Association study of candidate genes selected in QTL regions for immune responses in chickens.

Maria Siwek

University of Technology and Life Sciences,
Bydgoszcz, POLAND

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Introduction

Ultimate goal of QTL research

QTL → QTN

Detection of Different Quantitative Trait Loci to Keyhole Lympet Hemocyanin and *Mycc* in Two Unrelated Populations of

M. Siwek,*[1] A. J. Buitenhuis,* S. J. B. Cornelissen,* M. G. I

Available online at www.sciencedirect.com

SCIENCE (

ELSEVIER

Developmental and Comparativ

Detection of QTL for innate: No
LPS and LTA in two indepen

Maria Siwek *, Bart Buitenhuis, Sandra Cc
Richard Crooijmans, Martien Groen

Animal Breeding and Genetic Groups, Wagen

Received 27
Available onlin

A quantitative trait locus for a primary antibody response to keyhole limpet hemocyanin on chicken chromosome 14—Confirmation and candidate gene approach

* M. Nieuwland,† A. Witkowski,‡ G. Zięba,‡ G. Minozzi,§
F. Knol,# and M. Bednarczyk*

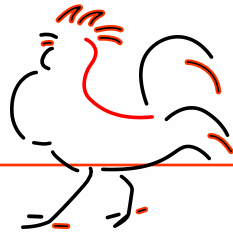
GENETICS

Quantitative trait loci associated with the humoral innate immune response in chickens were confirmed in a cross between Green-Legged Partridgelike and White Leghorn

A. Sławińska,*[1] A. Witkowski,† M. Nieuwland,‡ G. Minozzi,§ M. Bednarczyk,* and M. Siwek*

**Department of Animal Biotechnology, University of Technology and Life Sciences, Mazowiecka 28, 85-225 Bydgoszcz, Poland; †Department of Biological Bases of Animal Production, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland; ‡Adaptation Physiology Group, Department of Animal Sciences, Wageningen Institute of Animal Sciences, Wageningen University, 6700 AH Wageningen, the Netherlands; and §Parco Tecnologico Padano, Via Einstein, Polo Universitario, Lodi 26900, Italy*

Goal



The goal of this study was to verify *in silico* candidate gene selection with SNP genotyping and association study

Material

Green-legged partidgelike (Zk)

- native chicken breed
- closed population
- unselected
- **FACT: resistant to harsh environment**

QQ

White Leghorn (WL)

- commercial breed
- selected towards high egg production
- **ASSUMPTION: less resistant to harsh environment**

qq

Experimental population

F_0 : Zk x WL

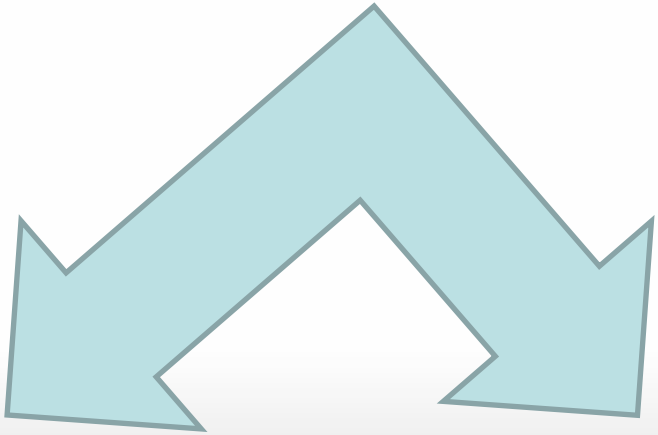
F_1 : (Zk x WL) x (Zk x WL)

F_2 : **500** individuals

Phenotypic traits

Immune responses

innate



adaptive

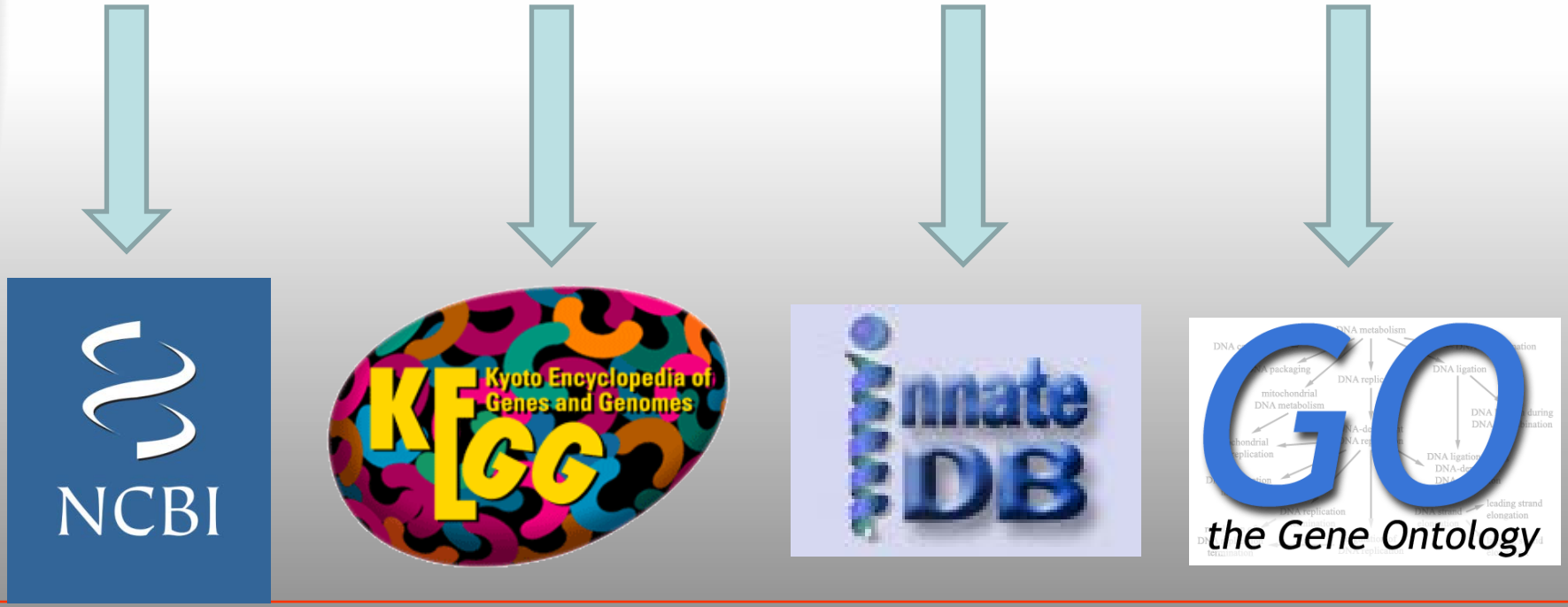
Antibody response towards lipopolisaccharide **(LPS)**

Antibody response towards lipotechoic acid **(LTA)**

Primary antibody response towards keyhole lypmhet heamocyanin **(KLH)**

Methods: candidate genes

In silico analysis of positional and biological candidate genes in QTL regions was performed using:



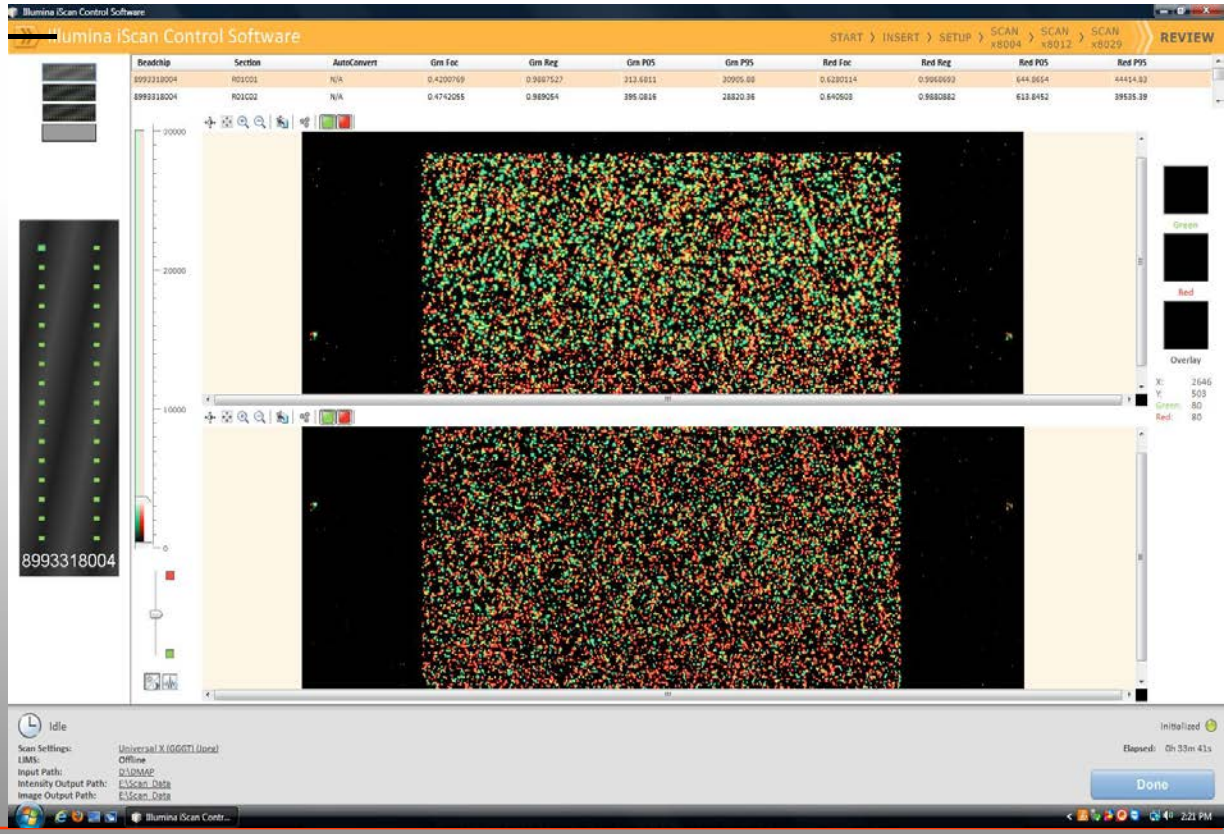
Methods: SNP selection

Ensembl, Biomart tool

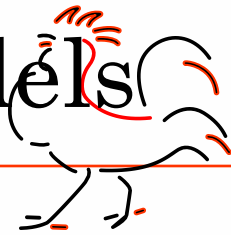
iCom, quality check on myillumina.com

Methods: Illumina SNP genotyping

- Custom SNP assay
- Golden gate genotyping
- Genome studio scoring



Methods: Association - statistical models



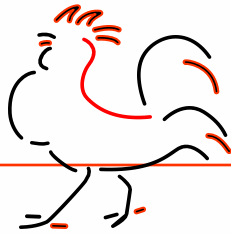
- **Additive effects – two models**
  - Mixed model

$$y = X^{*}b + Z^{*}g + e^{*}$$

  - The CAR score

$$\boldsymbol{\omega} = \mathbf{P}^{-1/2}\mathbf{P}_{Xy}$$

Results: Candidate genes



NCBI

605 Candidate genes on QTL regions located on three chromosomes : GGA9, GGA14, GGA18

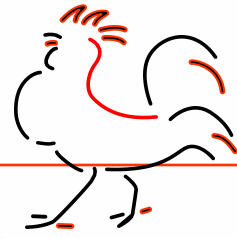
KEGG Kyoto Encyclopedia of Genes and Genomes

innateDB

GO the Gene Ontology

GGA14 (LTA, KLH) - 14 genes
GGA9 (LPS) - 8 genes
GGA18 (LPS) - 7 genes

Results: SNP markers



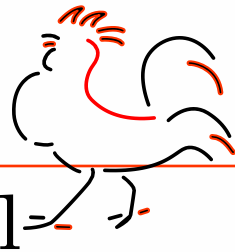
All together 2023 SNP markers:

- 1339 on GGA14
- 392 on GGA9
- 297 on GGA18

illumina®

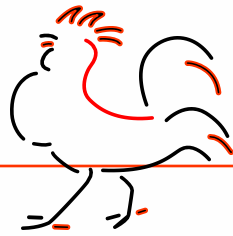
384 SNP markers

Results: SNP genotyping



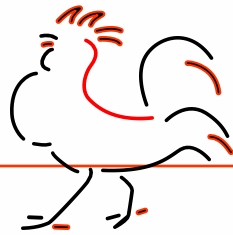
- 40 SNPs removed from the set due to technical failure
- 132 SNPs removed based on the MAF criterion
- 1 SNP removed based on the Call Rate
- **211 SNPs used in association analysis**
- Average MAF:
  - 0.17 (all SNPs)
  - 0.27 (selected SNPs)
- Average Call Rate:
  - 97.52% (all SNPs)
  - 97.80% (selected SNPs)

Results – mixed model



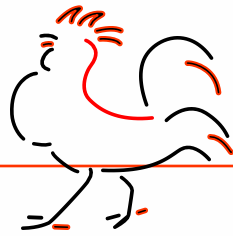
LPS	KLH/	LTA	LPS
GGA9	GGA14/	GGA14	GGA18
EPHB1 PROCR ST6GAL1	CARD11 IL9R MAPK8IP3 PDGFA PRCKB		CRLF3 FOXJ1
GPC1		IL9R MAP2K3 MAPK8IP3 PRCKB	FOXJ1 ITGB4 JMJD6
GPC1			ITGB4 UNCBD
	PGP		

Results – CAR score



LPS	KLH/	LTA	LPS
GGA9	GGA14 /	GGA14	GGA18
EPHB1 KLHL6 ST6GAL1	IL9R MAPK8IP3 PGP PRCKB TRAF7		CRLF3
EPHB1 GPC1 KLHL6 PROCR SOX14		TNFRSF13B MAPK8IP3 CARD11 IL9R	FOXJ1 ITGB4 JMJD6 MAP2K4 UNCBD
KLHL6	IL9R MAPK8IP3 PRCKB SOCS1		ITGB4

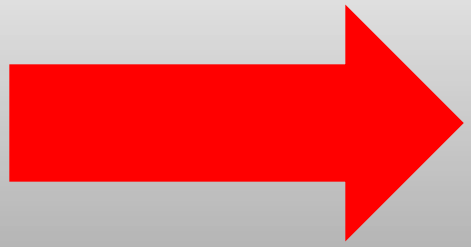
Conclusions

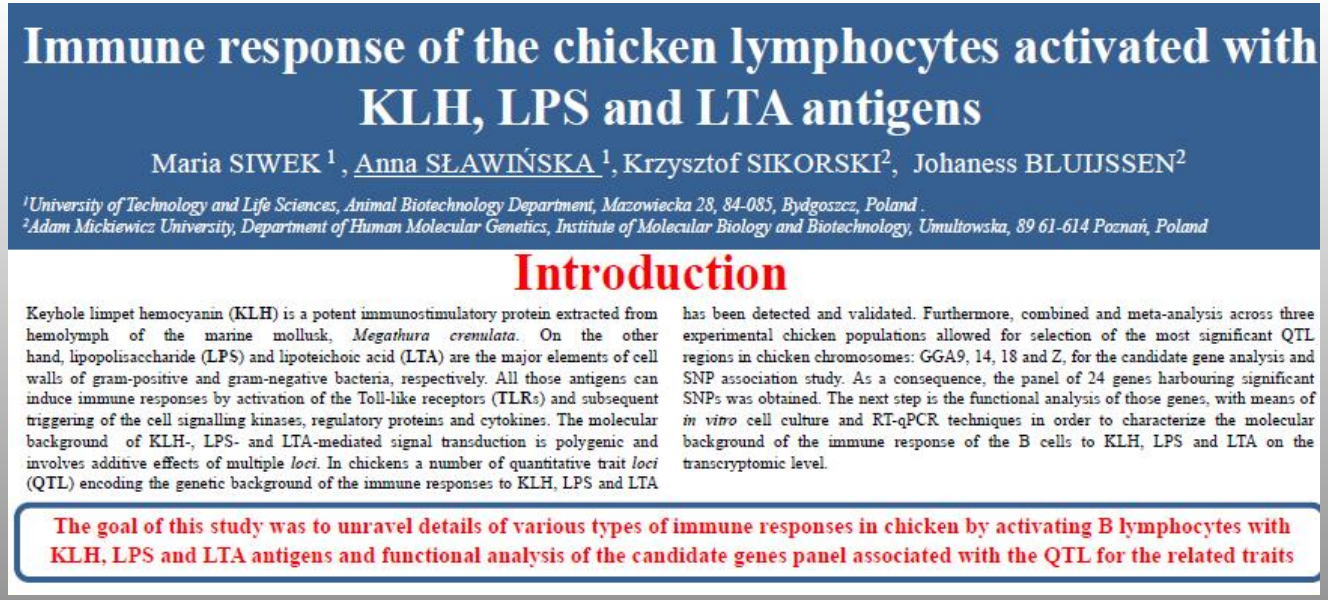


- Custom SNP assay was very informative
- Candidate genes selected *in silico* were successfully validated by SNP association
- Both statistical models applied to the data set show association with selected candidate genes

Future plans

- Analysis for dominant and epistatic effects for selected genes
- Validation study *in vitro* – chicken lymphocytes LPS/LTA/KLH stimulation – analysis of the effect at the mRNA and protein level- preliminary results at the poster





Immune response of the chicken lymphocytes activated with KLH, LPS and LTA antigens

Maria SIWEK [1], Anna SŁAWIŃSKA [1], Krzysztof SIKORSKI[2], Johaness BLUIJSSEN[2]

[1]University of Technology and Life Sciences, Animal Biotechnology Department, Mazowiecka 28, 84-085, Bydgoszcz, Poland .
[2]Adam Mickiewicz University, Department of Human Molecular Genetics, Institute of Molecular Biology and Biotechnology, Umultowska, 89 61-614 Poznań, Poland

Introduction

Keyhole limpet hemocyanin (KLH) is a potent immunostimulatory protein extracted from hemolymph of the marine mollusk, *Megathura crenulata*. On the other hand, lipopolisaccharide (LPS) and lipoteichoic acid (LTA) are the major elements of cell walls of gram-positive and gram-negative bacteria, respectively. All those antigens can induce immune responses by activation of the Toll-like receptors (TLRs) and subsequent triggering of the cell signalling kinases, regulatory proteins and cytokines. The molecular background of KLH-, LPS- and LTA-mediated signal transduction is polygenic and involves additive effects of multiple *loci*. In chickens a number of quantitative trait *loci* (QTL) encoding the genetic background of the immune responses to KLH, LPS and LTA has been detected and validated. Furthermore, combined and meta-analysis across three experimental chicken populations allowed for selection of the most significant QTL regions in chicken chromosomes: GGA9, 14, 18 and Z, for the candidate gene analysis and SNP association study. As a consequence, the panel of 24 genes harbouring significant SNPs was obtained. The next step is the functional analysis of those genes, with means of *in vitro* cell culture and RT-qPCR techniques in order to characterize the molecular background of the immune response of the B cells to KLH, LPS and LTA on the transcryptomic level.

The goal of this study was to unravel details of various types of immune responses in chicken by activating B lymphocytes with KLH, LPS and LTA antigens and functional analysis of the candidate genes panel associated with the QTL for the related traits

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