Institute, and is known as ‘Pig 26’. ‘Gene-editing’ involves researchers spinning the DNA of the piglet’s parents together to create a disease-resistant offspring.

Live pigs produced from genome edited zygotes

According to researchers, the piglet’s DNA was manipulated in the laboratory to make it resistant to African swine fever. The manipulated DNA was then introduced into the egg cell, allowing the piglet to develop.

Species-specific variation in RELA underlies differences in NF-κB activity: a potential role in African swine fever pathogenesis

Efficient TALEN-mediated gene knockout in livestock
Improving Sustainability

- Genomic identification and selection could be designed and applied to improve resistance in nucleus herds:
  - Reduce the production cost
  - Increase robustness
  - Improve welfare
- Consider identifying gene targets for gene editing or designer breeding
Acknowledgments

- PIG GENOME COORDINATION PROGRAM
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