



Integrating blood transcriptome and immunity traits to identify markers of immune capacity in pigs

Maroilly T., Blanc F., Lemonnier G., Lecardonnel J., Bidanel J.P., Rau A., Billon Y., Mercat M.J., Estellé J., Rogel-Gaillard C.

UMR1313 GABI, Génétique Animale et Biologie Intégrative, Jouy-en-Josas
<http://www6.jouy.inra.fr/gabi>



ABOUT IMMUNE CAPACITY

Session 21 on resilience:
« Problem-free with no vet costs »
« 'She' never (or rarely) gets ill »

Pathogen pressure:
viruses, bacteria, parasites

Farm system:
practices, feed diet, social interactions, etc.

Vaccination
Sanitary rules



Anti-infectious medication
(e.g. antibiotics)

Individual response variability

[(genome x epigenome) x microbiome] x environment

Death

Survival with loss of
performance

Survival with no loss of
performance

AIM OF THE STUDY: TO IDENTIFY MARKERS OF IMMUNE CAPACITY

Genetic and genomic study of immunity traits

- Animals with no clinical sign of infection
- No focus on a specific pathogen

Immunity Traits in Pigs: Substantial Genetic Variation and Limited Covariation

Laurence Flori^{1,2,3}, Yu Gao^{1,2,3}, Denis Laloë^{1,3}, Gaëtan Lemonnier^{1,2,3}, Jean-Jacques Leplat^{1,2,3}, Angélique Teillaud^{1,2,3}, Anne-Marie Cossalter⁴, Joëlle Laffitte⁴, Philippe Pinton⁴, Christiane de Vaureix⁵, Marcel Bouffaud⁶, Marie-José Mercat⁷, François Lefèvre⁵, Isabelle P. Oswald⁶, Jean-Pierre Bidanel^{1,3}, Claire Rogel-Gaillard^{1,2,3*}

Flori et al., PLOS One, 2011

The peripheral blood transcriptome reflects variations in immunity traits in swine: towards the identification of biomarkers

Núria Mach^{1,2*†}, Yu Gao^{3†}, Gaëtan Lemonnier^{1,2}, Jérôme Lecardonnel^{1,2}, Isabelle P Oswald^{4,5}, Jordi Estellé^{1,2} and Claire Rogel-Gaillard^{1,2*}

Mach et al., BMC Genomics, 2013

SUS_FLORA PROJECT: A MULTIDISCIPLINARY PARTNERSHIP



INRA, Génétique Animale et Biologie Intégrative, Jouy-en-Josas

T. Maroilley, J. Estellé, F. Blanc, G. Lemonnier, J. Lecardonnel, M. Moroldo, C. Denis, G. Piton, J-J. Leplat, S. Vincent-Naulleau, J-P. Bidanel



INRA, Virologie et Immunologie Moléculaires, Jouy-en-Josas

N. Bertho, I. Schwartz-Cornil

INRA, ISP, Tours

M. Berri



INRA, TOXALIM, Toulouse

I. Oswald, P. Gourbeyre, P. Pinton



Bioporc, La Motte au Vicomte

M-J. Mercat

INRA, GENESI

Y. Billon

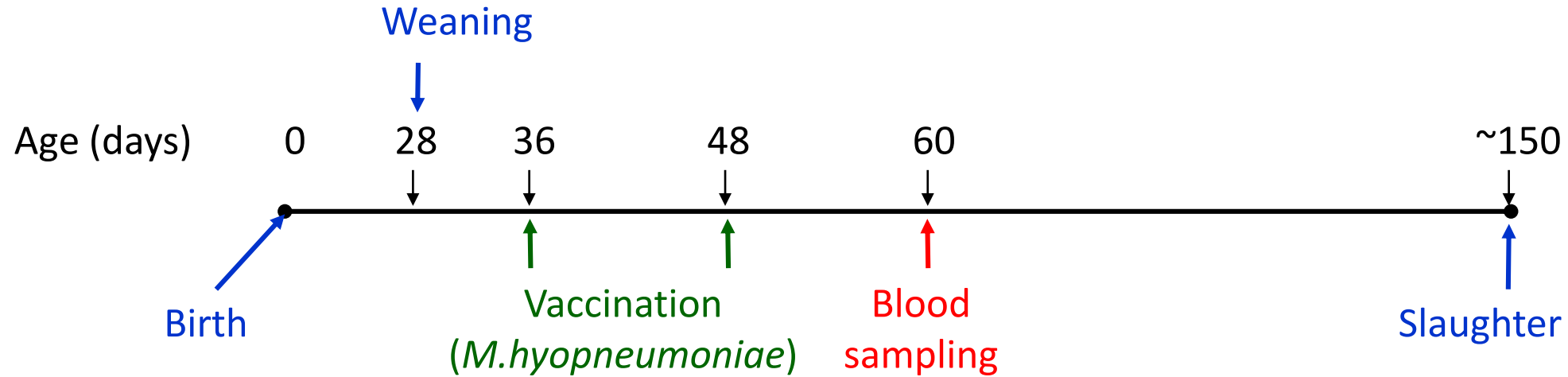


INRA MICALIS, Jouy

P. Lepage, F. Levenez, J. Doré

ANIMALS, SAMPLING AND DATA

- Large White breed
- 90 families
- 550 60-day-old piglets



Immunity parameters

- Direct measures from blood
- Responses to *in vitro* stimulation

Blood transcriptome

- Agilent microarrays
- Subset of 243 piglets

Genotypes

- 60K SNP Illumina chips

IMMUNITY PARAMETERS: DIRECT MEASURES FROM BLOOD

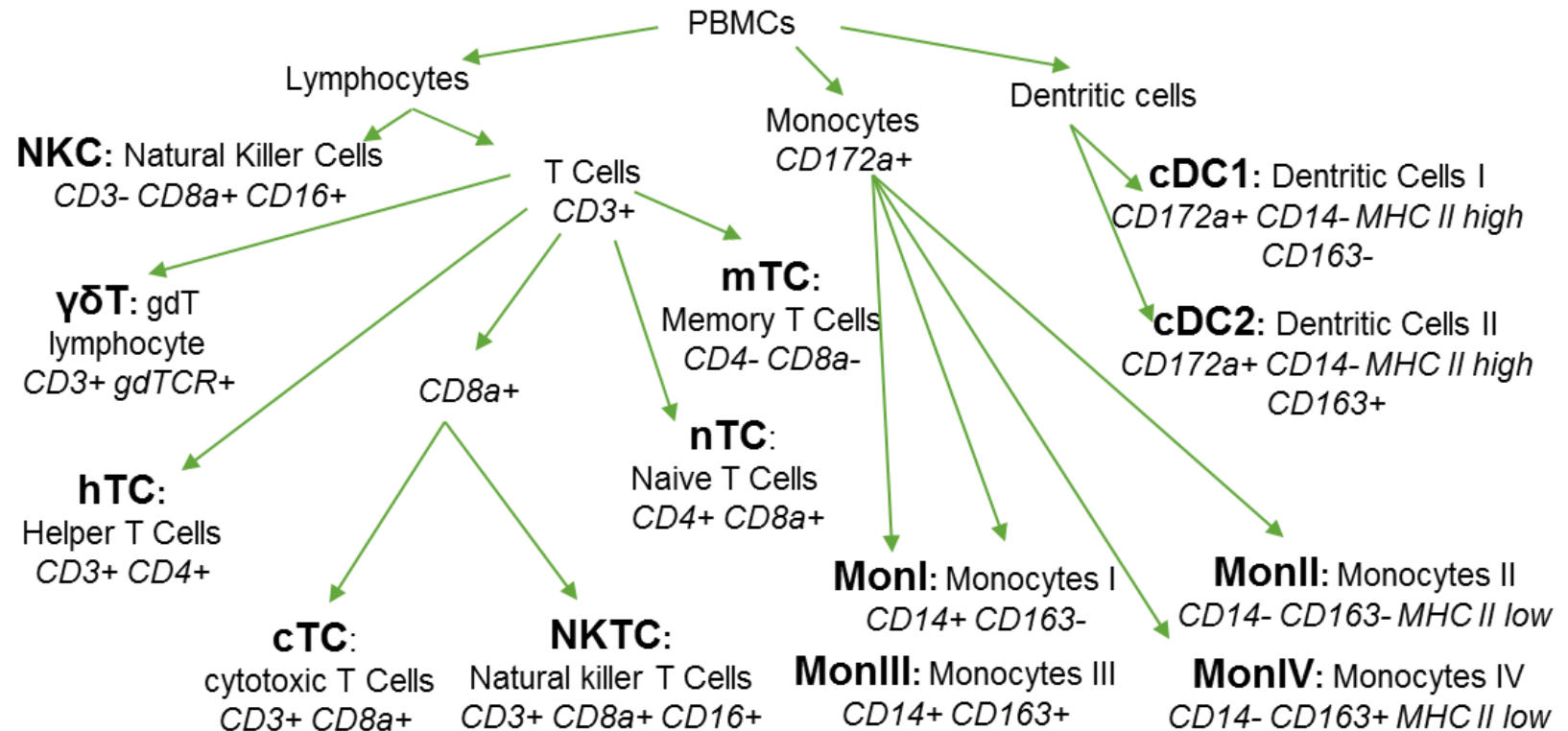
Seric molecules

- **IgGmh:** anti-*M-hyo* IgG
- **IgA:** Immunoglobulin A
- **HAPTO:** Haptoglobin
- **CRP:** C Reactive Protein

Hemogram

- **WBC:** White blood cells
- **LYM:** Lymphocytes
- **MON:** Monocytes
- **GRA:** Granulocytes
- **EOS:** Eosinophils
- **RBC:** Red blood cells
- **PTL:** Platelets
- **Hct:** Hematocrit
- **Hgb:** Hemoglobin
- **MCV:** mean cell volume
- **MCH:** mean cell hemoglobin
- **MCHC:** mean cell hemoglobin conc.

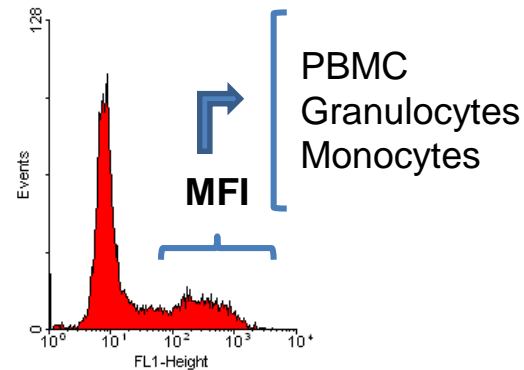
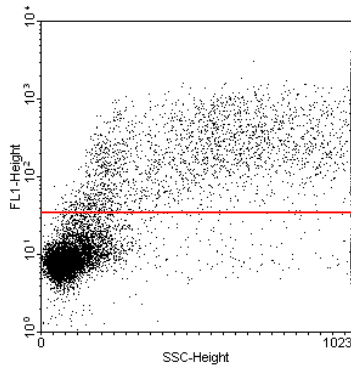
Cell sub-populations by flow cytometry



IMMUNITY PARAMETERS: RESPONSES TO *IN VITRO* STIMULATION

Phagocytosis capacity

| Cell system / IT | Number of phagocytosing cells | Number of phagocytosed bacteria per cell |
|------------------|-------------------------------|--|
| PBMC | PHAGO | PHAGO(MFI) |
| Granulocytes | PHAGOgra | PHAGOgra(MFI) |
| Monocytes | PHAGOmomo | PHAGOmomo(MFI) |



MFI = Mean Fluorescence Intensity

Production of cytokines

| Cell system | Total blood | | |
|---------------|-------------|-----|-----|
| | PIL | PI | LPS |
| Stimulation | | | |
| Time | 4h | 48h | |
| IL-2 | | | |
| IL-1 β | | | |
| IL-8 | | | |
| IL-10 | | | |
| IL-17 | | | |
| IFN- γ | | | |
| TNF- α | | | |

P = phorbol myristate acetate (PMA)

I = ionomycin

L = lipopolysaccharide (LPS)

GENETIC PARAMETERS AND COVARIATIONS OF IMMUNITY TRAITS

Estimation of factor effects (linear mixed models)

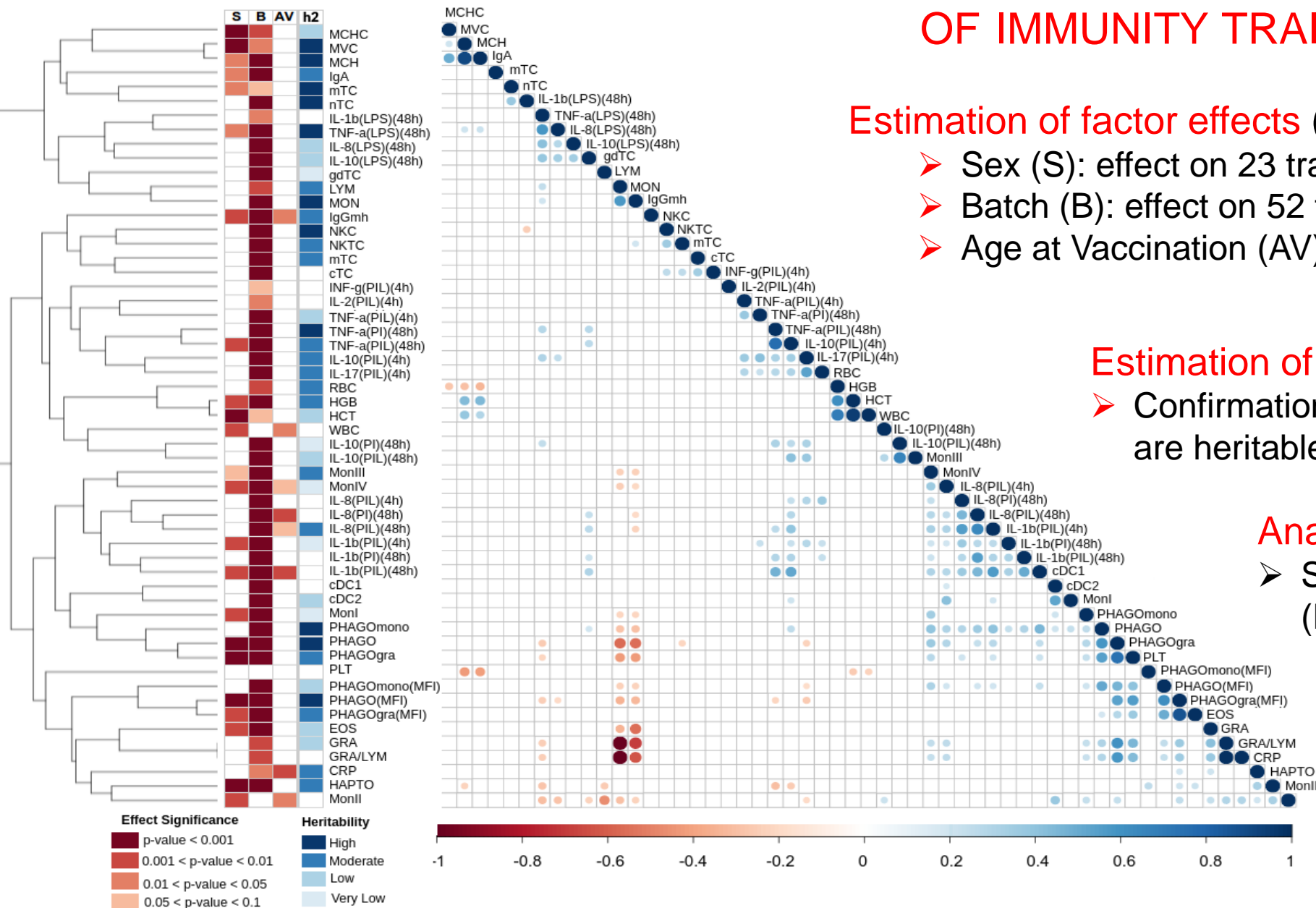
- Sex (S): effect on 23 traits (43%)
- Batch (B): effect on 52 traits (95%)
- Age at Vaccination (AV): effect on 8 traits

Estimation of heritabilities (h^2)

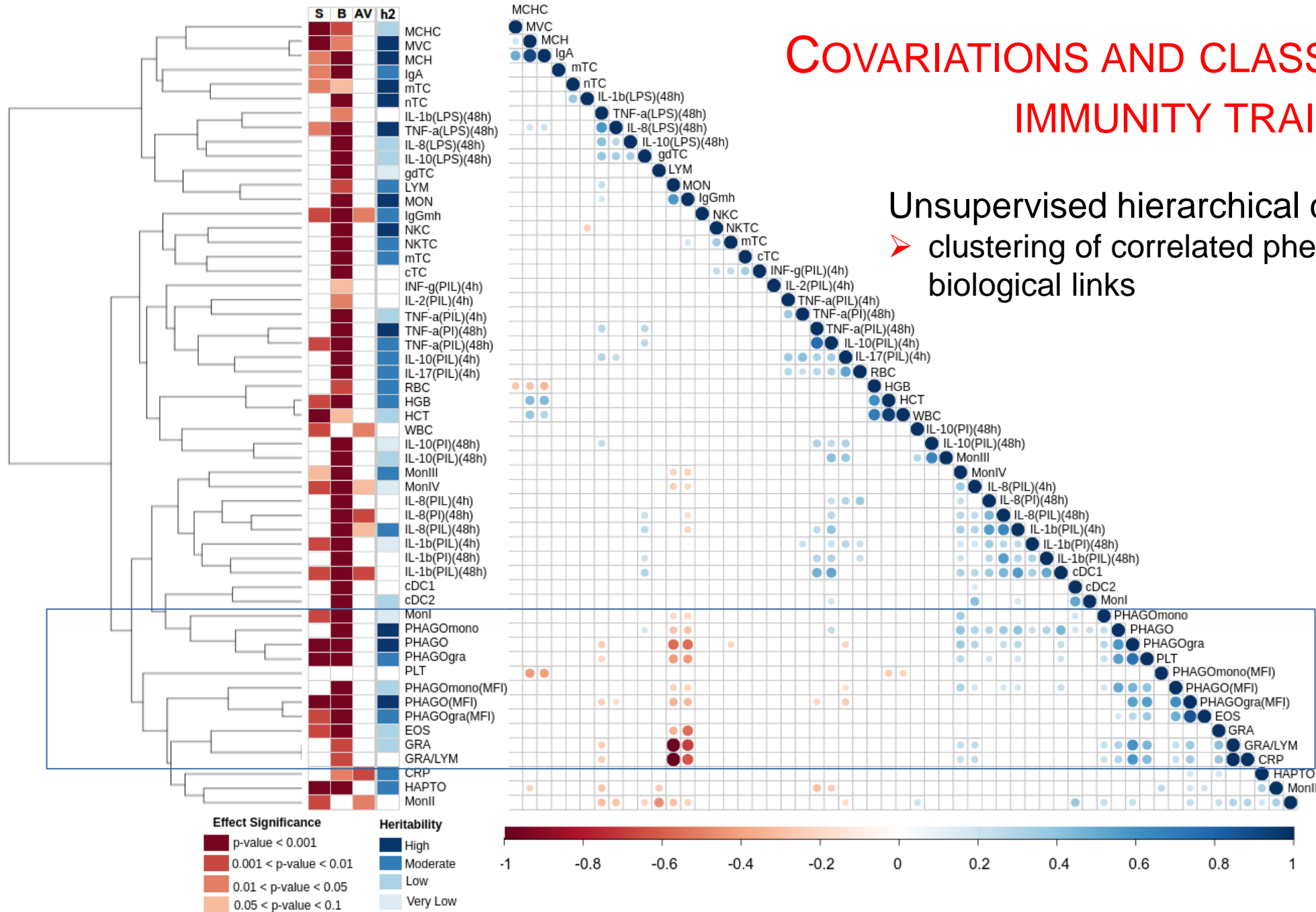
- Confirmation that many parameters are heritable

Analysis of covariations

- Spearman correlations (FDR<0.05)



COVARIATIONS AND CLASSIFICATION OF IMMUNITY TRAITS



Unsupervised hierarchical classification
 ➤ clustering of correlated phenotypes with biological links

Phagocytosis activity

COVARIATIONS BETWEEN GENE EXPRESSION IN BLOOD AND IMMUNITY TRAITS

Spearman correlation between gene expression (microarray data) and immunity trait measures (243 piglets); correction for multiple testing (FDR < 0.05)

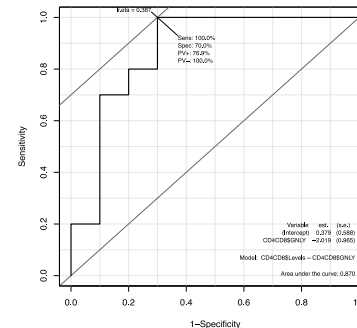
| | Blood Phenotype | Number of correlated genes |
|-----------------------------|------------------------|-----------------------------------|
| Cell sub-populations (FACS) | NKC | 200 |
| | NKTC | 73 |
| | $\gamma\delta$ TC | 38 |
| | MonIII | 2201 |
| | MonIV | 3 |
| | MonII | 2396 |
| | HGB | 4 |
| Hemogram | hTC | 3 |
| | cTC | 66 |
| | WBC | 237 |
| | LYM | 948 |
| | MON | 1283 |
| | GRA | 1106 |
| | EOS | 3 |
| | HCT | 1 |
| | MCH | 4 |

- Correlations found for 24 traits
 - 16 measured from blood
 - 8 measured after blood stimulation (response)

| | Response Phenotype | Number of correlated genes |
|--------------|---------------------------|-----------------------------------|
| Cytokines | IL-1 β (LPS)(48h) | 2 |
| | IL-8(PI)(48h) | 2 |
| | IL-8(PIL)(48h) | 75 |
| Phagocytosis | PHAGO | 5002 |
| | PHAGO(MFI) | 287 |
| | PHAGOgra | 1291 |
| | PHAGOmono | 3431 |
| | PHAGOmono(MFI) | 817 |

COVARIATIONS BETWEEN GENE EXPRESSION IN BLOOD AND IMMUNITY TRAITS

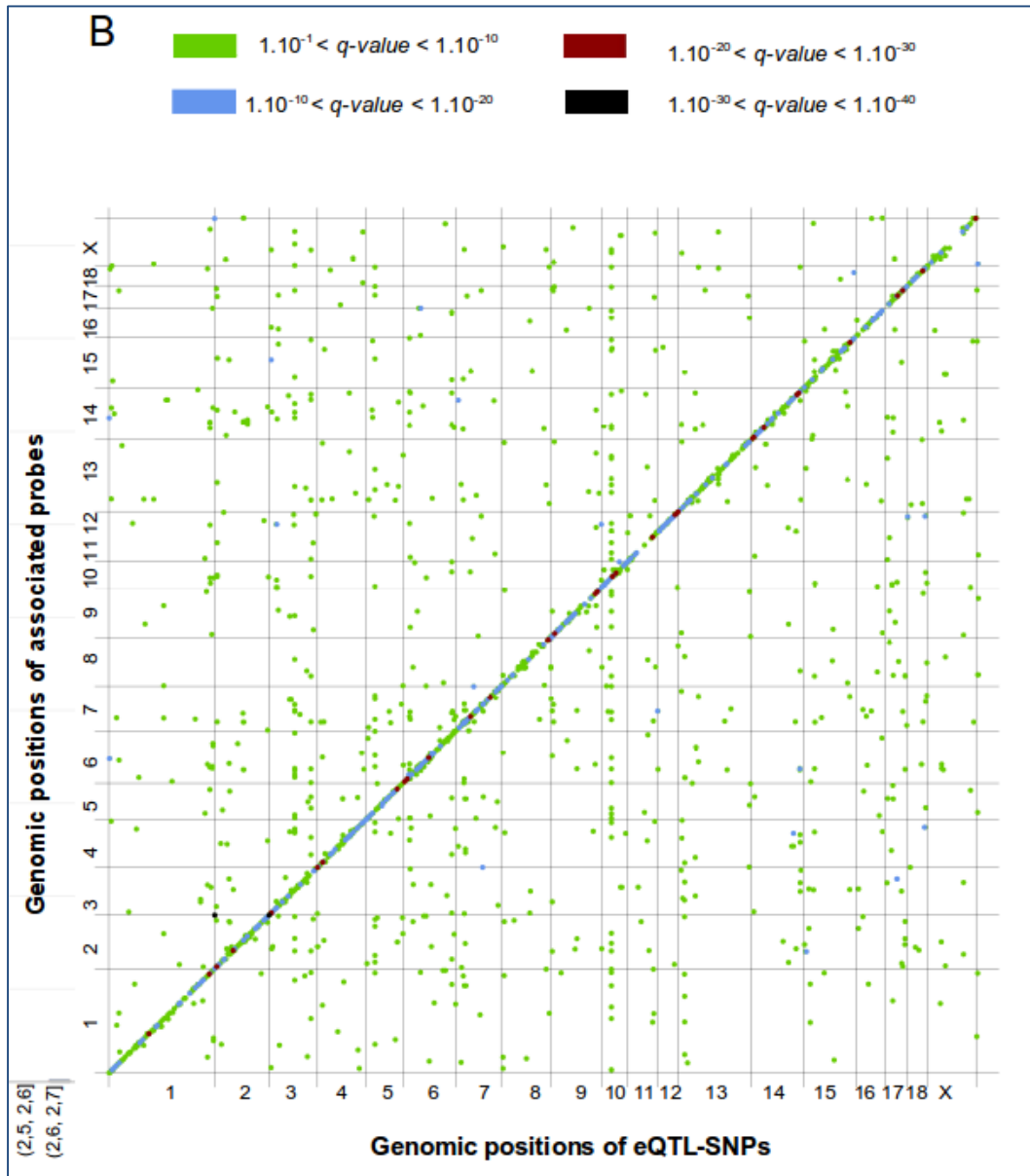
- 6,172 genes correlated with at least one immunity trait
 - immune system process 721 genes
 - regulation of innate immune system 148 genes
 - cellular response to stress 567 genes
 - regulation of immune system process 506 genes
- 989 genes found correlated with only one trait
- 5 genes found correlated with 13 immunity traits: *CCL3L1*, *CD163*, *KCNJ12*, *TRIM2*, *UBAC2*
- Genes already found as putative biomarkers in previous studies
 - For phagocytosis capacity: *GATM*
 - For cytotoxic CD8+ T cells: *GNLY* and *CXCR1*



GNLY: granulysin

Mach et al., 2013

GENETIC CONTROL OF PUTATIVE BLOOD BIOMARKERS



Few candidate biomarkers already shown to be genetically controlled for their transcription in blood by eGWAS.

Maroille et al. *BMC Genomics* (2017) 18:967
DOI 10.1186/s12864-017-4354-6

BMC Genomics

RESEARCH ARTICLE

Open Access

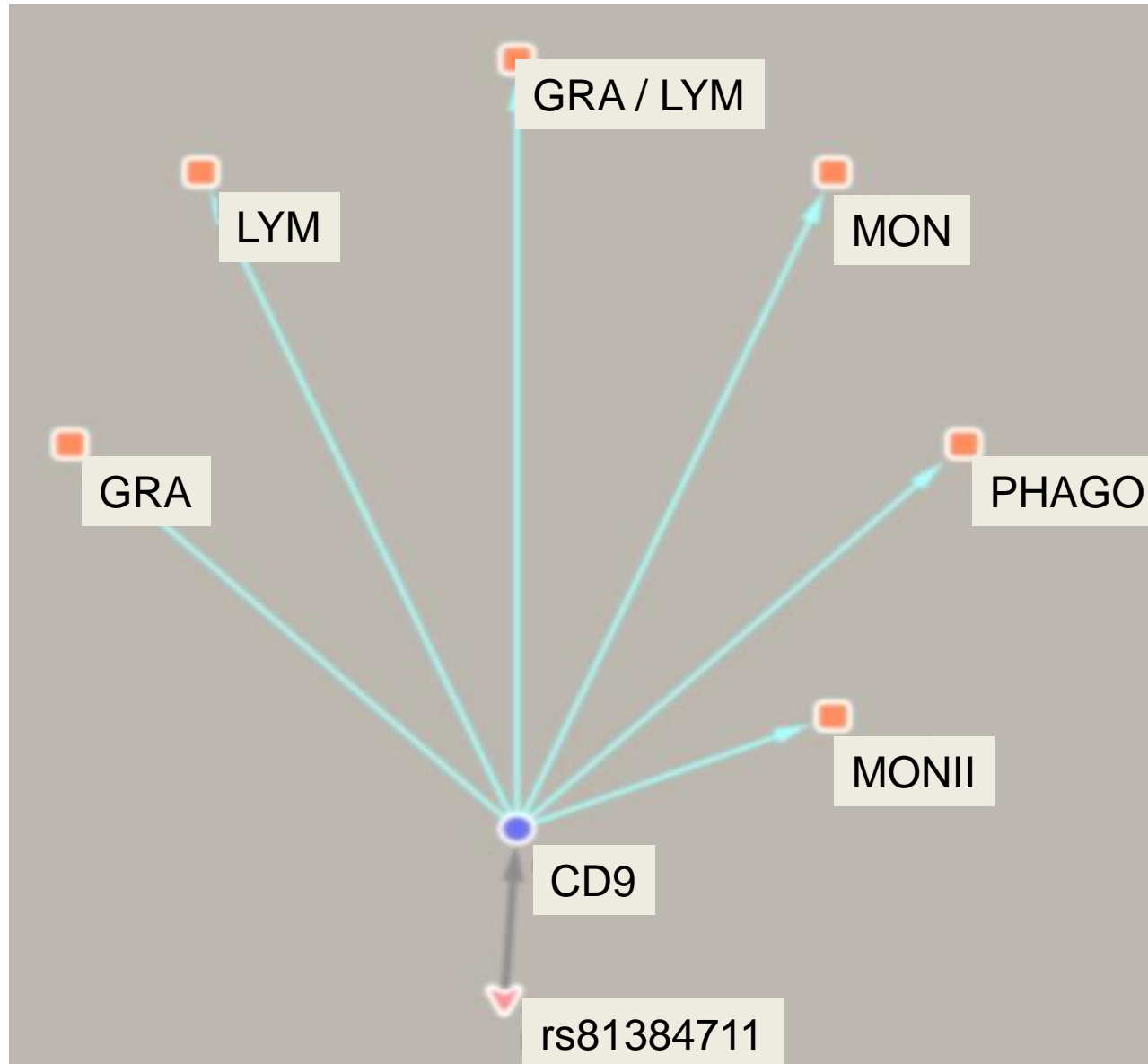


Deciphering the genetic regulation of peripheral blood transcriptome in pigs through expression genome-wide association study and allele-specific expression analysis

T. Maroille^{1*}, G. Lemonnier¹, J. Lecardonnel¹, D. Esquerré², Y. Ramayo-Caldas¹, M. J. Mercat³, C. Rogel-Gaillard^{1*} and J. Estellé^{1*}

Maroille et al., 2017

SNP RS81384711



- Association between the SNP rs81384711 (M1GA0007843; MAF=0.31) and the transcription level of *CD9* in the blood
- The SNP rs81384711 maps to an intron of the gene *CD9* (5:64,458,916).
- *CD9* encodes a cell surface glycoprotein involved in differentiation, adhesion and signal transduction (three critical mechanisms in cell-mediated immunity)
- Correlation between *CD9* transcription level and various immune traits related to cell-mediated immunity.

A promising genetic marker of cell-mediated immunity traits ?

PERSPECTIVES

- To identify candidate blood biomarkers that predict a combination of immune traits
 - sPLS based on covarying immunity traits (cluster) and genes found correlated with at least one trait (6,172 genes)
- To deepen analysis of putative blood biomarkers with transcription levels found under a genetic control (eQTLs)
 - Access to the underlying biology
 - Help to prioritize predictive markers (genetic markers and biomarkers)
- To have access to large scale test and validation pig populations
 - Various environments
 - Various genetics

Thank you for your attention

