

Variation in chicken (innate) immune genes

Pete Kaiser (IAH)



EADGENE



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Why study the genetics of chicken immunity?

To reduce the risk of food-borne zoonoses, reduce the use of antibiotics and feed additives, and reduce the level of pollutants from chickens

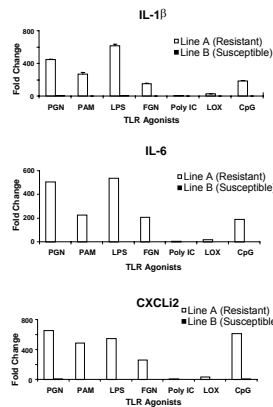
To deliver more sustainable breeding strategies that meet consumers' need for safe, high quality food and sustainable food production

Additionally we will gain a more detailed understanding of the mechanisms and pathways involved in chicken disease resistance

Salmonella

Cobb-Vantress lines:

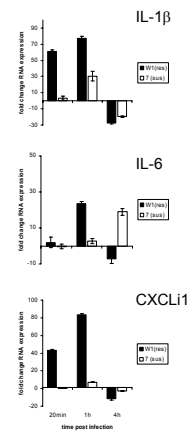
- differ in their resistance to *Salmonella* Enteritidis
- heterophils from line A produce a greater induced innate response than heterophils from line B, as illustrated by the cytokine and chemokine response following stimulation with TLR agonists



Salmonella

IAH lines:

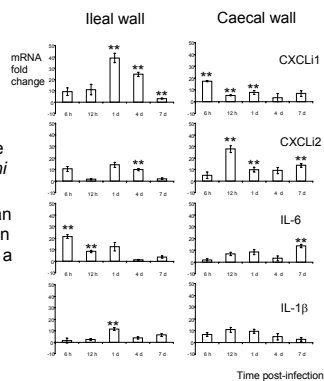
- differ in their resistance to *Salmonella* Typhimurium
- macrophages from line W1 produce higher levels of pro-inflammatory cytokines and chemokines than those from line 7₂



Campylobacter

IAH lines:

- differ in their resistance to *Campylobacter jejuni*
- infection does induce an innate response, both in terms of cytokines and a heterophil influx



Main experimental interest is to identify genes, genetic variation and molecular pathways that control and coordinate the immune response.

How do we map innate immune resistance genes?

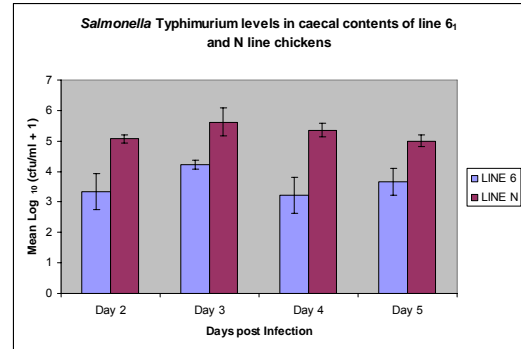
1. Candidate genes
2. Whole genome association (WGA)
3. eQTL - microarray studies; yields fresh candidates

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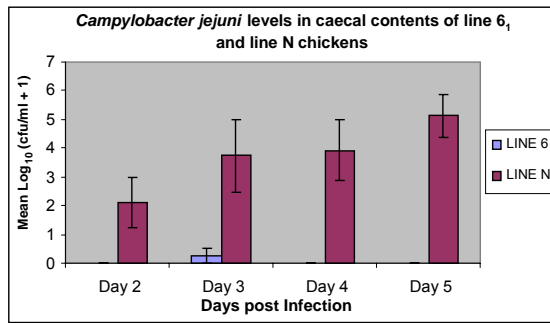
Inbred lines of chickens that differ in their resistance to gut pathogens;
i.e. low genetic heterogeneity with phenotypic differences

Disease resistance of IAH inbred lines														
line	Coccidiosis						Salmonellosis				IBV	IBDV	MDV	
	E. acervulina	E. praecox	E. necatrix	E. maxima	E. mitis	E. brunetti	S. typhimurium	S. gallinarum	S. enteritidis	S. pullorum	S. typhimurium colonisation	antibodies mortality	antibodies mortality	viral load
BrL	M	S	S	M	S	M								
WI	S	R	R	R	R	R	S	R	R	R		M	R	M
151	S	S	S	S	S	S	S	S	S	S		M	M	M
7	S	S	R	M	S	S	R	S	S	S		S	S	S
6	S	S	R	S	S	S	R	R	R	R	R	M	R	R
C	R	R	R	R	S	S	S	S	S	S		S	S	S
N	R	R	R	M	S	R	R	R	R	R	S	M	R	R
O	R	S	M	M	S	R	R	S	S	S		M	R	R
P												M	R	S
Sykes												S	M	S

IAH lines 6₁ and N – S. Typhimurium gut colonisation



IAH lines 6₁ and N – C. jejuni gut colonisation



Candidate Gene Approach

- human genome contains ~510 genes with the GO annotation "innate immunity"
- have identified ~480 of these in the chicken
- initially targeted ~100 of these
- line 6₁ and line N DNA sequenced for informative SNP ID
- check SNP homozygosity in parent line DNAs
- genotype SNPs in archived and novel backcross DNAs to identify association with disease resistance or susceptibility

Innate Immune Gene Panel					
TLR pathway:	Interleukins:	Chemokines:	Chemokine receptors:	Defensins/Gallinacins:	Other AMPs:
Tollip	IL-1beta	chCXCL1	chXCR1	GAL1	LEAP-2
IRAK-4	IL-10	chCCL15	chXCR1	GAL2	
MyD88	IL-19	chCCL16	chCCR4 (CCR2)	GAL3	Other PRRs:
TRAF6	IL-22	chCCL17	chCCR6 (CCR5)	GAL4	NOD1 (CARD4)
MAP3K7IP1 (TAB1)	IL-26	chCCL18		GAL5	IFIH-1 (MDA-5)
MAP3K7IP2 (TAB2)	IL-17A	chCCL19	TNFSF members:	GAL6	
MAP3K7 (TAK1)	IL-17B	chCCL10	TNFSF4 (OX40L)	GAL7	Interleukin receptors
TLR1/6/10 gene 1	IL-17D	chCCL11	TNFSF6 (FASL)	GAL8	IL-1R1
TLR2 type 1	IL-17F	chCCL12	TNFSF15 (VEG1)	GAL9	IL-1RL2
TLR2 type 2	IL-2	chCCL13	TNFSF8 (CD301)	GAL10	GP130
TLR3	IL-15	chCCL14	TNFSF5 (CD40L)	GAL11	
TLR4	IL-21	chCXCL1	TNFSF10 (TRAIL)	GAL12	Other genes:
TLR5	IL-12alpha	chCXCL2	TNFSF11 (RANKL)	GAL13	Caspase1
TLR7	IL-12beta	chCXCL3	TNFSF13B (BAFF)		MIF
TLR15	IL-4		TRAIL-L		N-RAM1
TLR21	IL-5				
IKK-alpha	IL-13				CSFs:
IKK-beta	IL-3				GM-CSF
IkappaB-alpha	IL-6				G-CSF
NF-kappaB1	IL-7				TGFs:
NF-kappaB2	IL-9				TGF-beta2
	IL-16				TGF-beta3

Red = informative SNPs found
Blue = uninformative SNPs found

SNP Discovery Sequencing



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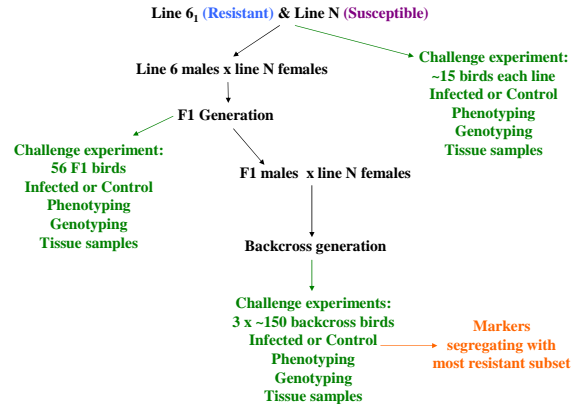
No SNP identified:

Some genes have identified no SNP despite large areas of gene sequenced. For example the TLR pathway:

- Tollip >2 kb
- TLR2 (type 1) >2 kb
- TLR2 (type 2) 1.7 kb
- TLR3 1.8 kb
- TLR4 >3 kb
- TLR5 >1 kb
- TLR7 >3 kb
- TLR15 1.8 kb
- TLR21 >2 kb
- IκBα >2 kb
- IKKα >2 kb
- NF-κB1 >1 kb

Most other genes only 400-500 bp sequenced so far

Outline: *Salmonella* and *Campylobacter* backcross and challenge experiments



eQTLs – Expression Microarray

Microarray analysis: whole genome cDNA array (ARK-Genomics compiled; contains Ensembl gene set plus BBSRC ESTs plus targetted immune function genes)

Comparing gene expression in lines 6₁ and N (collaboration with Rima Zoorob, CNRS). *Salmonella* arrays run and being analysed. *Campylobacter* arrays run next month.

Whole genome SNP Genotyping

Two chicken WGA SNP panels, each of approx 3000 markers

Illumina “GoldenGate” platform - high multiplex SNP genotyping

Analyze linkage disequilibrium and SNP heterogeneity between lines

Determine further informative SNPs between lines of interest

BeadStation 500 Powerful & Economical BeadArray™ Platform



- Confocal laser scanning system
- Compact bench top system
- >30 000 genotypes per day
- 0.5-5p per genotype (but £150,000 for the machine!)

Illumina SNPs - results so far...

- Both SNP sets have now been run, only first analysed
- Call frequency of 98% per SNP
- Call rate of 99.6% for each sample
- 390 informative line 6₁/N SNPs confirmed
- Potentially additional 51 markers
- Therefore we expect ~900 genome-wide SNPs for mapping disease resistance for this cross, AND ANY other cross between our inbred lines, from these two 3K SNP panels
- Compares to 100-150 informative microsatellites for any cross
- Also, INRA using another WGA of 9K on the same lines

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Short Term Objectives

Develop line 6/N Illumina SNP panel from:

- SNPs identified through candidate gene sequencing
- SNPs identified through whole genome panel
- Genes and pathways of interest from microarrays

Run Illumina genotyping array on backcross DNA samples
(focus on birds with low and high resistance phenotypes)

Analyse results for markers associated with disease
resistance phenotype

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