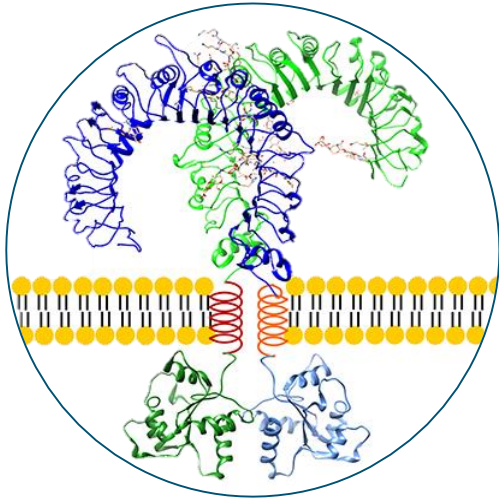


Investigation of a (putative) causal mutation in Toll-like receptor family member 1A in chickens

Animal Genetics and Disease 2019

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Co-authors & Acknowledgment



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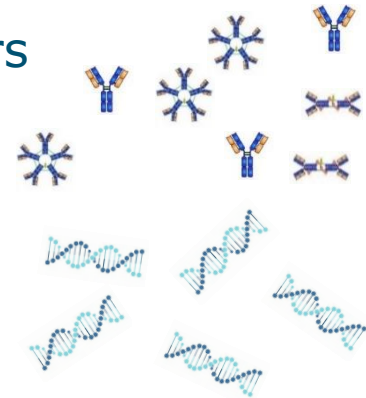
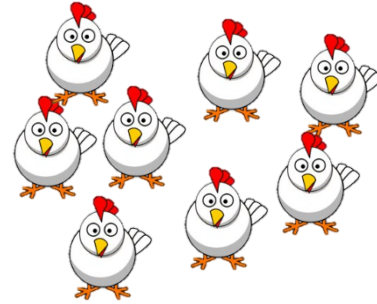
Jos van Putten



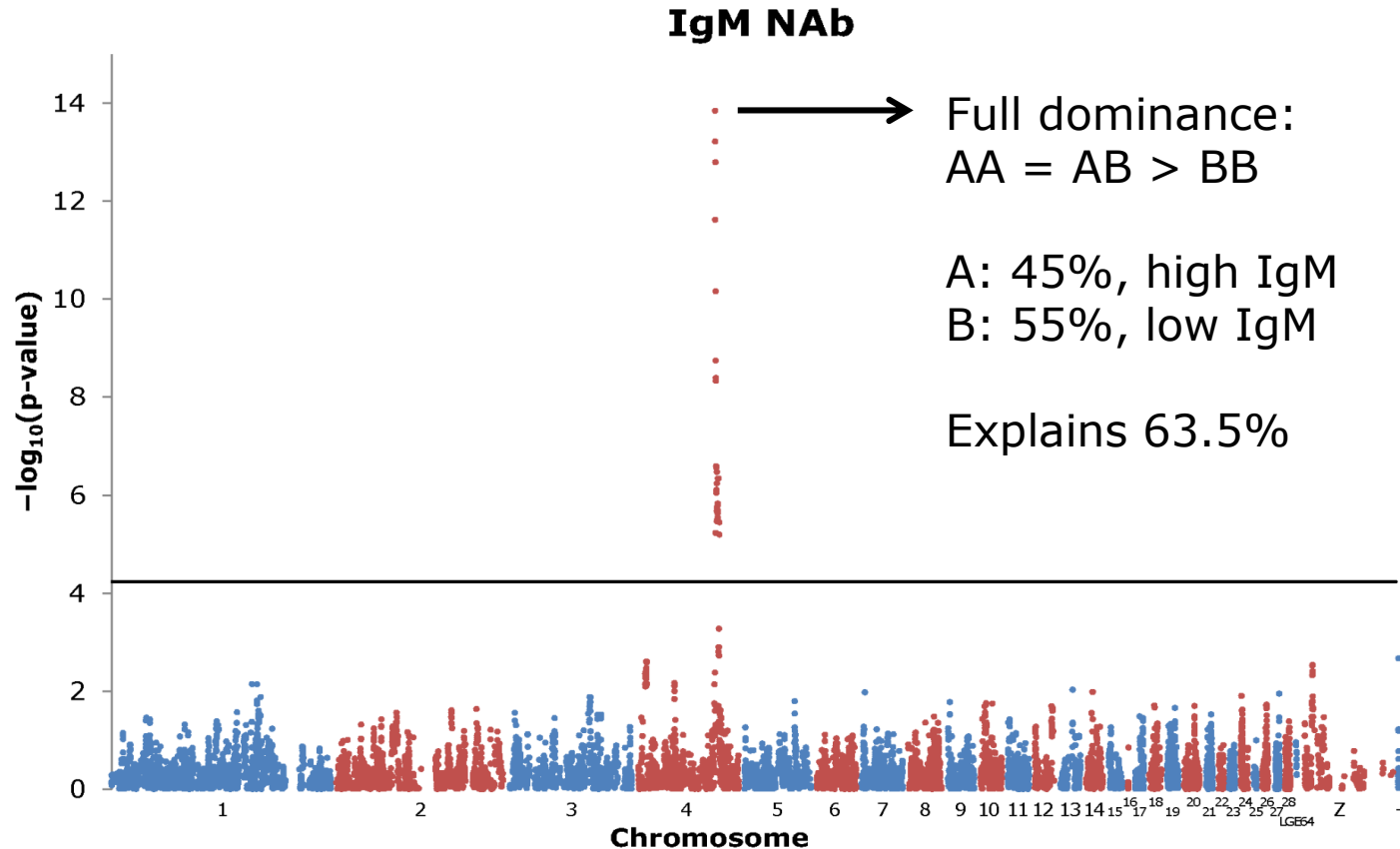
Genome-wide association study



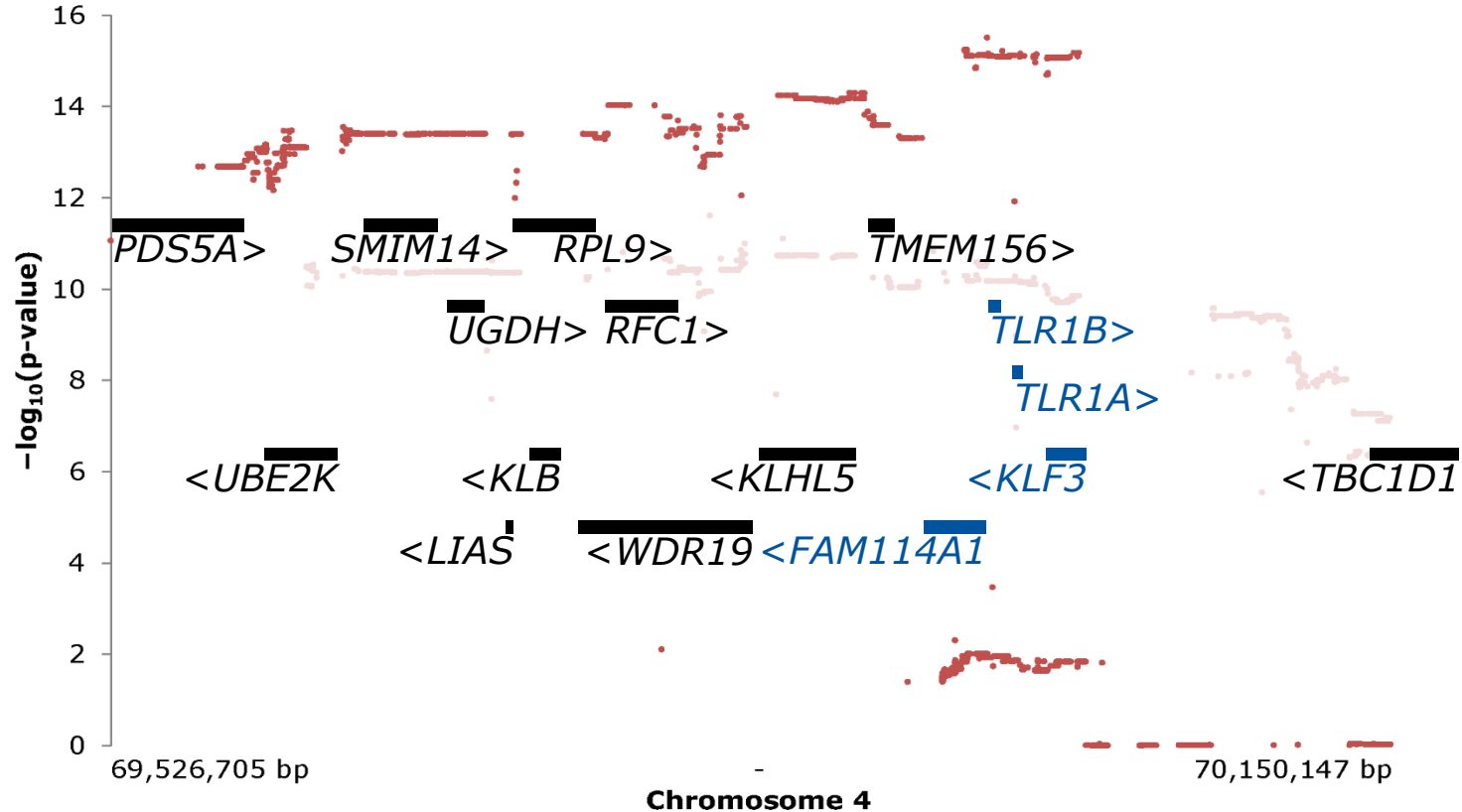
- 1,628 purebred white layer individuals (σ + η)
 - Around 16 weeks of age
- Total, IgM, IgA, IgG KLH-binding natural antibody titers
- 57,636 SNP
 - After quality control: 15,578 SNP



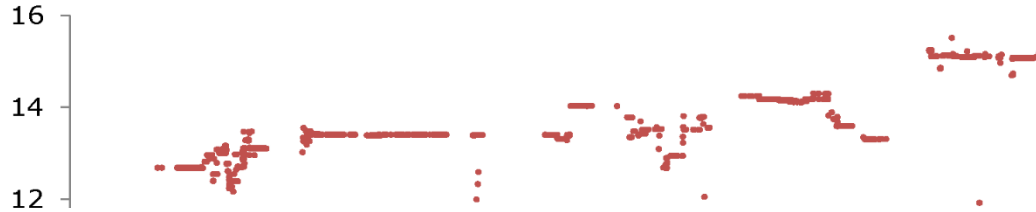
Manhattan plots IgM



Fine mapping of GGA4 for IgM NAb



Fine mapping of GGA4 for IgM NAb

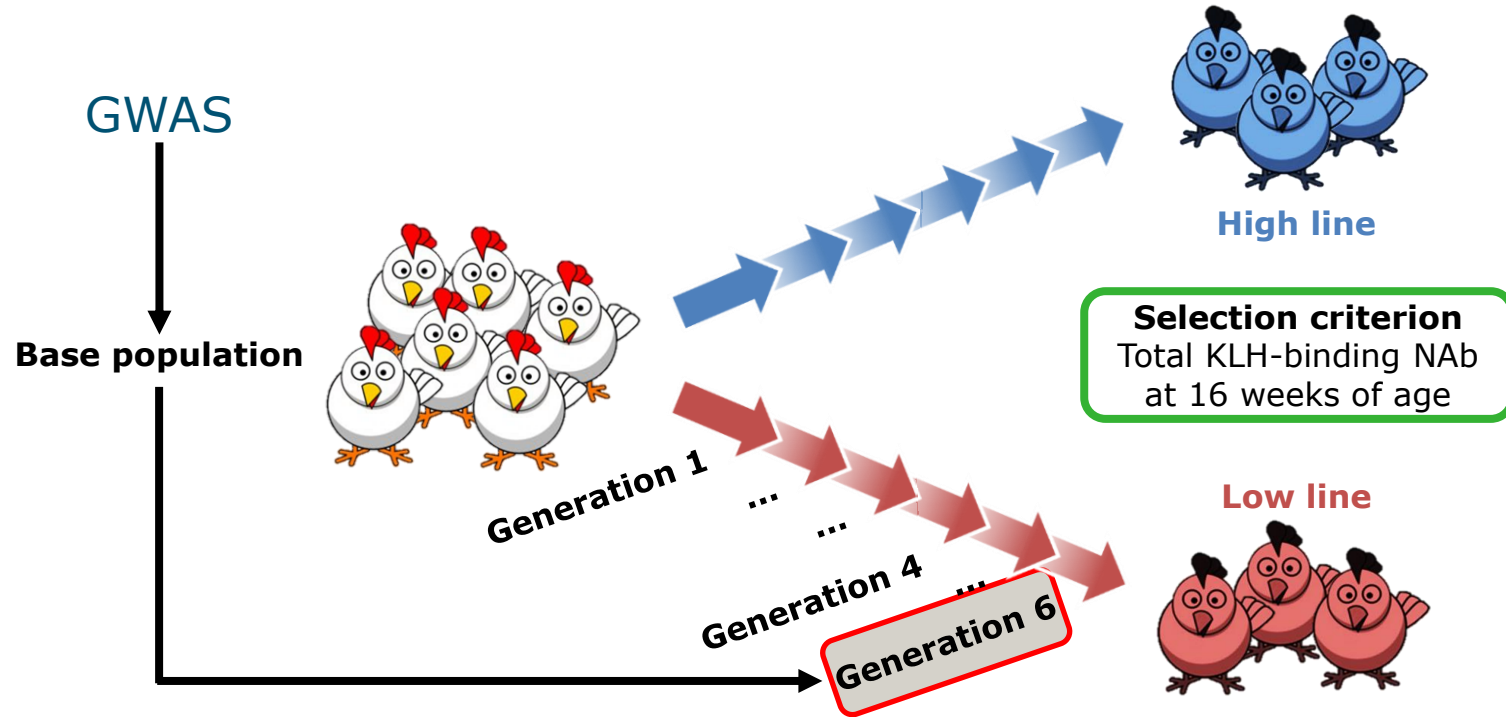


Genomic Region Containing Toll-Like Receptor Genes Has a Major Impact on Total IgM Antibodies Including KLH-Binding IgM Natural Antibodies in Chickens

 Tom V. L. Berghof^{1,2*},  Marleen H. P. W. Visker¹,  Joop A. J. Arts²,  Henk K. Parmentier²,  Jan J. van der Poel¹,  Addie L. J. Vereijken³ and  Henk Bovenhuis¹



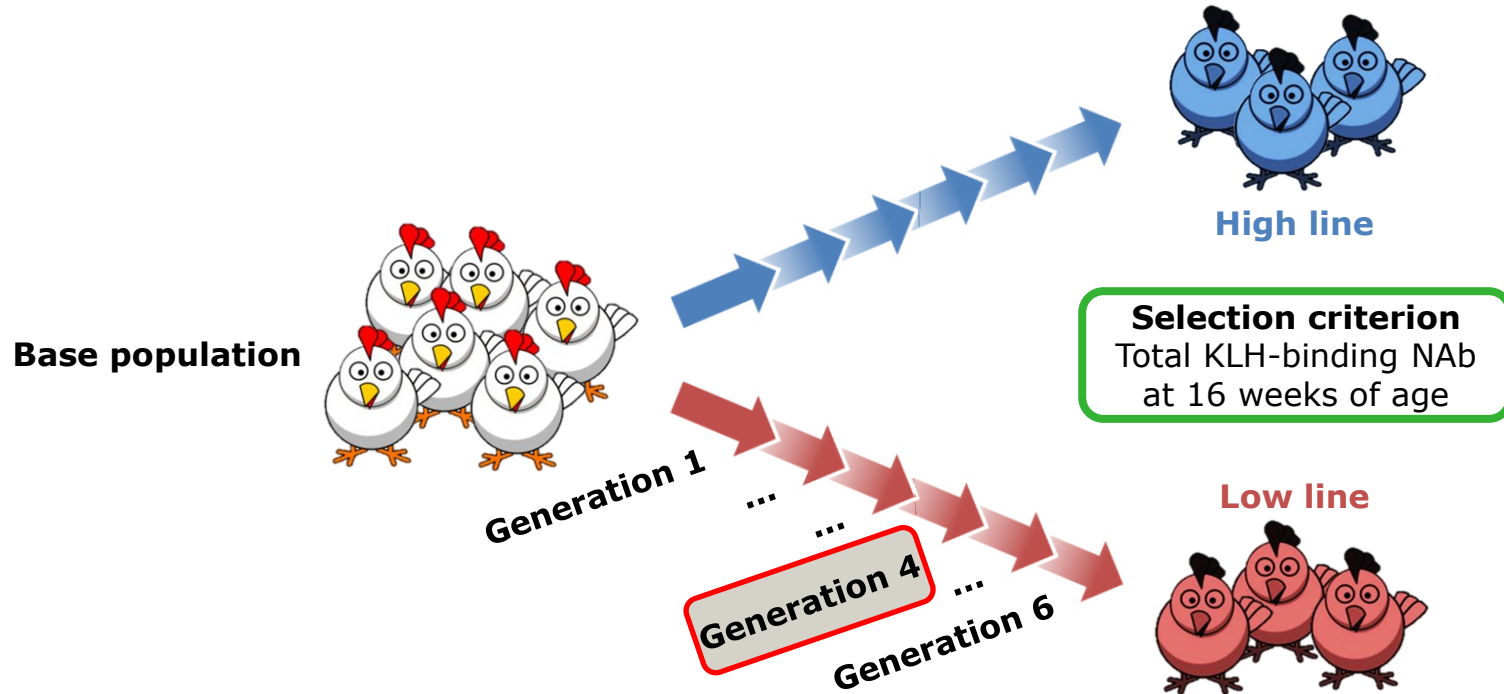
Selection experiment on natural antibodies



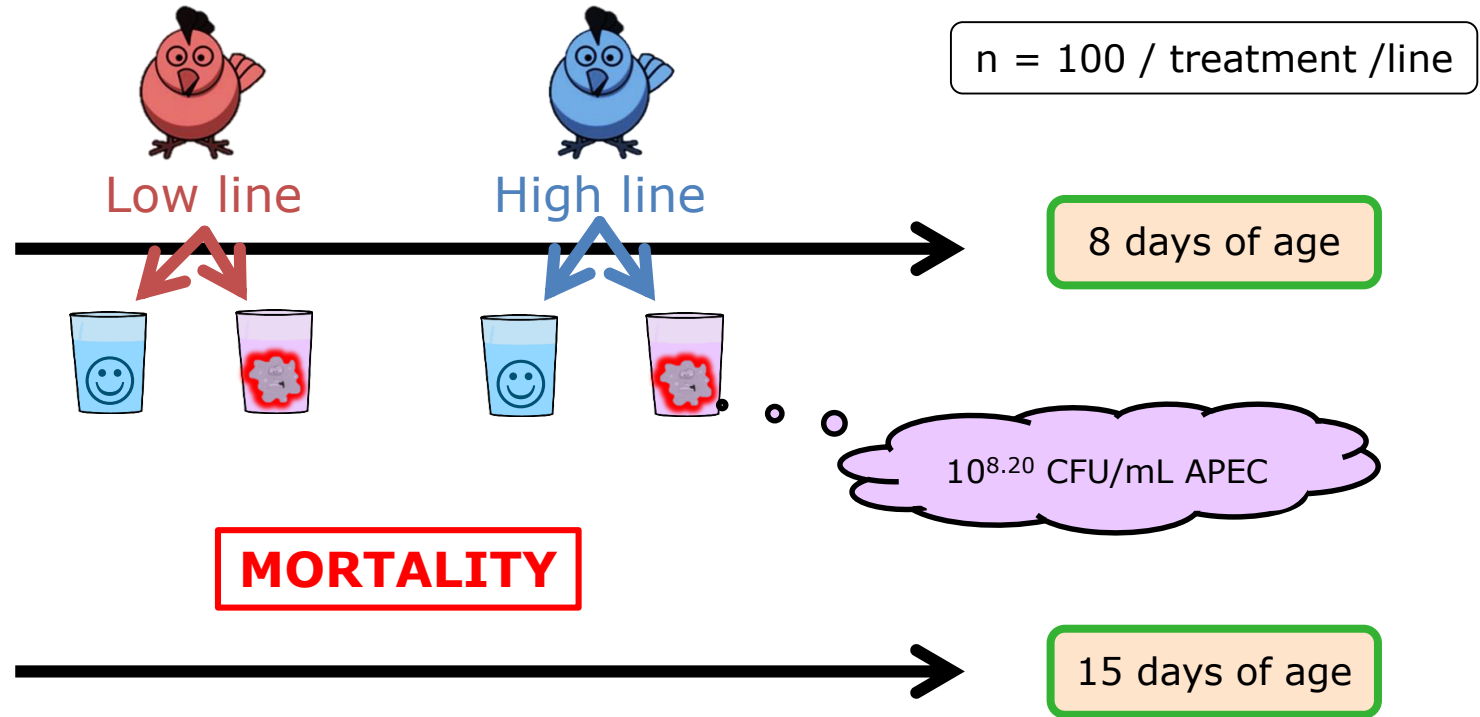
Allele frequency changes

Population		A-variant (dominant, high IgM)	B-variant (recessive, low IgM)
GWAS		0.45	0.55
Generation 6	High line	0.64	0.36
	Low line	0.07	0.93

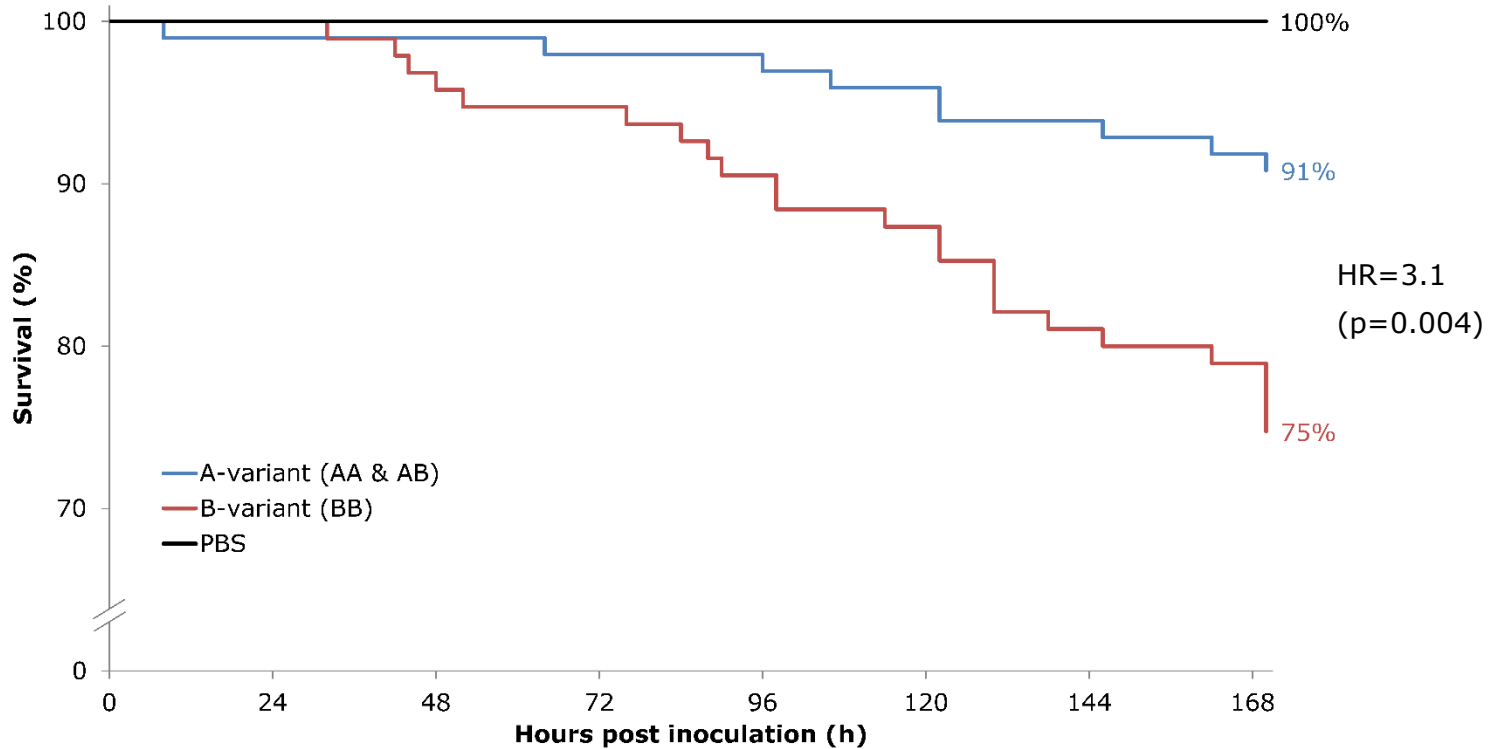
Selection experiment on natural antibodies



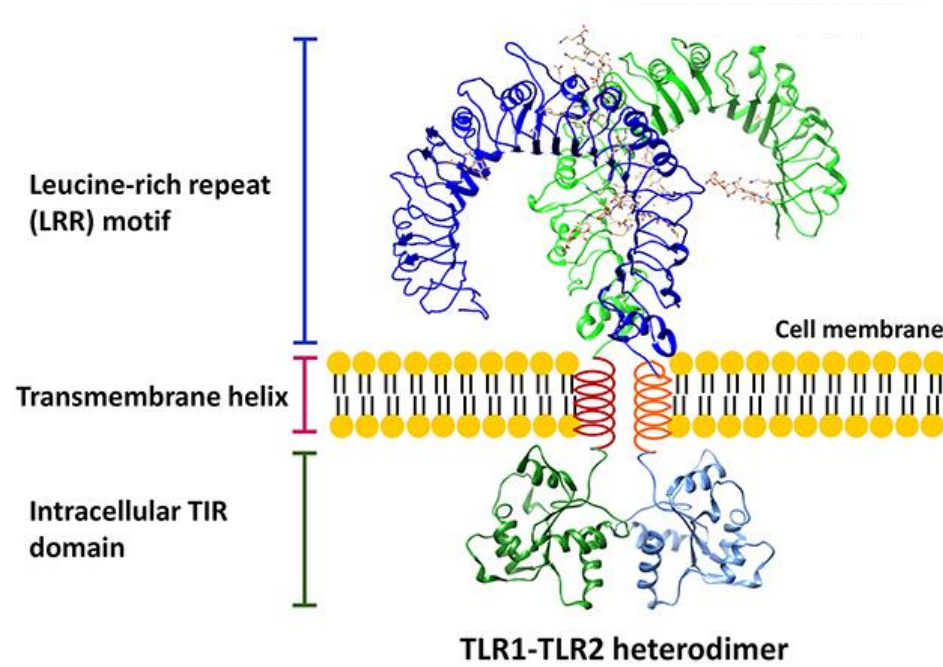
Infection experiment



Mortality per variant (over lines)



Toll-like receptor (TLR)

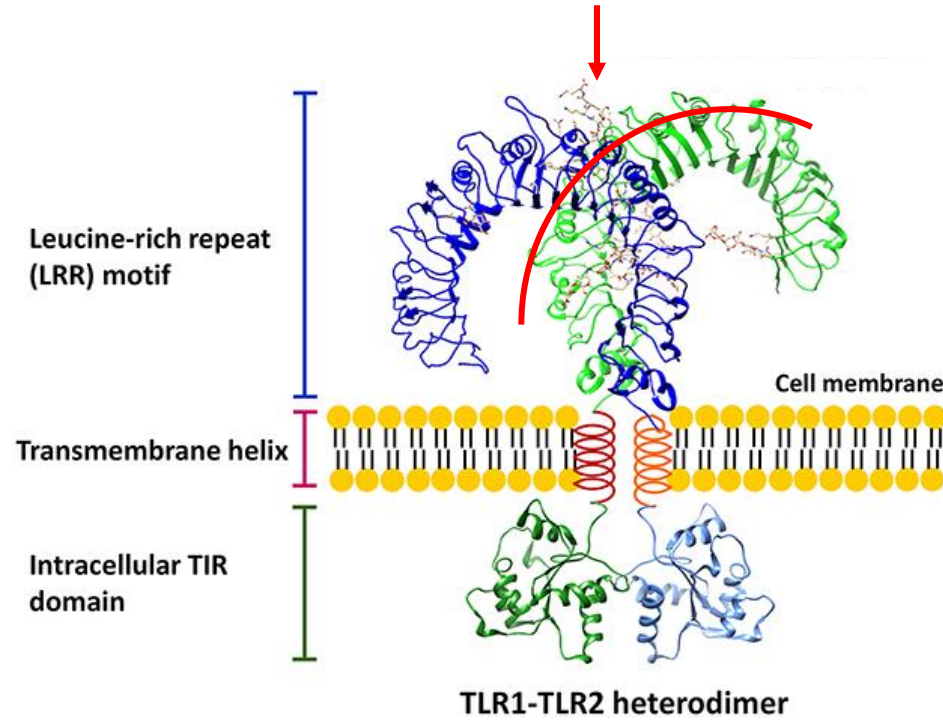


- Evolutionary conserved
- Recognize PAMP
 - f.e. TLR4 → LPS
- Genetic variants associated with (risk for) diseases¹
 - *Escherichia coli*?

¹ for overviews: Schröder et al., 2005, Lancet Infect Dis;

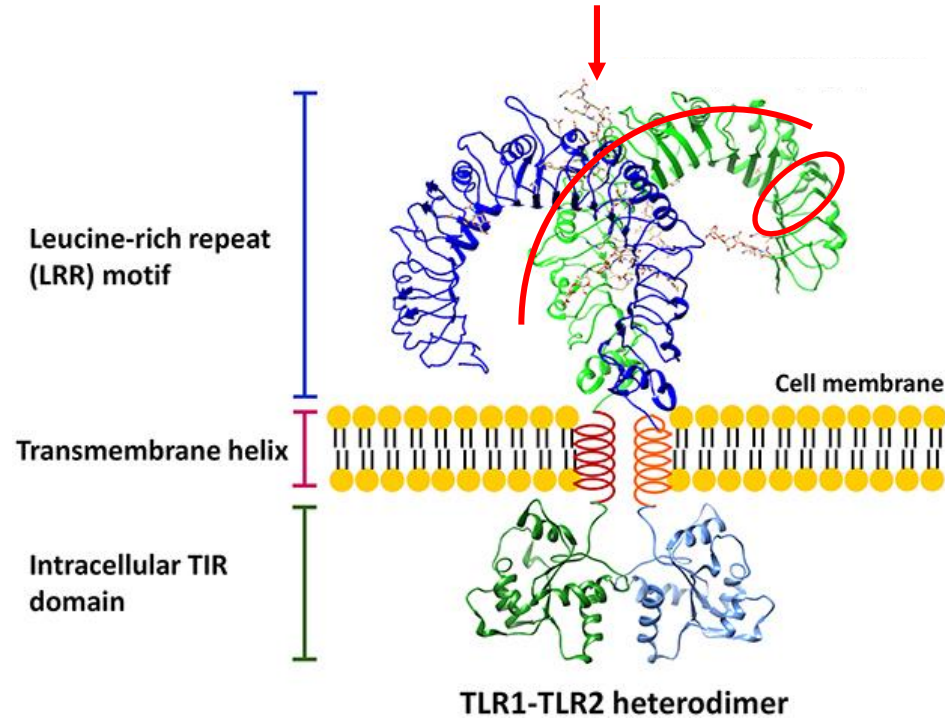
Netea et al., 2012, Nat Immunol; Medvedev, 2013, Interferon Res

Toll-like receptor family member 1A (*TLR1A*)



- Heterodimer with TLR2(A/B)
- LRR6-16 for recognition of¹:
 - Bacterial lipoproteins
 - Mycoplasma
 - Related (synthetic) structures

Putative causal mutation



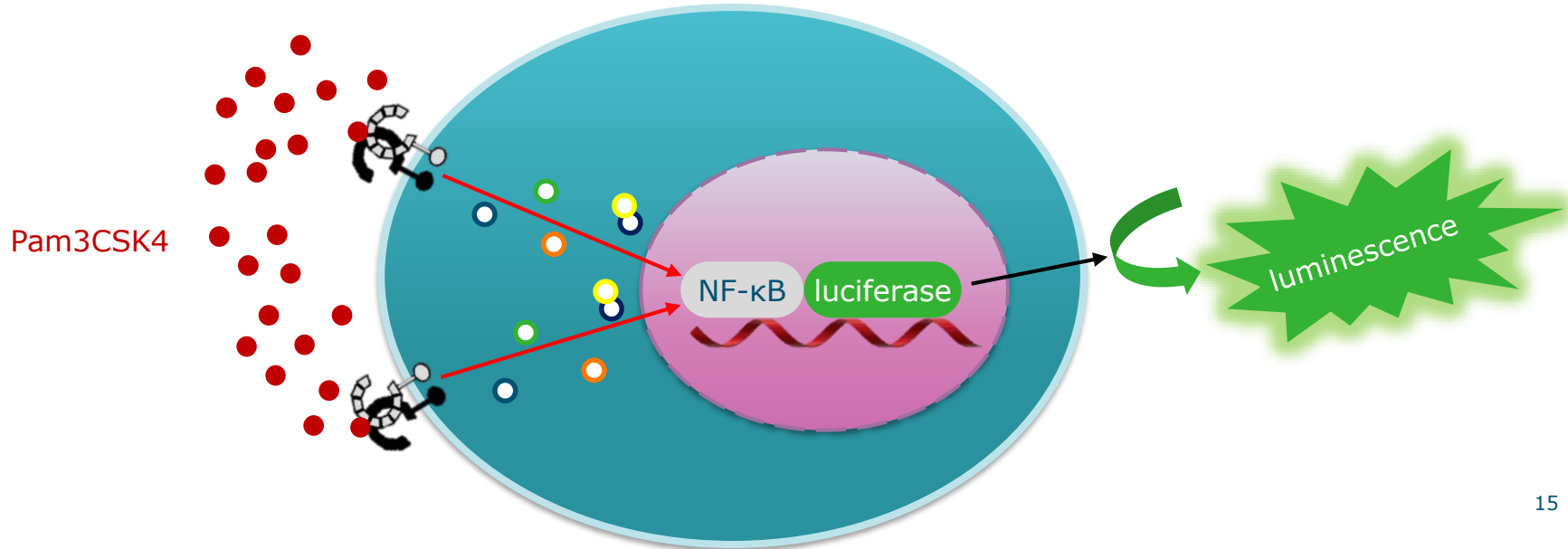
- Protein position 126 in LRR4
- C/G polymorphism
→ Phe/Leu AA substitution
- Dominant variant: C/Phe
Recessive variant: G/Leu

→ No evidence that mutation will affect ligand recognition

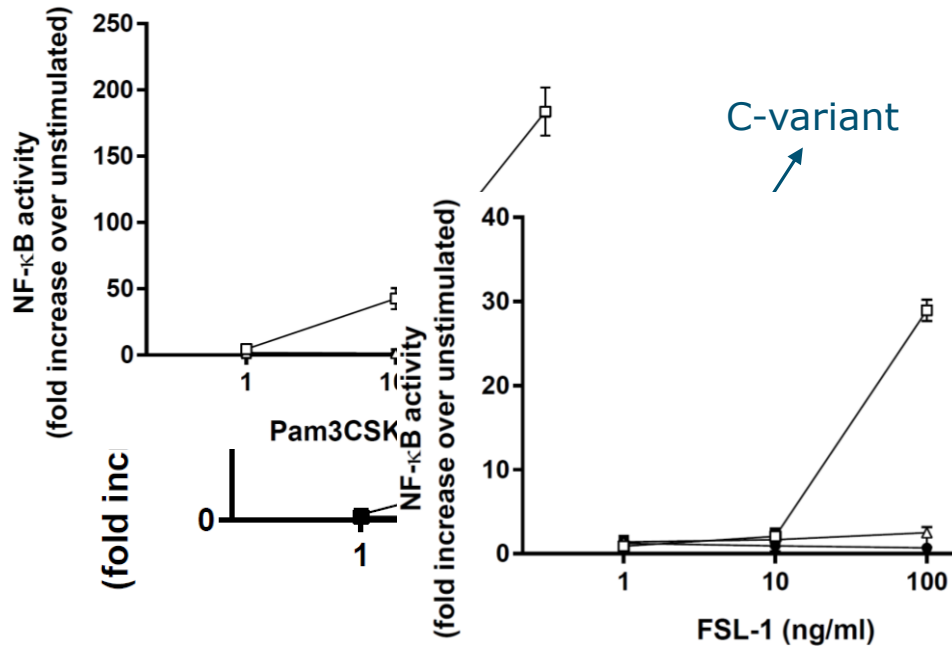
TLR1A activation in vitro



- HeLa cells with luciferase reporter
- Transfected with *TLR2B* and *TLR1A* variants



No (downstream) TLR1A activation for G-variant



- Tested in triplo
- Replicated
- Similar results in HEK cells
- Similar responses observed for FSL-1 and LTA

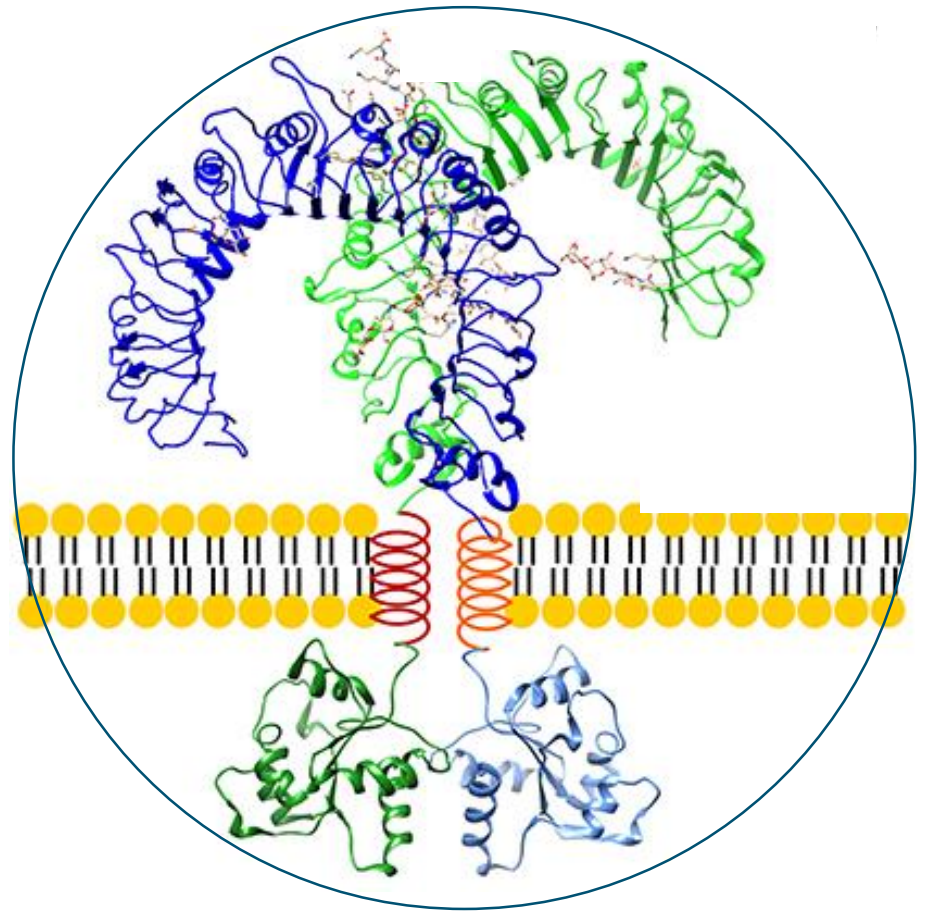
Causal mutation confirmed

- C-variant (Phe) is functional. G-variant (Leu) seems non-functional.
 - G-variant is deleterious mutation: “natural knock-out”¹
 - C compensates G in heterozygotes: full dominance (GWAS)
- G-variant not found in any other chicken line, human or mouse TLR
 - Currently testing (Voogdt et al., in preparation)
- Working mechanism remains to be elucidated

Take-home messages

- Mutation in *TLR1A* confirmed
→ Natural knock-out?!
- TLR1A influence IgM (natural) antibody levels and might influence disease resistance

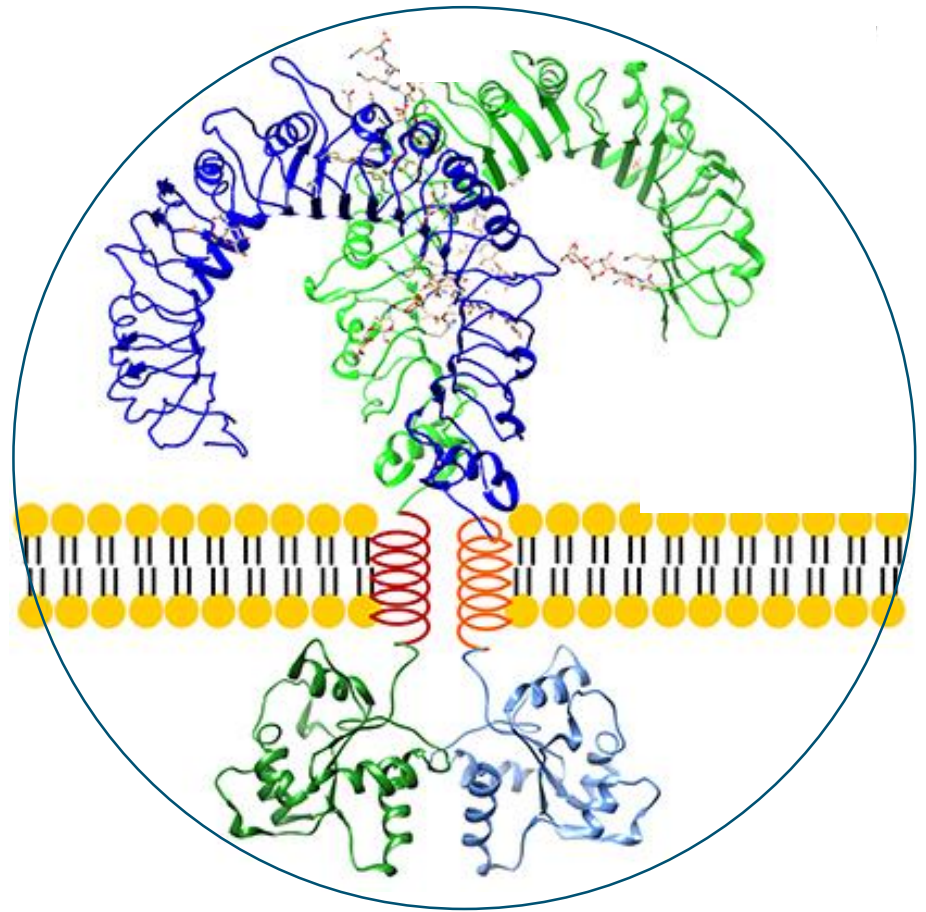
QUESTIONS?



Take-home messages

- Mutation in *TLR1A* confirmed
→ Natural knock-out?!
- TLR1A influence IgM (natural) antibody levels and might influence disease resistance

QUESTIONS?



Effect of causal mutation

On TLR activation

- Ligand recognition
- Dimerization
- Co-receptor binding

On IgM antibody levels

- IgM production by B-cells
- Naïve B-cells proliferation
- IgM plasma cell proliferation
- Differential downstream signal transduction in the NF- κ B pathway
- Indirect via other immune cells

