Prospects of studying animal life histories to identify undetected genetic defects: the example of BLIRD

F. Besnard, A. Guintard, M. Cano, C. Hozé, H. Leclerc, M. Boussaha, C. Grohs, J. Jourdain, A. Clément, A. Barbat, S. Fritz, C. Escouflaire, J. Rivière, C. Péchoux, C. Danchin-Burge, A. Relun, V. Plassard, M.-A. Arcangioli, S. Mattalia, D. Boichard, G. Foucras and **A. Capitan**

EHRC Conference

Dublin, 27 April 2023

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Context

Recurrent outbreaks of recessive defects

Classical approach: positional cloning

Surveillance networks → Clinical investigations → Mapping → Sequençing

Not exhaustive

Specific symptoms

Embryonic deaths
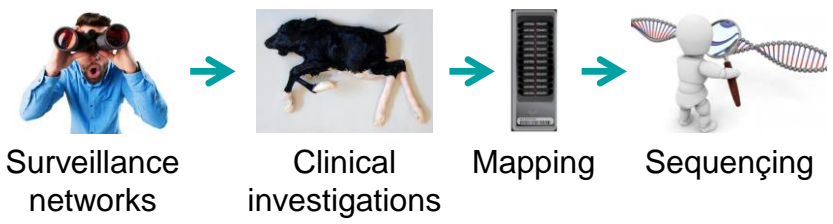Metabolic or Immune defects

Dvpt of alternative strategies:

Deficit in homozygotes
VanRaden *et al.*, 2011
Fritz *et al.*, 2013

Leverage large genotype/WGS data sets

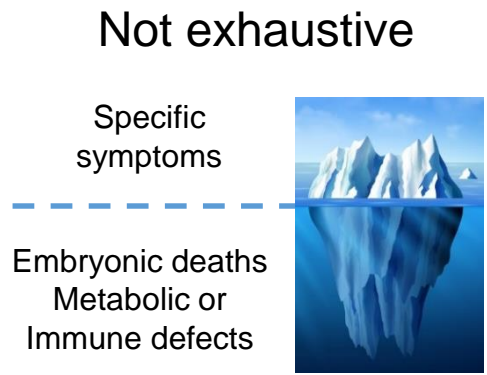Start with no or raw phenotypes

Reverse genetics
Charlier *et al.*, 2016
Michot *et al.*, 2016

Identification of numerous new loci

GWAS on proxy phenotypes accounting for dominance
Reynolds *et al.*, 2021

But not efficient in case of incomplete penetrance

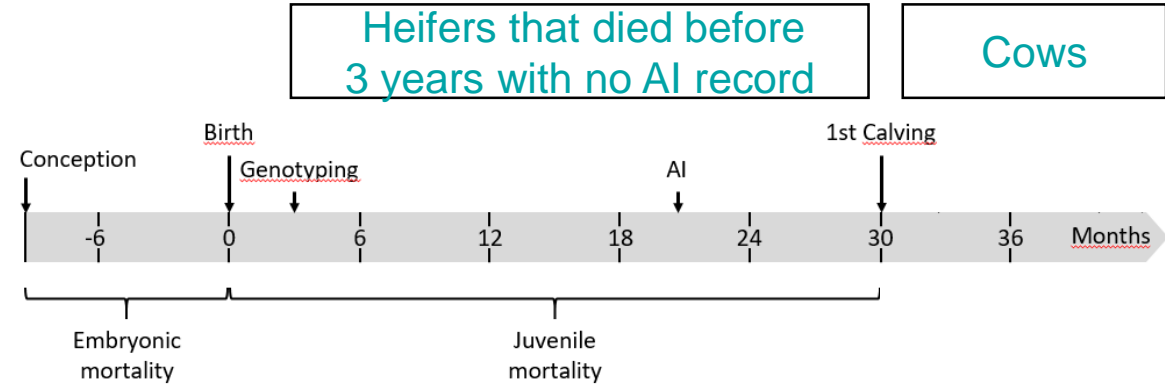**or incomplete LD (case of *de novo* mutations)**

Homozygous Haplotype Enrichment/Depletion mapping (HHED)

Principle:

to combine searches for depletion/enrichment in hmz in groups of animals with distinct life trajectories

Heifers that died before 3 years with no AI record

Cows

Animals:

Breed	Dead heifers	Cows
Holstein	8203	291529
Montbéliarde	6198	141343
Normande	2254	56095
Total	16655	488967

With sire + dam/MGS genotyped

Methods:

Windows of 20 mrk (Illumina BovineSNP50)
Nobs ≥10 in dead heifers
(Nobs-Nexp)/Nexp ≥ 25% in dead heifers
(Nobs-Nexp)/Nexp ≤ -25% in cows

Loose filters to account for incomplete penetrance or LD between haplo/mut.

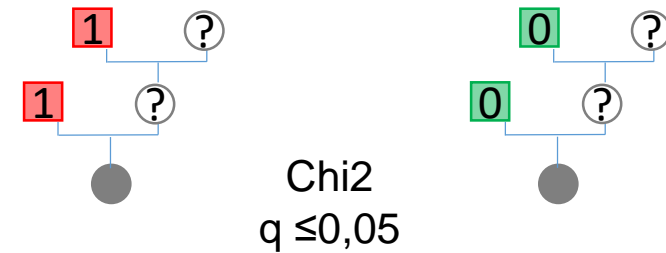# Results of HHED mapping
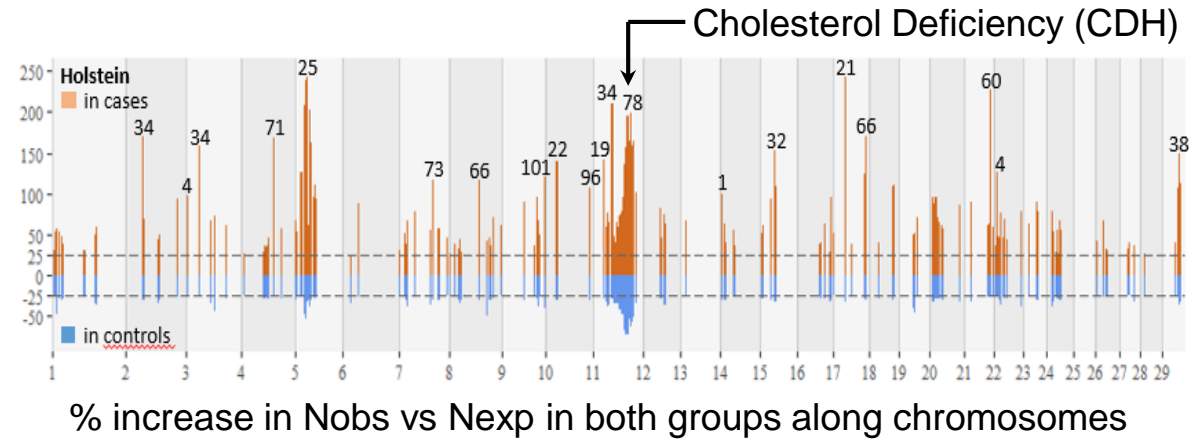
Many loci detected

↓

Focus on the top20 per breed
+
Analysis of the life trajectories
of 1 to 6 millions females
with sire and MGS genotyped

↓

34/60 validated !

1.5 ≤ haplo freq ≤ 7.6%

~0.5 to 1% of calves are hmz
for at least one of these haplotypes
of increased juvenile mortality

Cholesterol Deficiency (CDH)

% increase in Nobs vs Nexp in both groups along chromosomes
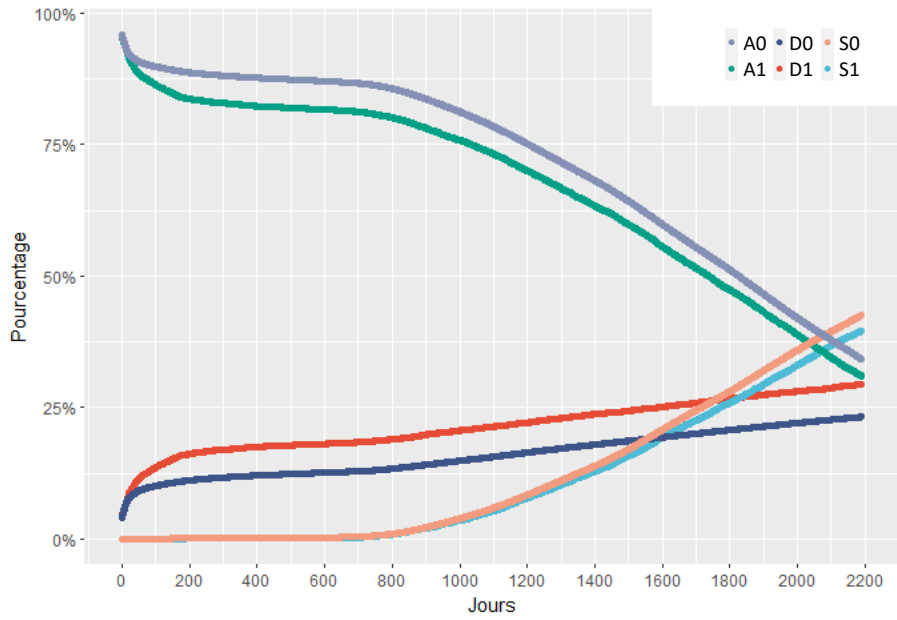
Chi2
q ≤0,05

13/20 loci

11/20 loci

10/20 loci
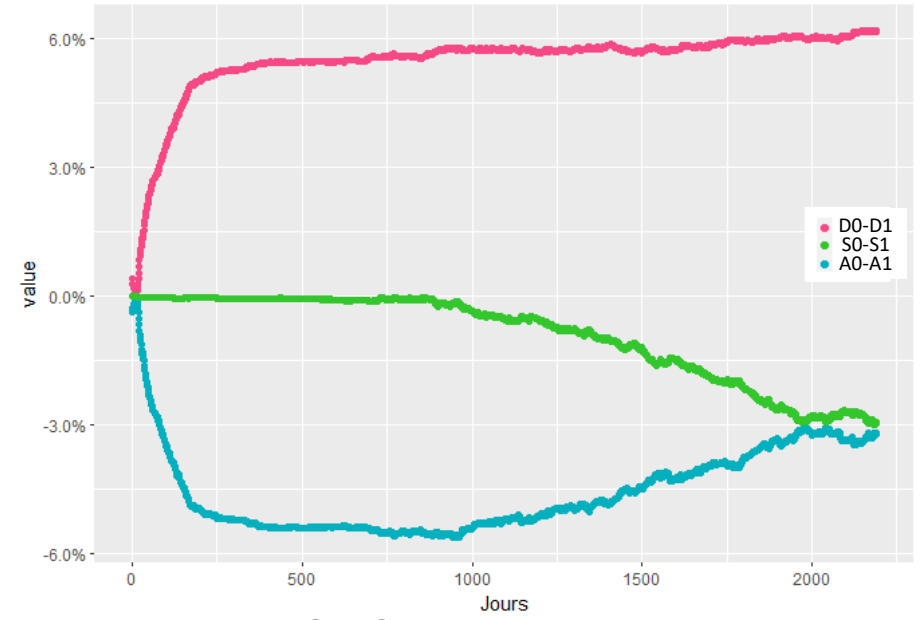
Study of survival curves for at-risk vs control mating

example of CDH in Holstein

Daily proportion of animals that died (D), were slaughtered (S) or are still alive (A) over a period of 6 years for control (0) and at risk (1) mating

D0-D1, S0-S1 and A0-A1 differences in proportions on a daily basis

Days for which 25, 50, 75 and 100 % of the maximum deviation between each difference was reached → 12 parameters

Difference	Max deviation	75%	50%	25%
A0-A1	945	139	73	33
D0-D1	2180	156	81	34
S0-S1	0	0	0	0

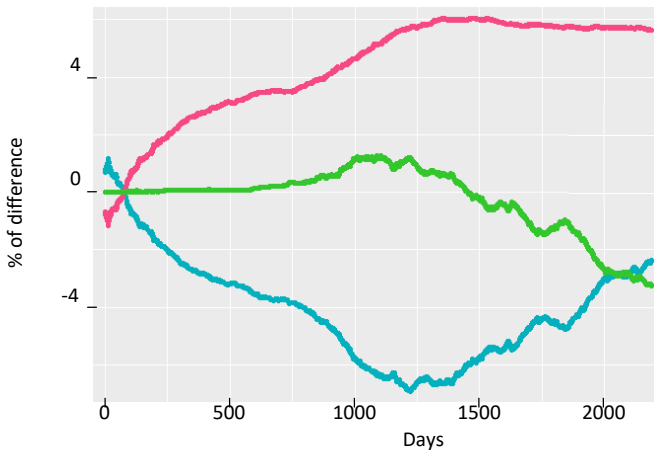# Principal component analysis and hierarchical clustering

34 haplotypes
12 parameters

→ three main survival curve profiles

CDH

Dim 2 (19.43%)

Dim 1 (52.58%)

Inertia gain

Cluster Dendrogram

% of difference

Days

D0-D1
S0-S1
A0-A1
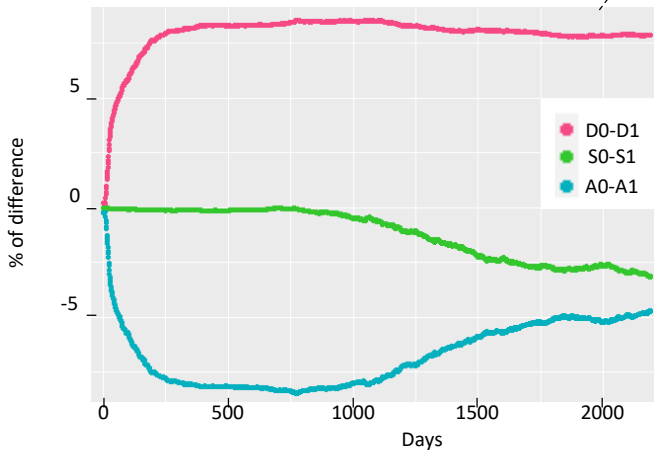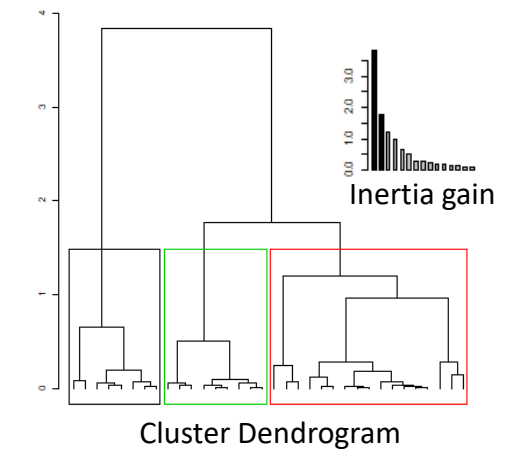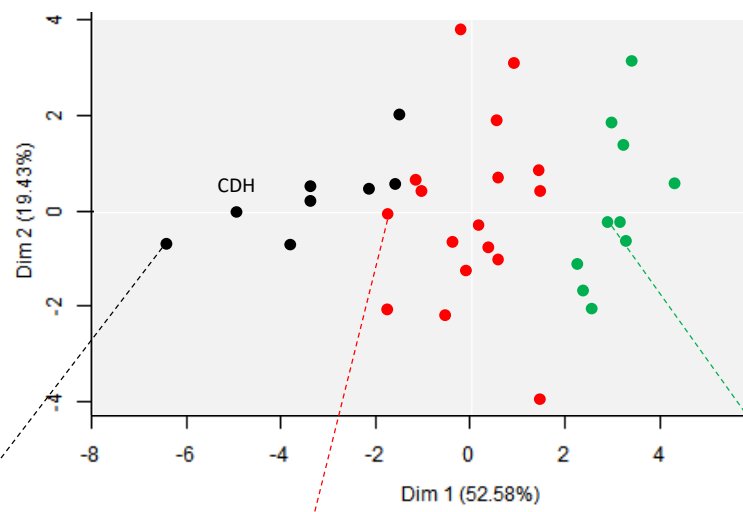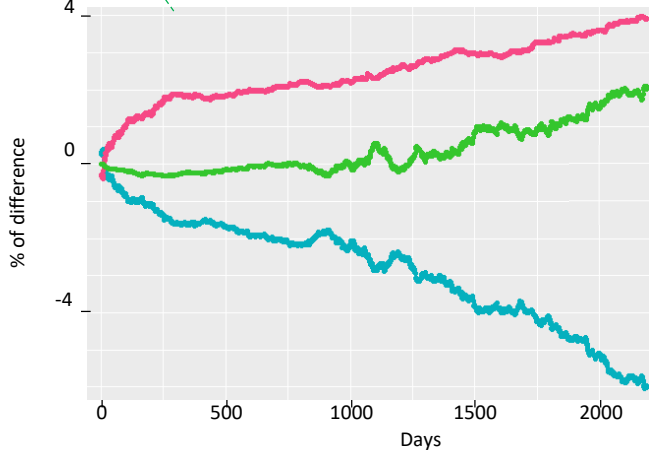
Early juvenile mortality

Progressive juveniles and subadult mortality

Increased mortality and premature culling throughout life

Search for candidate variants

1184 genomes incl.
333 Holstein
160 Montbeliarde
160 Normande
& 18 additional breeds
>5 carriers per locus

Filters:
Breed-specific variants
Haplo x variant $R^2 \geq 0.5$
Predicted to be deleterious:
SIFT≤0.05, stop, frameshift,…

EuroGMD chip / regular add-on

Strong candidates for 9/33 loci

Expected clinical signs:
neurological, metabolic & immune
unknown/no live mutant

Clinical characterization

MTCP   ALOP   BLIRD

European Holstein & Red Holstein Conference
IHFA 2023

INRAe
université PARIS-SACLAY
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GABI
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EURO Genomics
for reliable cattle breeding
Oniris
École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation
Nantes Atlantique

The 1000 bull genomes project

Funding:
ANRT
ASSOCIATION NATIONALE RECHERCHE TECHNOLOGIE

APIS-GENE
Investir Innover Valoriser
anr

EURO Genomics
for reliable cattle breeding

MGI
Mouse Genome Informatics

THE UNIVERSITY OF SYDNEY
OMIA
ONLINE MENDELIAN INHERITANCE IN ANIMALS

50 YEARS OMIM
Human Genetics Knowledge for the World

Example of BLIRD in Holstein

Preliminary analysis (annotation, conservation,…)
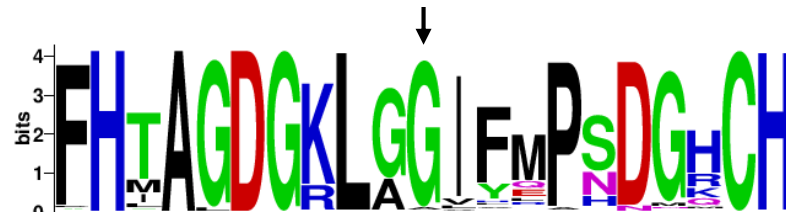
Functional candidate variant:
Point mutation affecting the Integrin β7 protein

ITGB7 and ITGA4 form the ITGα4β7 receptor, which is essential for the migration of T lymphocytes to the intestinal mucosa

The candidate variant affects a conserved residue and is predicted to impair the binding of ITGB7 with ITGA4
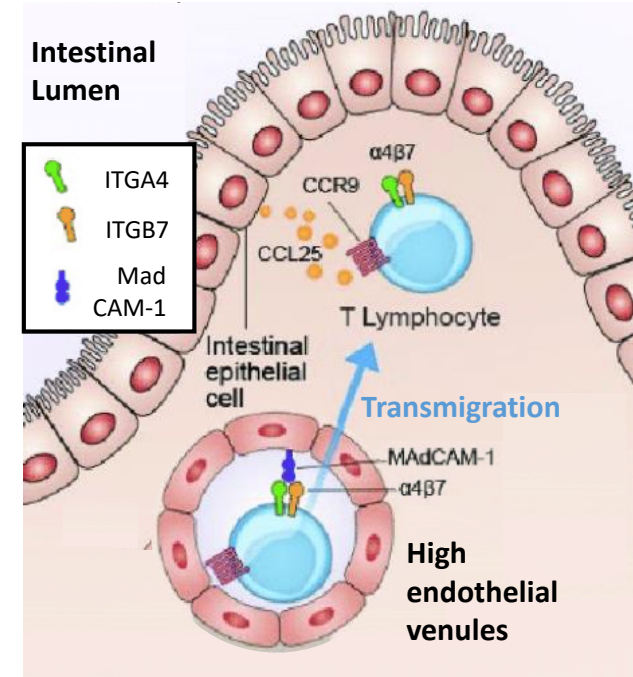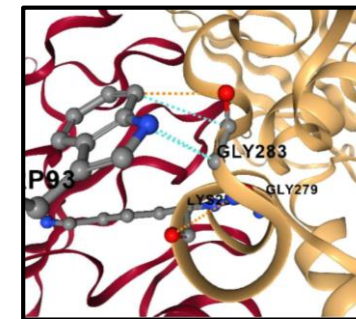
Bovine ITGB7 p.G375S

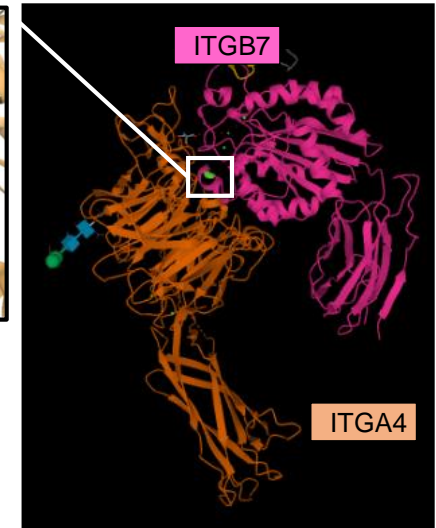Alignment of 199 orthologs incl. invertebrates

Homozygous mutant cows are predicted to show clinical signs similar to ITGB7 KO mice : hypoplasia of gut-associated lymph tissue, abnormal response to infection and increased parasitism

Human
ITGB7 p.G283S

Change in affinity:
Δ = - 0,818 kCal.mol-1

Modelisation using crystallographic structure

Funding:

Example of BLIRD in Holstein

Insights from large scale genotyping

f = 4.6% (367k genotypes)
Incomplete LD: R^2=0.66

	Haplo_0	Haplo_1	Haplo_2	Total
ITGB7_0	329852	3558	6	333416
ITGB7_1	6259	26283	110	32652
ITGB7_2	27	162	447	636
Total	336138	30003	563	**366704**

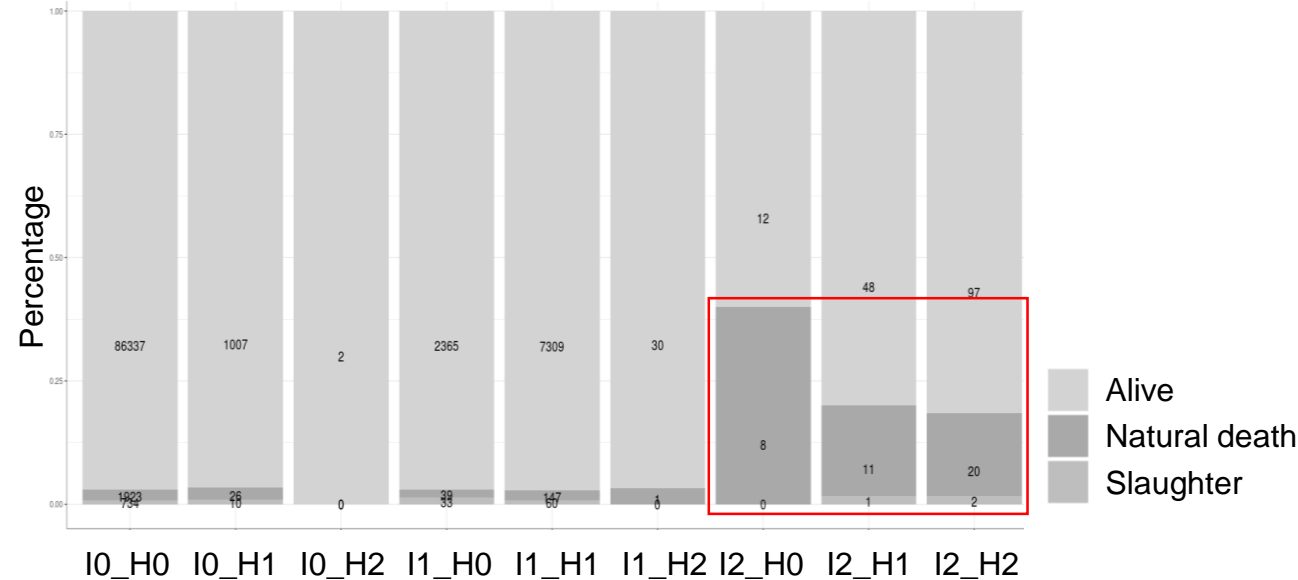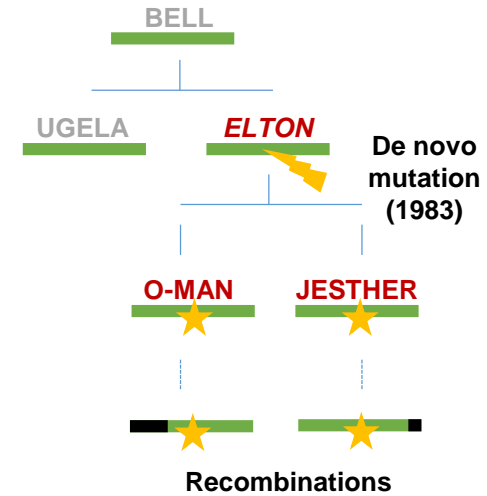~80% double homozygotes (Haplo_2 & ITGB7_2)

The mutation occurred in 1983 on a relatively frequent haplotype

High mortality rates in homozygous mutants regardless of their haplotype status

→ Support the causality of the mutation

Female survival in the first 2 years by genotype combinations
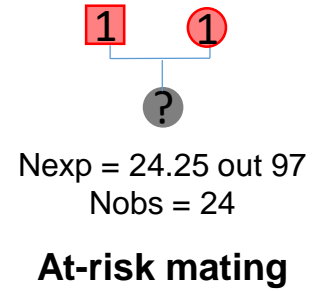
Example of BLIRD in Holstein

***In silico* phenotypic characterization based on <u>true</u> genotypes at *ITGB7* variant**

High genotype accuracy
but variant on the chip since 2019

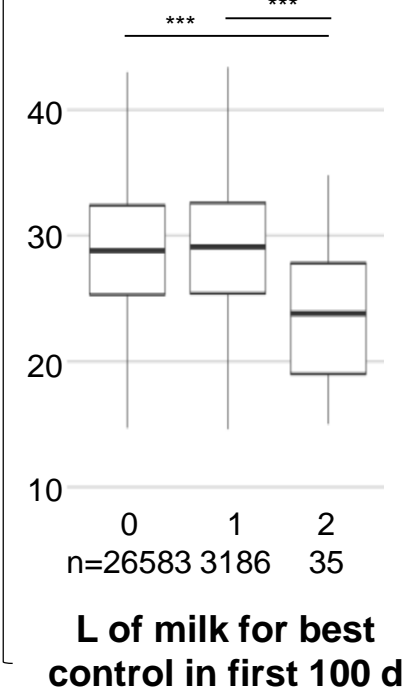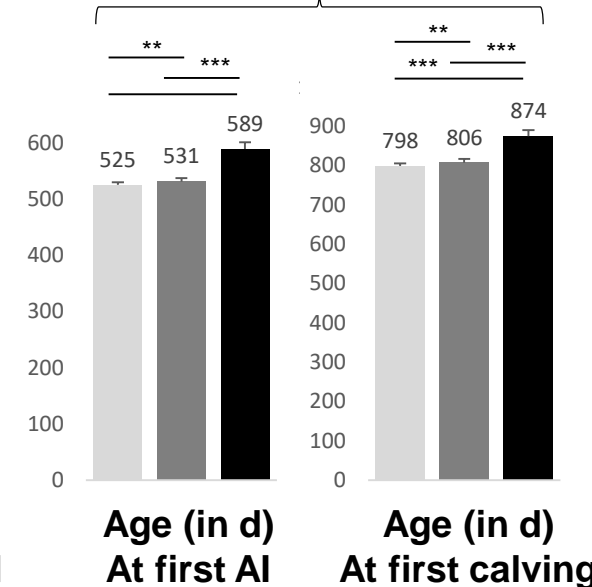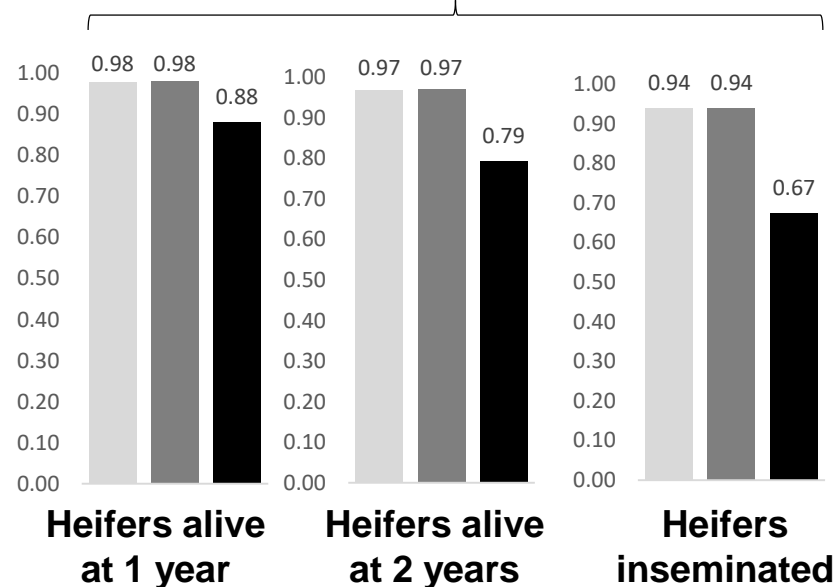→ low nb of hmz mutants old enough
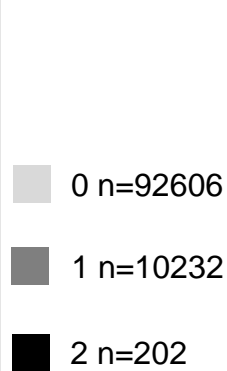for some statistics (born > 2 years ago)

No depletion in hmz
→ No effect at ~ 3 months

Nexp = 24.25 out 97
Nobs = 24

At-risk mating

Lower milk
production

Increased mortality / poor health

Retarded growth

0 n=92606

1 n=10232

2 n=202

Heifers alive at 1 year	Heifers alive at 2 years	Heifers inseminated
0.98 0.98 0.88	0.97 0.97 0.79	0.94 0.94 0.67

Age (in d) At first AI	Age (in d) At first calving
525 531 589	798 806 874

40

30

20

10

0   1   2
n=26583 3186 35

**L of milk for best
control in first 100 d**

Funding:

Example of BLIRD in Holstein

In silico phenotypic characterization based on <u>true</u> + <u>imputed</u> genotypes
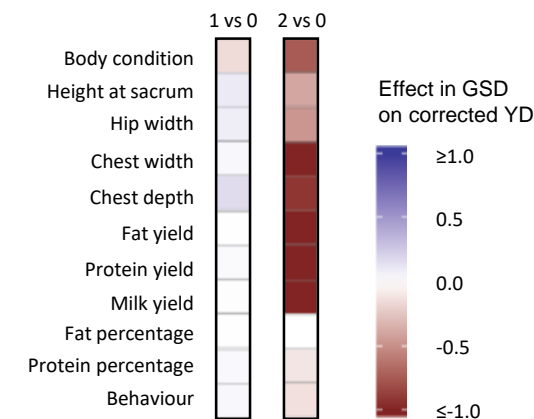
Imputation using pedigree information & distant markers
Analysis on 860k animals with imputed genotypes (2010-now)
+ 367k animals genotyped for ITGB7 variant (2019-now)

Increased mortality & premature culling

Strong negative effects on most of the traits
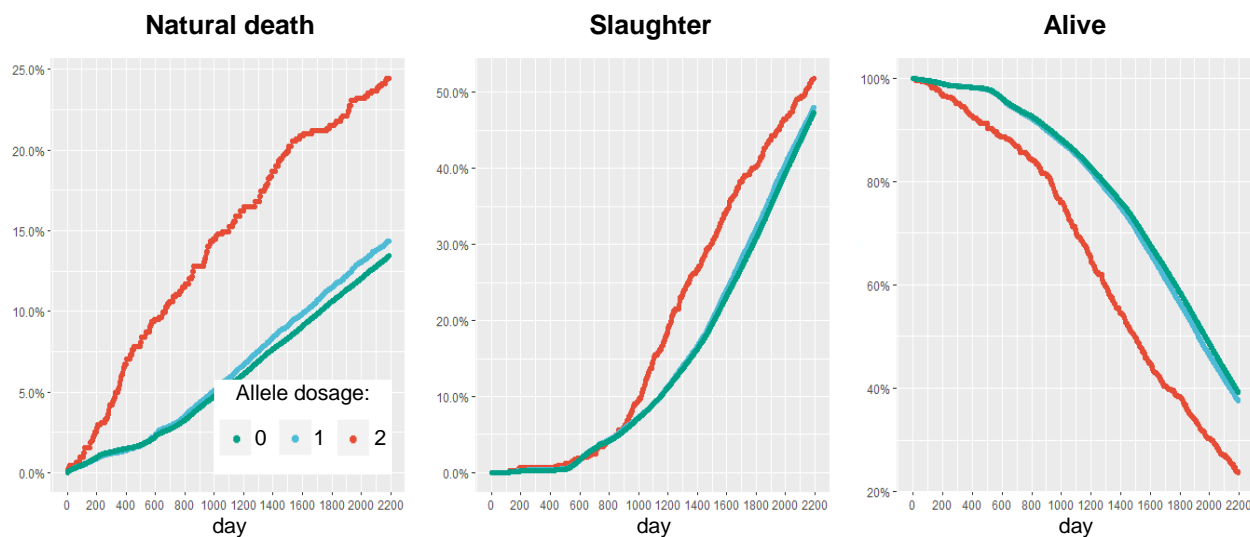
Survival over a six-year period

Effects on traits

Nb of imputed genotypes:
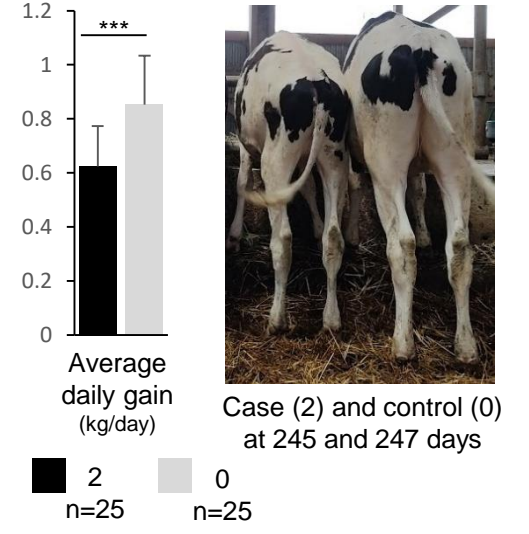0: 382425; 1: 51321; 2: 1320

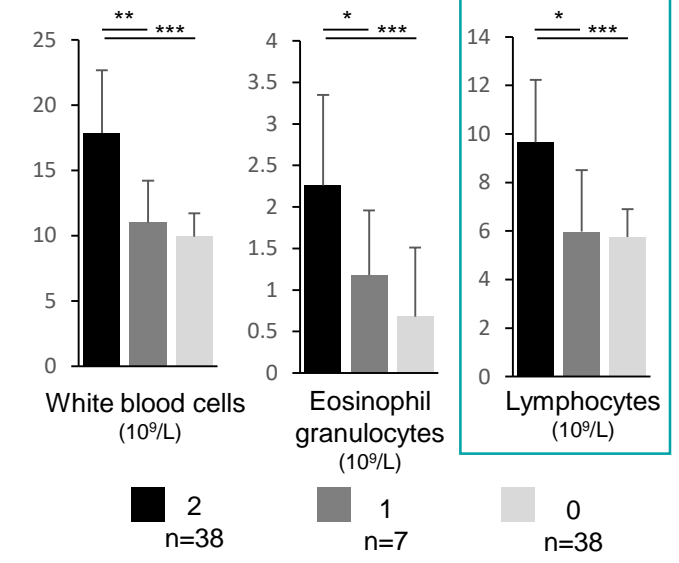# Example of BLIRD in Holstein

On field characterization

Clinical examination :
38 case-control pairs per herd
aged 3 months to 4 years (+ 7 htz)

Case (2) and control (0)
at 245 and 247 days

Average daily gain (kg/day)

2 n=25   0 n=25

Reduction by 27 % of the
daily gain btw 6-24 months

White blood cells (10⁹/L)   Eosinophil granulocytes (10⁹/L)   Lymphocytes (10⁹/L)

2 n=38   1 n=7   0 n=38

Significant modification of blood
parameters in hmz mutants

Necropsy of 4 heifers:
Small lymph nodes
and Peyer's patches
in the intestine

Expected in case of inability of T lymphocytes
to migrate to the intestine and increased parasitism

→ **BLIRD** : **B**ovine **L**ymphocyte **I**ntestinal
Retention **D**efect (G. Foucras, ENVT)

Conclusion and prospects

Our strategy works well !

33 new recessive loci in 3 breeds:
Affect 0.5 to 1% of the population
Cause increased juvenile mortality, reduced lifespan & production

Improve our knowledge:

Molecular bases of imbreeding depression

Challenges:

Managment in selection

Identification and functional validation
of the causative variants for all loci

Analysis of additional life stages

**Require massive operational forces
Need to initiate international collaborations**

Thank you for your attention

Any question ?

Thanks to colleagues and partners

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