What potential of genome-wide integrative approaches to predict vaccine responses?


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

**BEFORE**

Individual variabilities of baseline parameters

**Vaccination**

Individual variabilities of vaccine responses

**AFTER**

source of predictive markers?

- Genetic markers (DNA variability)
- Biomarkers (e.g. in the blood)

**WP 13**

Individual variability of immunocompetence: search for markers predictive of vaccine responses

- *M. hyopneumoniae* (Stellamune)
- Swine flu virus (Respiporc)
- *Eimeria maxima*
Animals and vaccines

WP13

Pigs (Large White)

48 families

Vaccination

M. hyo  swine flu virus  none (controls)

# animals  190  192  64

WP5

Chickens (broilers)

N = 96

Commercial broilers (COBB 500) vaccinated with wild-type *Eimeria maxima*, followed by challenge with homogeneous strain.
Available data

Genotypes
Affymetrix AXIOM
- Pig: 658K SNPs
- Chicken: 580K SNPs

Family pedigrees

Vaccine response phenotypes

Production phenotypes
- Body weight

Blood transcriptome
- RNA-Seq (pigs, chickens)
- miRNA-Seq (pigs)
Experimental design for pigs

Weaning / vaccination

- blood
  - hemogram + DNA
  - miRNA + transcriptome
  - PBMC
  - serology

 Booster

- blood
  - serology

- Blood
  - serology
  - hemogram
  - PBMC

Age (days)

Days post-vaccination

0 6 14 21 28 35

before vaccination

Biomarkers and genetic markers

after vaccination

Follow-up of the vaccination response

This project has received funding from the European Union’s Horizon 2020 program for research technological development and demonstration under Grand Agreement no. 633184
**M-hyo vaccine:**
variability of individual responses

Commercial ELISA (IDEXX M hyo Ab test)

**M. hyo Ab response (S/P)**

- **controls**
- **vaccinated**

Pigs at 49d: 3 wpv

- **POS**
- **NEG**

Days post-vaccination:

- 28
- 34
- 42
- 49
- 56
- 63
- 146

Vaccine and booster:

- **sera diluted 1/40**
- **sera diluted 1/4**

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**M-hyo vaccine:**

estimation of genetic parameters

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### Vaccine response phenotypes

- **controls**
- **vaccinated**

#### Heritability

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>h²</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>56d</td>
<td>190</td>
<td>0.34</td>
<td>0.15</td>
</tr>
</tbody>
</table>

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**M-hyo vaccine:**

**genome wide association studies (GWAS)**

- **3wpv**
  - controls
  - vaccinated

**SSC4: 10201158-11076588 (< 1Mb)**

- **sera diluted 1/40**
- **sera diluted 1/4**

- RepeatABEL R package
- Linear mixed model
- **Fixed effects:** age at weaning (24 to 31d), batch (n=5), sex
- Random effects: litter (n=48), genomic kinship matrix
- **Genome assembly:** Sscrofa11.1

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**M-hyo vaccine:**

*a candidate associated region on SSC4*

**SSC4: 10201158-11076588 (< 1Mb)**

- **ASAP1**: ArfGAP with SH3 domain, ankyrin repeat and PH domain 1
  - The gene has been reported to be expressed in immune tissues; with a role in regulating the migration of dendritic cells; associations have been reported with platelet counts in humans

- **FAM49B**: family with sequence similarity 49 member B
  - GWAS in humans have revealed associations between variants of *FAM49B* and lymphocyte/white blood cell counts

- **GSDMC**: Gasdermin C
  - GWAS in humans have revealed associations between variants of *GSDMC* and monocyte counts

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### M-hyo vaccine: blood transcriptome before vaccination

82 pigs

<table>
<thead>
<tr>
<th>Trait</th>
<th>N per group</th>
<th>DE genes (FDR &lt; 0.1)</th>
<th>DE genes (FDR &lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Up in group B</td>
<td>Down in group B</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. hyo-IgG at day 56</td>
<td>38 high</td>
<td>612</td>
<td>603</td>
</tr>
<tr>
<td></td>
<td>44 low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. hyo-IgG at day 49</td>
<td>55 NEG</td>
<td>392</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>14 POS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

27 shared genes were found DE between extreme animals for *M. hyo*-specific IgG levels detected at both time points (day 49 and day 56).

RNA-Seq (> 20M reads / sample)  
edgeR package (3.18.1 version)
**M-Hyo vaccine: biological functions enriched by genes**

**DE in blood between high and low Ab producers**

Top-13 functions significantly enriched for the 22 shared genes with symbols (Ingenuity Pathway Analysis, Qiagen).

<table>
<thead>
<tr>
<th>Functions Annotation</th>
<th>p-Value</th>
<th>Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity of lymphoid tissue</td>
<td>1,62E-08</td>
<td>APBB1IP,FYB1,IL2RG,KAT6A,LCP2,MTOR,RUNX2,TET2</td>
</tr>
<tr>
<td>Quantity of leukocytes</td>
<td>2,22E-07</td>
<td>APBB1IP,FYB1,IL2RG,IRF2,KAT6A,LCP2,MTOR,PREX1,RUNX2,TET2</td>
</tr>
<tr>
<td>Quantity of lymphatic system cells</td>
<td>5,79E-07</td>
<td>APBB1IP,FYB1,IL2RG,IRF2,KAT6A,LCP2,MTOR,RUNX2,TET2</td>
</tr>
<tr>
<td>Leukopoiesis</td>
<td>1,03E-06</td>
<td>ACIN1,CTCF,IL2RG,IRF2,LCP2,MTOR,PREX1,RUNX2,TET2</td>
</tr>
<tr>
<td>Quantity of lymphoid organ</td>
<td>1,29E-06</td>
<td>FYB1,IL2RG,KAT6A,LCP2,RUNX2,TET2</td>
</tr>
<tr>
<td>Quantity of hematopoietic progenitor cells</td>
<td>1,37E-06</td>
<td>FYB1,IL2RG,KAT6A,LCP2,MTOR,RUNX2,TET2</td>
</tr>
<tr>
<td>Morphology of lymphoid organ</td>
<td>3,05E-06</td>
<td>IL2RG,IRF2,KAT6A,LCP2,MTOR,RUNX2,TET2</td>
</tr>
<tr>
<td>Hematopoiesis of mononuclear leukocytes</td>
<td>3,81E-06</td>
<td>ACIN1,CTCF,IL2RG,IRF2,LCP2,MTOR,PREX1,RUNX2</td>
</tr>
<tr>
<td>Quantity of lymphocytes</td>
<td>3,95E-06</td>
<td>APBB1IP,FYB1,IL2RG,IRF2,KAT6A,LCP2,MTOR,RUNX2</td>
</tr>
<tr>
<td>Differentiation of hematopoietic progenitor cells</td>
<td>5,30E-06</td>
<td>CTCF,IL2RG,LCP2,MTOR,RUNX2,TET2</td>
</tr>
<tr>
<td>Quantity of B lymphocytes</td>
<td>5,56E-06</td>
<td>APBB1IP,IL2RG,IRF2,KAT6A,MTOR,RUNX2</td>
</tr>
<tr>
<td>Cell spreading of peripheral T lymphocyte</td>
<td>5,78E-06</td>
<td>FYB1,LCP2</td>
</tr>
<tr>
<td>Quantity of T lymphocytes</td>
<td>5,93E-06</td>
<td>APBB1IP,FYB1,IL2RG,IRF2,KAT6A,LCP2,RUNX2</td>
</tr>
</tbody>
</table>

Blood cell counts and differentiation
### Summary for pigs

<table>
<thead>
<tr>
<th></th>
<th>M-Hyo vaccine response</th>
<th>Swine flu vaccine response</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N=190</td>
<td>N=94</td>
</tr>
<tr>
<td>Heritability</td>
<td>Moderate (0.34 +/- 0.15)</td>
<td>not applicable</td>
</tr>
<tr>
<td>Genetic markers</td>
<td>Candidate SNPs on SSC4</td>
<td>Candidate SNPs on SSC13 and SSC14</td>
</tr>
<tr>
<td>Blood biomarkers</td>
<td>DE genes before vaccination (27 shared at 2 time points)</td>
<td>DE genes before vaccination (N=24)</td>
</tr>
</tbody>
</table>
Experimental design for chickens

- Place Birds
- Wing Tag, Weigh

Day
- PI w/ 100 Em
- Challenge 50K Em

Day
- Oocyst Counting

Blood/ serum
- Transcriptome
- IL-10 ELISA

Blood
- Whole blood FACS
- IL-10 ELISA

Blood
- Whole blood FACS
- IL-10 ELISA
- Spleen
- Genotyping

Before vaccination
- Transcriptomic signatures and biomarkers

After vaccination
- Follow-up of the vaccination responses and genetic markers
**Eimeria vaccine/challenge: animal responses**

- Vaccination led to significantly increased serum IL-10 levels.
- High degree of individual variation in body weight gain as well as in vaccine response (serum IL-10 levels)

<table>
<thead>
<tr>
<th>Trait code</th>
<th>Trait name</th>
<th>Number of animals with phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>BWG_9dpv</td>
<td>Body weight gain (%), 9 days after vaccination</td>
<td>94</td>
</tr>
<tr>
<td>BWG_7dpc</td>
<td>Body weight gain (%), 7 days after challenge</td>
<td>94</td>
</tr>
<tr>
<td>LS</td>
<td>Lesion score</td>
<td>94</td>
</tr>
<tr>
<td>Log10_Parasite</td>
<td>Parasite load (log10)</td>
<td>96</td>
</tr>
<tr>
<td>IL10_0dpv</td>
<td>IL10 measure, at vaccination</td>
<td>57</td>
</tr>
<tr>
<td>IL10_6dpv</td>
<td>IL10 measure, 6 days after vaccination</td>
<td>39</td>
</tr>
<tr>
<td>IL10_7dpc</td>
<td>IL10 measure, 7 days after challenge</td>
<td>76</td>
</tr>
</tbody>
</table>
**Eimeria vaccine/challenge: blood transcriptome before vaccination**

<table>
<thead>
<tr>
<th>Trait</th>
<th>n per group</th>
<th>DE genes (FDR &lt; 0.1)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A up in group B</td>
<td>B down in group B</td>
</tr>
<tr>
<td><strong>IL-10 6 dpv</strong></td>
<td>11 high</td>
<td>14 low</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Body weight gain 9 dpv</strong></td>
<td>15 high</td>
<td>15 low</td>
<td>44</td>
<td>161</td>
</tr>
<tr>
<td><strong>IL-10 7 dpc</strong></td>
<td>15 high</td>
<td>15 low</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Body weight gain 7 dpc</strong></td>
<td>15 high</td>
<td>15 low</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td><strong>Log10 parasite</strong></td>
<td>15 high</td>
<td>15 low</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Lesion Score</strong></td>
<td>41 score 0</td>
<td>27 score 1 or 2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

RNA-Seq (> 20M reads / sample)  
edgeR package (3.18.1 version)
Read counts were normalized using the count per million (cpm) approach: only genes for which at least 30 chickens showed >1 cpm and >10 reads
- 13,904 genes included in the eGWAS
- 3,312 significant associations (FDR<10%) between 699 genes and 1,819 SNPs
## Eimeria vaccine/challenge: eQTLs and genes DE before vaccine

<table>
<thead>
<tr>
<th>Trait</th>
<th>logFC</th>
<th>PValue</th>
<th>FDR</th>
<th>Gene*</th>
<th>Associated regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>BWG_9dpv</td>
<td>-2.403</td>
<td>5.08E-07</td>
<td>0.00067</td>
<td>ID25925_XLOC_000613</td>
<td>14:10108226</td>
</tr>
<tr>
<td>BWG_7dpc</td>
<td>3.833</td>
<td>1.81E-07</td>
<td>0.00263</td>
<td>ID32565_XLOC_012879</td>
<td>2:444034 ; 2:144165527</td>
</tr>
<tr>
<td>BWG_9dpv</td>
<td>3.842</td>
<td>2.04E-08</td>
<td>0.00014</td>
<td>ID32565_XLOC_012879</td>
<td></td>
</tr>
<tr>
<td>BWG_7dpc</td>
<td>1.510</td>
<td>2.76E-05</td>
<td>0.05558</td>
<td>ID34549_XLOC_016493</td>
<td>14:10108226</td>
</tr>
<tr>
<td>BWG_9dpv</td>
<td>1.666</td>
<td>1.01E-04</td>
<td>0.02161</td>
<td>ID45455_XLOC_036372</td>
<td>6:28785026-28804673</td>
</tr>
<tr>
<td>BWG_7dpc</td>
<td>0.881</td>
<td>6.95E-06</td>
<td>0.03356</td>
<td>ID45455_XLOC_036372</td>
<td></td>
</tr>
</tbody>
</table>

Four genes showed both a DE expression and a significant genetic control by eGWAS

* INRA annotation

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### Summary for chickens

<table>
<thead>
<tr>
<th></th>
<th><em>Eimeria 7 dpv</em></th>
<th><em>Eimeria 9 dpc</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>N=96</td>
<td>N=96</td>
</tr>
<tr>
<td><strong>Heritability</strong></td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td><strong>Genetic markers</strong></td>
<td>Candidate SNPs by eGWAS</td>
<td>Candidate SNPs by eGWAS</td>
</tr>
<tr>
<td><strong>Blood biomarkers</strong></td>
<td>DE genes before vaccination (N=205)</td>
<td>DE genes before vaccination (N=24)</td>
</tr>
</tbody>
</table>
Conclusion

- The genetic control of vaccine response has been confirmed at least in pigs and is likely due to many loci spread along the whole genome.
  - Few candidate genetic markers to be tested

- As a proof of concept, we have shown significant covariations between gene expression in blood before vaccination and vaccine responses, both in pigs and in chickens
  - Blood can be used as a source of biomarkers predictive of vaccine responses: list to be drawn for further tests

- Genetic control of gene expression in blood (eGWAS)
  - Toward the identification of candidate biomarkers for which expression is under a genetic control
Thank you for your attention