A new Holstein Haplotype affecting calf survival

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Background

- Reports from German industry
- Calves with chronic diarrhea
  - No response to any treatment
- Test on pathogens: negative
- Secondary diseases like pneumonia and oedemas
- Underdevelopment (in body weight)
- Died between 3 weeks and 6 months after birth
- Identical twins out of split embryo, raised on two farms, revealed identical phenotype

- Pedigree analyses revealed common ancestors
- Indication of genetic background
Pathological examination

- Three animals
  - „Normal“ at birth
  - Age of death: 1-5 months
  - Weight at death: 36.5 – 59 kg
  - Normal height
- No apparent cause of death
- Most probably affected calves starved
- Pathological findings: All fat reserves are used up including the fat of the spinal cord

⇒ Indication of disorder of fat metabolism
Blood analyses

- Parameter of fat metabolism are conspicuous

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<tbody>
<tr>
<td>Case 1</td>
<td>0.17</td>
<td>0.1</td>
<td>&lt;0.02</td>
<td>0.05</td>
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<tr>
<td>Case 2</td>
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<td>Case 3</td>
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<tr>
<td>Case 4</td>
<td>0.41</td>
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<tr>
<td>Control 1</td>
<td>1.53</td>
<td>1.36</td>
<td>0.19</td>
<td>0.16</td>
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<tr>
<td>Control 2</td>
<td>2.46</td>
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<td>0.68</td>
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<td>Control 3</td>
<td>2.21</td>
<td>1.84</td>
<td>0.43</td>
<td>0.36</td>
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<tr>
<td>Control 4</td>
<td>2.5</td>
<td>2.38</td>
<td>0.33</td>
<td>0.29</td>
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- Affected animals show significantly reduced values of lipid blood levels
- Also reduced values for control 1
  ➜ Codominant inheritance?
Identification of disease-associated region

- Genome-wide association study based on Illumina 54K SNP-Chip genotypes
  - 23 cases (common phenotype and died)
  - 11,177 control animals (survived first year of life)
    - Data available from the reporting system in Germany

⇒ strong association on BTA11
Homozygosity mapping BTA 11

- Non-affected animals need to be heterozygous/alternative homozygous

- Affected animals (= cases) must have a common homozygous region within the associated region
Haplotype frequency in population

- Haplotype is identified
- Haplotype analyses reveal
  - 234 homozygous animals (80% dead)
  - 14,093 heterozygous animals (8.7% of all genotyped animals)

Widespread use of carriers’ sperm → Strong increase of frequency
Effect of risk matings

- Increase of frequency since 2000 → high probability of risk matings
- Analysis based on 6.9 million female calf records

After day 30:
association haplotype calf mortality

- mating = carrier x carrier
- carrier x non-carrier
- non-carrier x carrier
- non-carrier x non-carrier
Effect of haplotype on blood cholesterol levels

- Comparing heterozygous carriers (14) versus “free” animals (141)
  - Blood cholesterol levels were routinely measured
  - 50K-SNP-Chip genotypes → information about haplotype status

- Cholesterol level of heterozygous carriers clearly lower (1.65 vs. 2.30)
Economic importance

- ~3,400 homozygous animals born per year in Germany
  - Uniform mating of all bulls
  - Carrier frequency: 8.7 %
  - 1,800,000 Holstein calves born per year in Germany

- 1.3 million Euro loss per year
  - 400€ per calf (value of calf, average lifetime: 85 d, medical treatment)

- Actual loss could be higher
  - Widespread, intensive use of carrier bulls
  - Heterozygous animals → disadvantages in fat metabolism?

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Homozygous animals alive

Healthy animals

Cases

Approx. 26 animals older >1 year found computed as homozygous for the haplotype

Haplotype test accuracy < 100%
Reasons for homozygous animals alive

- **All died homozygous animals trace back double to Maughlin Storm**
- **Survived homozygous animals got at least one allele from Comestar Laurie Sheik**

→ **Within the identified haplotype there must exist two variants**
  - one carrying the causal mutation, the other not
Identification of causal mutation

Sequence based analyses

- Request of all position in the genome including causal mutation
  - Whole genome re-sequencing

- 43 animals with whole genome re-sequencing data
  - 5 Red dairy cattle
  - 38 Holstein (black and red)
    - 1 affected animal

- Chromosome 11
  - Chromosome including the causal mutation
  - 755,920 variants detected
    - (2303 positions on 54K-SNP-Chip \(\rightarrow\) 0.3%)

\(\rightarrow\) How to pinpoint the causal mutation?
Sequence based analyses (II)

1) BB

Affected animal \(†\)

\(→\) affected animal must be homozygous

2) | BB | AB | AA |
--- | --- | --- | --- |
Affected animal \(†\) | -- | Remaining 42 animals (Holstein and RDC)

- No carrier (AB) included in German data
- Allele frequency (B) \(≤ 0.045\)

3) Associated homozygous region in the genome

4) Monogenic defect

- Filtering of genic variants

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Chromosome 11

- 174,373 variants
- 3,360 variants
- 498 variants
- 20 variants
Sequence based analyses (III)
Collaboration with Technical University Munich

- Access to data of 1000 Bull Genomes Project (Run 4)
  - ~ 1,150 animals re-sequenced
  - 288 Black Holstein animals, 23 Red Holstein animals
  - 37.92 Million variants detected

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<thead>
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<th></th>
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<th>AB</th>
<th>BB</th>
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<tr>
<td>Remaining animals (Holstein and other breeds)</td>
<td>Prominent Storm offspring</td>
<td>Affected animal †</td>
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⇒ Potential variants (n=20) could not be confirmed, because …
  - False-positive carriers in Holstein ⇒ do definitely not carry this defect
  - Occurrence in other breeds ⇒ Holstein specific defect
Identification of causal mutation

- Most probably the causal mutation is located in a gap in the reference sequence

- Currently hard to find the causal mutation
  - but new reference sequence announced for 2015/2016
Summary and outlook

- Phenotype: non-curable diarrhea in calves & cholesterol deficiency
  - genetic disposition

- Identified haplotype is clearly associated with calf mortality
  - Results based on complete calf survival data of entire German Holstein population

- Haplotype has high impact on worldwide Holstein population

- Accuracy of haplotype test for prediction of carrier status: 80%
  - Two identical 50K-based haplotypes: affected ↔ healthy

- Causal mutation has not yet been identified

- Improvement of haplotype test
  - More sequence data of ancestors
  - High-density genotyping of ancestors
  - …..
Thank you.

Many thanks to Masterrind for the intensive cooperation and financial support.