Genomic region containing Toll-like receptor genes has a major effect on IgM natural antibodies in chickens

Avian Genetics & Immunity

Tom V.L. Berghof, MSc

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Co-authors/Collaborations

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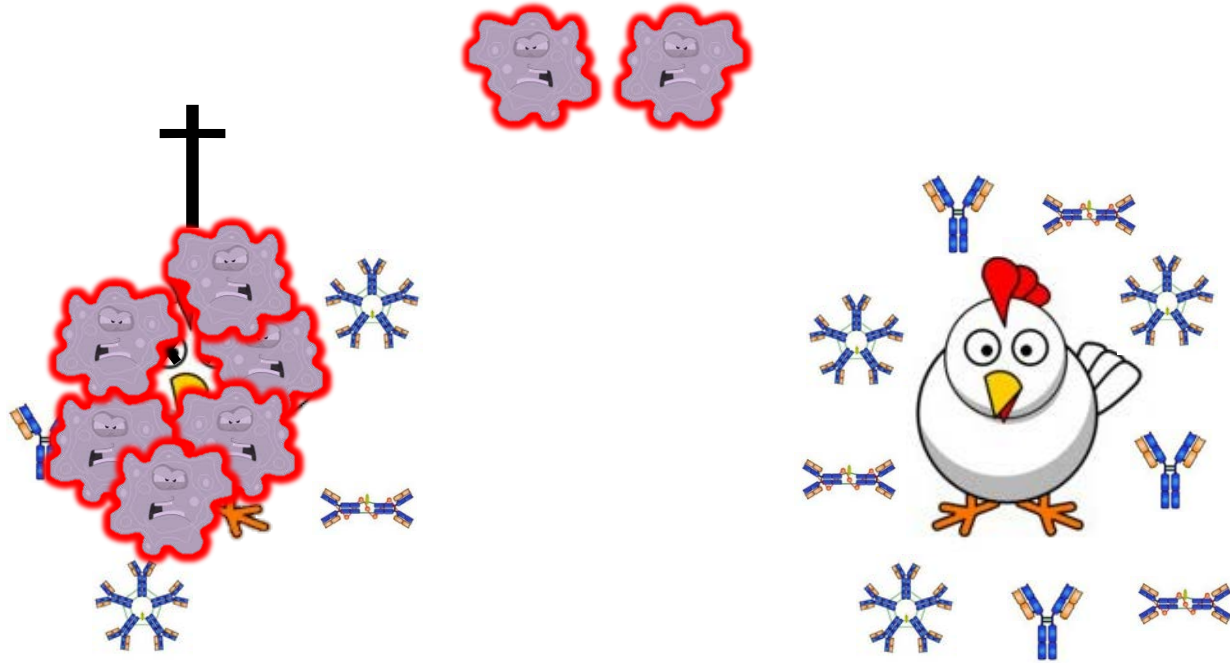- Joop Arts   (ADP)

Organisations
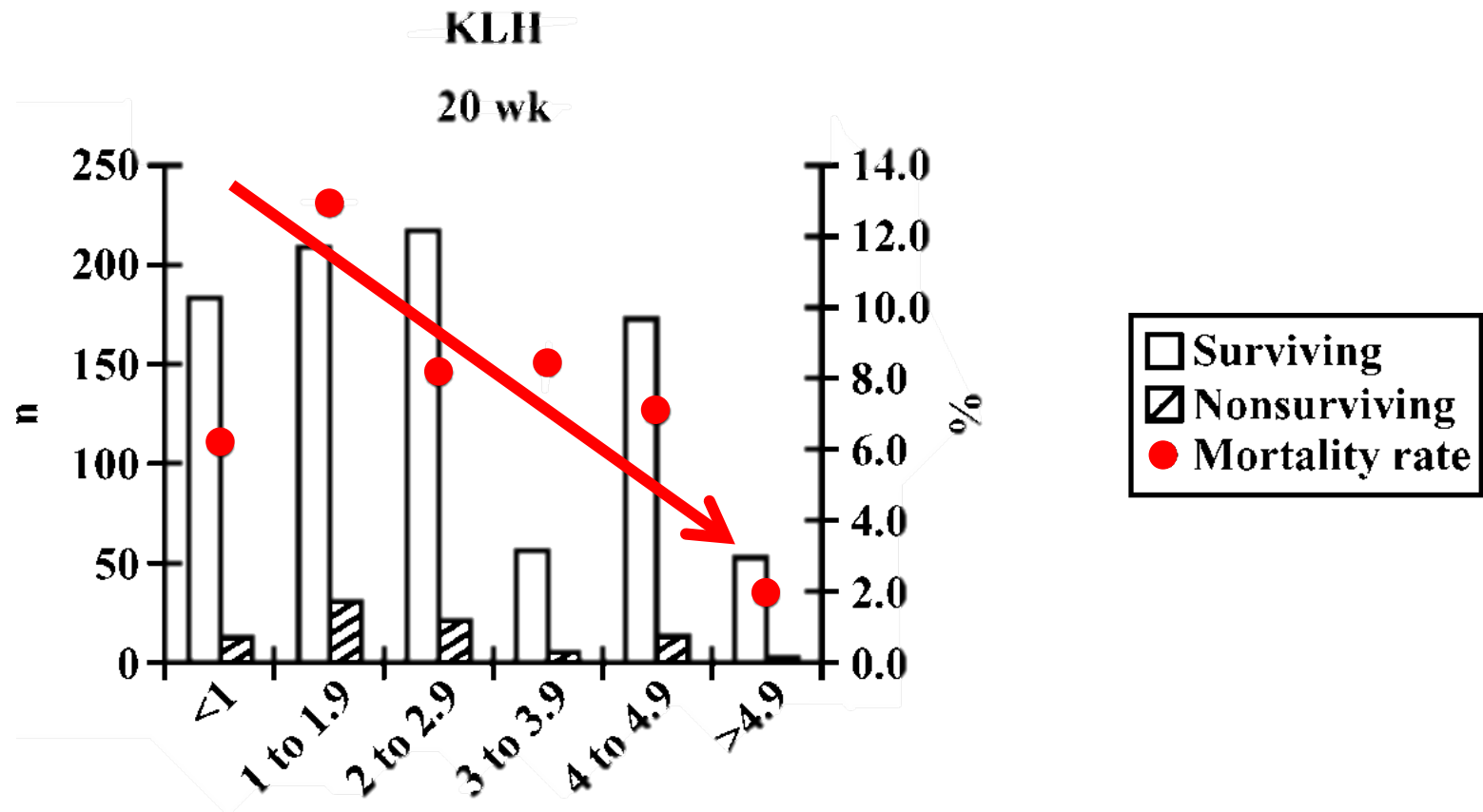- NWO-TTW
- Hendrix Genetics
  - Addie Vereijken

Natural antibodies?

Natural antibodies!

KLH
20 wk

Surviving
Nonsurviving
Mortality rate

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
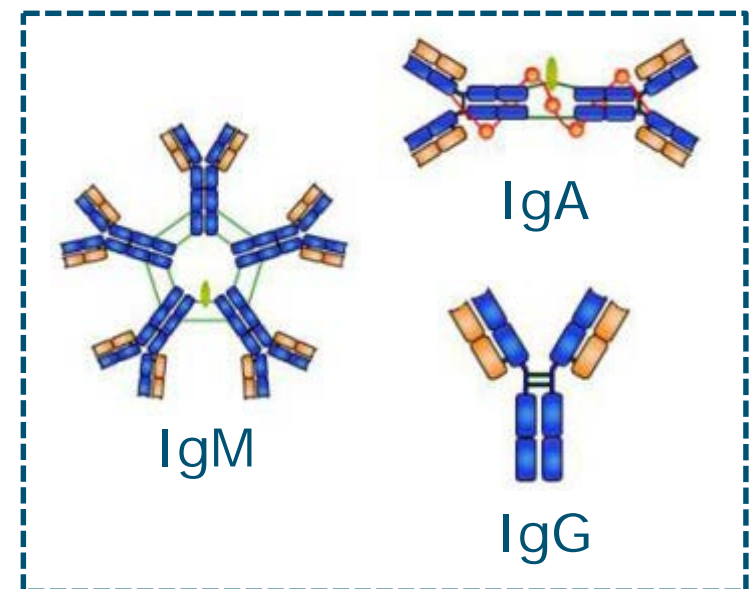
Keyhole Limpet Hemocyanin (KLH)

Natural antibodies binding KLH

Antigen binding antibodies present in individuals without exposure to this antigen

Keyhole Limpet Hemocyanin (KLH)

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

IgA

IgM

IgG

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Objective PhD thesis

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

Selection criterion
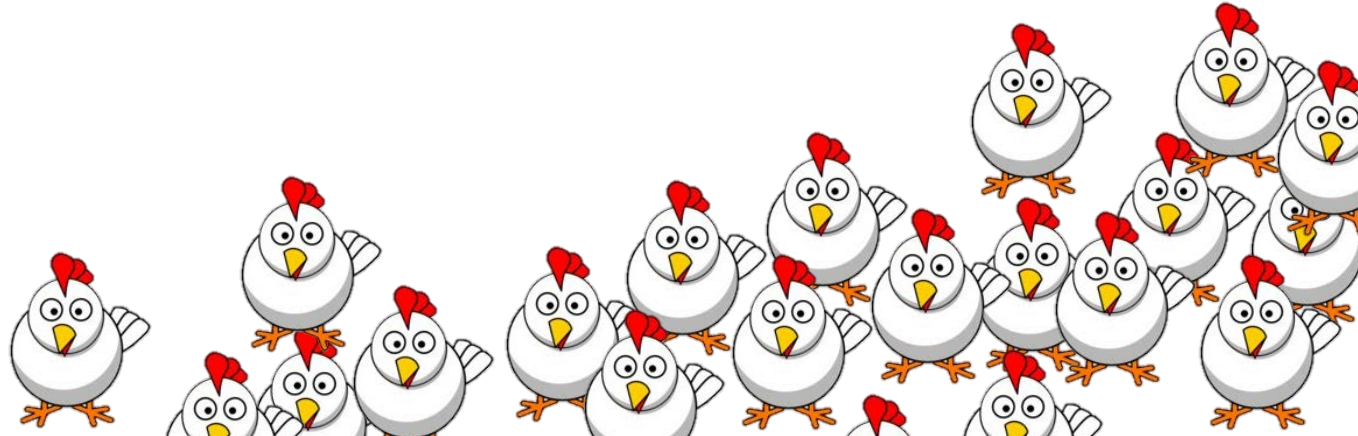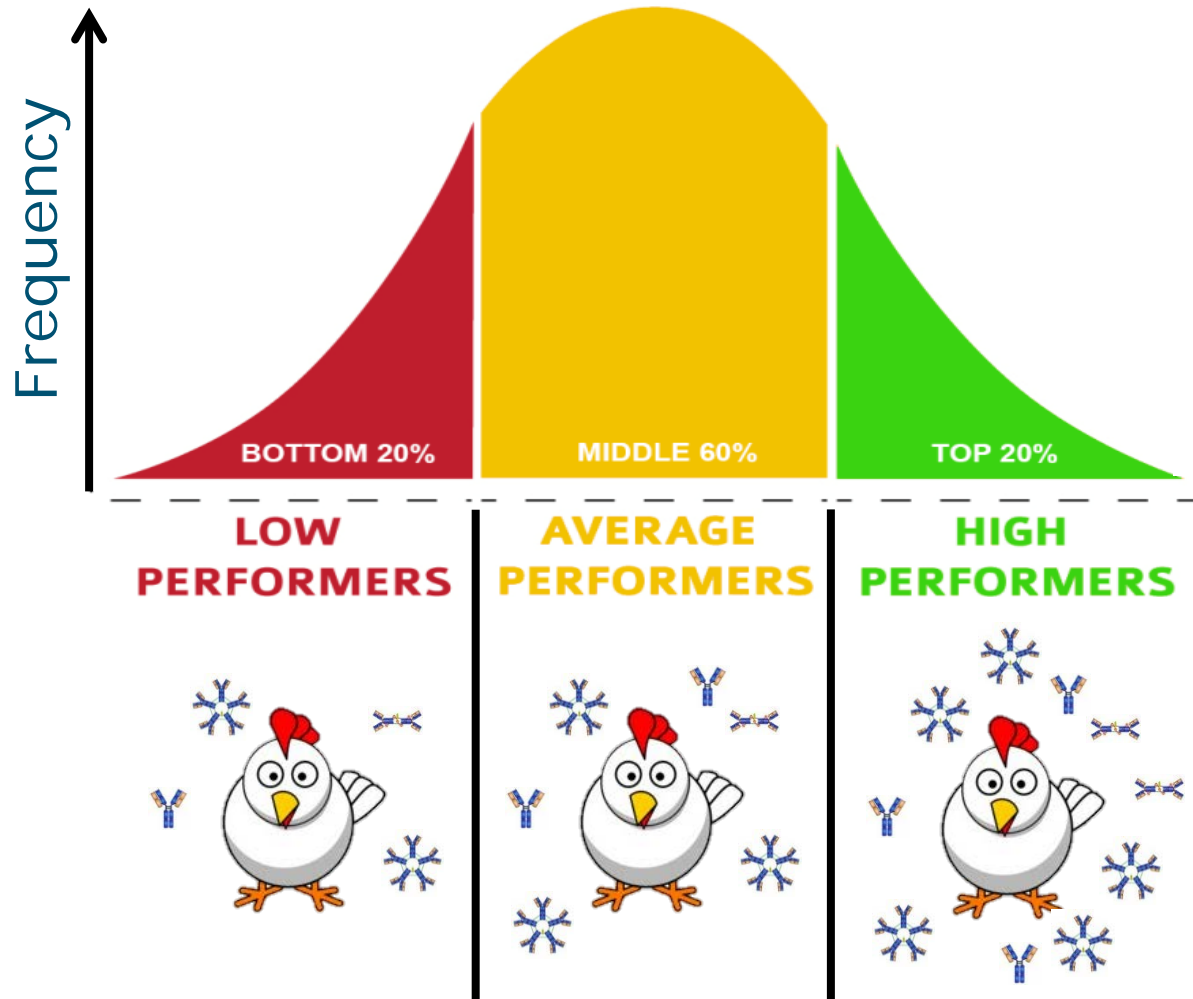IgTotal natural antibodies binding KLH
at 16 weeks of age

AND identify its genetic background

Phenotypic variation

Genetic variation

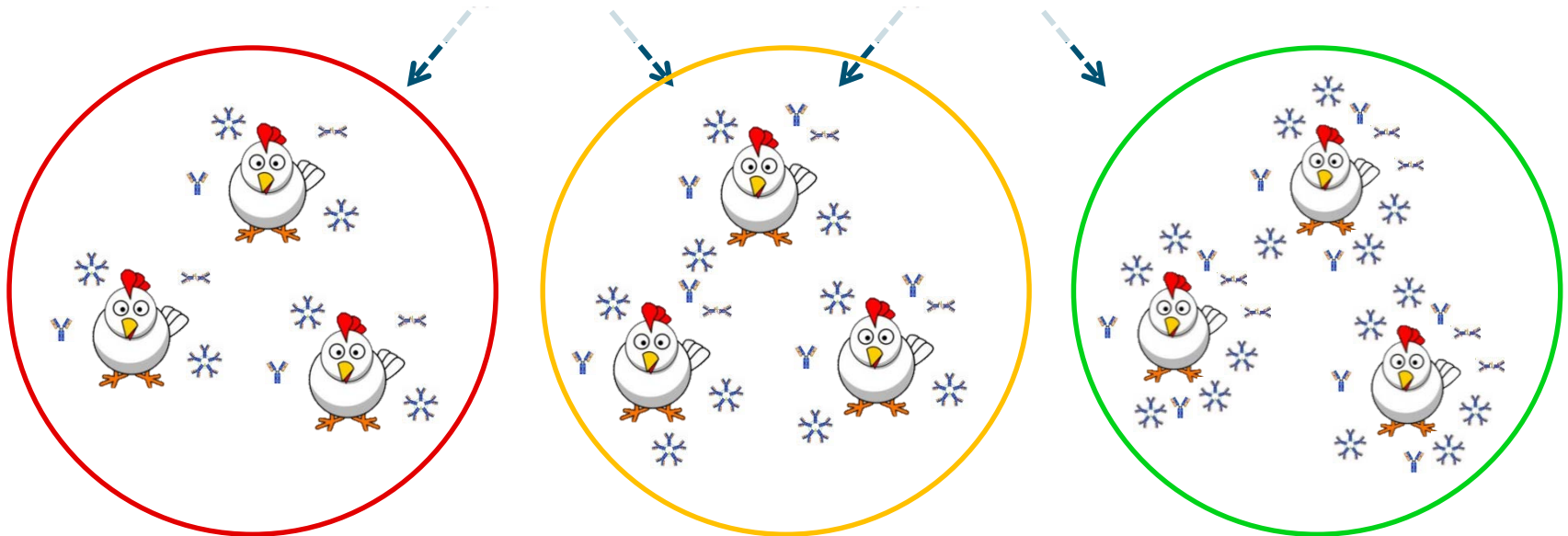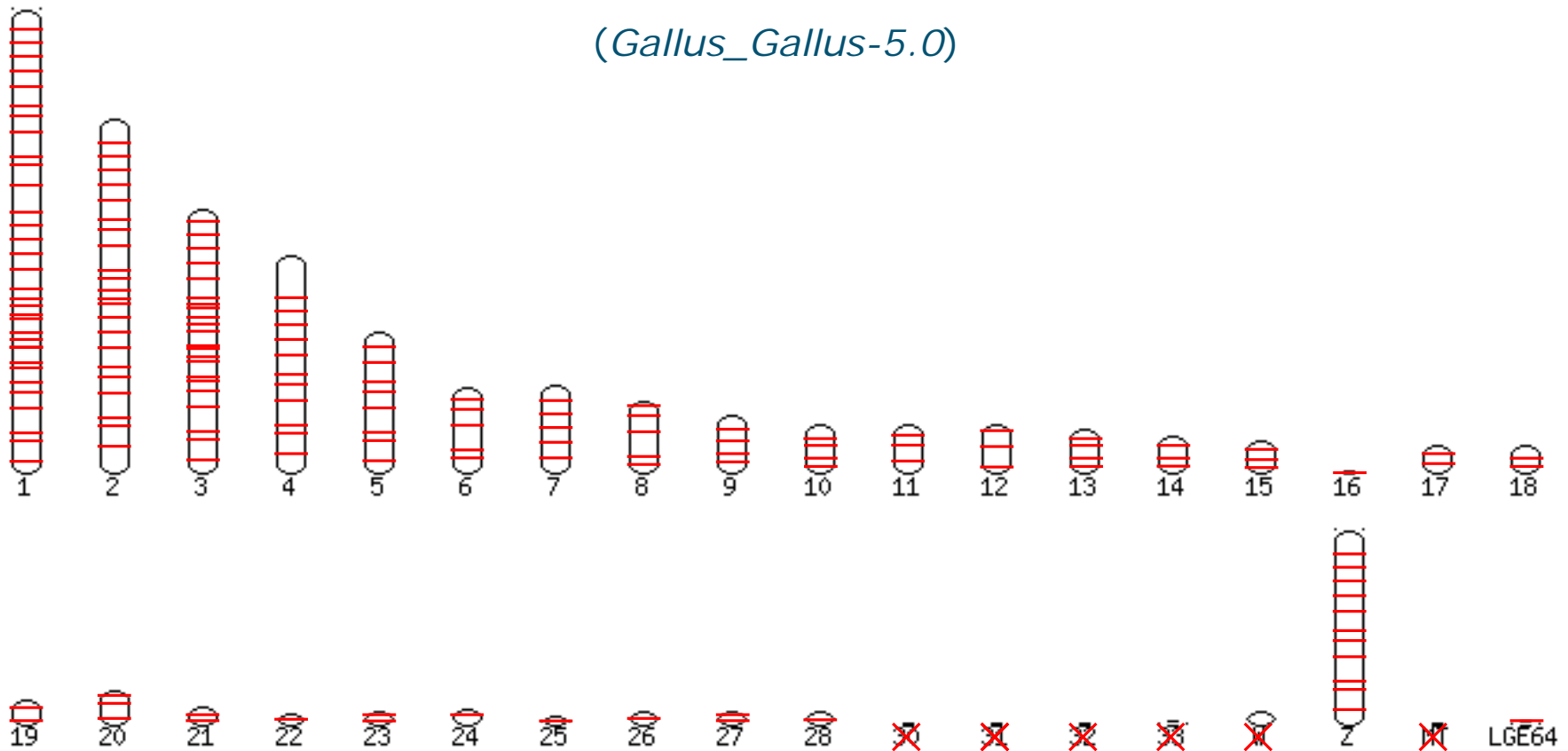| NAb type	IgTotal	IgM	IgA	IgG
Heritability	0.12	0.14	0.10	0.07

Berghof et al., 2015, PLoS ONE

LOW PERFORMERS   AVERAGE PERFORMERS   HIGH PERFORMERS

Genome-wide association study (GWAS)

Chicken genome

(*Gallus_Gallus-5.0*)

Genome-wide association study

- ~1,600 chickens (♂ + ♀) ~16 weeks of age

- IgTotal, IgM, IgA, and IgG titers

- 57,636 SNP

  → Quality control: 15,578 useable SNP

- Single SNP GWAS

Associated regions

- 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   → -

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Manhattan plot IgM

IgM NAb
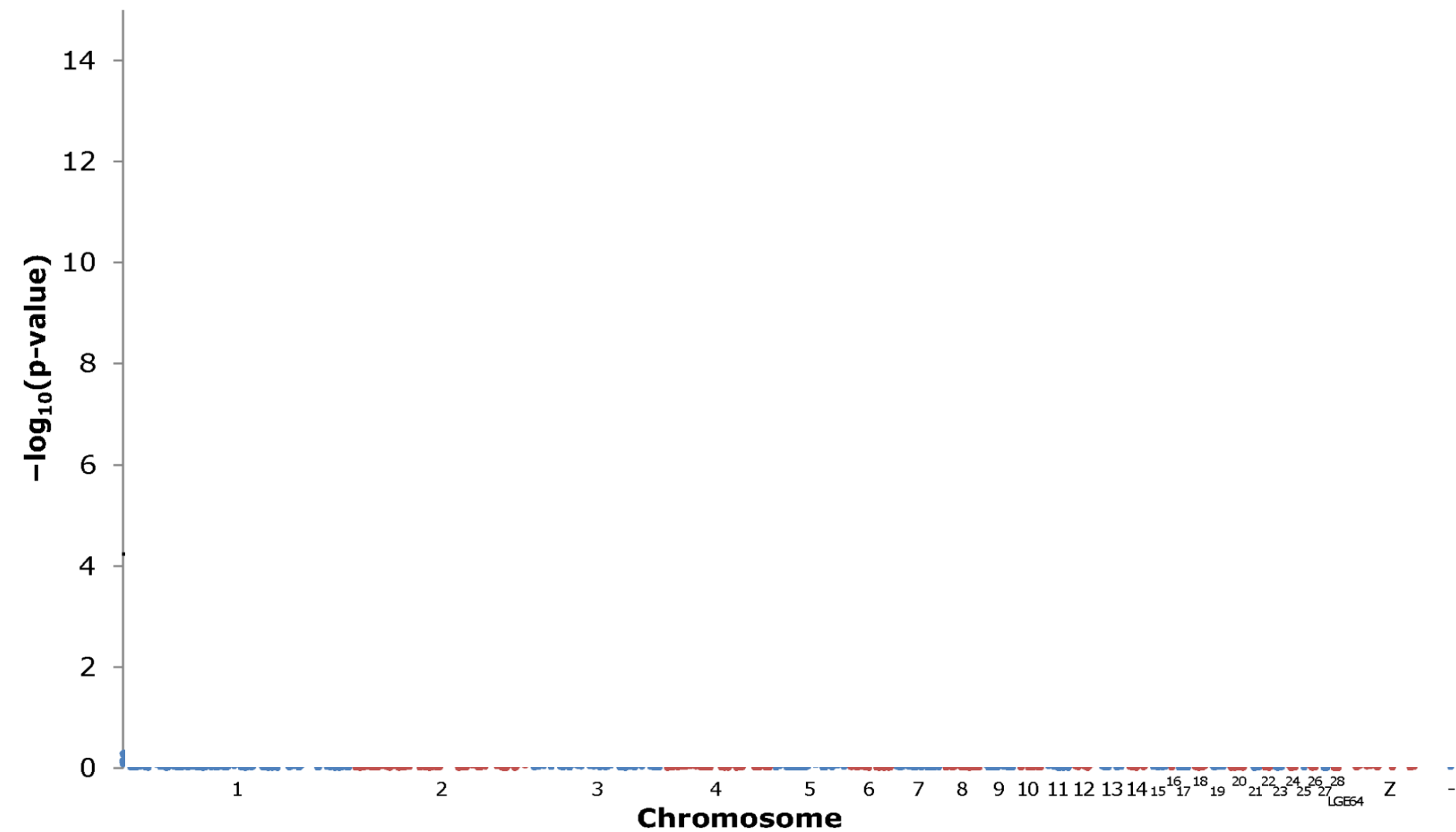
Fine mapping of GGA4 for IgM NAb

Fine mapping of GGA4 for IgM NAb

Fine mapping of GGA4 for IgM NAb

3,153 SNP → 28 SNP = AA change
1 SNP = highest impact scores

TLR1A

Ensembl VEP (SIFT) + PolyPhen2

Chromosome 4

69,526,705 bp

70,150,147 bp
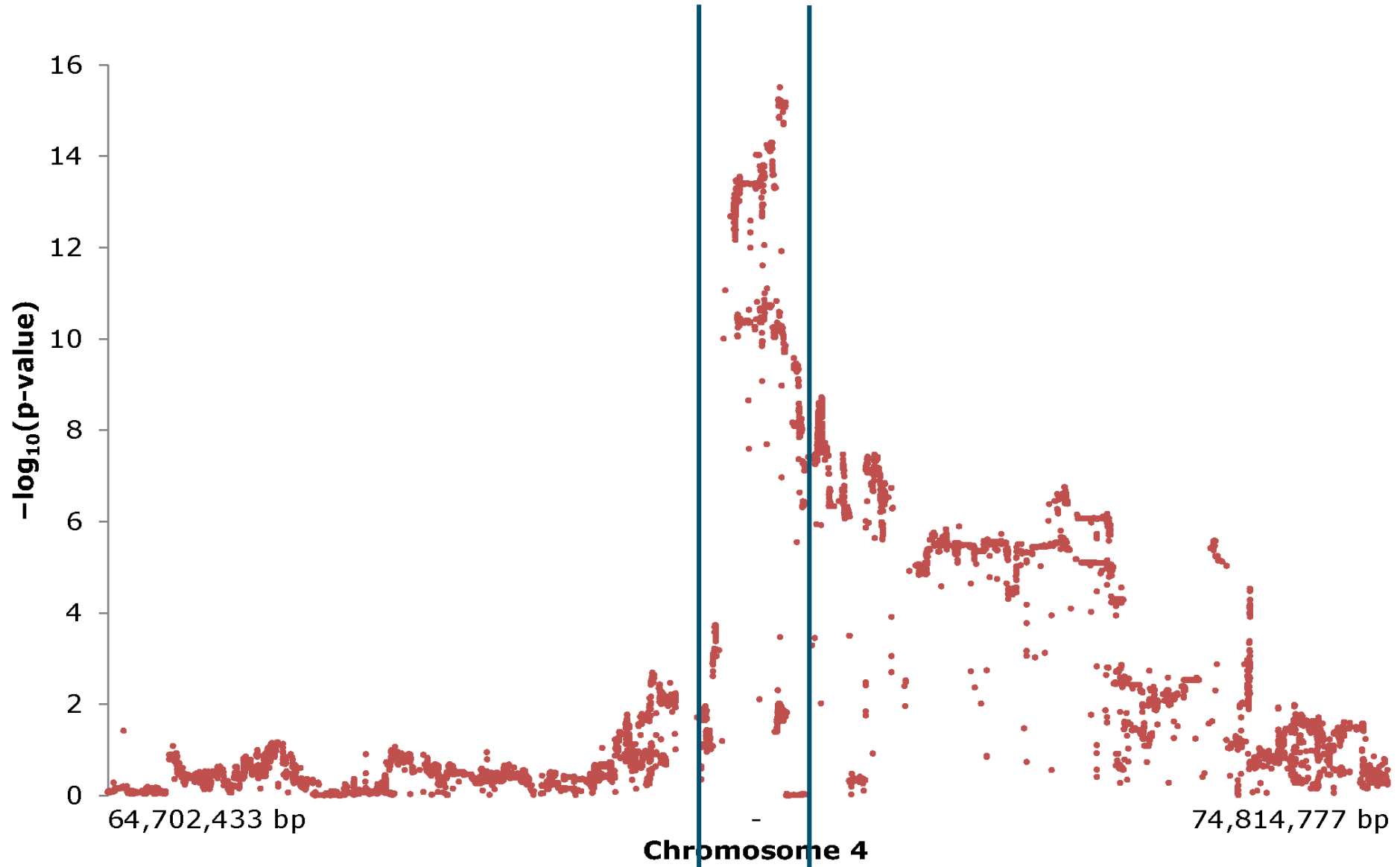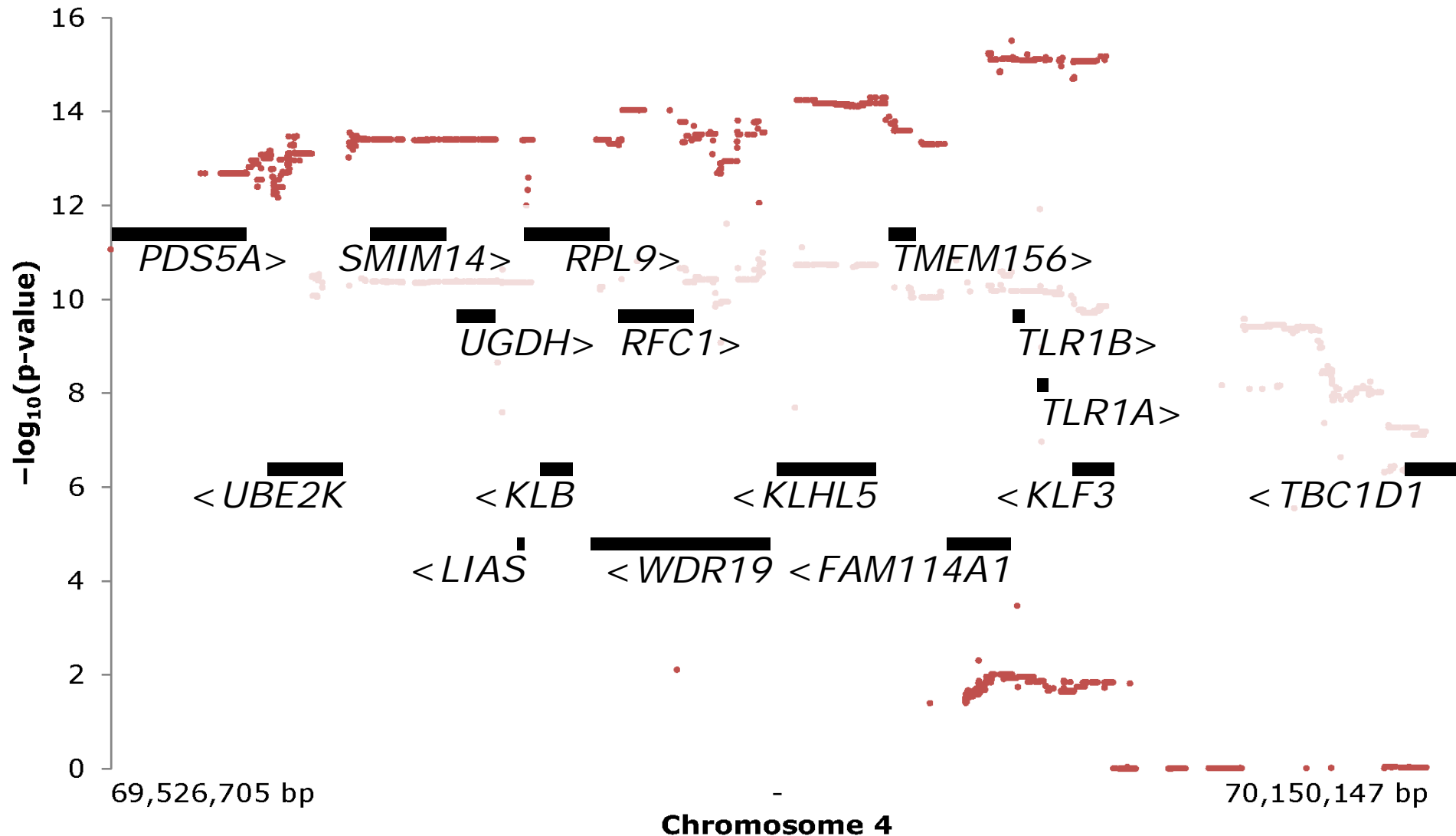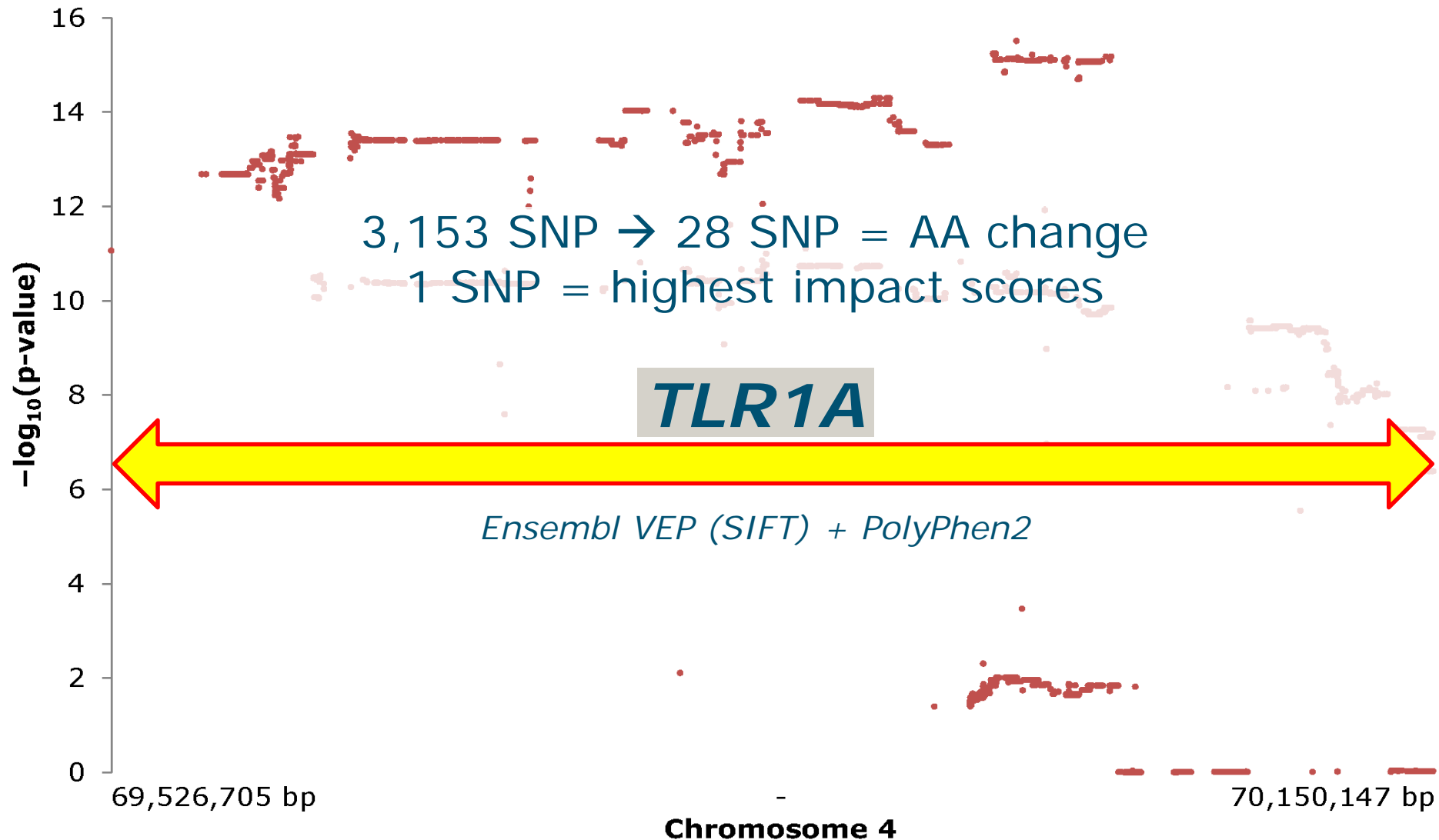
GWAS summary

■ Genetic variation underlying IgM NAb

- Most likely candidate: *TLR1A*
- Full dominance
- Explains ~60% of genetic variation

■ No significant associations for IgTotal, IgA, and IgG

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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

Thanks for your attention!!!
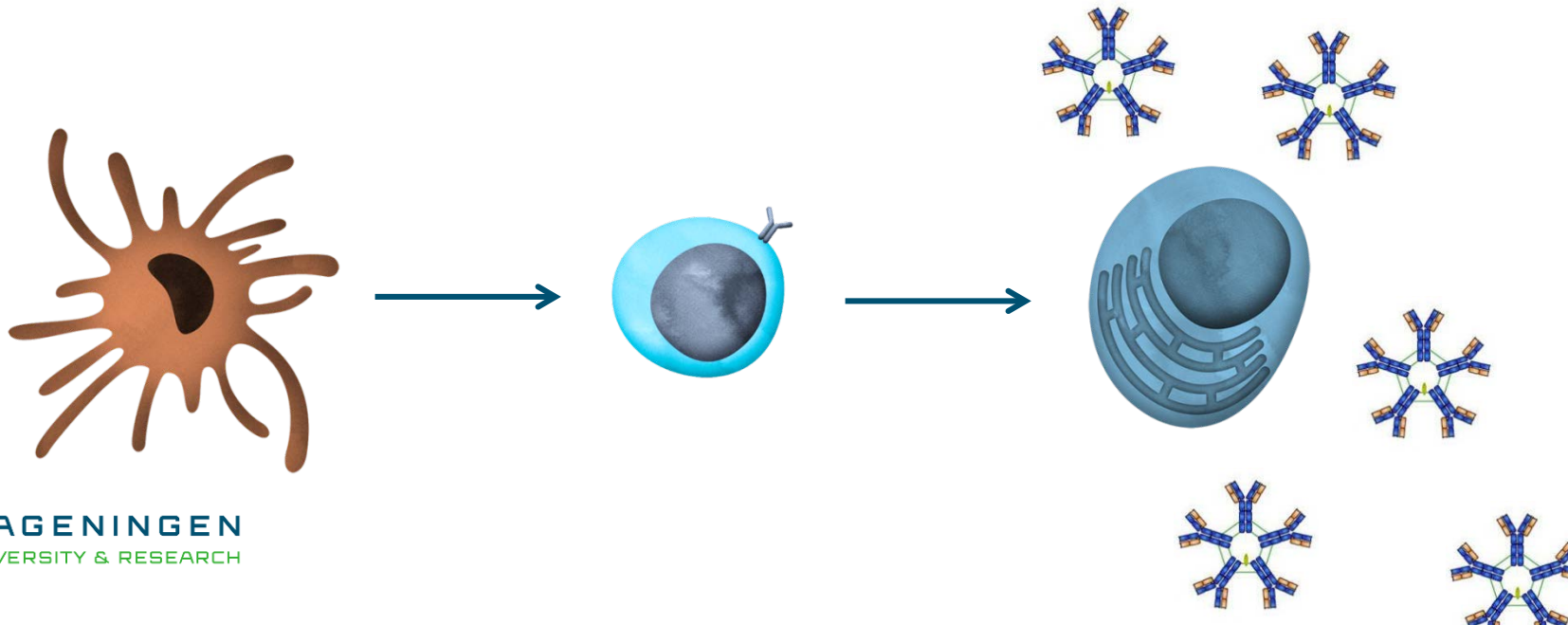
1. <u>KLH-binding IgM natural antibodies</u> and

<u>total IgM antibody concentrations</u> are influence 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

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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

Huang et al., 2011, BMC Evol Biol

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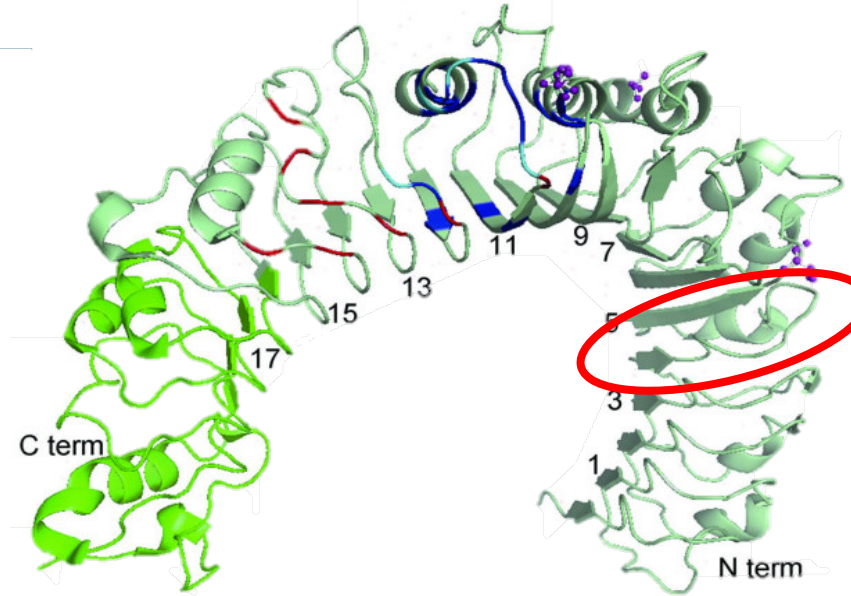
A role of TLR1A in (natural) antibody levels
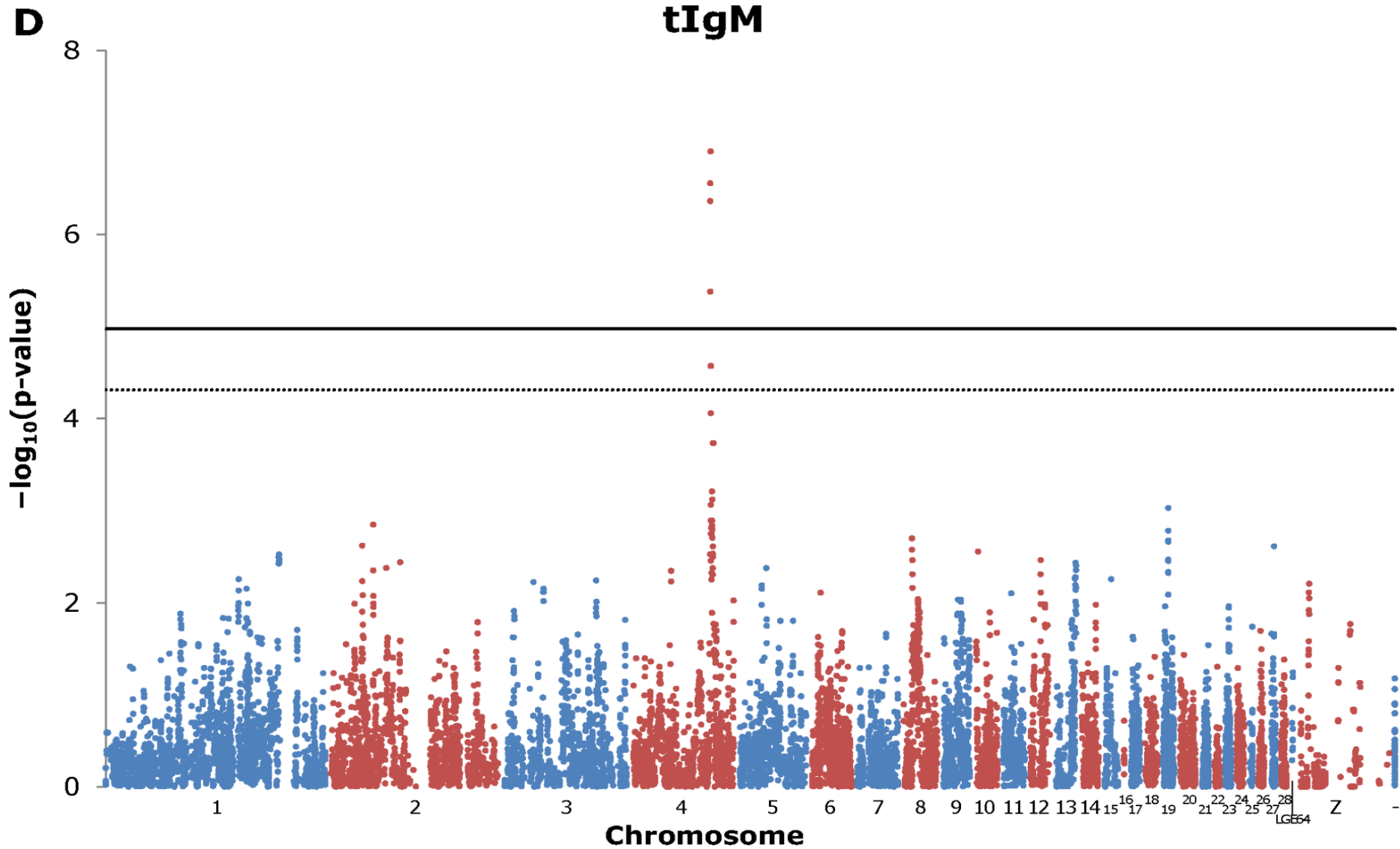
- B cell ontogeny

  - 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%)

Manhattan plot IgM antibody concentration

GGA4, 70Mbp
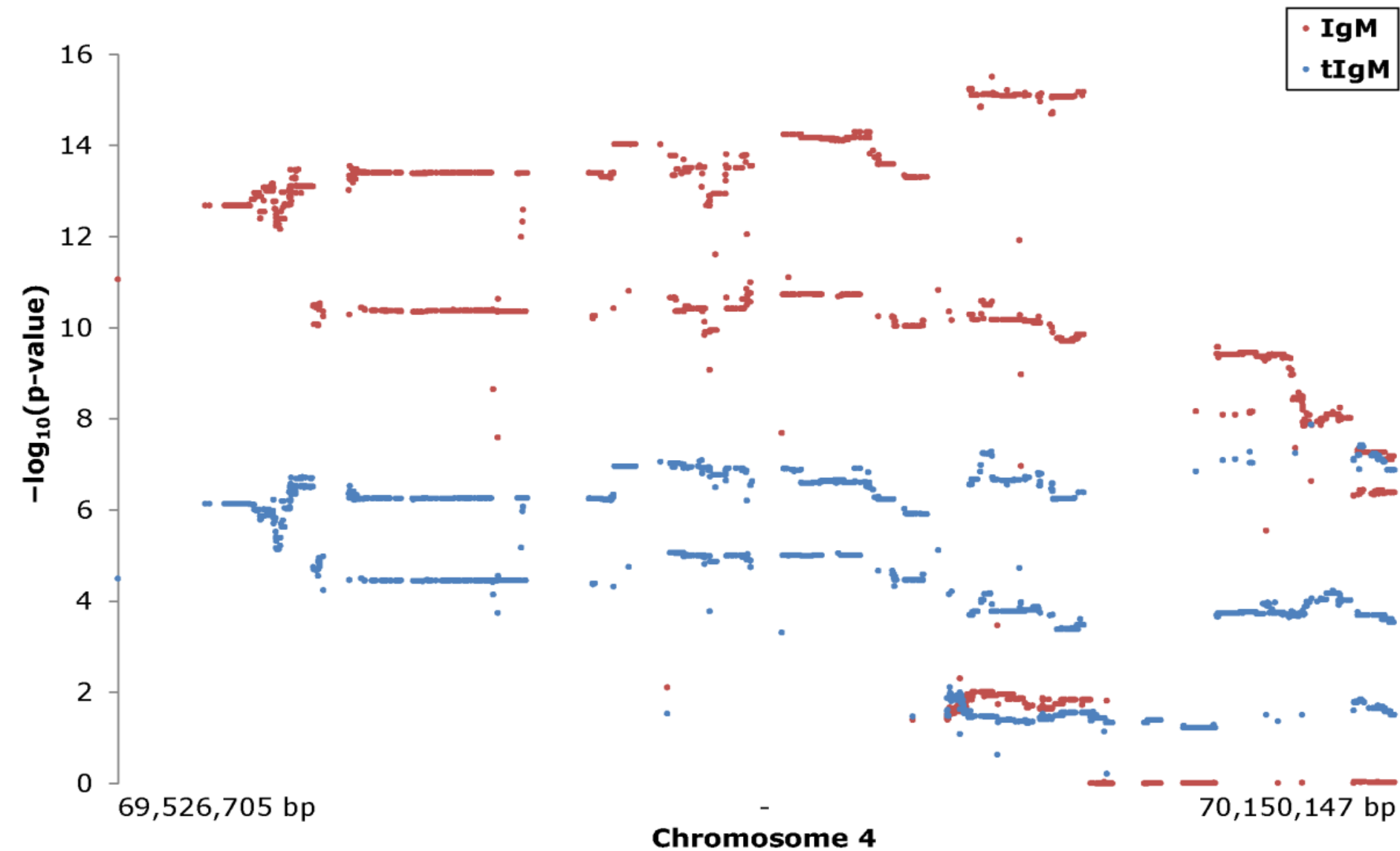
GWAS total antibody concentrations

- 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

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Heritabilities

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

Berghof et al., 2015, PLoS ONE

Ab type	IgTotal	IgM	IgA	IgG
Heritability	0.08	0.23	0.22	0.06
Mat. Env.	-	-	-	-

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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	

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	

Genetic correlations

Phenotypic correlations

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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

Genotypes

n = 488   2,740 SNP

70 key ancestors

120 key ancestors

57,636 SNP

WGS

n = 1,140   11,173 SNP

(of 52,232 SNP)
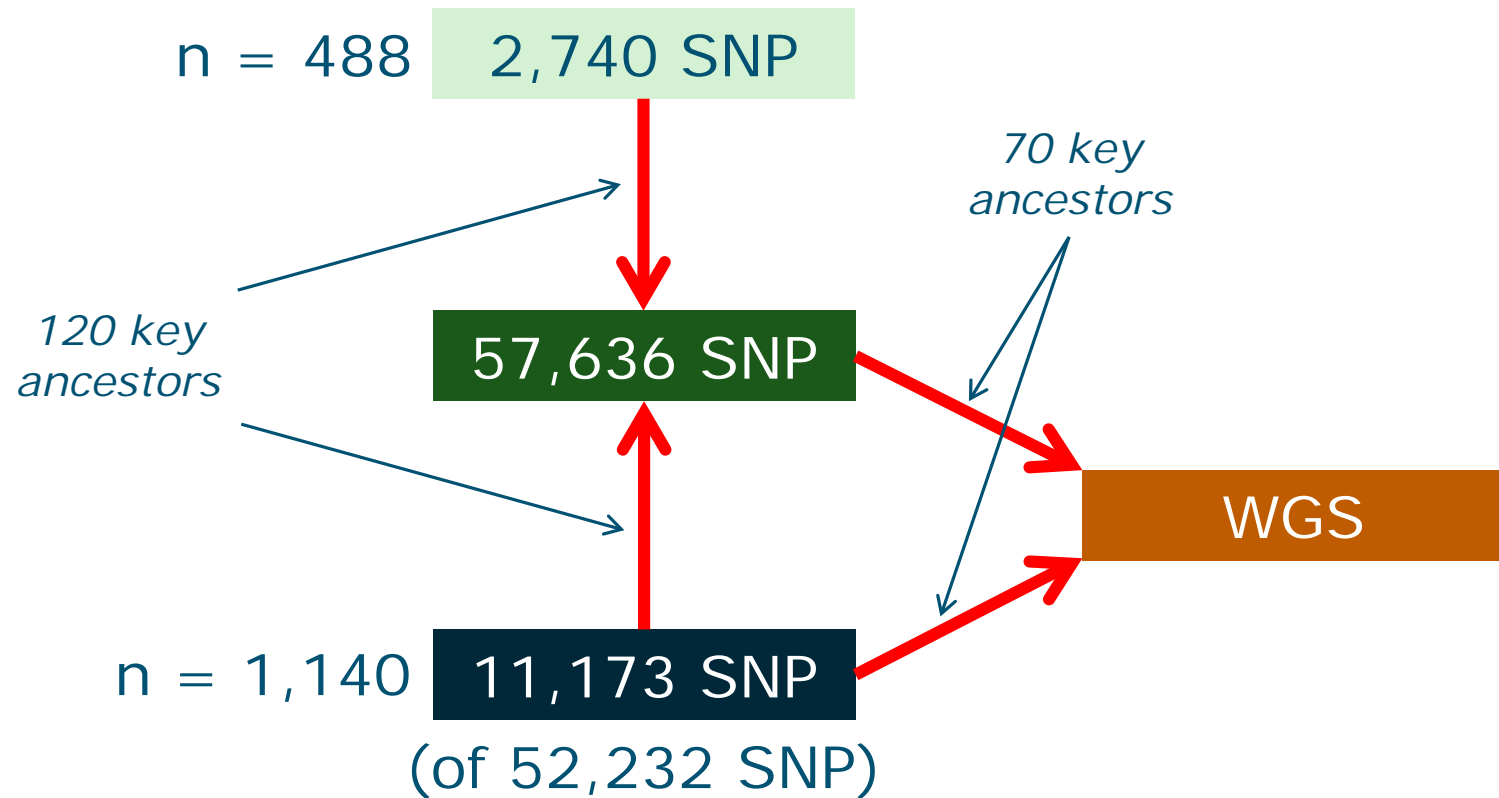
Illumina, imputed with Beagle 4.0 (≥97%),
average sequence depth = 12.4 (2.1)

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Gene association

1 gene

3 genotype classes (SNP)

AA AB BB