Genome-wide associations of immune-associated traits in dairy cows

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Introduction

- Low-cost high-throughput genotyping technology
- Higher accuracy breeding values
- Increased understanding of genetic control of economically important phenotypes (Pryce et al., 2010)
- Genome-wide association studies (GWAS) used to help researchers dissect the genetics of complex traits in many species
Introduction

Inclusion of immune response phenotypes in selection indices may be a viable option to decrease disease and improve animal health

(Abdel-Azim et al., 2005; Thompson-Crispi et al., 2012a; Mallard et al., 2015)
Objective

Can we determine genomic regions significantly associated with immune-associated traits?
Methods - Data collection

- **2010 - 2015 Sampling periods**
- **2013 - 2015 3x daily milk samples**
- **Genetic Line**
- **Diet**
- **Analysed for 17 immune-associated traits**
### Methods - Data collection

<table>
<thead>
<tr>
<th>Trait</th>
<th>No. Records</th>
<th>Mean</th>
<th>Std. Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>% PBMC</td>
<td>2,266</td>
<td>58.39</td>
<td>10.24</td>
</tr>
<tr>
<td>% Eosinophils</td>
<td>2,266</td>
<td>3.61</td>
<td>3.43</td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>2,265</td>
<td>44.25</td>
<td>12.35</td>
</tr>
<tr>
<td>% Monocytes</td>
<td>2,265</td>
<td>13.99</td>
<td>8.25</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>2,266</td>
<td>37.76</td>
<td>10.10</td>
</tr>
<tr>
<td>% CD4⁺</td>
<td>2,232</td>
<td>25.52</td>
<td>6.28</td>
</tr>
<tr>
<td>% CD8⁺</td>
<td>2,260</td>
<td>11.29</td>
<td>3.42</td>
</tr>
<tr>
<td>CD4⁺:CD8⁺ ratio</td>
<td>2,232</td>
<td>2.38</td>
<td>0.73</td>
</tr>
<tr>
<td>% NKp46⁺</td>
<td>2,262</td>
<td>2.32</td>
<td>1.58</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trait</th>
<th>No. Records</th>
<th>Mean</th>
<th>Std. Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hp (milk)</td>
<td>3,561</td>
<td>83.87</td>
<td>369.79</td>
</tr>
<tr>
<td>NAbKLH (blood)</td>
<td>2,687</td>
<td>0.94</td>
<td>0.30</td>
</tr>
<tr>
<td>NAbLPS (blood)</td>
<td>3,570</td>
<td>1.15</td>
<td>0.52</td>
</tr>
<tr>
<td>TNF-α (blood)</td>
<td>3,568</td>
<td>1,841.74</td>
<td>6,435.26</td>
</tr>
<tr>
<td>Hp (milk)</td>
<td>2,667</td>
<td>0.97</td>
<td>6.39</td>
</tr>
<tr>
<td>NAbKLH (milk)</td>
<td>2,667</td>
<td>0.81</td>
<td>0.35</td>
</tr>
<tr>
<td>NAbLPS (milk)</td>
<td>2,667</td>
<td>0.34</td>
<td>0.24</td>
</tr>
<tr>
<td>TNF-α (milk)</td>
<td>2,667</td>
<td>103.29</td>
<td>375.23</td>
</tr>
</tbody>
</table>
Methods - Genotyping

• 80% of cows genotyped
  – Illumina BovineSNP50 BeadChip

• Remaining cows genotyped
  – GeneSeek GGP Bovine 150k BeadChip

• 34,143 common SNPs
Methods – Quality control

- **SNP QC applied**
  - minor allele frequency (MAF) < 0.01
  - call rate < 95%

- **Sample QC applied**
  - call rate < 85%

Post QC:
- **1,637 SNPs removed**
- **535 samples × 32,506 SNPs**
- **13 samples removed**
Methods - GWAS

- EBVs calculated via:
  \[ y = Xa + Z_1b + Z_2c + e \]

- De-regressed following Garrick et al. (2009)

- dEBVs used in single marker regression via:
  \[ y = \mu + Xg + e \]

Fixed effects included:
- diet group
- genetic group
- lactation week
- assay technique
- \( Y \) x \( Z \) interaction
- random permanent environmental
- random additive genetic
- random residual

\[ y \] = trait observations
\[ EBV \] = de-regressed following Garrick et al. (2009)
\[ dEBV \] = used in single marker regression via:
\[ y = \mu + Xg + e \]

- Cow fitted as random effect
  - permanent environmental effect of cow
  - also fitted (repeated measures)

\[ c > 0 \] is the fraction of genetic variance not explained by markers.
Methods - GWAS

- **EBVs** calculated via:
  
  \[ y = Xa + Z_1b + Z_2c + e \]

- De-regressed following **Garrick et al. (2009)**

- **dEBVs** used in single marker regression via:
  
  \[ y = \mu + Xg + e \]

- \( y \) : vector of dEBV
- \( \mu, g, e \) : overall mean, effect of SNP (coded 0, 1, 2) and residual error, respectively
- \( X \) : design matrix allocating records to SNP effects

**Bonferroni correction**

\[ = \frac{0.05}{\text{number of SNPs}} \]

**False Discovery Rate (FDR)**

\[ = \frac{\text{number of tests} \times P\text{-value}}{\text{number of significant SNPs}} \]
Methods – Functional analysis

- **Ensembl**\(^1\) *Bos taurus* UMD3.1 assembly
- Genes within ±500kbp of SNP significantly associated with immune-associated traits
- Database for Annotation, Visualization and Integrated Discovery (DAVID\(^2\)) v6.8

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\(^1\) https://www.ensembl.org, Yates et al. (2016)
\(^2\) https://david.ncifcrf.gov, Huang et al. (2009)
Results – Genetic analysis
Results – GWAS
## Results – Functional analysis

- 9 clusters containing 122 genes were observed
- Significant associations with biological pathways also observed such as systemic lupus erythematosus

*Clusters and Enrichment Scores*

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Number of genes</th>
<th>Enrichment Score</th>
<th>Cluster names</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>3.6</td>
<td>phospholipases</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>3.1</td>
<td>Interleukins</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>2.4</td>
<td>Histone cluster proteins</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>1.1</td>
<td>olfactory receptor</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>1.0</td>
<td>homeobox</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>0.4</td>
<td>zinc finger protein</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>0.3</td>
<td>myelin associated glycoprotein</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>0.3</td>
<td>transmembrane proteins</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>0.3</td>
<td>transmembrane proteins</td>
</tr>
</tbody>
</table>

‡ geometric mean (-log scale) of member's p-values in corresponding annotation cluster, used to rank biological significance. The top ranked annotation groups most likely have consistent lower p-values for their annotation members (Huang et al., 2009)
Conclusions

- Interesting and potentially novel genomic regions observed
- Highlighted several SNP significantly associated with various immune-associated traits
  - notably SNP associated with natural antibodies on chr17
- Identified potentially useful SNPs for enriching genotyping
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Thanks for listening!
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