RP1: AN EXAMPLE OF REVERSE GENETICS APPROACH TO DESCRIBE COMMON RECESSIVE DEFECTS

C. Grohs GABI, INRA, AgroParisTech,

Université Paris Saclay

Outbreaks of recessive defects as a consequence of animal selection

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- We all carry hundreds of recessive mutations (Some are deleterious)
- Animal populations: few founders and strongly selected, conducing to low diversity and large genetic drifts
- Increase of frequency of some deleterious mutations: « the cost of domestication »

→ Creation of dedicated observatories (ONAB in France) to manage outbreaks: detection, collection, characterization

ONAB Stakeholders

HD genotyping, homozygosity mapping and sequencing to characterize genetic defects

❖ Thousands of markers can be genotyped for 24 to 96 samples at the same time using ILLUMINA bead chips

❖ Homozygosity mapping can be carried out with as few as 2-5 cases and leads to the identification of a 1-2 Mb candidate region

❖ With lots of avalaible sequences, the causative variant can be found in a few months

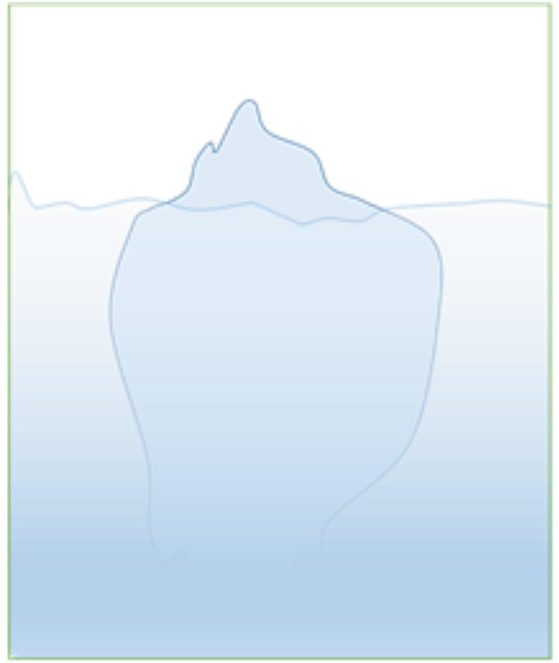
❖ So far, 130 cattle defects have been characterized *vs* > 4900 in human

Some defects are lilely to be missed

- Embryonic mortality: Charlier *et al. (2016)* estimated by simulation that each cattle might carry 0.5 recessive Embryonic Lethality (EL) mutation

- Non specific symptoms: immune or metabolic defects e.g. diarrheas (like CDH)

- We have a lot of genotyping and sequencing data that permit a new strategy: **reverse genetics** to identify the underlying mutations



The tip of the iceberg

Reverse genetics

❖ We used the data of the « 1000 bull genomes » project, we investigated WGS from 15 bovine breeds (with >20 sequenced animals)

❖ Thousands of variants can be filtered with deleterious annotation, but they are rare and breed specific: due to their low frequency, mutations are not yet a danger for the population

❖ We investigated common variants (MAF >5%), shared by at least two breeds and with a deleterious annotation, because of their larger potential impact at the population level

Reverse genetics strategy

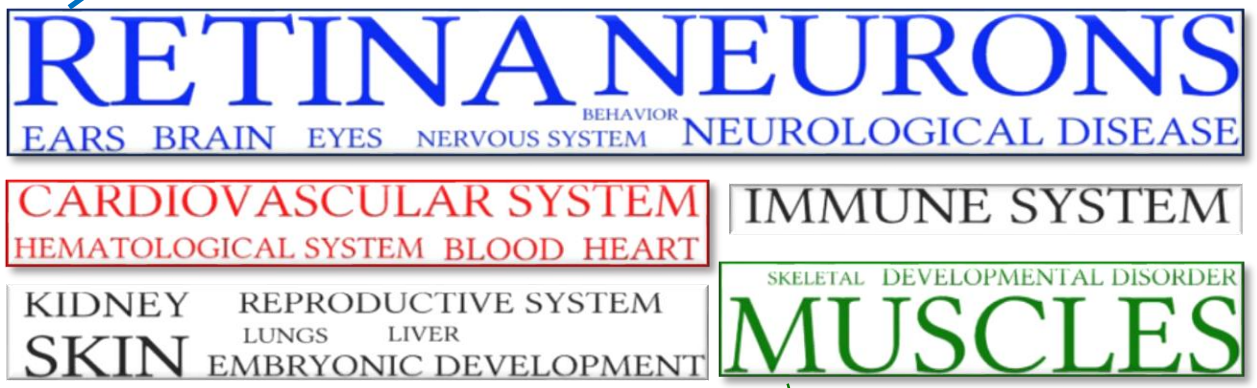
From the sequencing data of the main contributors of each breed :

- ❖ Annotate the deleterious mutations
- ❖ Include these potential variants on the EuroG10K custom chip
- ❖ Characterize the mutation on the population genotyped for Genomic Selection
  - ✓ EL: Homozygote haplotypes deficiency
  - ✓ Effect on recorded traits: Imputation and GWAS
  - ✓ Specific monitoring of animals born from matings at risk
  - ✓ Embryos production, genotyping, animals monitoring

Results

- 2,489 putative deleterious variants in 1,923 genes
- Analysis of gene enrichment with Ingenuity Pathway Analysis showed:

NERVOUS SYSTEM, VISION, SENSITIVE SYSTEM…
41.5% of functional annotations (**17.7%** related to retina)

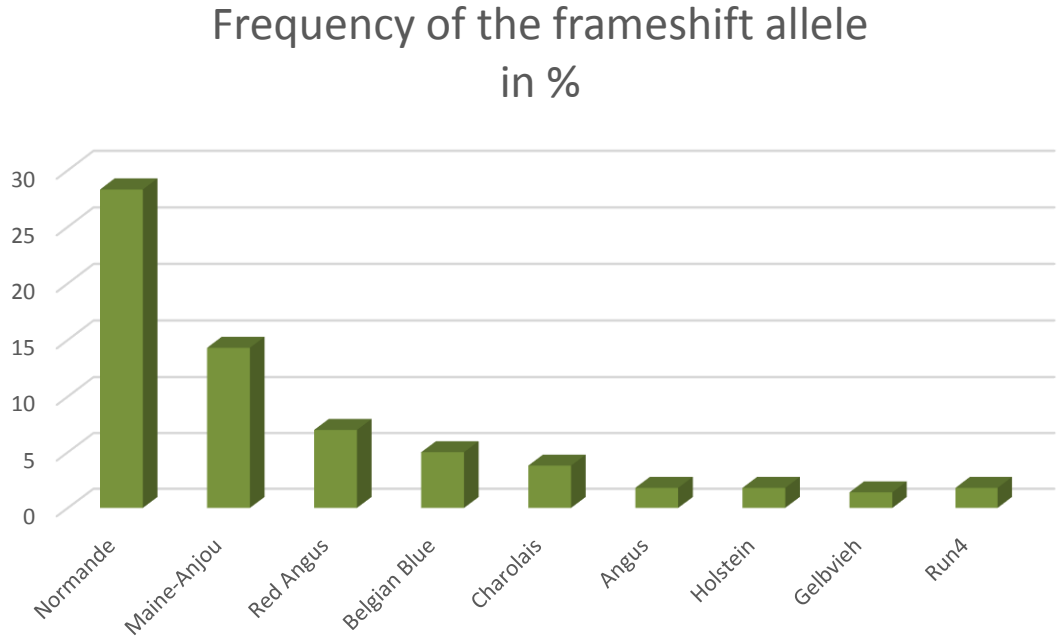


HEART, CIRCULATORY SYSTEM
12.8% functional annotations

MUSCLE, SKELETON
12.3 % functional annotations

Results

- To assess the phenotypic consequences of this phenomenon, we studied one mutation in RP1 gene
  - ✓ observed in many breeds
  - ✓ predicted to affect the retina



RESEARCH ARTICLE Open Access

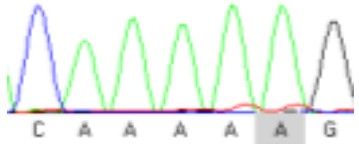
A reverse genetic approach identifies an ancestral frameshift mutation in *RP1* causing recessive progressive retinal degeneration in European cattle breeds

Pauline Michot[1,2], Sabine Chahory[3], Andrew Marete[1,4], Cécile Grohs[1], Dimitri Dagios[3], Elise Donzel[3], Abdelhak Aboukadiri[1], Marie-Christine Deloche[1,2], Aurélie Allais-Bonnet[2,5], Matthieu Chambrial[6], Sarah Barbey[7], Lucie Genestout[8], Mekki Boussaha[1], Coralie Danchin-Burge[9], Sébastien Fritz[1,2], Didier Boichard[1] and Aurélien Capitan[1,2*]

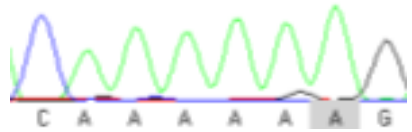
Retinis Pigmentosa-1 gene (RP1)

Addition of A in a stretch of 5A

Wt/Wt



Mut/Mut



- This gene encodes a microtubule-associated protein expressed in the retina, playing a role in the differentiation and organization of the photoreceptors

- Involved in Retinitis Pigmentosa 1 Syndrome : Retinal dystrophy → progressive loss of rod and cone photoreceptors (night and daytime vision) resulting in blindness after 4 years of age

- Well described in human and mouse

- No clear economic impact but animal welfare is addressed

Clinical and functional analysis

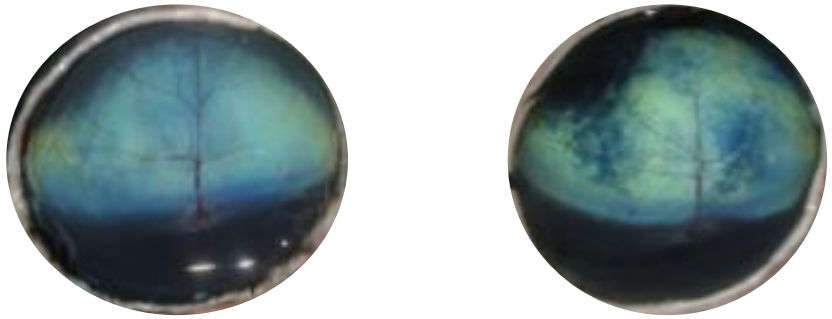
- Phenotyping of 23 cows from the INRA experimental farm, of the 3 possible genotypes (blind test)

- Ocular examination
  - ✓ Threat response
  - ✓ Pupillary light reflexes
  - ✓ Eye fundus
  - ✓ Electroretinography test

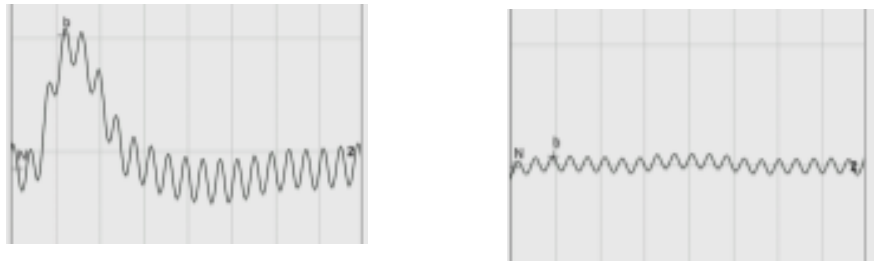
- Histological analysis of the retina

Control Affected

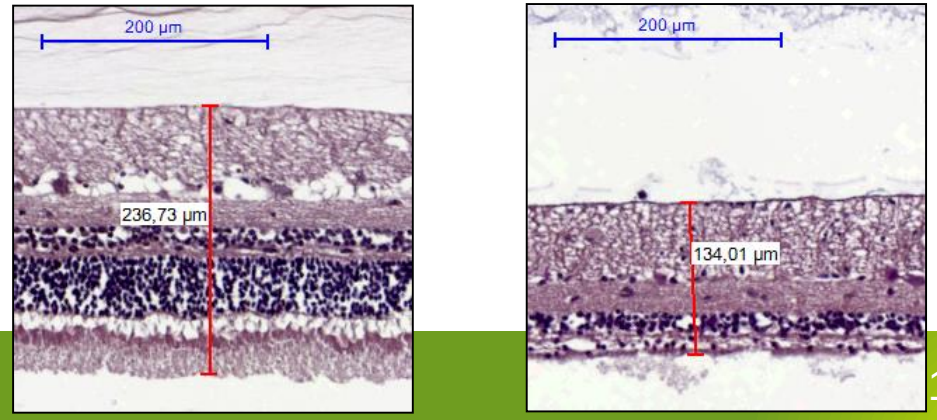
Eyes fundus (n=6)



Electroretinograms (n=2)



Histology of the retinis (n=6)



In Conclusion

❖ Current Resources (technology, data and strategy) allow to identify recessive abnormalities without a described phenotype

❖ Hundreds of harmful mutations can easily be detected in cattle, including mutations that do not affect production traits,

…but it is still essential to validate the effect of an identified mutation

❖ Finally, we worked on some mutations, but hundreds of them remain to be investigated, especially in breeds that are not common in France.

→It would be useful to collaborate in a **“phenotyping network”**

Thank you for your attention