Identification and characterization
of two new recessive embryonic lethal mutations in Holstein cattle


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Acknowledgements
Introduction

Breeds are genetically small populations

Average inbreeding rate ~ 1% /generation

Regular emergence of recessive defects

Observatories + homozygosity mapping

Efficient tools to detect novel defects and map associated mutations in a short period of time with a limited number of cases
Introduction

However, this process rely on the observation of clinical cases affected animals with distinctive symptoms

Two approaches

Depletion in homozygous genotypes
(VanRaden et al., 2011)

Look for haplotypes with a lack in homozygous animals

Search for causative mutations using WGS

Reverse genetics
(Charlier et al., 2016)

Identify deleterious mutations heterozygous in sires’ WGS and predict their effects

Add them on SNP bead chip

Clinical examination of homozygous

→ Identification of 2 lethal embryonic mutations

~ 1000 markers
120 000 animals genotyped per year
Material

- Previous study in 2013 by Fritz et al. on 48,000 Holstein
- ~150,000 Holstein genotyped (with sire and MGS or sire and dam genotyped)
- Process of genomic evaluation
- (1/3 50K, 2/3 EuroG10k Illumina Beadchips and imputation)

- Sliding windows of 20-markers
- Comparison (Chi-2) between numbers of observed and expected homozygotes
- Consideration of haplotypes with:
  \[ \frac{N_{obs}}{N_{exp}} < 0.25 \text{ and adjusted } p\text{-value} < 0.01 \]
Results

- Detection of previously identified BY, HH1, HH3, HH4 and HH5, no detection of HH2
- Observed homozygotes for haplotypes are heterozygous for the causative mutations

- Detection of a new region HH6 (freq 1.7%)
  0 observed vs 31 homozygotes expected → embryonic lethal?

- Most influential and ancient carrier is Mountain (BIS-MAY S-E-L MOUNTAIN ET)
- Fine-mapping using intra familial recombinations → interval of 1.1 Mb

<table>
<thead>
<tr>
<th>Name</th>
<th>BTA</th>
<th>Interval (UMD3.1 Mb)</th>
<th>Haplotype freq. (%)</th>
<th>Nexp</th>
<th>Nobs</th>
<th>Chi² test</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH3</td>
<td>8</td>
<td>94.5-95.6</td>
<td>3.1%</td>
<td>332</td>
<td>3</td>
<td>7.4 x 10⁻⁹¹</td>
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<tr>
<td>HH4</td>
<td>1</td>
<td>0.1-1.4</td>
<td>4.4%</td>
<td>301</td>
<td>8</td>
<td>5.9 x 10⁻⁸²</td>
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<tr>
<td>BY</td>
<td>21</td>
<td>20.0-21.2</td>
<td>2.7%</td>
<td>124</td>
<td>1</td>
<td>6.2 x 10⁻³⁰</td>
</tr>
<tr>
<td>HH5</td>
<td>9</td>
<td>94.8-96.4</td>
<td>1.9%</td>
<td>117</td>
<td>8</td>
<td>3.6 x 10⁻²⁸</td>
</tr>
<tr>
<td>HH1</td>
<td>5</td>
<td>63.0-65.6</td>
<td>1.7%</td>
<td>57</td>
<td>1</td>
<td>1.1 x 10⁻¹¹</td>
</tr>
<tr>
<td>HH6</td>
<td>16</td>
<td>27.8-32.0</td>
<td>1.1%</td>
<td>31</td>
<td>0</td>
<td>1.7 x 10⁻⁴</td>
</tr>
</tbody>
</table>

Charlier et al. 2012; Fritz et al., 2013; McClure et al., 2014; Daetwyler et al. 2014; Adams et al., 2016; Schütz et al., 2016).
Identification of candidate mutation

- Use of 186 Holstein bull’s whole genome sequences from Run4 of the 1000 bull genome consortium (10 HH6C and 176 HH6F)

- Correlation between status on haplotypes and genotypes for the variants in the reduced interval

- Candidate mutation: chr16 g.29773628A>G affects the initiator codon of the gene SDE2 Telomere Maintenance Homolog
Identification of candidate mutation

- Function of SDE2 protein
  - Maintain genomic stability during mitosis
  - KO causes chromosomal rearrangements and losses in Fission Yeast

- Entirely conserved among eukaryotes:
  Mutant SDE2 protein truncated by 83 AA

Wildtype protein
Mutant protein (predicted)

B. taurus (Cow)
H. sapiens (Human)
G. gallus (Chicken)
P. sinensis (Chinese softshell turtle)
D. rerio (Zebrafish)
D. elegans (Fruit fly)
C. interstinalis (Vase tunicate)
O. sativa (Rice)
S. pombe (Fission yeast)

8-AA motif conserved
Using the Eurogenomics chip (Friday 1:30)

- 1000 markers designed for deleterious mutations heterozygous in sires’ genomes
  - Detection of mutations intrabreed
  - Focus on embryonic lethal

- A four base pair deletion in CENPU gene downstream the splice donor site (6 +/- VS 1141 +/-)

Chr27:14168128  ATTACCTACT  ATTACCT

- Specialized chromatin domain which play a key role in mitosis
- Close to the splice donor site of CENPU
- Modifications of the primary structure of the protein

Validation ongoing

Mice

Homozygotes for a knock-out allele exhibit embryonic lethality between E7.5 and E9.5, small embryo size and thickened visceral endoderm.
Large scale genotyping

- No homozygous to the mutation
  
  On EuroGenomics beadchip since 2015
  
  0 homozygous observed (out of 100 100 animals genotyped)
  
  Freq=0.9%

- Comparison with the depletion in homozygous analysis
  
  The haplotype (HH7) associated with this mutation shows significant depletion in homozygous without Bonferroni correction !

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<th>Chi² test</th>
<th>Bonferroni corrected</th>
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<tr>
<td>HH7</td>
<td>27</td>
<td>13.0-14.4</td>
<td>1.2%</td>
<td>16</td>
<td>0</td>
<td>2.0 x 10⁻⁶</td>
<td>18.6</td>
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</table>

Recombining

Ancestral version of the haplotype predating the mutation event

- No homozygote for HH7 haplotype or candidate mutation
Effects on fertility of HH6 and HH7

- Decrease in fertility observed in mating among carriers

Analysis of Conception and Non Return Rates suggests that embryos die before 35 days of gestation.

Estimated values were closed to the expected effect under the assumption of complete lethality in homozygous embryos.
Discussion

- Reverse genetics approach:
  Study gene function
  Lot of false-positives: (Charlier et al.)
  - 15% deleterious
  - 6% missense are true

- Depletion in homozygous
  Few false-positive
  Need a huge population of genotyped animals to identify haplotypes with a frequency higher than 1%

→ Complementarity of the two approaches

Thank you for your attention