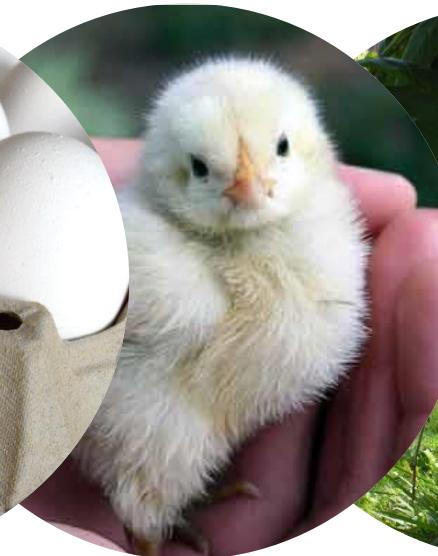


# A major effect of genomic region containing Toll-like receptor genes on IgM (natural) antibodies in chickens

Animal Genetics & Disease

Tom V.L. Berghof, MSc



# Co-authors/Collaborations

## Wageningen University & Research

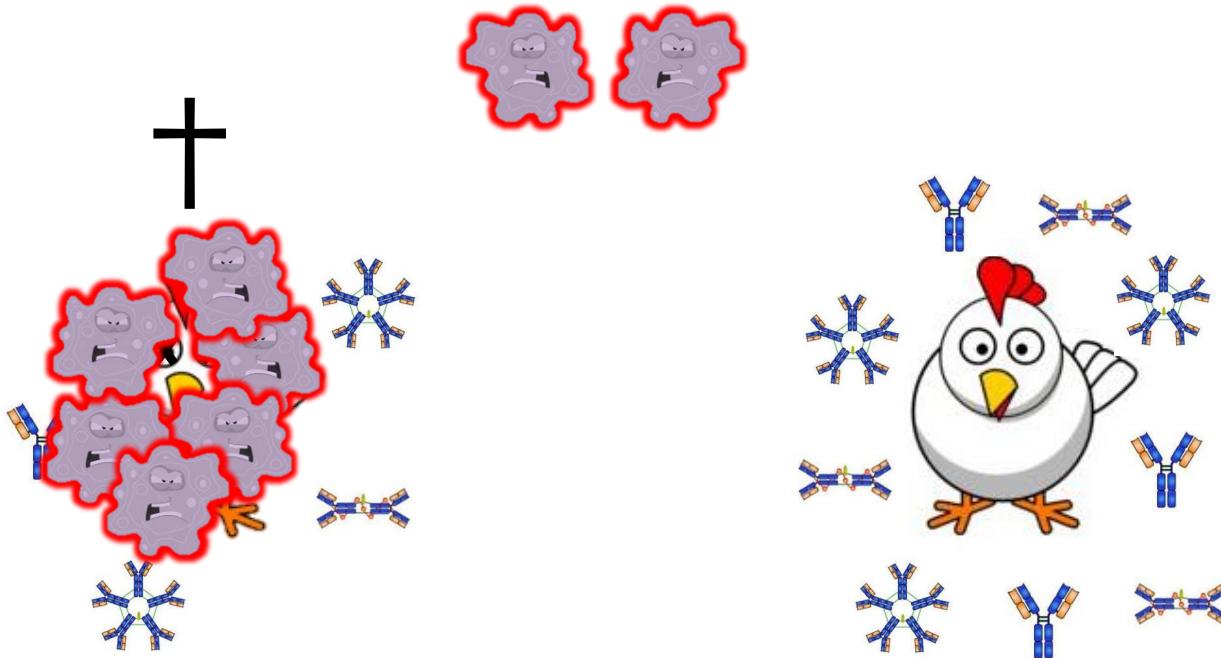
- Henk Parmentier (ADP)
- Jan van der Poel (ABG)
- Henk Bovenhuis (ABG)
- Marleen Visker (ABG)
- Joop Arts (ADP)

## Organisations

- NWO-TTW
- Hendrix Genetics
  - Addie Vereijken

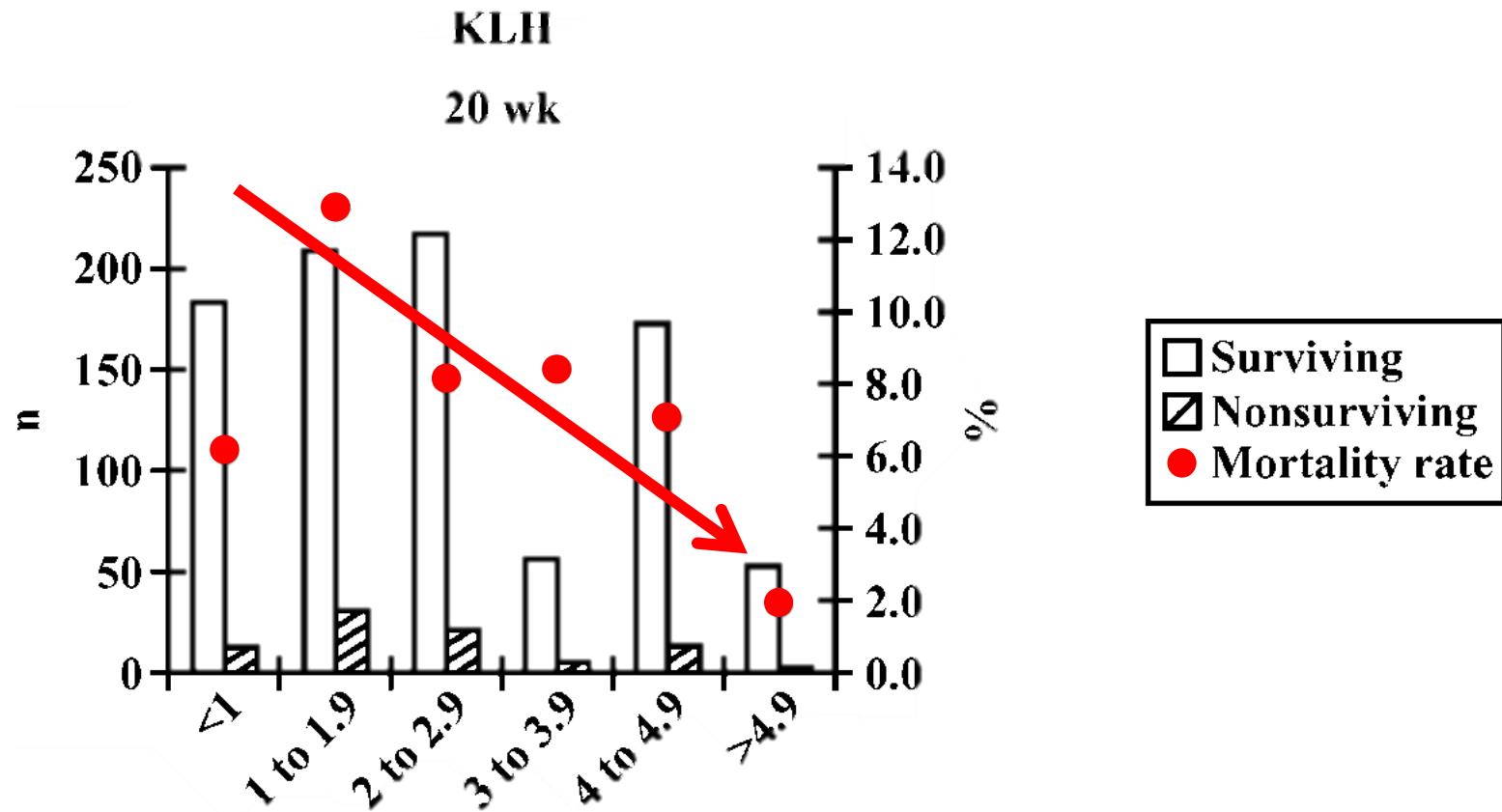


# Natural antibodies?



# Natural antibodies!

Star et al., 2007, Poultry Science



# Natural antibodies

Antigen binding antibodies present in individuals without exposure to this antigen

# Natural antibodies binding KLH

Antigen binding antibodies present in individuals without exposure to this antigen

Keyhole Limpet Hemocyanin (KLH)



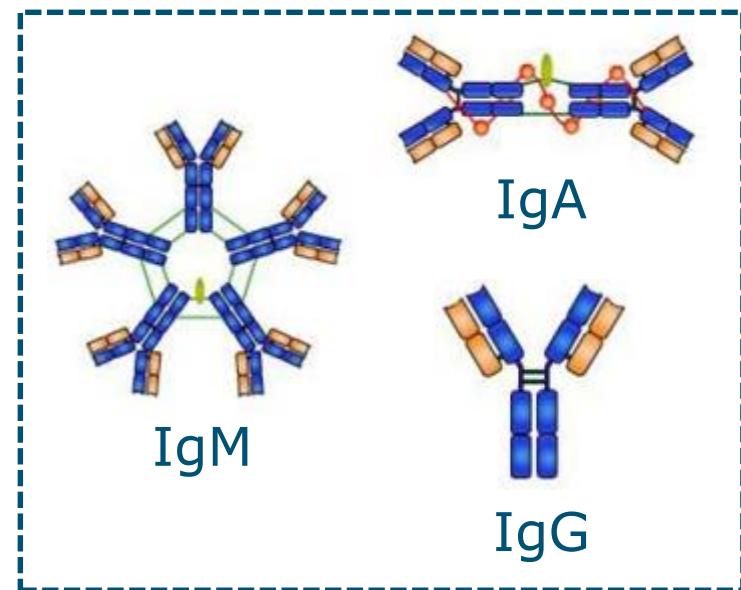
# Natural antibodies binding KLH

Antigen binding antibodies present in individuals without exposure to this antigen

## Keyhole Limpet Hemocyanin (KLH)

### ■ Plasma titers

- IgTotal → Total levels (IgTotal)
- IgM
- IgA
- IgG



# Objectives PhD thesis

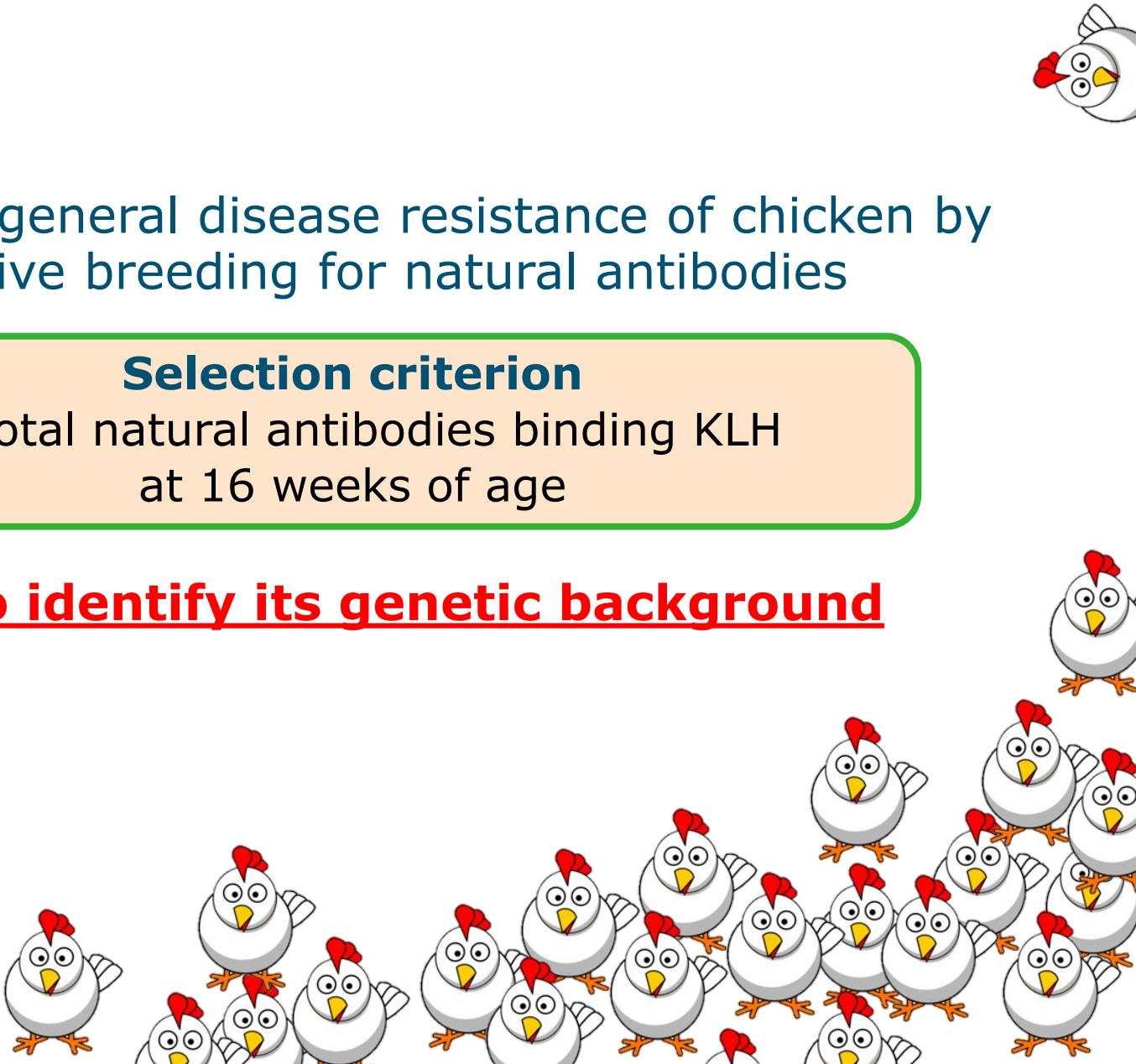


To improve general disease resistance of chicken by selective breeding for natural antibodies

## **Selection criterion**

IgTotal natural antibodies binding KLH  
at 16 weeks of age

**AND to identify its genetic background**



# To identify the genetic background...

- Genetic parameter  
~3,600 purebred white layers ( $\sigma + \varphi$ ) at 16 weeks

	IgTotal	IgM	IgA	IgG
Heritability	0.12	0.14	0.10	0.07
Mat. Env.	-	0.06	-	-

(Berghof et al., 2015, PLoS ONE)

- Genome-wide association study  
~1,600 purebred white layers ( $\sigma + \varphi$ ) around 16 weeks  
57,636 SNP → Quality control: 15,578 useable SNP

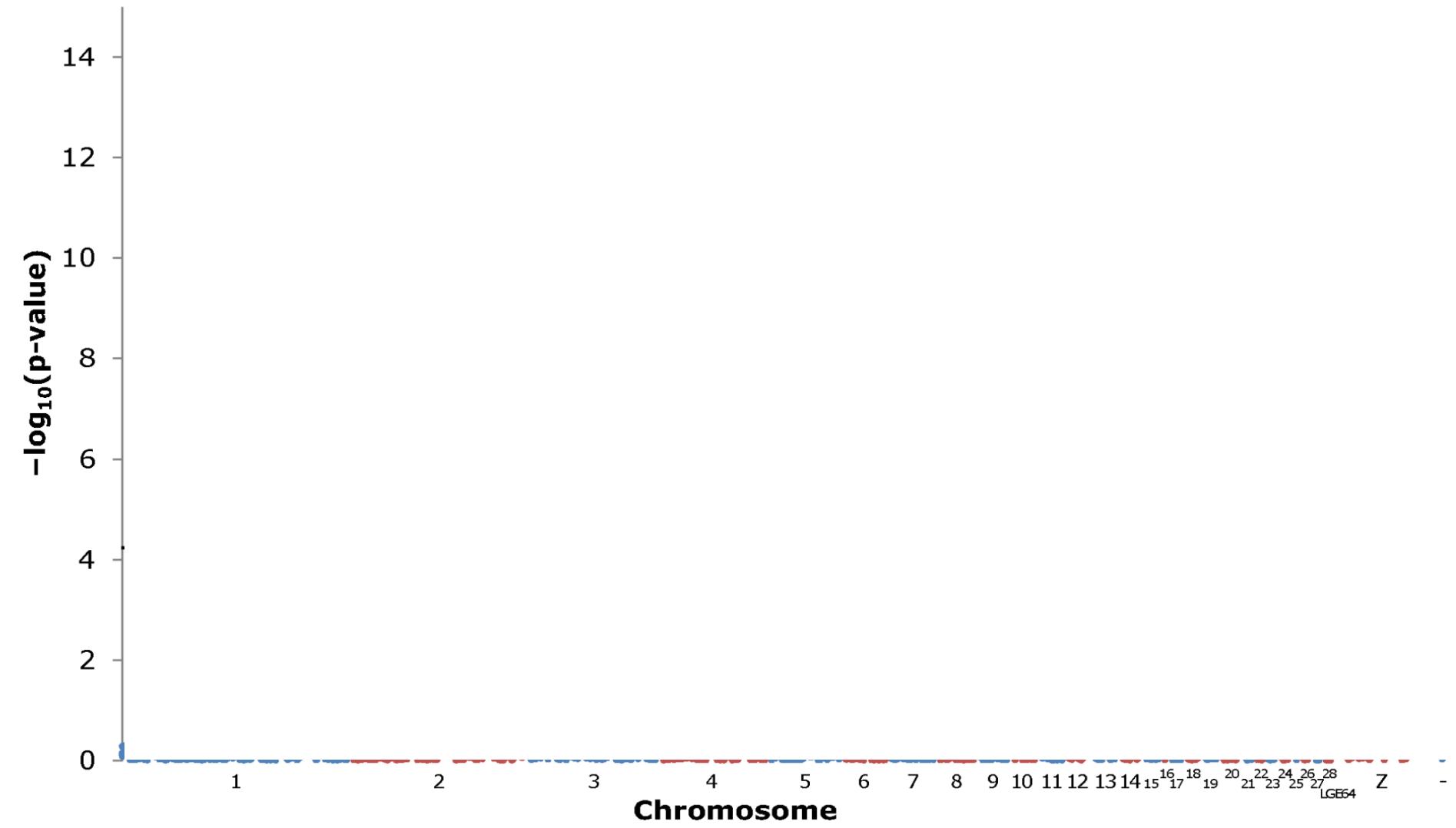
# Associated regions

KLH-binding natural antibodies:

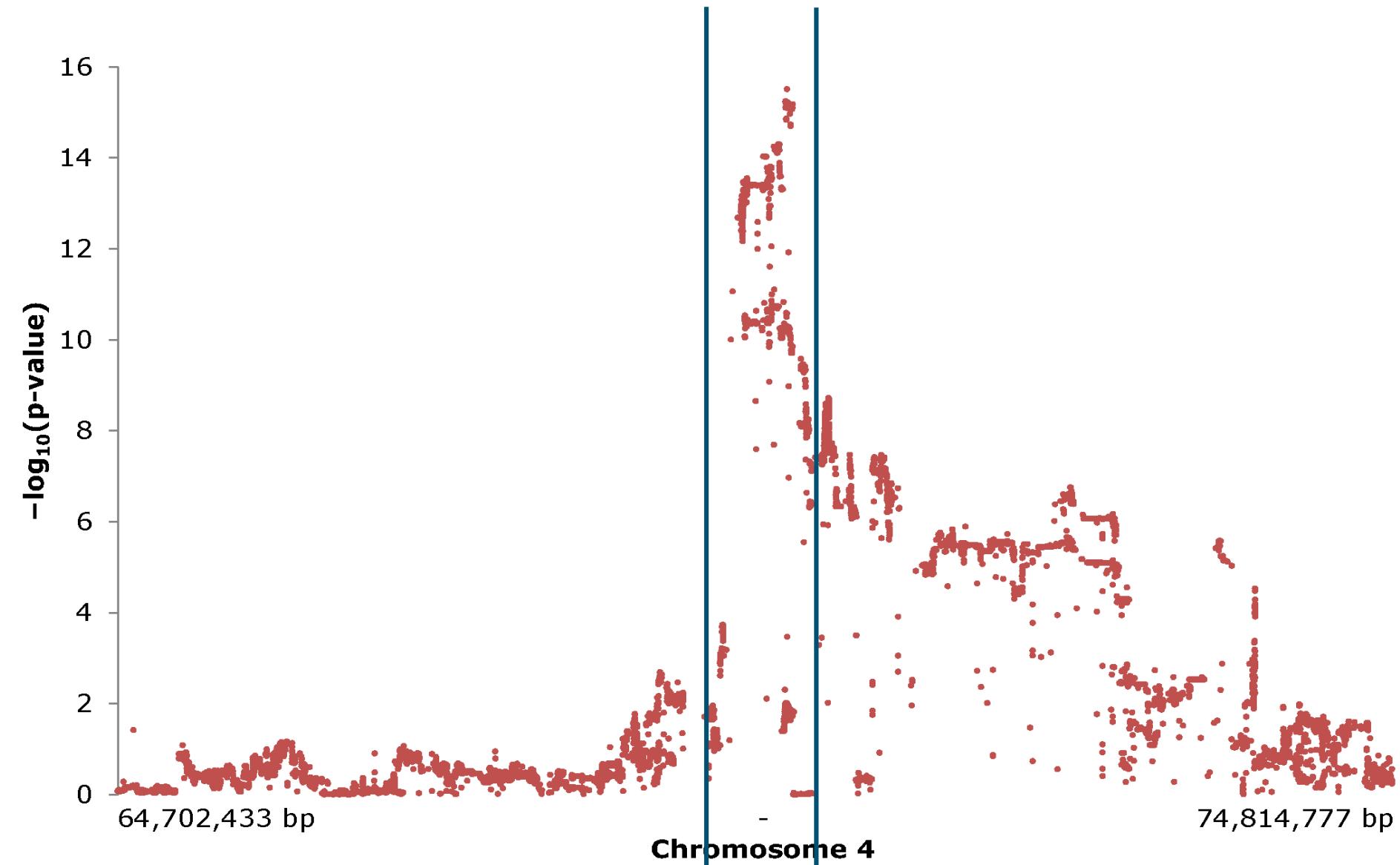
- IgTotal      → GGA4, 70 Mbp (3 SNP; p<0.10)
- IgM           → GGA4, 70 Mbp (35 SNP; p<0.05)
  
- IgA           → GGA9, 12 Mbp (2 SNP; p<0.10)  
                → GGA18, 10 Mbp (3 SNP; p<0.10)
  
- IgG           → -

# Manhattan plot IgM

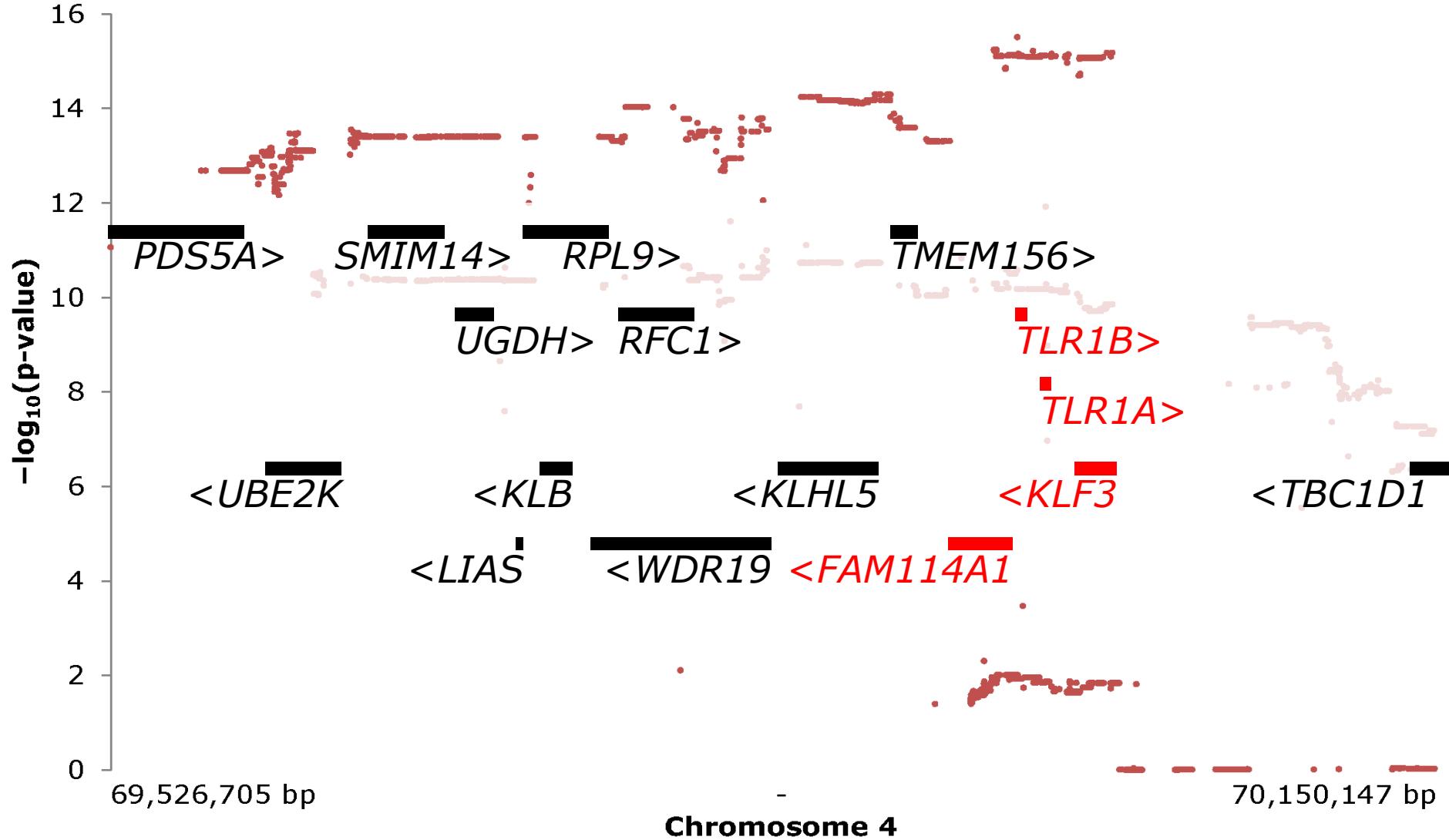
**IgM NAb**



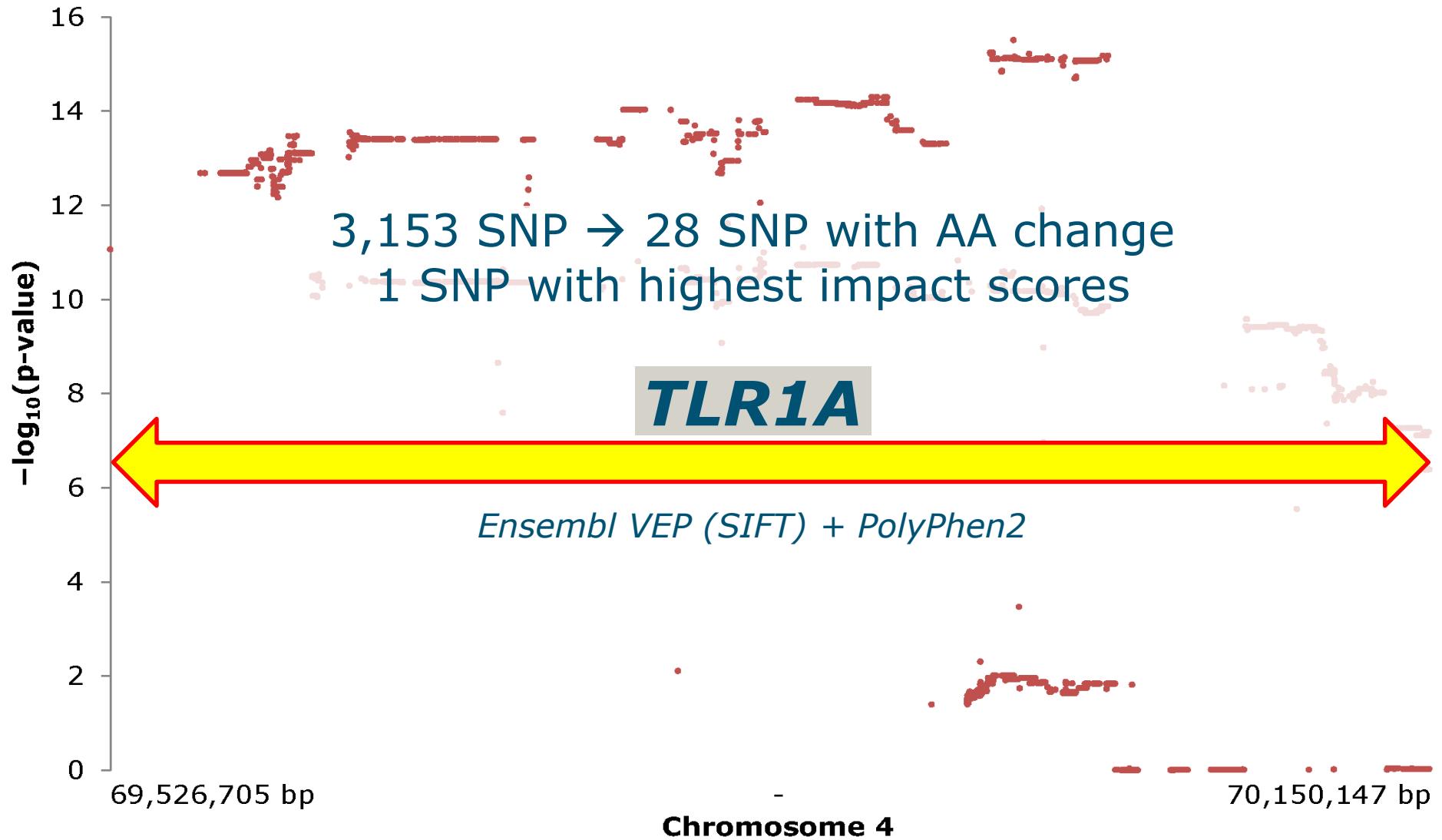
# Fine mapping of GGA4 for IgM NAb



# Fine mapping of GGA4 for IgM NAb



# Fine mapping of GGA4 for IgM NAb



# GWAS summary

- Genetic variation underlying IgM NAb
  - Most likely candidate: *TLR1A*
  - Full dominance
  - Explains ~60% of genetic variation
- No significant associations for IgTotal, IgA, and IgG

# GWAS total antibody concentrations

- Associations of total antibody concentration of

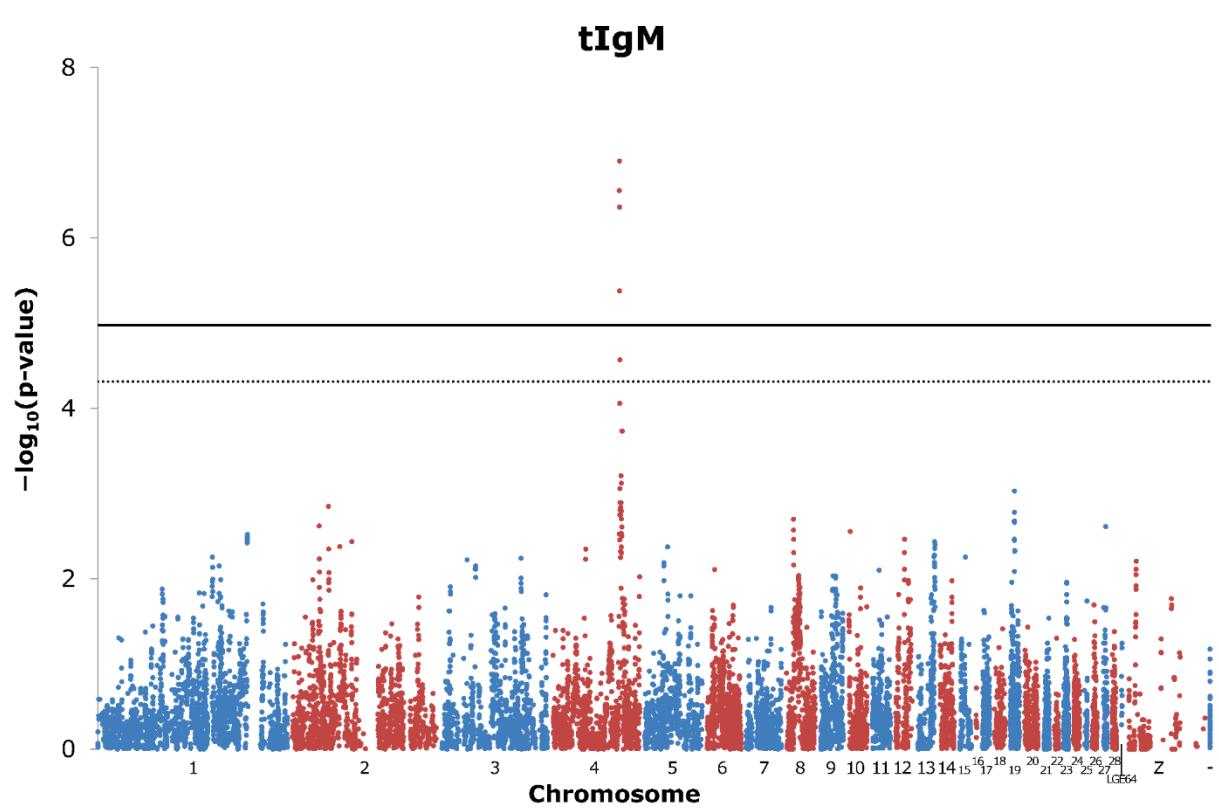
- IgM

- IgA

- IgG

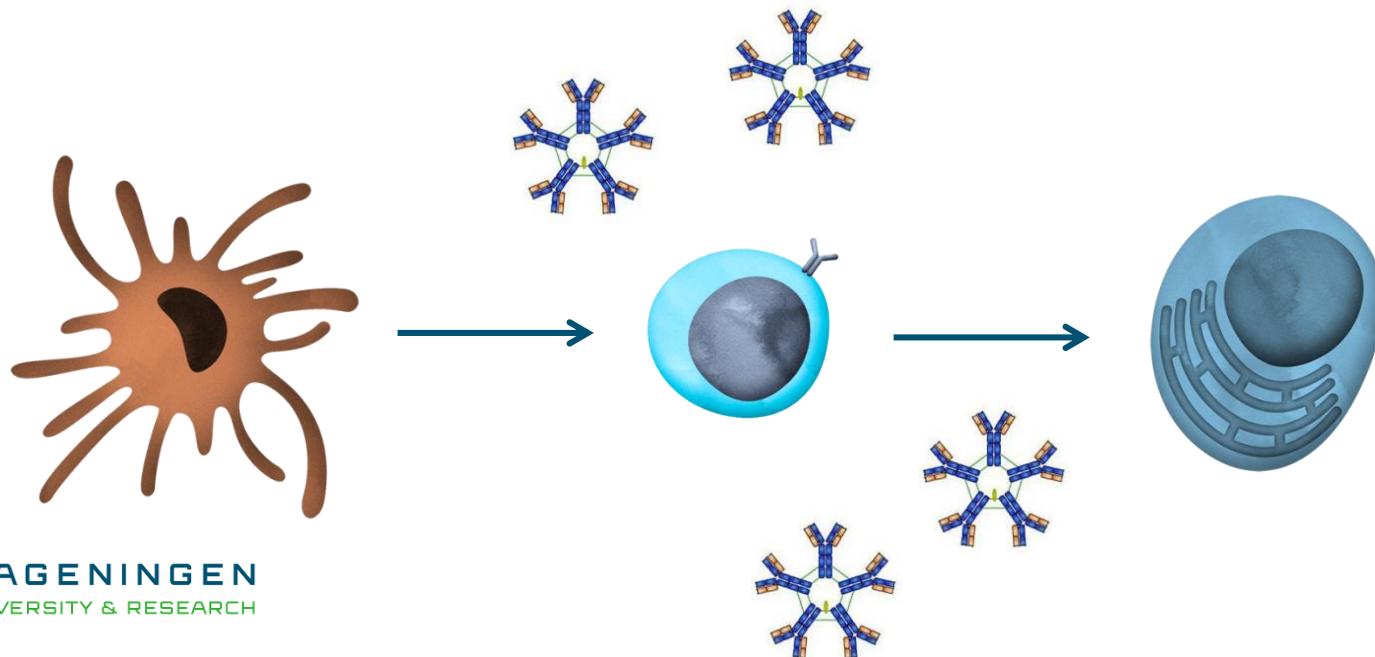
+

- Total Ig



# TLR1A is an important player in (natural) antibody levels

- IgM production
  - B cell maturation
  - B cell proliferation
  - B cell survival
- } Directly: B cells  
Indirectly: f.e. macrophages



# Take from me at session!!!

1. IgM (natural) antibodies are influenced by a genomic region on GGA4 at ~70Mbp.
2. This region contains 16 candidate genes.
3. Prediction tools suggest mutation in *TLR1A*.

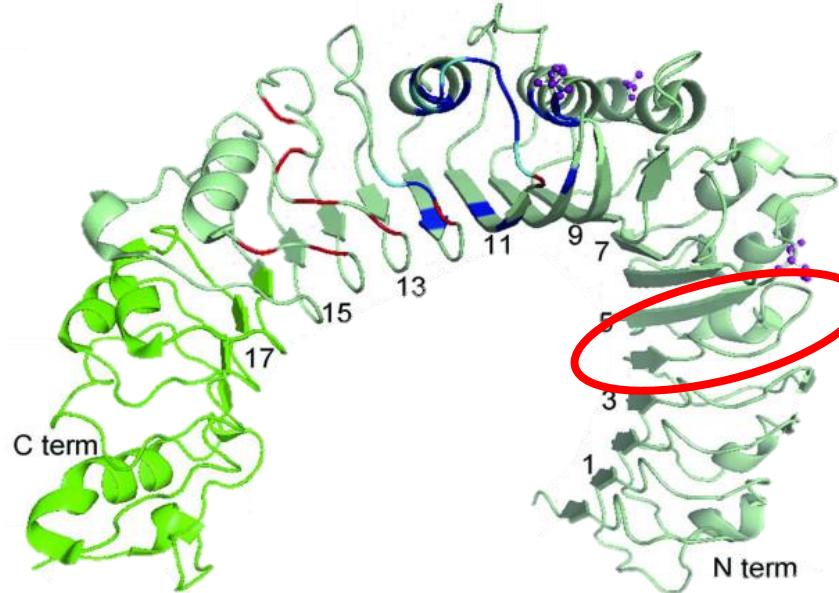
Questions???

[tom.berghof@wur.nl](mailto:tom.berghof@wur.nl)



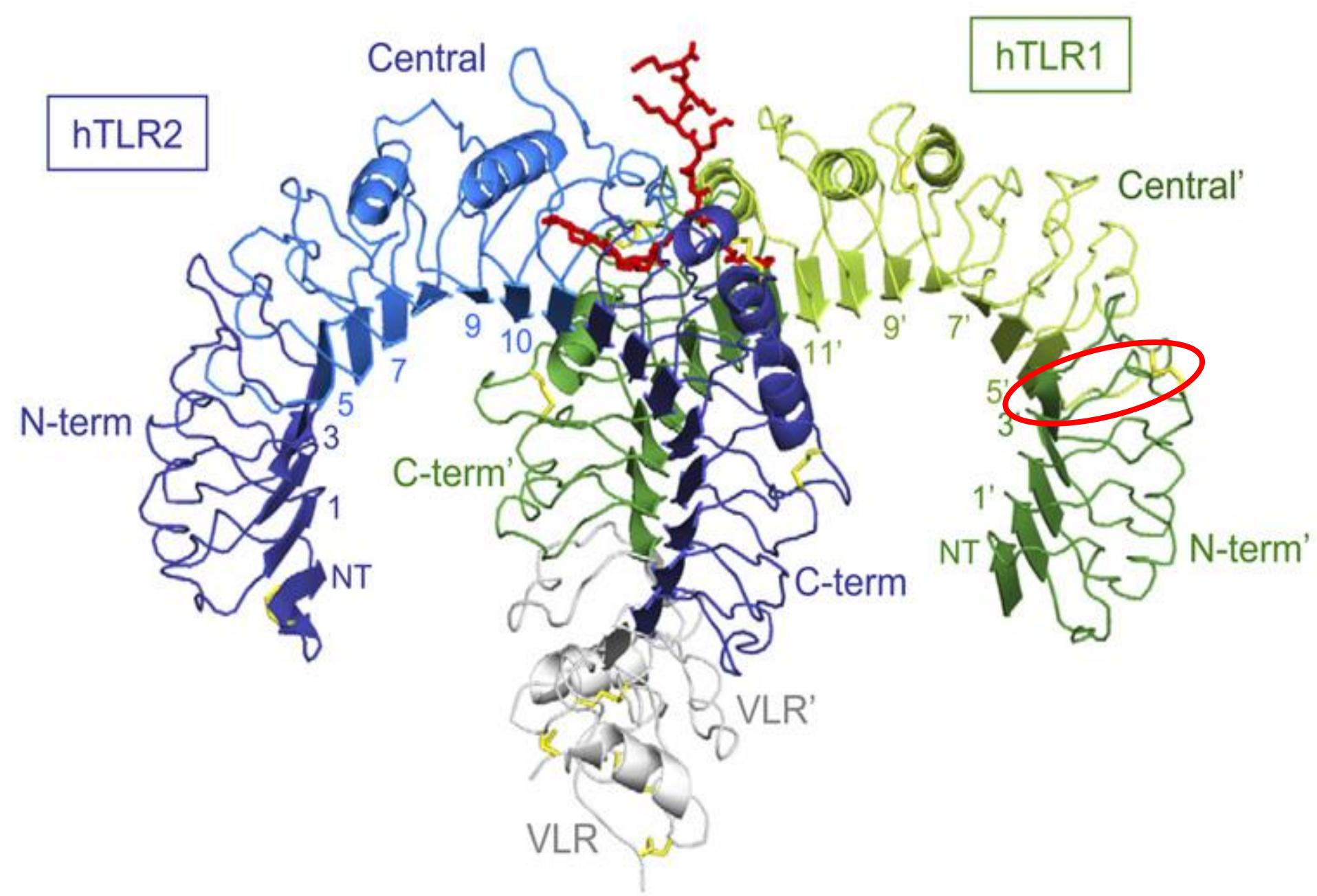
# TLR1A

- Recognizes peptidoglycan
- GGA4, 69,965,939 bp
- C/G SNP
- phenylalanine (F)/leucine (L) AA substitution
- AA position 126 → LRR4 (Keestra et al., 2007, J Immunol)
  - Ligand recognition
  - Dimerization with TLR2
  - Mediation with coreceptors



human TLR1

Jin et al., 2007, Cell



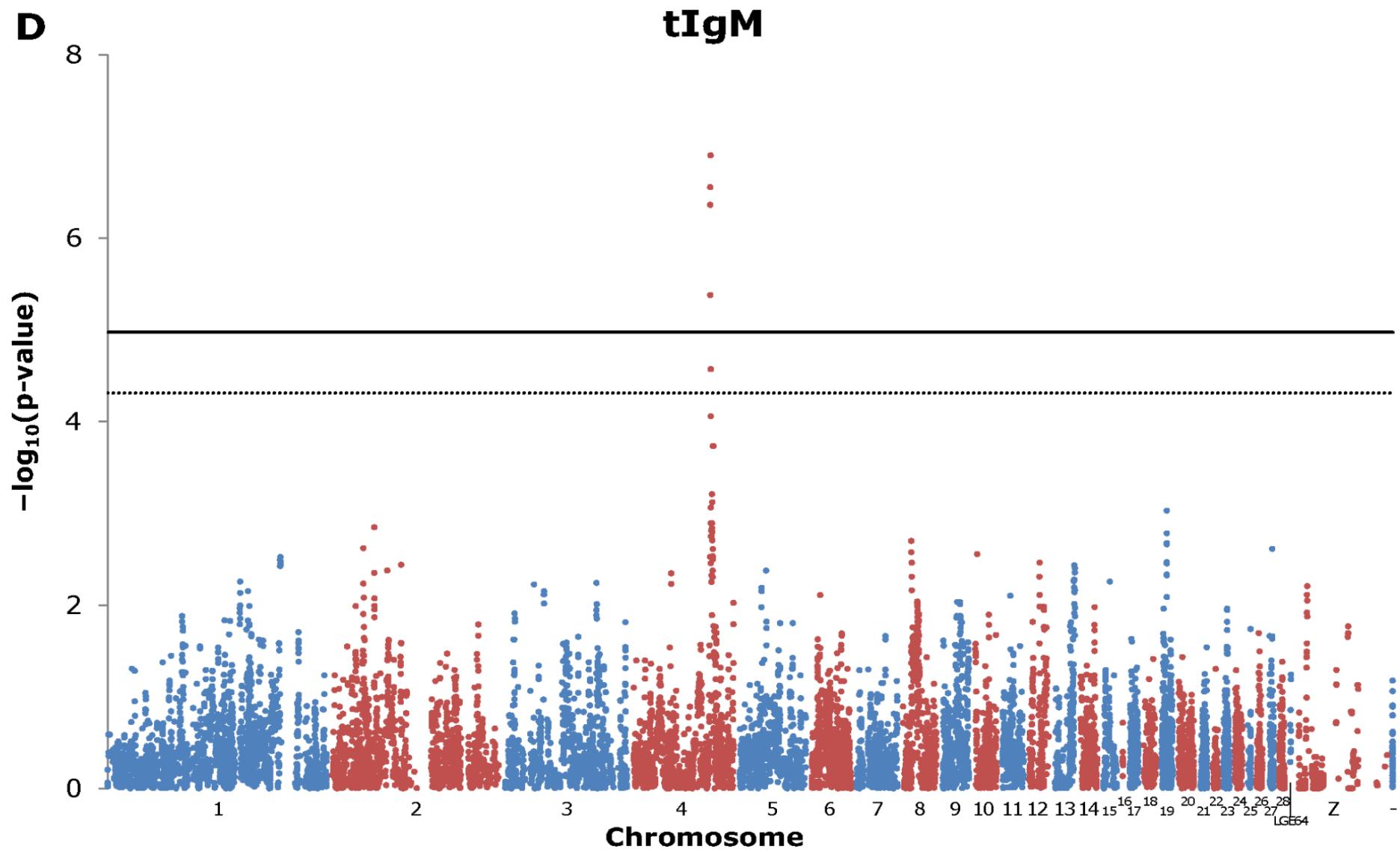
# A role of TLR1A in (natural) antibody levels

- B cell ontogeny
  - Pre-bursal stage
  - *Hatch*
  - Bursal stage
  - Post-bursal stage
  
- B cell types
  - Short-live B cells (~60%)
  - Longer-lived B cells (~35%)
  - Spleen-originating B cells (~5%)

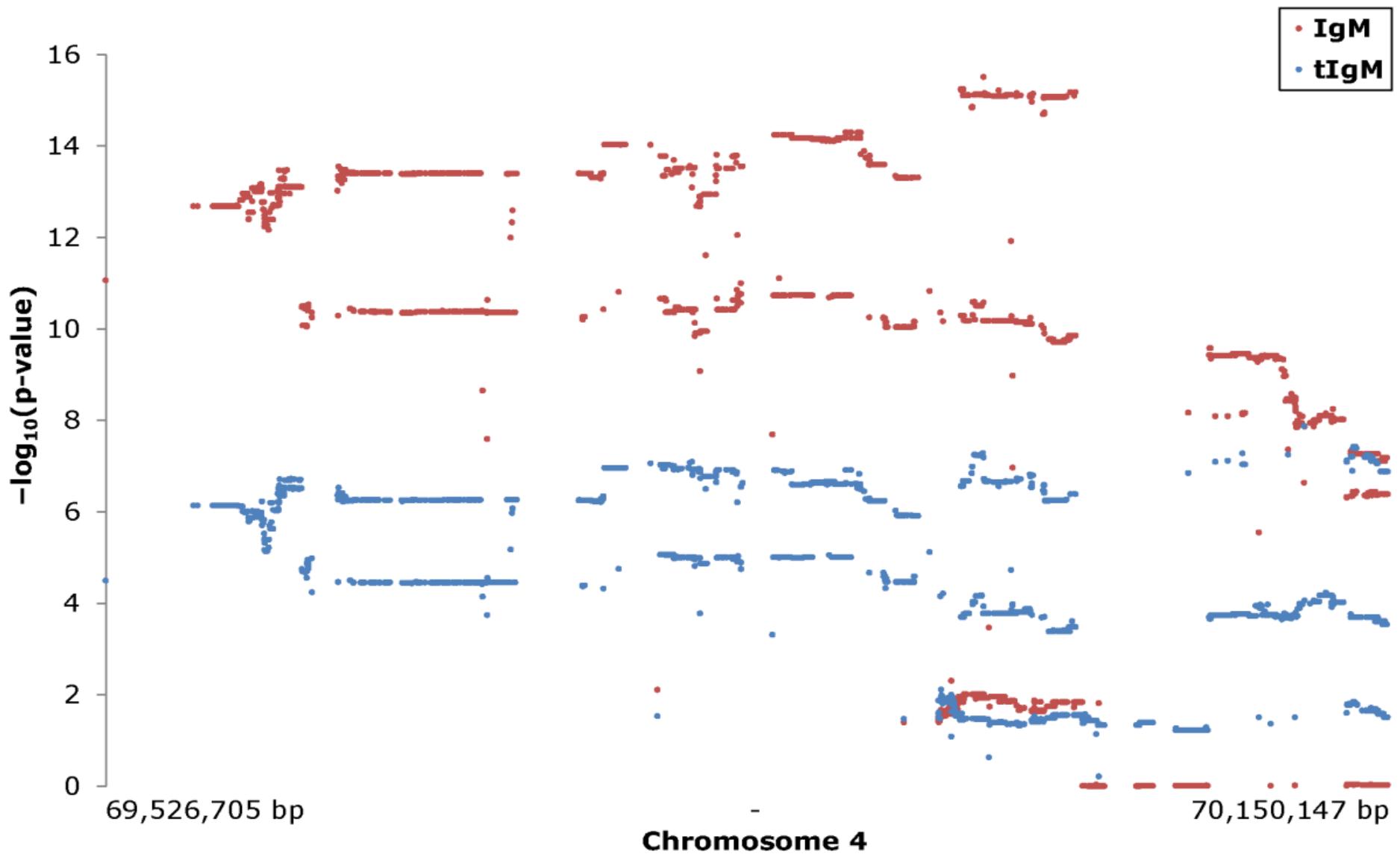
# GWAS total antibody concentrations

- Genetic variation underlying IgM NAb
  - Most likely candidate: *TLR1A*
  - Full dominance
  - Explains ~13% of genetic variation
  - ~40 µg/mL
- No significant associations for IgTotal, IgA, and IgG

# Manhattan plot IgM antibody concentration

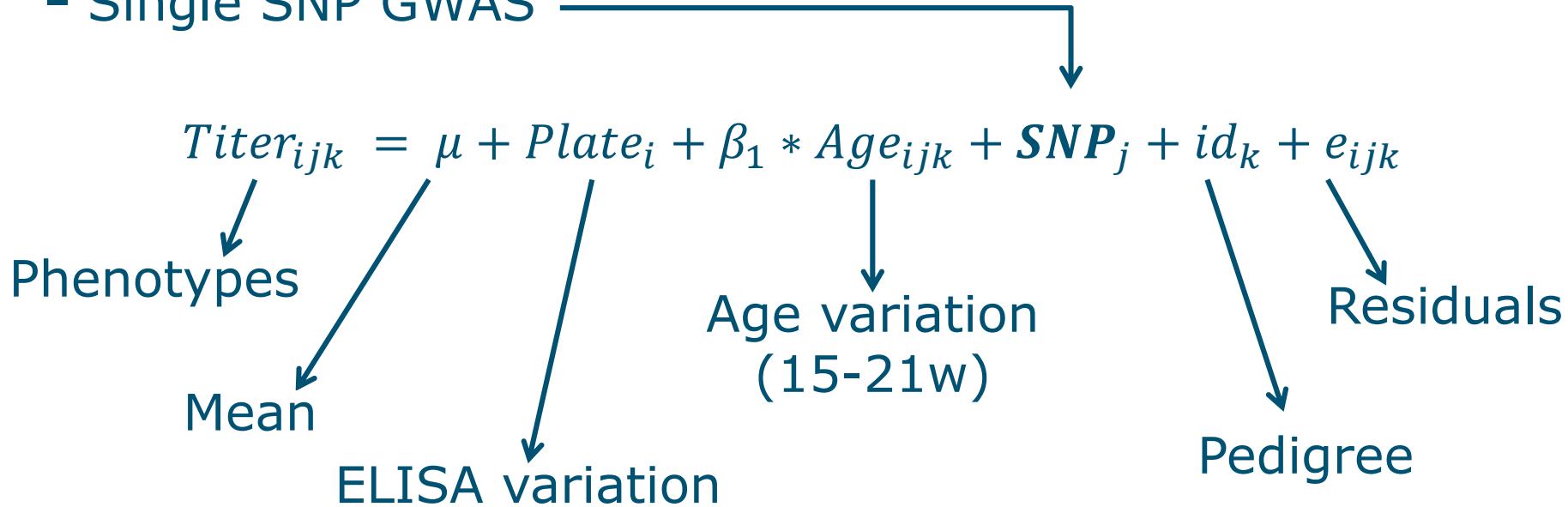


# GGA4, 70Mbp



# Genome-wide association study

- ~1,600 chickens ( $\sigma + \Omega$ ) ~16 weeks of age
- IgTotal, IgM, IgA, and IgG titers
- 57,636 SNP  
→ Quality control: 15,578 useable SNP
- Single SNP GWAS



# Heritabilities

NAb type	IgTotal	IgM	IgA	IgG
<b>Heritability</b>	0.12	0.14	0.10	0.07
<b>Mat. Env.</b>	-	0.06	-	-

Berghof et al., 2015, PLoS ONE

Ab type	IgTotal	IgM	IgA	IgG
<b>Heritability</b>	0.08	0.23	0.22	0.06
<b>Mat. Env.</b>	-	-	-	-

# Correlations NAb and Ab

	Genetic correlation	Phenotypic correlation
IgTotal	-0.27	0.03
IgM	0.91	0.41
IgA	0.38	0.26
IgG	-0.61	0.08

# Correlations

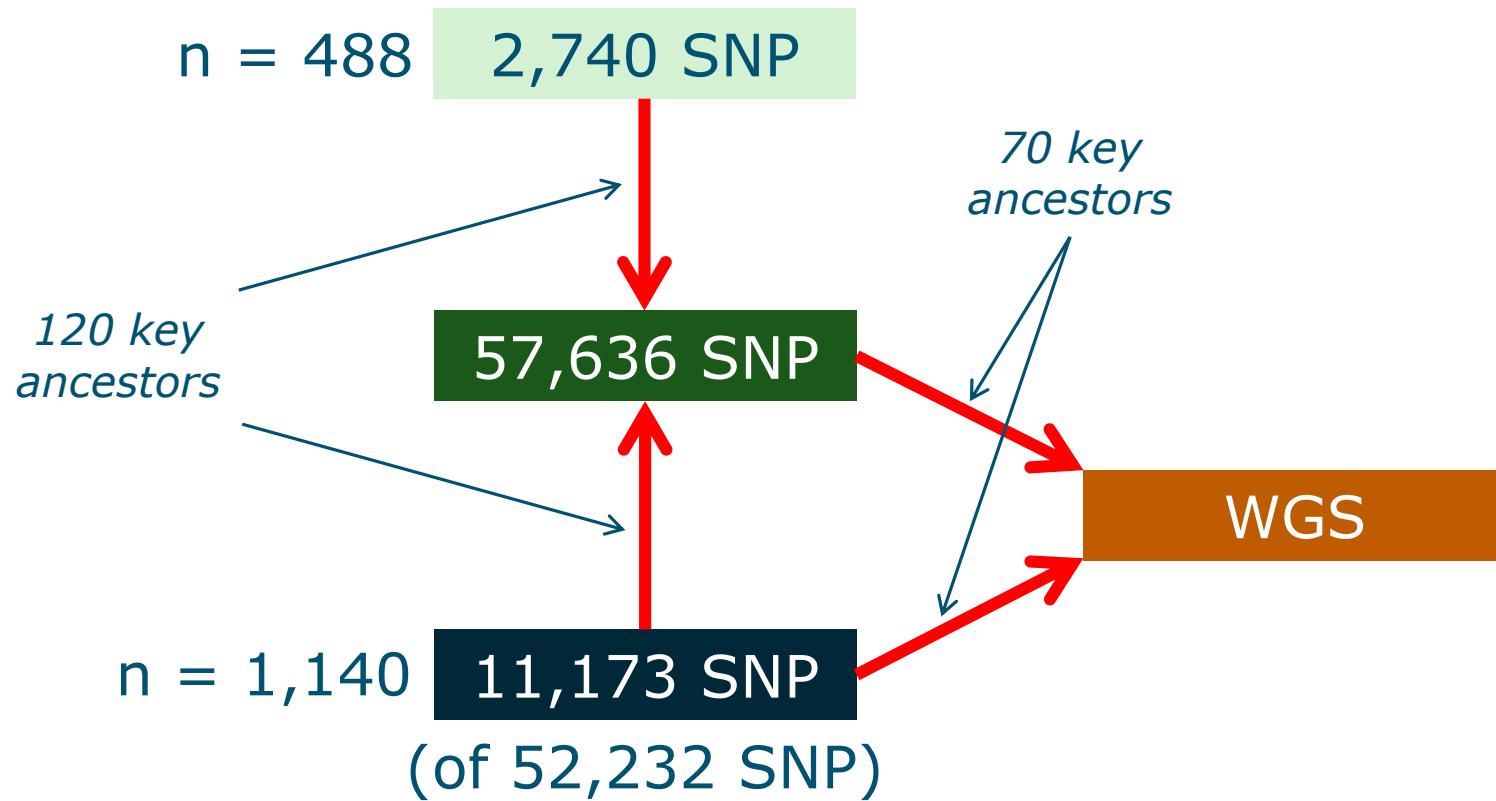
Genetic correlations

NAb	IgTotal	IgM	IgA	IgG
IgTotal		0.55	0.30	0.81
IgM	0.97		0.33	0.26
IgA	0.92	0.81		0.22
IgG	0.96	0.86	0.87	

Phenotypic correlations

Ab	IgTotal	IgM	IgA	IgG
IgTotal		0.39	0.19	0.99
IgM	0.24		0.18	0.29
IgA	0.72	0.17		0.13
IgG	0.99	0.12	0.62	

# Genotypes



Illumina, imputed with Beagle 4.0 ( $\geq 97\%$ ),  
average sequence depth = 12.4 (2.1)