




## Approaches to filling that "Phenotype Gap"

Liz Glass




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

## Infectious disease resistance

To discover genetic markers or polymorphisms that control variation in a trait, you need two linked features:  
**Phenotype**  
**Genotype**




With genome sequences, massive parallel sequencing, SNP chips, etc etc we may have the technologies for Genotypes solved. Possible that pedigrees are no longer required either. It is also relatively easy to collect DNA (blood/tissue/hair follicle/swab).  
**But:**  
**Phenotype Gap –**  
 Additional complexities relating to infectious disease resistance traits (compared to production traits like growth)

## Complexity of disease resistance traits




ENVIRONMENT (age/sex/maternal antibody/housing/nutrition/exposure/previous exposure)

	Not exposed	Exposed Not infected	Infected: Innate immunity clears	Infected: Acquired immunity protects	Infected: Acquired immunity insufficient	Pathogen
TIME	-ve	-ve/+ve	+ve/-ve	+ve	+ve	Symptoms & Measurable effects
	None	Asymptomatic	Few	More	Pathology Chronic Death	
	GENES (Many of small effect)					
	Variable & time dependent OUTCOME					




## Phenotype Requirements

- **Phenotype:**
  - Need appropriate & relevant measure
  - In appropriate cell or tissue or whole animal
  - At the appropriate time
  - Labour intensive
  - Collect data on large numbers of individuals








## What do breeders/farmers want?

- Healthy fertile livestock that produce well under all environments
- And have resistance to many (all) pathogens
- Feasible?

Phenotype	Advantage	Disadvantage
Symptoms only E. g. veterinarian observation	not invasive	Need observers Time dependent/Not precise/End point Field data/epidemiology
Pathogen presence	Better precision	Require samples/field data/epidemiology
Inherent, basal immunity	No challenge	require appropriate samples: DNA candidate genes & or in vitro tests Underlying infections unknown
Innate immunity:	Closer relationship to underlying genetics?	Requires samples as above
Response capability Response	Pathogen Nonspecific Specific May orchestrate adaptive	Appropriate in vitro tests Short-lived - hours to days Also requires pathogen test
Adaptive immunity:		Samples: appropriate tests and time points Less close relationship to underlying genetics?
Response capability Response	Pathogen specific Longer lived: weeks to lifetime	Few tests of adaptive immunity capability In vitro/ex vivo "correlates of protection" often unknown
In vivo response to challenge	Experimental: Precise Field: Cheaper?	Pathogen specific; Expensive! Logistics! Less precise

### Solutions?

Solution	Advantage	Disadvantage
Vaccine response	Some vaccines given routinely Pathogen dose/timing is constant Blood sample(s) Response reflects pathogen infection	Can't measure resistance to pathology/death Many diseases: no vaccines May not reflect pathogen infection Acquired immunity/pathogen specific In vitro/ex vivo "correlate of protection" often unknown Blood sample may be inappropriate tissue
Candidate genes	No challenge Blood sample SNP test: causal mutation	Many! SNPs - many!
Inherent basal immunity	No challenge Blood sample	Blood sample may be inappropriate tissue Labour intensive; what test?

### Approach: inherent/basal immunity

- Traits measurable from blood sample
- Need High-throughput tests
- Repeatable
- Correlate with growth
- Heritable in different environments
- = Natural Killer cell & monocyte levels:
  - Flow cytometry for cell specific markers
- = acute phase proteins ( $\alpha_1$  acid glycoprotein)
  - Specific antibody test

### Innate immune traits

**Natural Killer cells:**  
Surveillance, Alarm!! Kill!!  
Cells infected with **Intracellular pathogens** e.g. viruses, bacteria, fungi, protozoa  
Recognise "altered"/"missing" MHC class I  
Contain toxic granules  
Produce cytokines: e.g. Interferon  $\gamma$

**INFLAMMATION**  
INFECTION

**Monocytes Macrophages:**  
Surveillance, Alarm!!  
Ingest and kill many types of pathogen  
Produce pro-inflammatory cytokines

### Heritability

Study: between 647 and 1549 pigs were tested across 7 farms, mainly Large White breed; few Landrace; apparently healthy

Trait	Overall $h^2$ (Se)	SPF	Non-SPF
White blood cells	0.28 (0.08)	0.29 (0.13)	0.28 (0.11)
% NK cells	0.35 (0.09)	0.46 (0.12)	0.07 (0.08)
% Monocytes	0.28 (0.09)	0.26 (0.11)	0.26 (0.13)
AGP (APP)	0.48 (0.1)	0.49 (0.14)	0.48 (0.14)
Daily Gain	0.25 (0.06)	0.40 (0.07)	0.13 (0.07)

Conclusions:

- All traits are heritable i.e. significant proportion of the variation is explicable by genetics
- NK cell variation under non-SPF conditions is essentially environmental, variation in monocytes and AGP are similar under SPF & non-SPF

### Correlations with daily gain

Trait	SPF Genetic r	SPF Environ r	Non-SPF Genetic r	Non-SPF Environ r
White blood cells	-0.06 (0.2)	-0.03 (0.05)	-0.69 (0.36)	-0.10 (0.05)
% NK cells	-0.08 (0.2)	-0.14 (0.05)	-0.68 (0.29)	-0.16 (0.06)
% Monocytes	-0.46 (0.23)	0.17 (0.05)	0.27 (0.39)	-0.02 (0.05)
AGP (APP)	-0.53 (0.2)	-0.49 (0.05)	-0.72 (0.22)	-0.48 (0.04)

Conclusions:

- Strong significant relationships: all negative
- High levels = poor growth
- Relationships may reflect subclinical infections
- NK cells, monocytes and particularly AGP may make good phenotypic markers for selecting pigs better able to resist infection

### Genetic markers: porcine SNP chip (4167 SNPs)

SSC

Mainly even coverage across genome

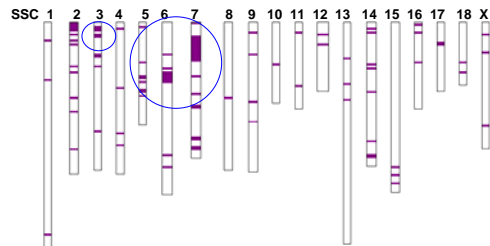
## Correlation of SNP markers with traits

960 pigs: mostly from previous studies:  
 Many traits including NK, monocytes, AGP

1. trait = genetic + environment + error (residual)  
 correlate each SNP v residuals of each trait
  2. re-test significant SNP - mixed model analysis  
 trait = genetic + environment + error + SNP xxx
- 155 SNPs were significant



## Positions of significant SNPs



## SSC 3: Significant SNPs

TRAIT:	Mb	Candidate GENES Mb
NK cell:	10.76	CD11b 14.06
	14.49	CD11c 14.28
Monocyte:	13.89	CD11a (LFA-1) 14.78
	14.38	



## SSC5: significant SNPs

TRAIT:	Mb	Candidate GENES Mb
NK cell	51.39	Natural Killer Cell Complex
	53.55	NKG2D 60.0
	58.45	Ly49 60.2
	63.61	CD69 61.0
	63.62	



## SSC 6: significant SNPs

TRAIT:	Mb	Candidate GENES Mb
AGP	44.6	Leukocyte Receptor Cluster
	47.82	DAP10 & 12 44.7
NK cell	51.08	IRF3 52.8
APP	52.0	LILR 54.4
	52.8	KIR2DL1 54.4
		FCAR 54.4
		NCR1 54.4

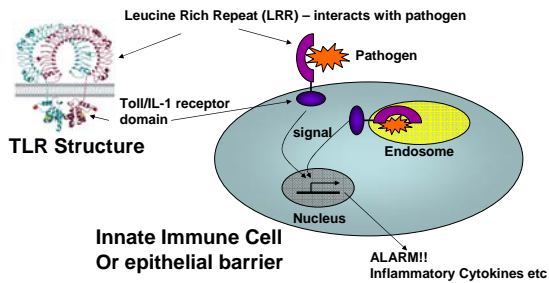


## Approach: Candidate genes

- Genes expressed during early host-pathogen interaction
- Conservation between species, so essential for survival
- Yet variable because of pathogen pressure
- Pattern recognition receptors (PRR)
- "Sense the enemy within"
- Recognise pathogen specific "molecular patterns"
- Expressed on innate immune cells
- Signal alarm/bridge between innate & acquired
- Major family group of PRR: Toll-like receptors
  - Comparative analysis has identified non-synonymous SNPs that are positively selected (i.e. advantageous) within the ligand binding domains
  - Most likely to be functionally relevant & therefore potential markers for disease resistance (Jann et al, 2008)



## TLR structure & function



## Comparative Genomics

- Mapped all TLRs (10) & downstream signalling molecules in cattle, pigs, sheep
  - Compared to humans/mice
  - Identified all the chromosomal regions associated with disease resistance (QTL)
  - Do they overlap?
  - Differential expression? (transcriptomic data)
  - functional relevance?



## Most likely candidates

Gene	function	expression	QTL overlap/associations
<b>TLR6</b>	Detection of ligands originating from gram-positive bacteria	mouse strains of divergent general disease resistance status	<ul style="list-style-type: none"> <li>• Clinical Mastitis in cattle</li> <li>• Susceptibility to <i>Listeria</i> infections in mice</li> </ul>
<b>TIRAP</b>	Adapter protein mediating signalling after TLR-ligand binding	mouse strains of divergent disease resistance status post infection with <i>Trypanosoma congolense</i>	<ul style="list-style-type: none"> <li>• Somatic cell score in cattle</li> <li>• Tuberculosis severity in mouse</li> <li>• Association with Tuberculosis in human</li> </ul>
<b>MyD88</b>	Adaptor protein mediating TLR signalling after TLR-ligand binding	mouse strains of divergent disease resistance status post infection with <i>Trypanosoma congolense</i> and <i>Yersinia enterocolitica</i> and uninfected animals	<ul style="list-style-type: none"> <li>• <i>Trypanosoma</i> resistance in cattle</li> <li>• <i>P. chabaudi</i> Malaria tolerance in mice</li> <li>• <i>B. burgdorferi</i> associated Arthritis in mice</li> </ul>
<b>IRF3</b>	Activation of IFN- $\beta$	mouse strains of divergent disease resistance status post infection with <i>Yersinia enterocolitica</i>	<ul style="list-style-type: none"> <li>• Nematodirus FEC in sheep</li> <li>• Clinical mastitis in cattle</li> <li>• Coxsackie virus B3 sensitivity in human</li> <li>• Tuberculosis resistance in mice</li> <li>• Immune traits in pigs</li> </ul>

Jann et al, 2009, BMC Genomics



## Future Perspectives

- More genome sequences
  - Need more functional annotation especially as innate immune gene families vary between species
- More SNPs and larger SNP chips
  - Need more high-throughput relevant parameters
  - Need more disease-resistance trait studies
- Phenotypes
  - Innate immune related: may provide resistance to many pathogens
  - Need more high-throughput relevant parameters
  - Need more disease-resistance trait studies
- Pig study
  - relevant to other species?
  - Phenotypes & genotypes do they predict?
  - Mechanisms?
- Candidate genes
  - Expressed in innate immune system
  - Within and between species comparisons: identify relevant SNPs
  - Comparative genomics may help predictions



## Collaborators & Funders

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- Dirk Werling, RVC
- George Russell, MRI
- Tracey Coffey, IAH Compton
- Jayne Hope, IAH, Compton

