



IT-Solutions for
Animal Production

A new Holstein Haployppte 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

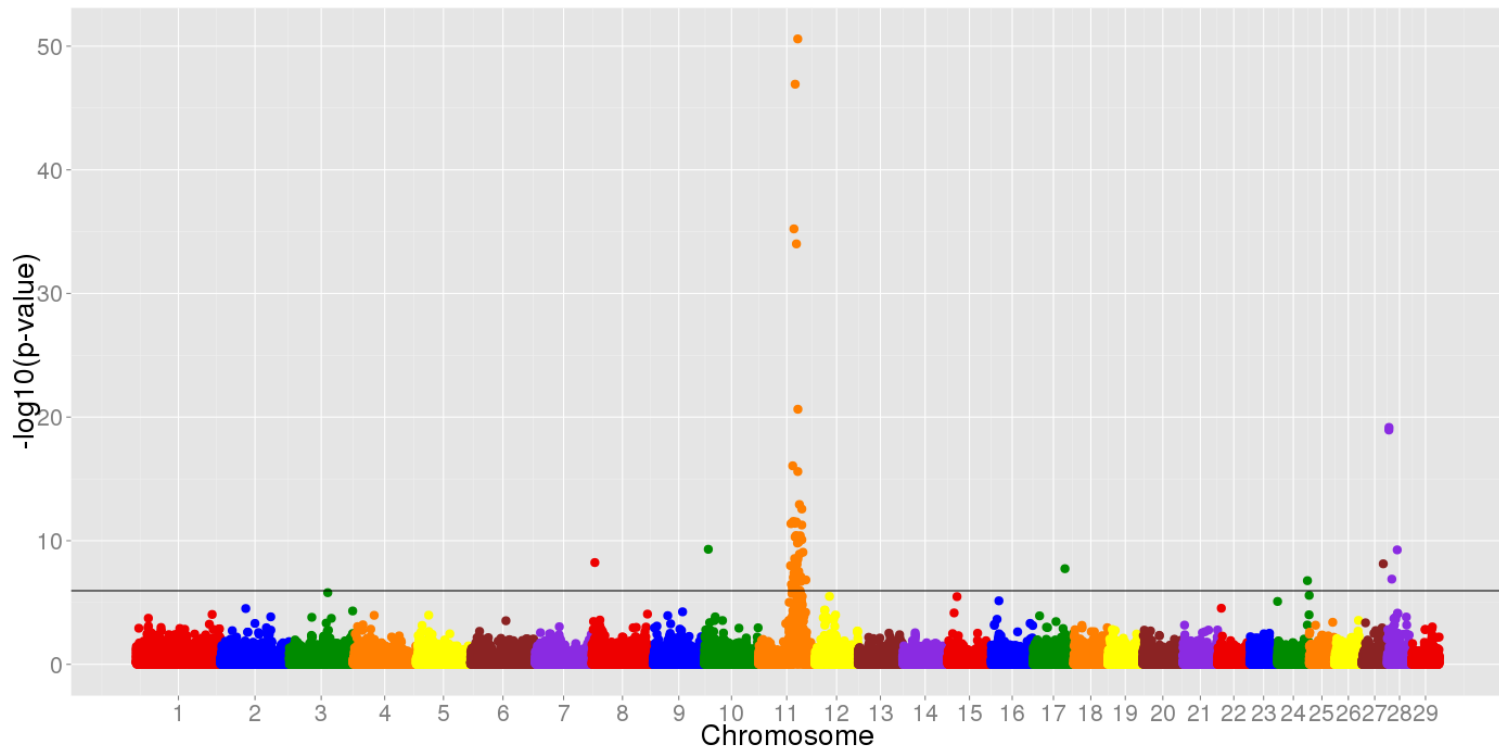
Animal	Cholesterol [mmol/l]	HDL [mmol/l]	LDL [mmol/l]	Triglyceride
Case 1	0.17	0.1	<0.02	0.05
Case 2	0.15			
Case 3	0.12			
Case 4	0.41			
Control 1	1.53	1.36	0.19	0.16
Control 2	2.46	1.85	0.68	0.40
Control 3	2.21	1.84	0.43	0.36
Control 4	2.5	2.38	0.33	0.29

- Affected animals show significantly reduced values of lipid blood levels
- Also reduced values for control 1
- ➔ Codominant inheritance?



Identification of disease-associated region

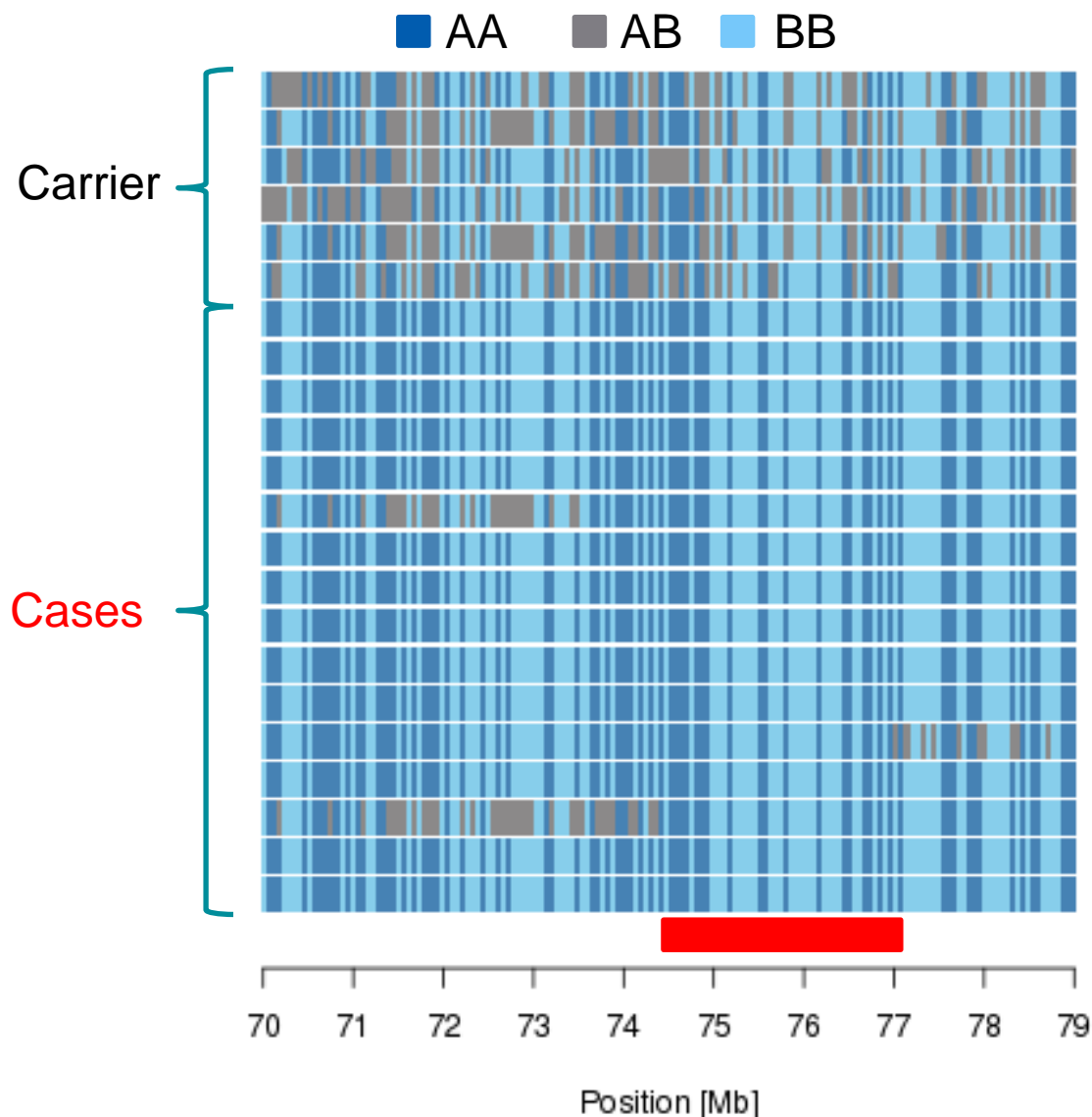
- Genome-wide association study based on Illumina 54K SNP-Chip genotypes
 - 23 cases (common phenotype and died)
 - 11.177 control animals (survived first year of life) ➤ Case-Control-Study
 - Data available from the reporting system in Germany



➔ strong association on BTA11



Homozygosity mapping BTA 11



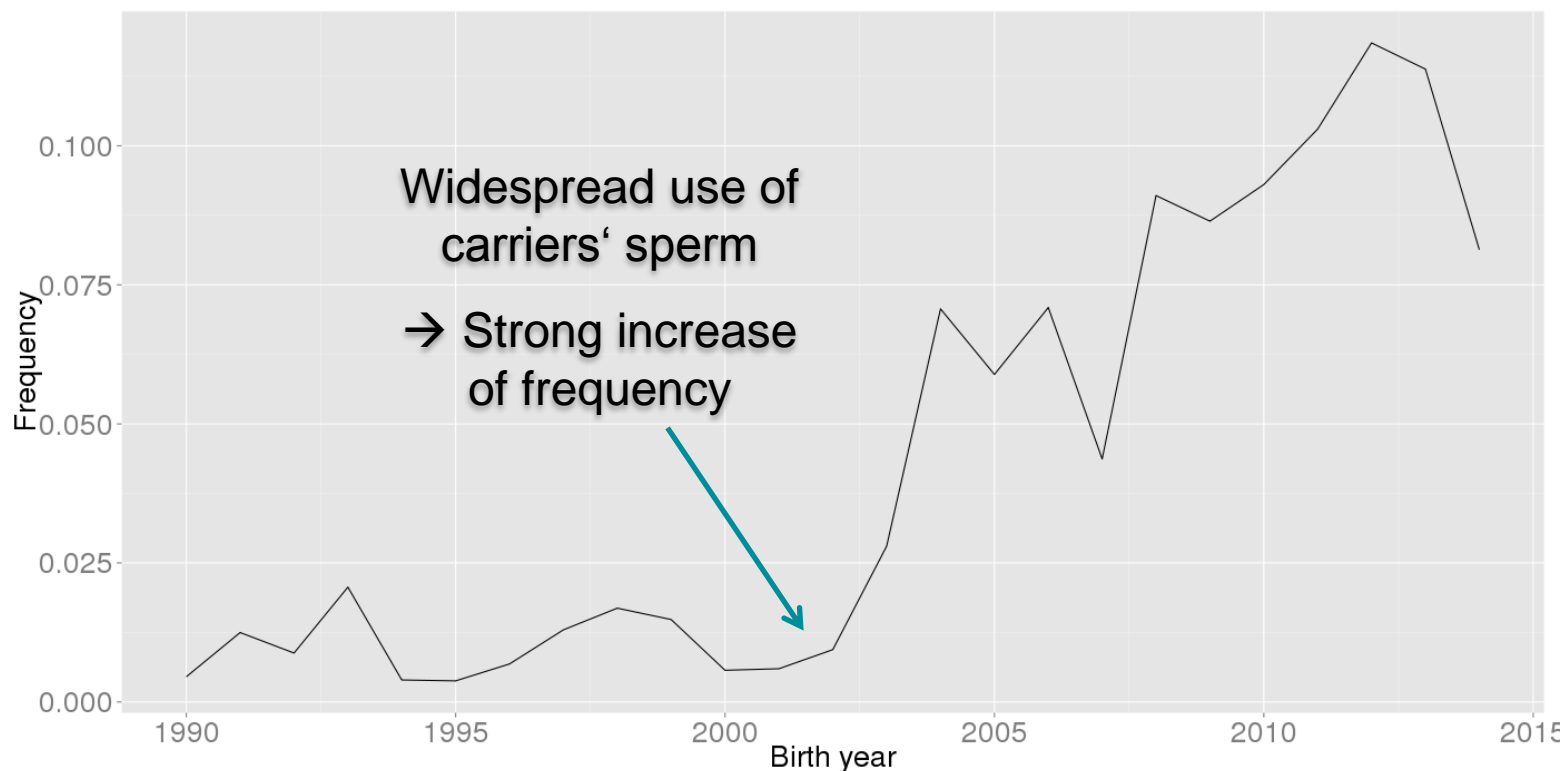
- Non-affected animals need to be heterozygous/alternative homozygous

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



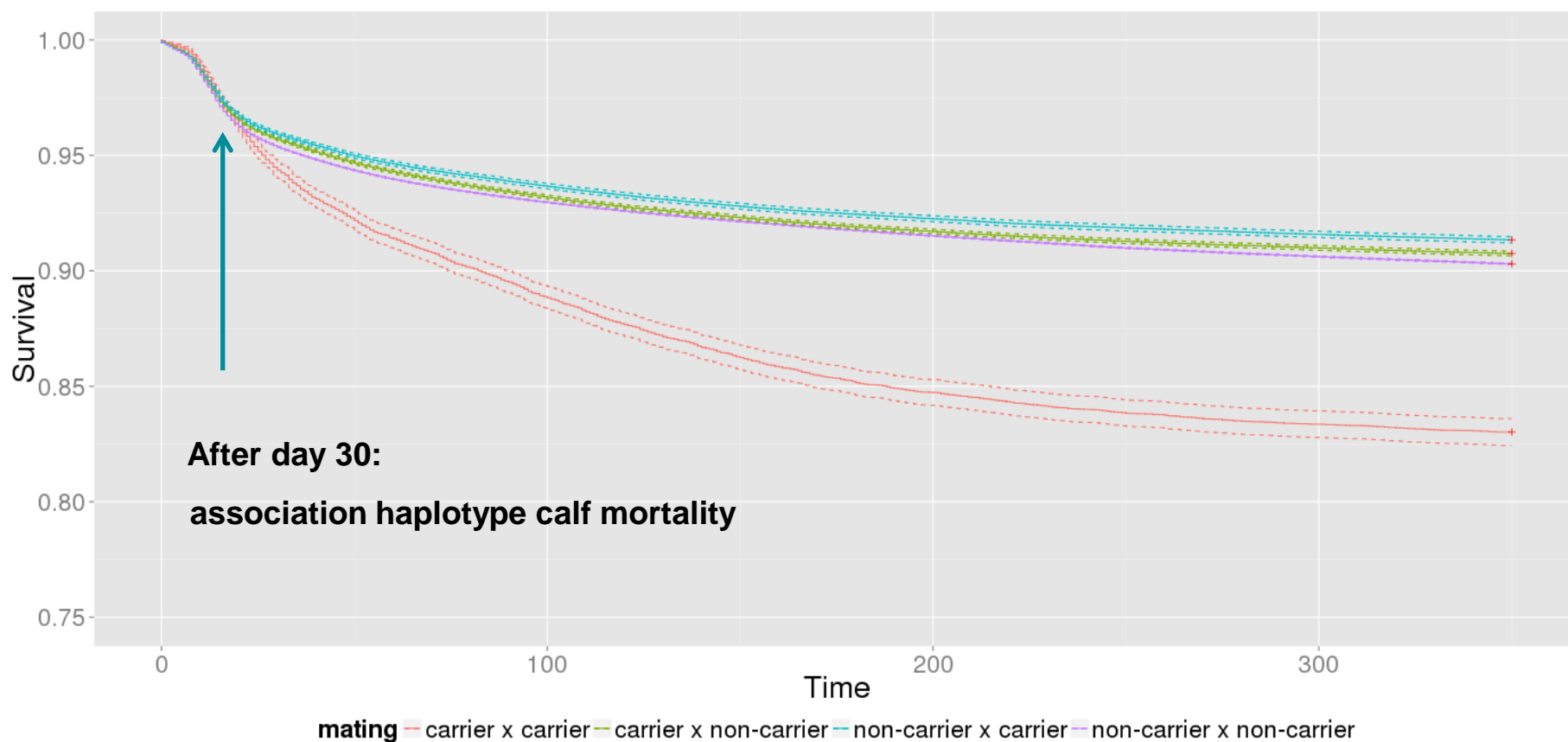
Haplotype frequency in population

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



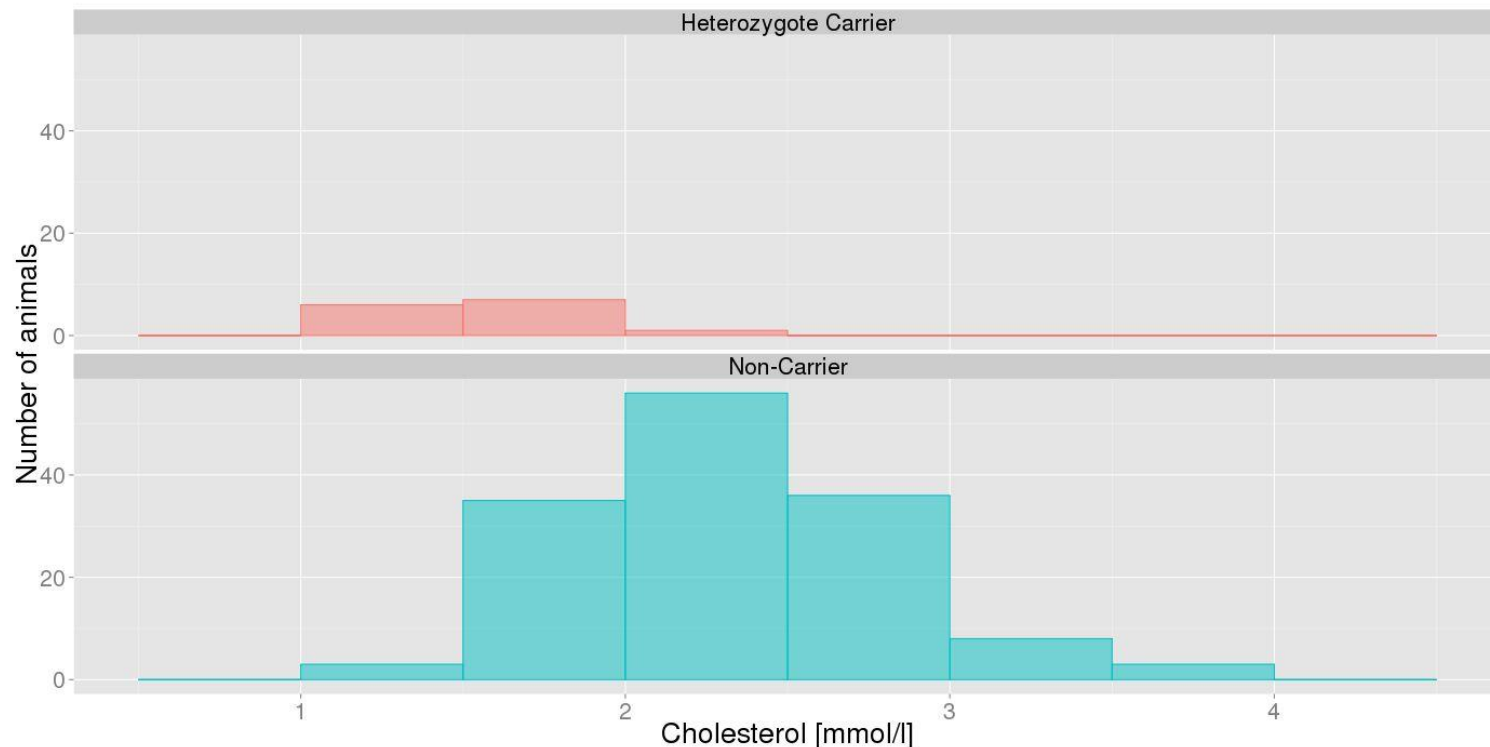
Effect of risk matings

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



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

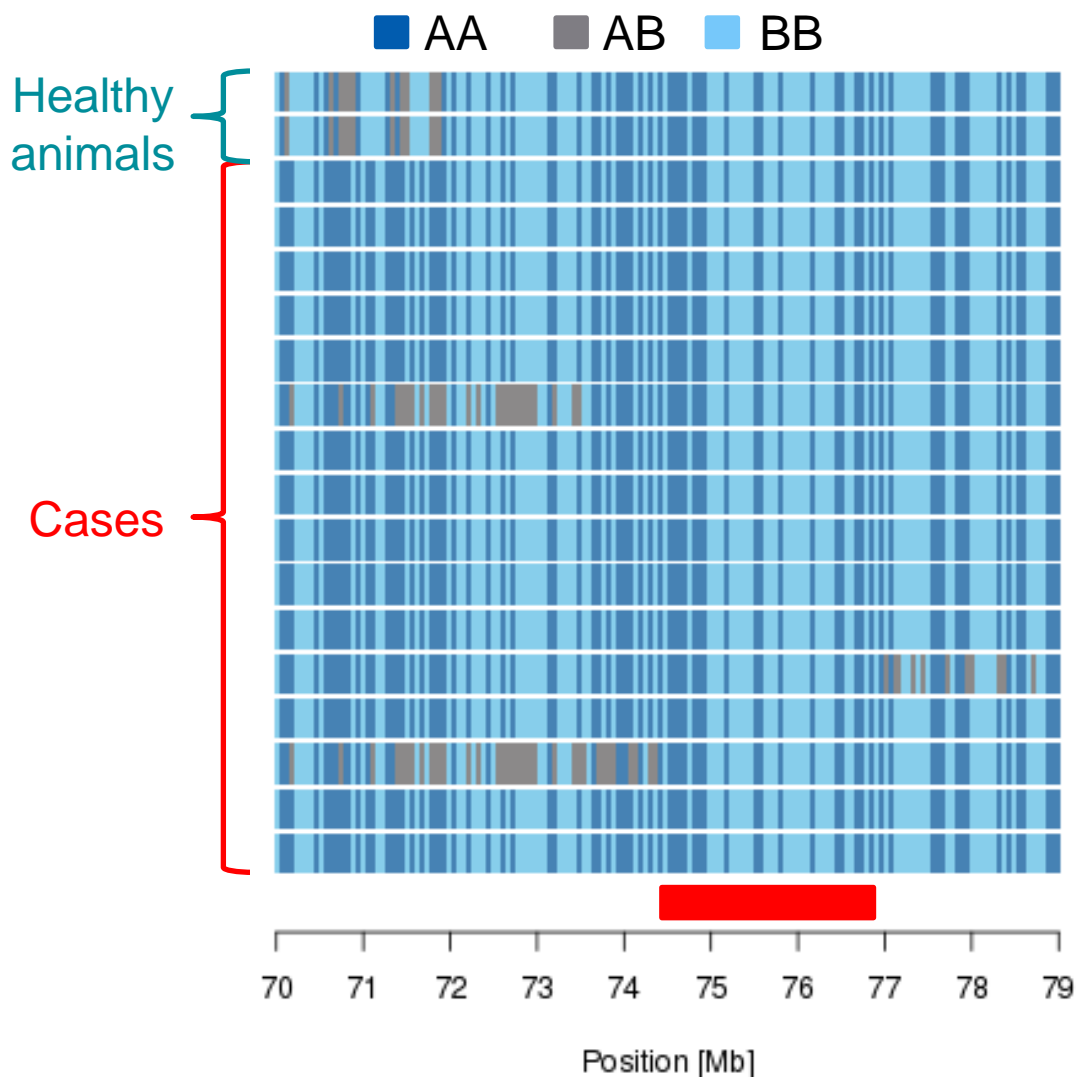
- 1.3 million Euro loss per year
 - 400€ per calf (value of calf, average lifetime: 85 d, medical treatment)

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

Animal	Cholesterol [mmol/l]	HDL [mmol/l]	LDL [mmol/l]	Triglyceride
Case 1	0.17	0.1	<0.02	0.05
Carrier	1.53	1.36	0.19	0.16
Control 1	2.46	1.85	0.68	0.40



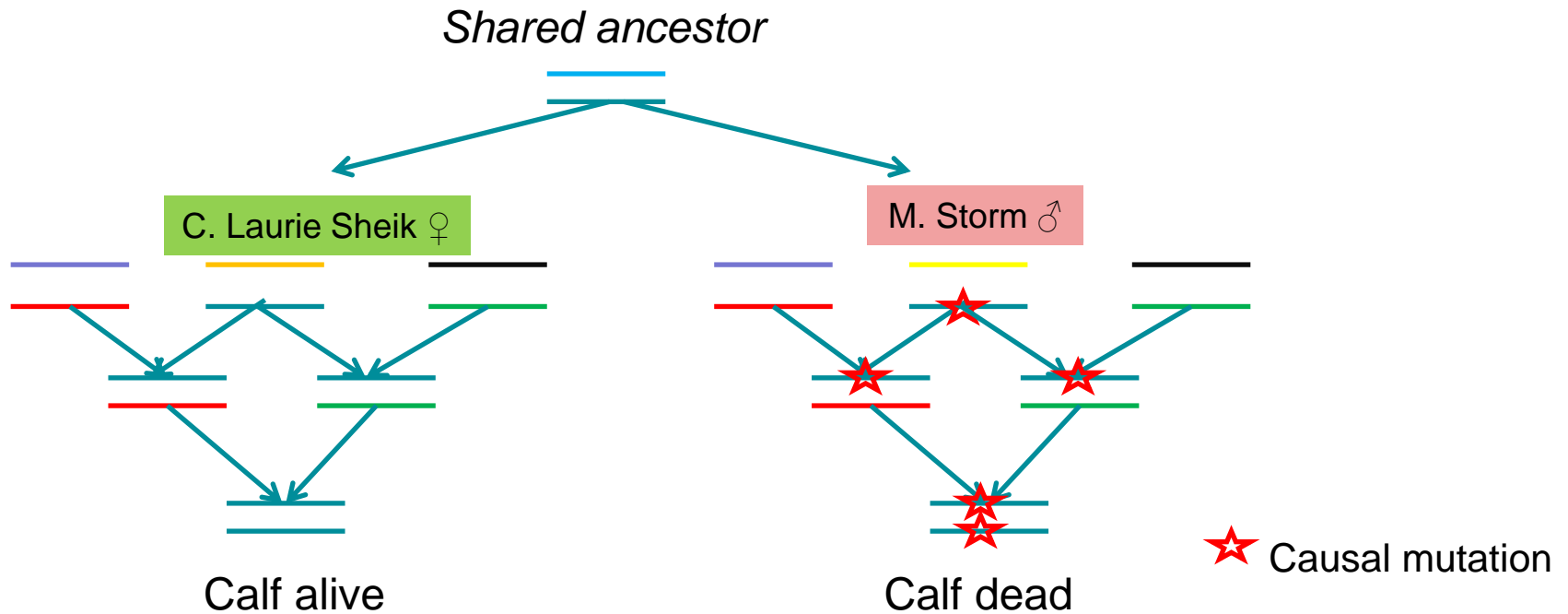
Homozygous animals alive



- 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 → 0.3%)

→ **How to pinpoint the causal mutation?**



Sequence based analyses (II)

Chromosome 11

1)

BB
Affected animal †

→ affected animal must be homozygous

→ 174,373 variants

2)

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

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

→ 3,360 variants

3) Associated homozygous region in the genome

→ 498 variants

4) Monogenic defect

→ 20 variants

- Filtering of genic variants



Sequence based analyses (III)

Collaboration with Technical University Munich

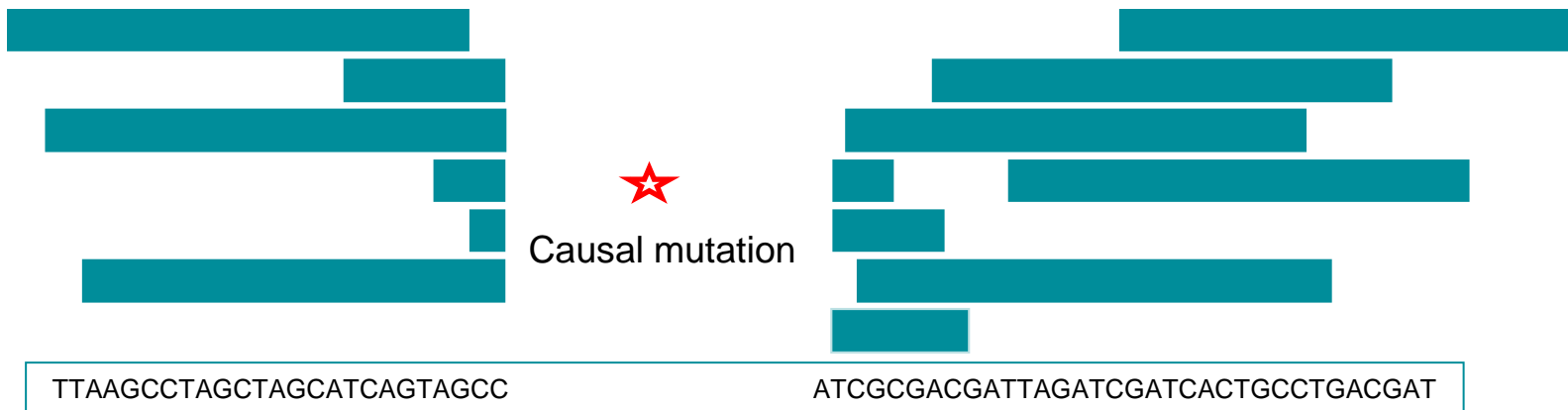
- Access to data of 1000 Bull Genomes Project (Run 4)
 - ~ 1,150 animals re-sequenced
 - 288 Black Holstein animals, 23 Red Holstein animals
 - 37.92 Million variants detected

AA	AB	BB
Remaining animals (Holstein and other breeds)	Prominent Storm offspring	Affected animal †

- ➔ 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
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Thank you.



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