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

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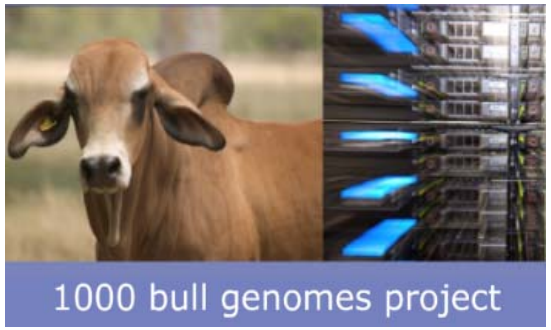
# Acknowledgements

APIS-GENE
Invest Innovate Exploit

ANR AGENCE NATIONALE DE LA RECHERCHE



# Introduction

Breeds are genetically small populations

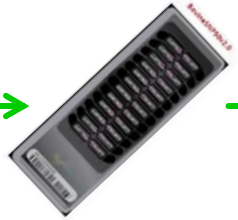
Average inbreeding rate ~ 1% /generation

Regular emergence of recessive defects

Observatories + homozygosity mapping

In front of a typical pedigree …

Phenotype



Genotype

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

CVM (A. Gentile)

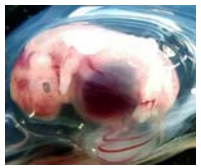
# Introduction

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

~ 1000 markers
120 000 animals genotyped per year

→ Identification of 2 lethal embryonic mutations

# 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 :

Nobs/Nexp <0.25 & adjusted p-value <0.01

# Results

| Name | BTA | Interval (UMD3.1 Mb) | Haplotype freq. (%) | Nexp | Nobs | Chi² test |
|---|---|---|---|---|---|---|
| HH3 | 8 | 94.5-95.6 | 3.1% | 332 | 3 | $7.4 \times 10^{-91}$ |
| HH4 | 1 | 0.1-1.4 | 4.4% | 301 | 8 | $5.9 \times 10^{-82}$ |
| BY | 21 | 20.0-21.2 | 2.7% | 124 | 1 | $6.2 \times 10^{-30}$ |
| HH5 | 9 | 94.8-96.4 | 1.9% | 117 | 8 | $3.6 \times 10^{-28}$ |
| HH1 | 5 | 63.0-65.6 | 1.7% | 57 | 1 | $1.1 \times 10^{-11}$ |
| HH6 | 16 | 27.8-32.0 | 1.1% | 31 | 0 | $1.7 \times 10^{-4}$ |

Charlier *et al.* 2012; *Fritz et al., 2013; McClure et al., 2014; Daetwyler et al. 2014; Adams et al., 2016; Schütz et al., 2016*).

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

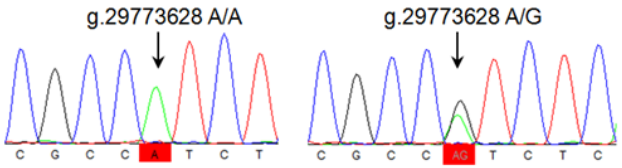
- Detection of a new region HH6 (freq 1.7%)
  0 observed vs 31 homozygotes expected → embryonic lethal ?

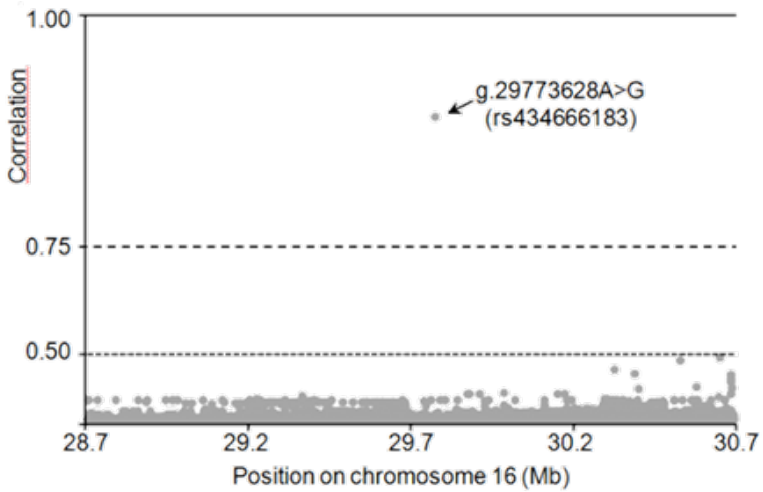
- Most influential and ancient carrier is Mountain (BIS-MAY S-E-L MOUNTAIN ET)
- Fine-mapping using intra familial recombinations → interval of 1.1 Mb

# 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



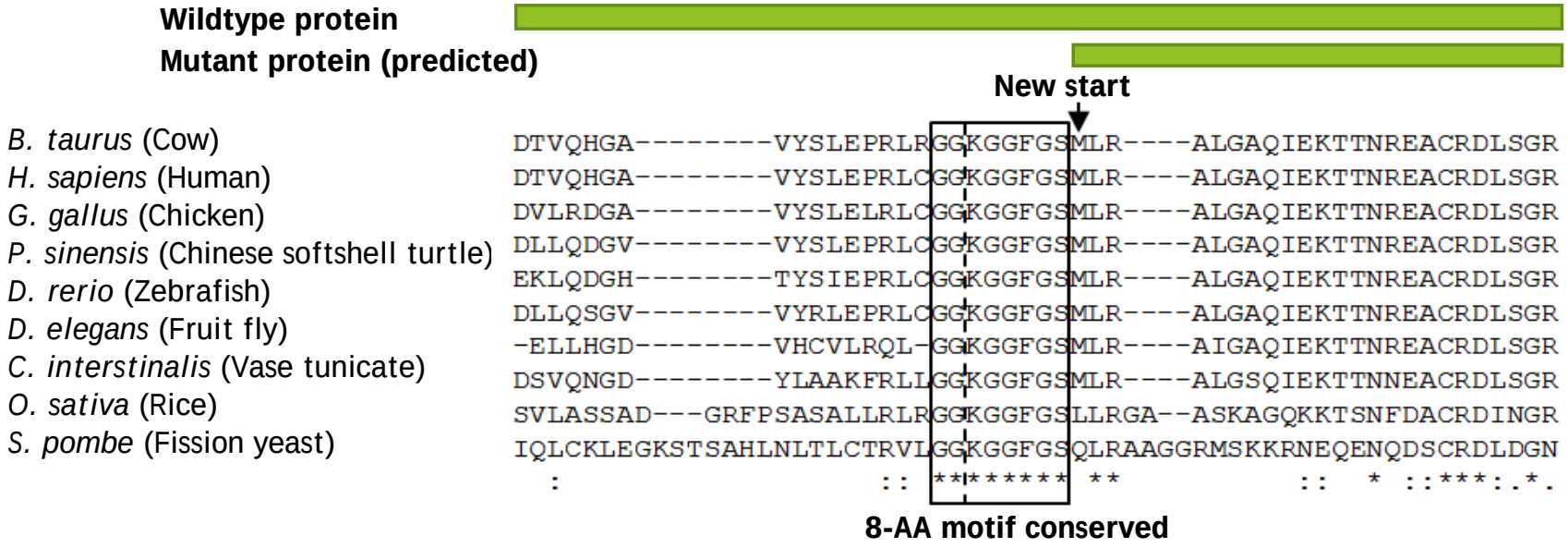




- Candidate mutation : chr16 g.29773628A>G affects the initiator codon of the gene *SDE2 Telomere Maintenance Homolog*

# 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



# Reverse genetic approach identifies HH7

**Experience from large scale use of the EuroGenomics custom SNP chip in cattle**

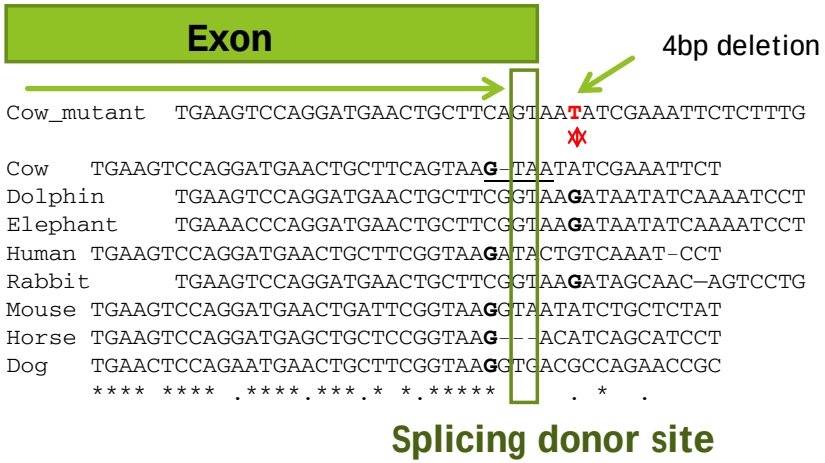
*D. Boichard[1], M. Boussaha[1], A. Capitan[1,2], D. Rocha[1], C. Hozé [1,2], M.P. Sanchez[1], T. Tribout[1], R. Letaief[1], P. Croiseau[1], Grohs C. [1], W. Li[3], C. Harland[3], C. Charlier[3], M.S. Lund[4], G. Sahana[4], M. Georges[3], S. Barbier[5], W. Coppieters[3], S. Fritz[1,2], & B. Guldbrandtsen[4]*

- **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 ATTACTTACT ATTACT

- 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 !!

| Name | BTA | Interval (UMD3.1 Mb) | Haplotype freq. (%) | Nexp | Nobs | Chi² test | Bonferronni corrected |
|---|---|---|---|---|---|---|---|
| HH7 | 27 | 13.0-14.4 | 1.2% | 16 | 0 | $2.0 \times 10^{-6}$ | 18.6 |

Recombining

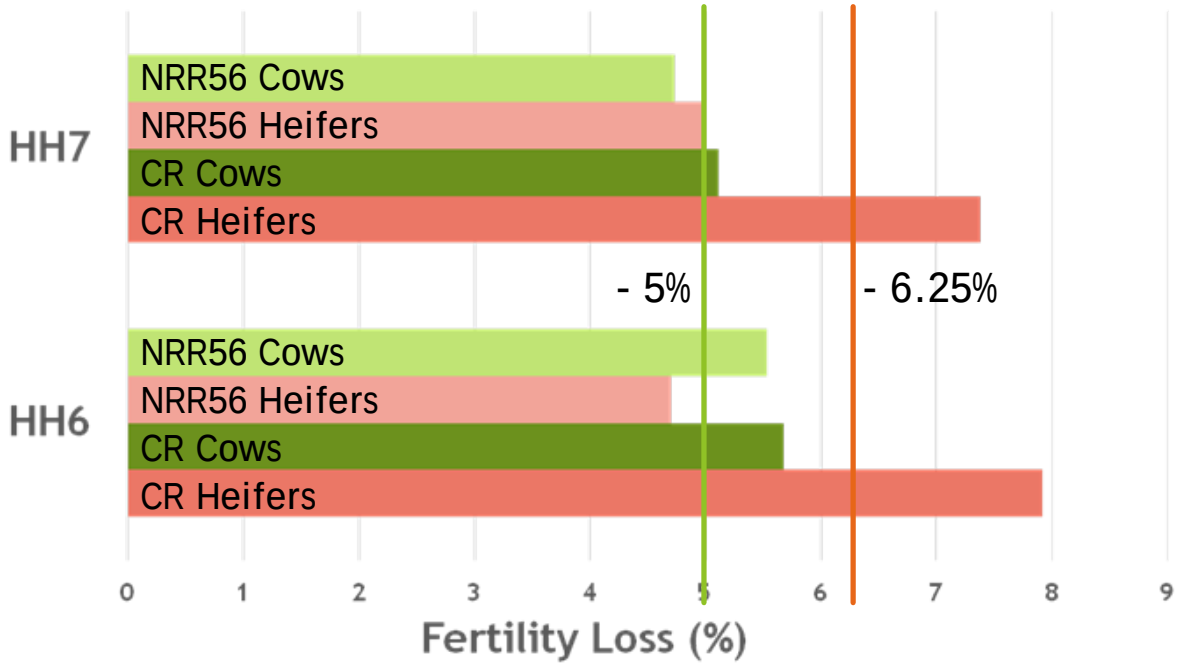
Ancestral version of the haplotype predating the mutation event

| | +/+ | HH7/+ | HH7/HH7 | Total |
|---|---|---|---|---|
| +/+ | 98224 | 20 | 0 | 98244 |
| +/DEL | 164 | 1692 | 0 | 1856 |
| DEL/DEL | 0 | 0 | 0 | 0 |
| Total | 98388 | 1712 | 0 | 100100 |

▶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%

Linkage Disequilibrium

→ **Complementarity of the two approaches**

**Thank you for your attention**