Identification of recessive lethal mutations in sheep using homozygosity deficiency

Stéphane Fabre and Maxime Ben Braiek

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Avec la contribution financière du compte d'affectation spéciale développement agricole et rural CASDAR
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Why identifying recessive lethal/deleterious mutations?

- Most individuals carry roughly 100 loss-of-function alleles in their genome, and among them one to five are predicted to be deleterious (MacArthur et al., 2012; Georges et al., 2019)
- In livestock, due to possible inbreeding, overuse of selected reproducers, or by genetic drift, the probability of generating homozygous carriers could increase

→ Production issues : declining fertility, declining offspring production.

→ Animal (and breeder) welfare issues : increasing health problems, culling decision, facing mortality.

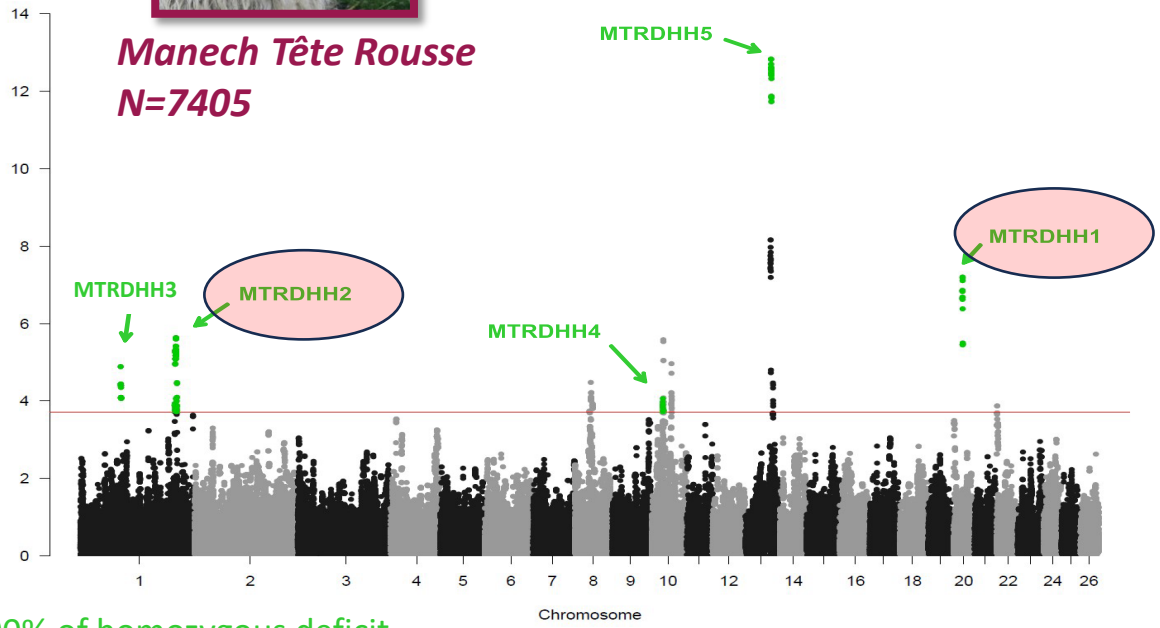
***Working hyp**: Homozygous animals carriers of such mutations are never genotyped in genomic selection programmes (not born, neonate lethality, not candidate)*

Screening for deficiency of homozygous haplotypes (DHH) in genotyped populations

Based on 50k SNP genotypes from dairy sheep genomic selection programmes (mainly males)

Lacaune dairy line
N=35221

Manech Tête Rousse
N=7405



Haplotype with 75-100% of homozygous deficit

13 independent haplotypes (based on LD) → Segregation of 13 putative recessive deleterious/lethal mutations:

8 in Lacaune ***5 in Manech Tête Rousse***

How to explain these DHH? Recessive lethal mutations? Counter-selection of morphological/health defects?

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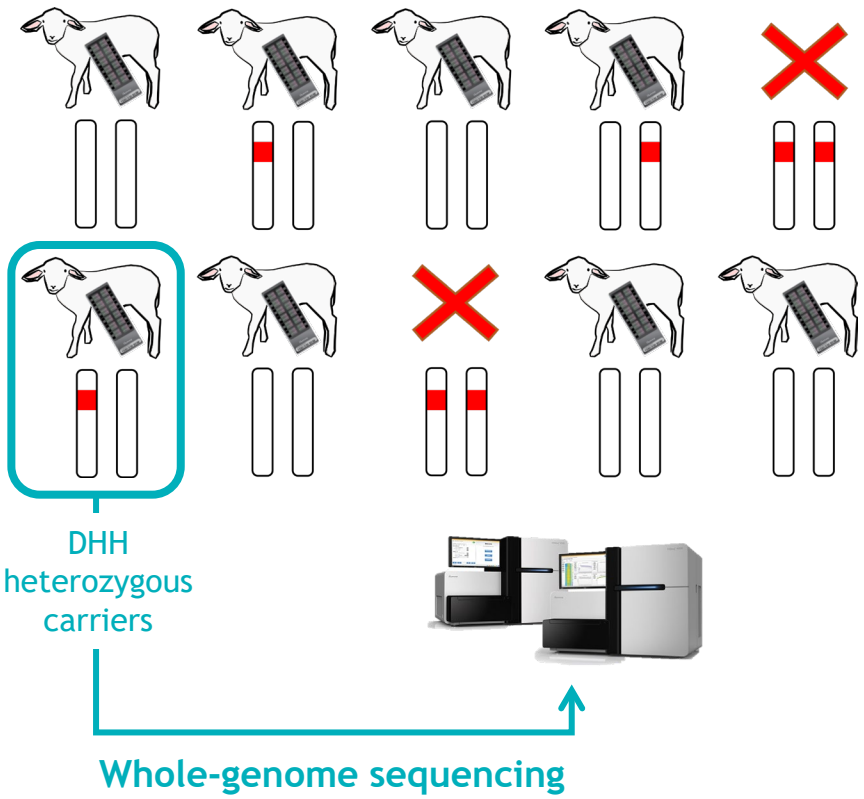
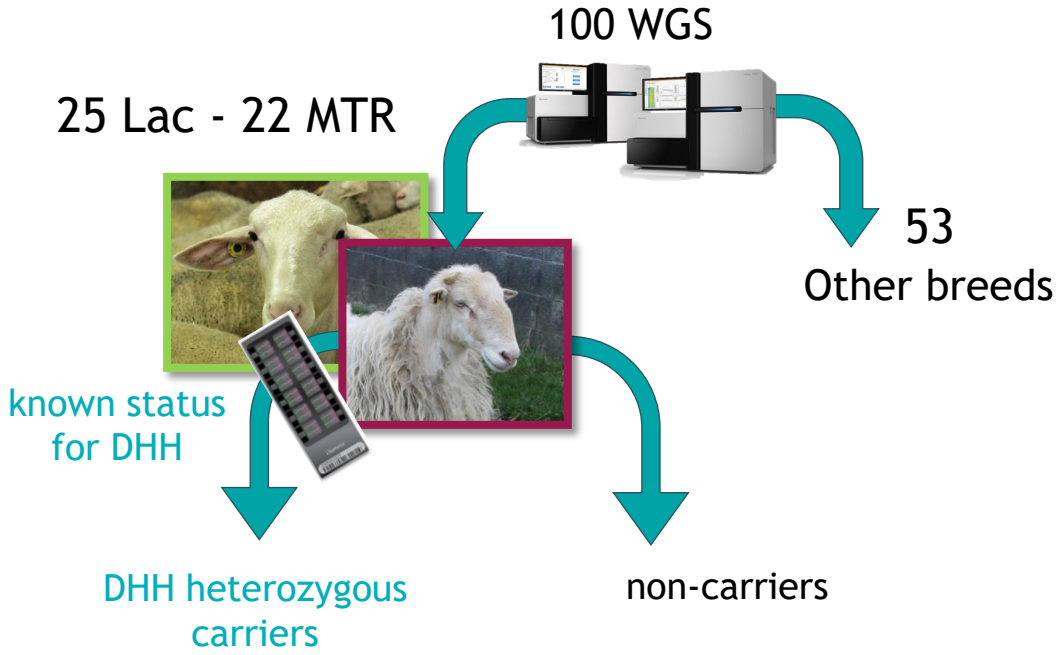
Identifying mutations by whole-genome sequence analyses

***Working hyp**: Strong LD between the haplotype and the mutation*





Nextflow Sarek

Alignment on Oar_rambouillet_v1.0

Variant Calling (SNP, Small InDels)

Functional annotation

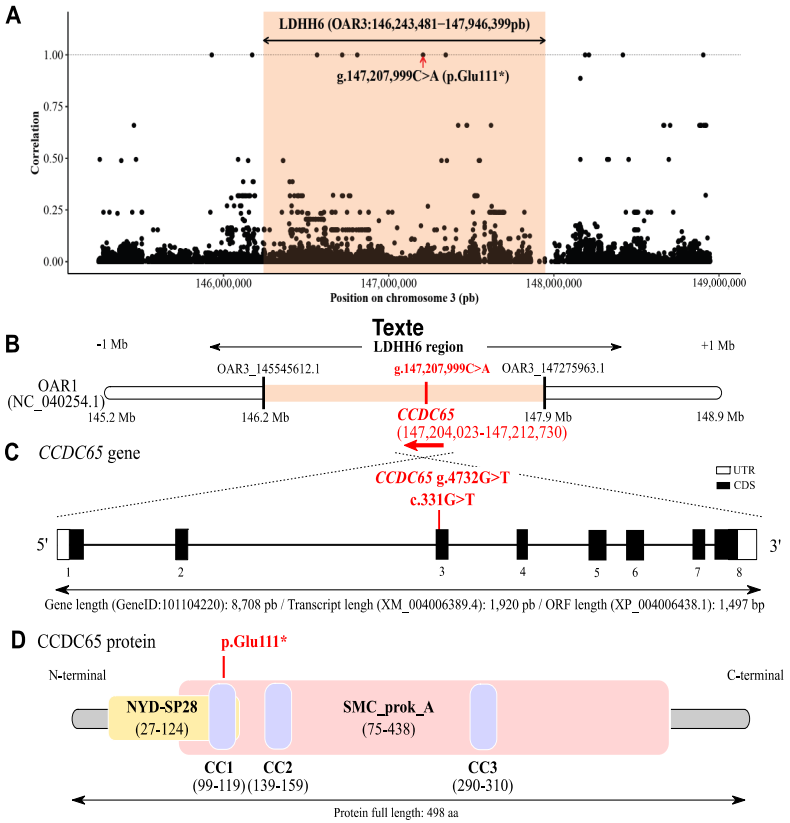
Extraction of variants located in **DHH regions ± 1 Mb**

→ Pearson correlation between DHH status (0,1 or 2) and allele dosage for biallelic SNPs and InDels (0/0, 0/1 or 1/1)

Identification and characterization of 3 causal mutations

LDHH6

Nonsense variant (G>T) in CCDC65
Coiled-Coil Domain Containing 65



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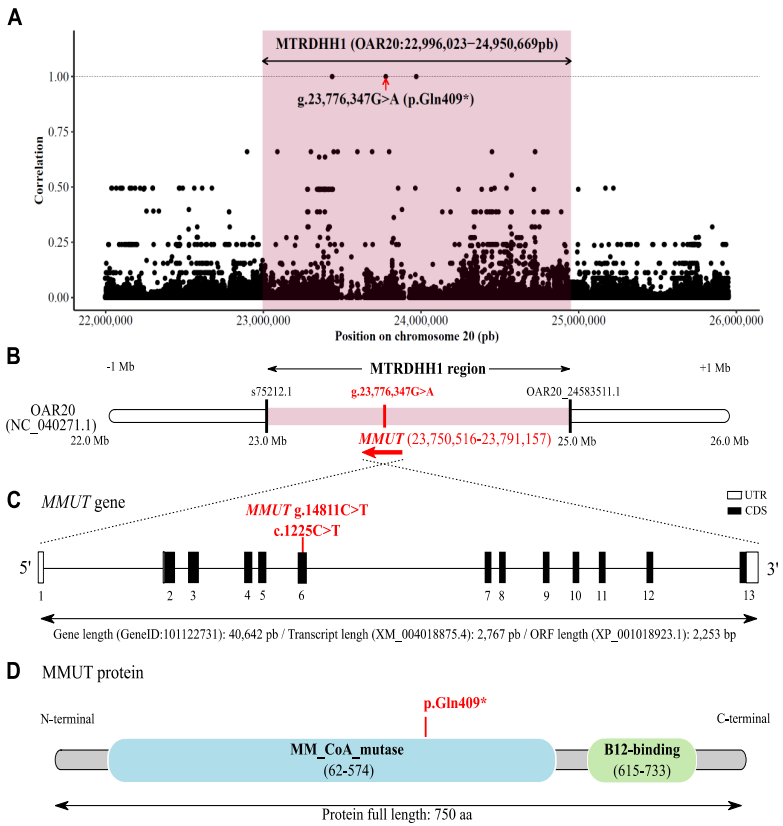
MTRDHH1

Nonsense variant (C>T) in MMUT
Methylmalonyl Coenzyme A mutase



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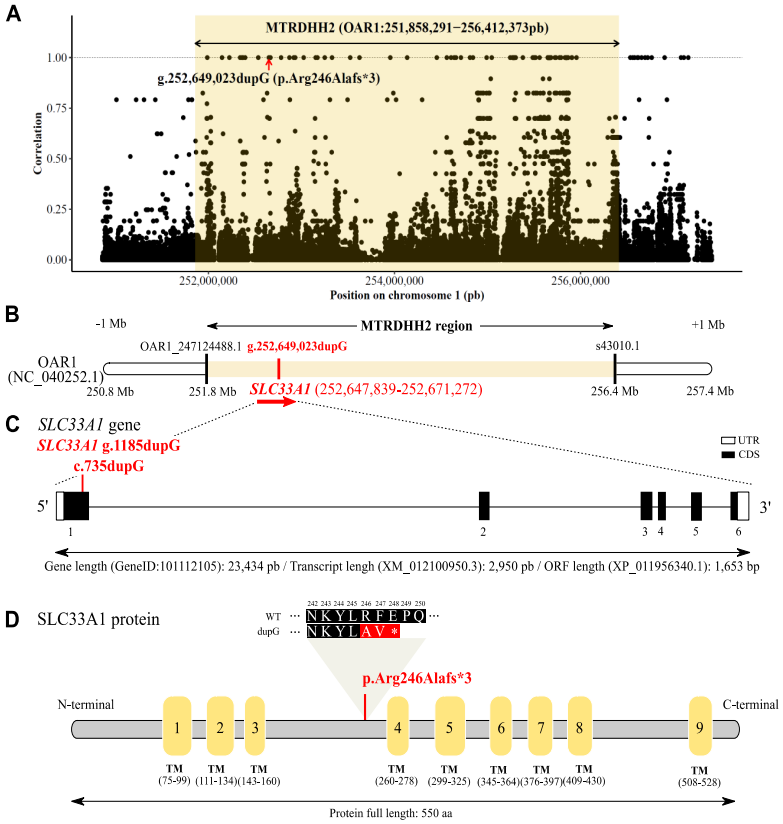
MTRDHH2

Duplication (G>dupG) in SLC33A1
Solute Carrier Family 33 Member 1



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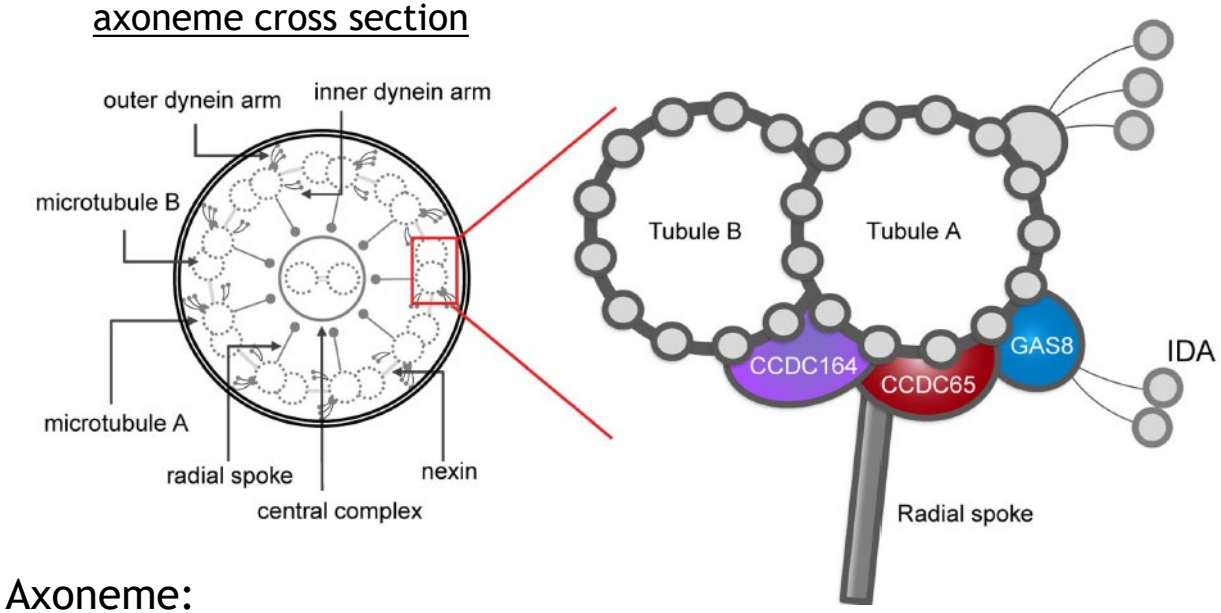
Loos-of-function mutations → altered phenotype?

Nonsense variant in *CCDC65* gene

axoneme cross section



Axoneme:

- ✓ 9 doublets of microtubules (A and B) + 2 (central complex)
- ✓ Nexin (slide between microtubules doublets)
- ✓ Dynein (microtubule curvature)
- ✓ N-DRC→ 3 subunits (CCDC164, CCDC65 and GAS8)

⇩

Cilia and flagellar motility

CCDC65 mutation in human associated with primary ciliary dyskinesia (OMIM #615504):

- ✓ chronic airway infections
- ✓ male infertility

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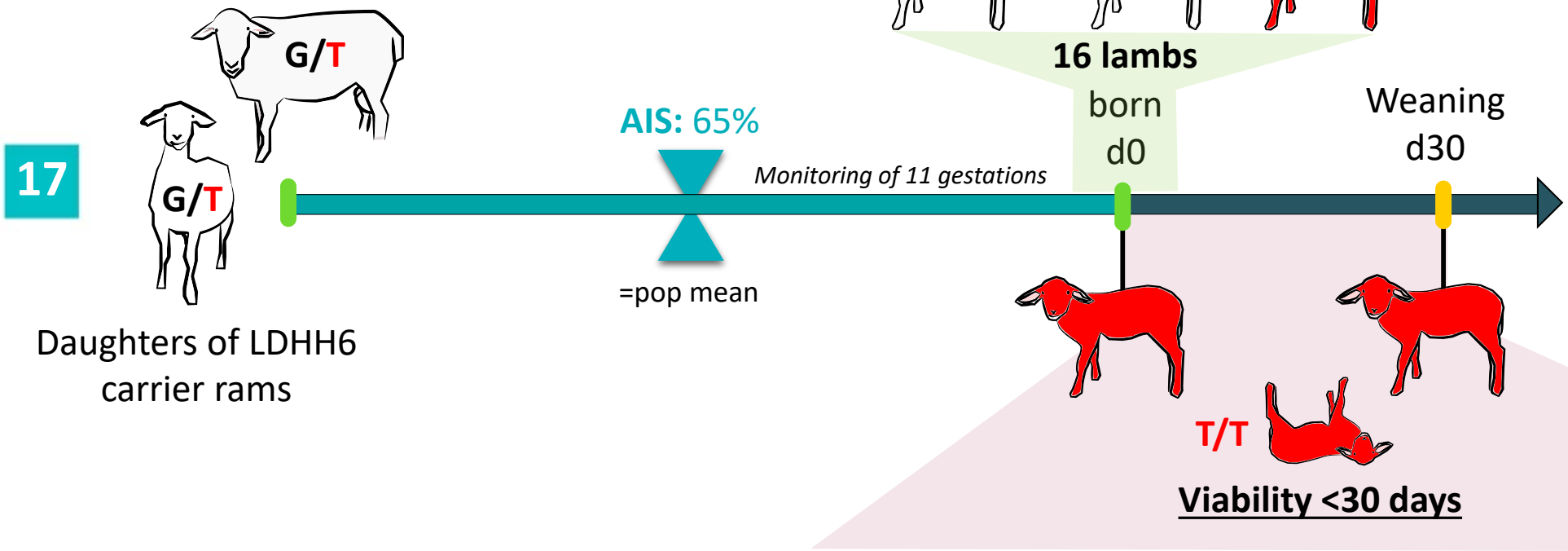
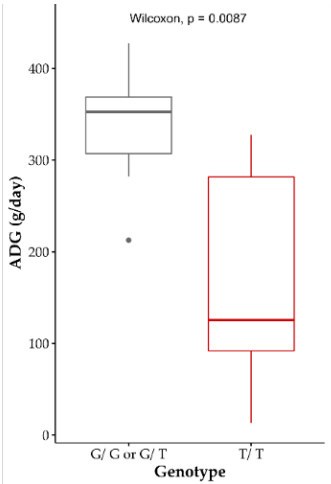
CCDC65 at-risk mating



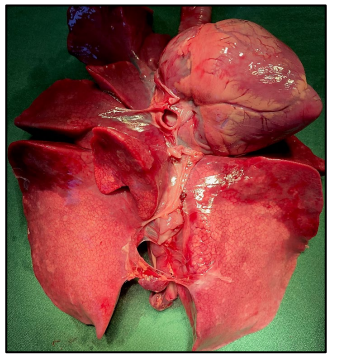
Daughters of LDHH6 carrier rams

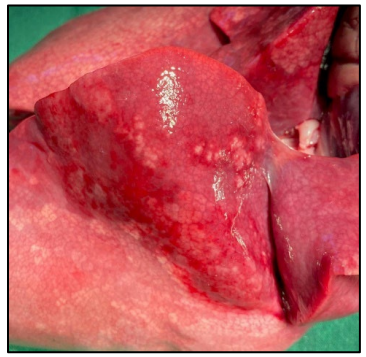
Underdevelopment



Respiratory failure:

- Tachypnea : 156±35 vs. 10-30mpm
- Acute pulmonary infection
- Lung hepatization





→ suspicion of acute enzootic pneumonia

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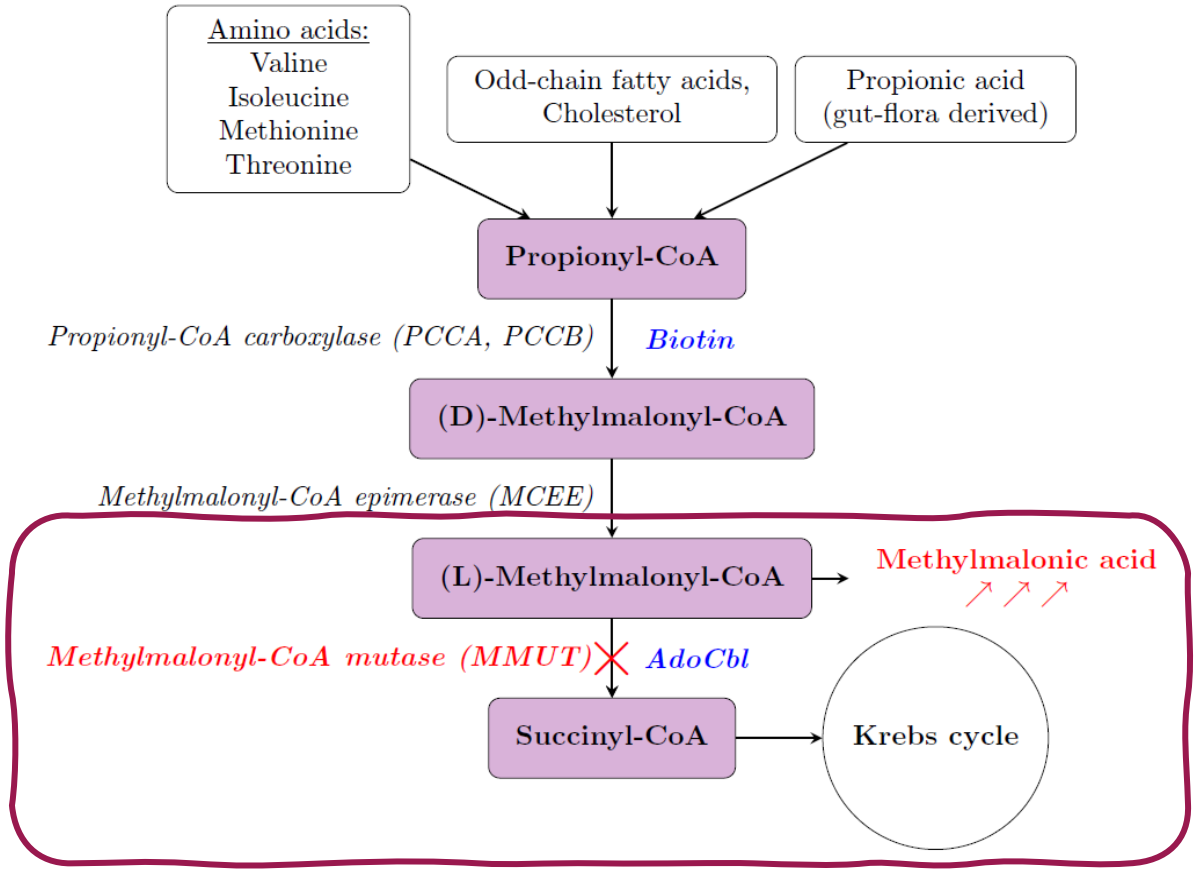
Nonsense variant in *MMUT* gene



MMUT: vitamin B12-dependent enzyme which catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA (mitochondria)

MMUT mutation in human
- ✓ Methylmalonic aciduria (OMIM#251000)

MMUT knock-out in mouse (MGI:3026845)
- ✓ Neonatal, postnatal lethality
- ✓ Methylmalonic aciduria

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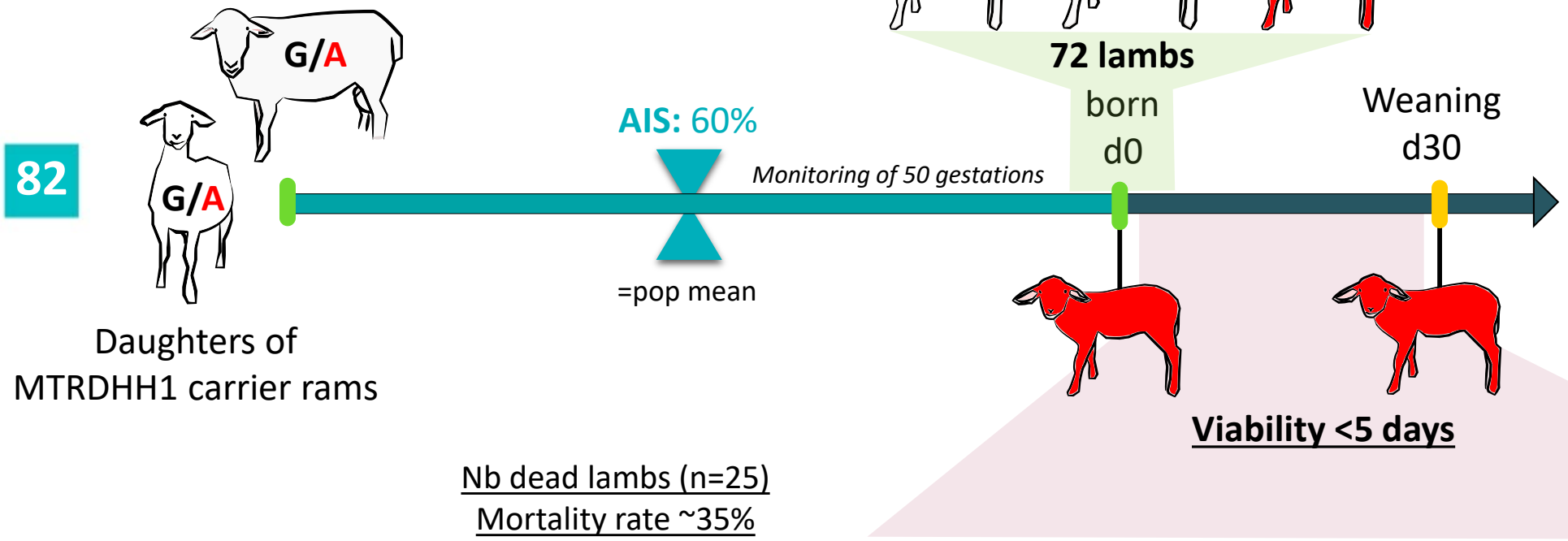
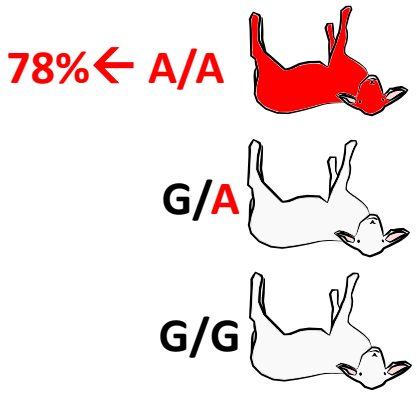
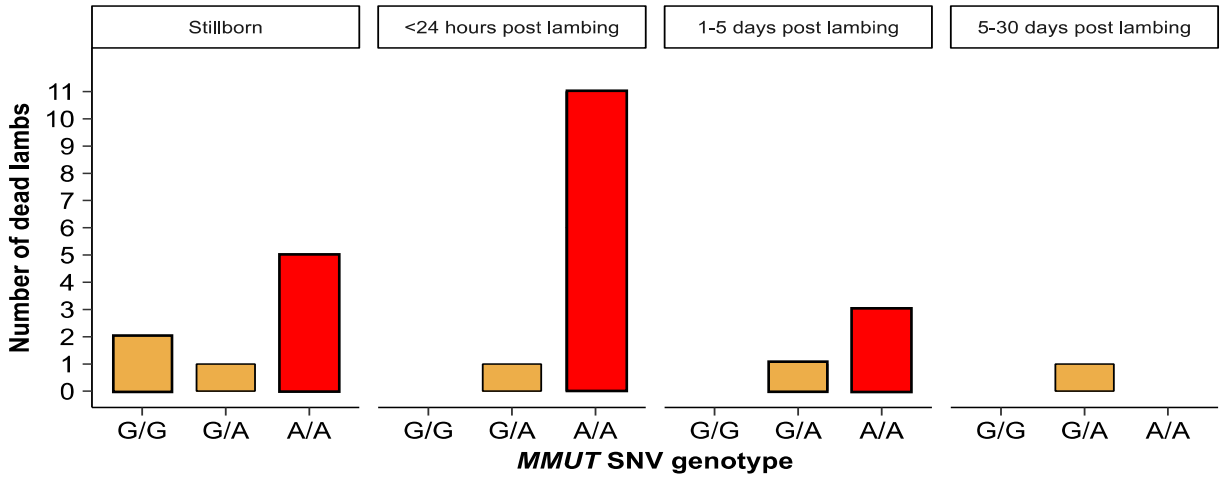
MMUT at-risk mating

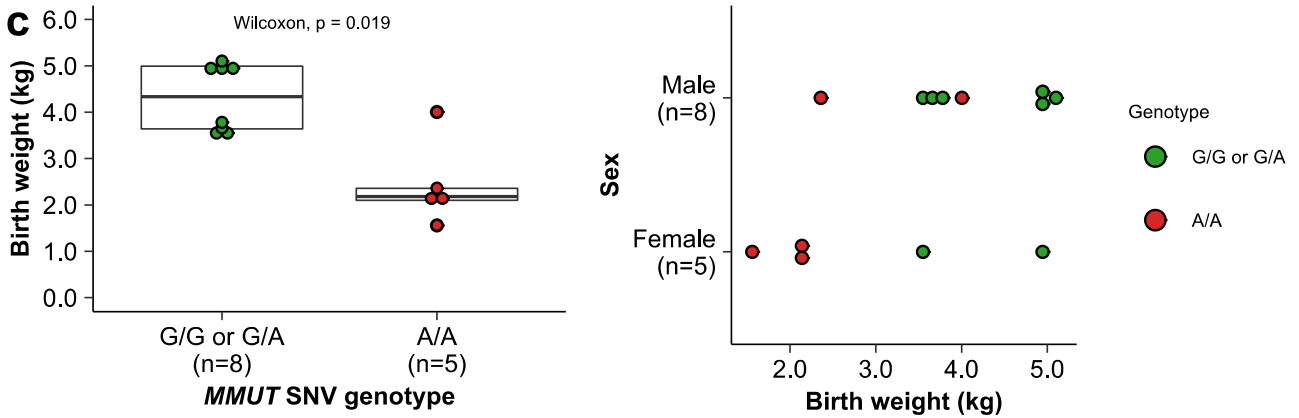


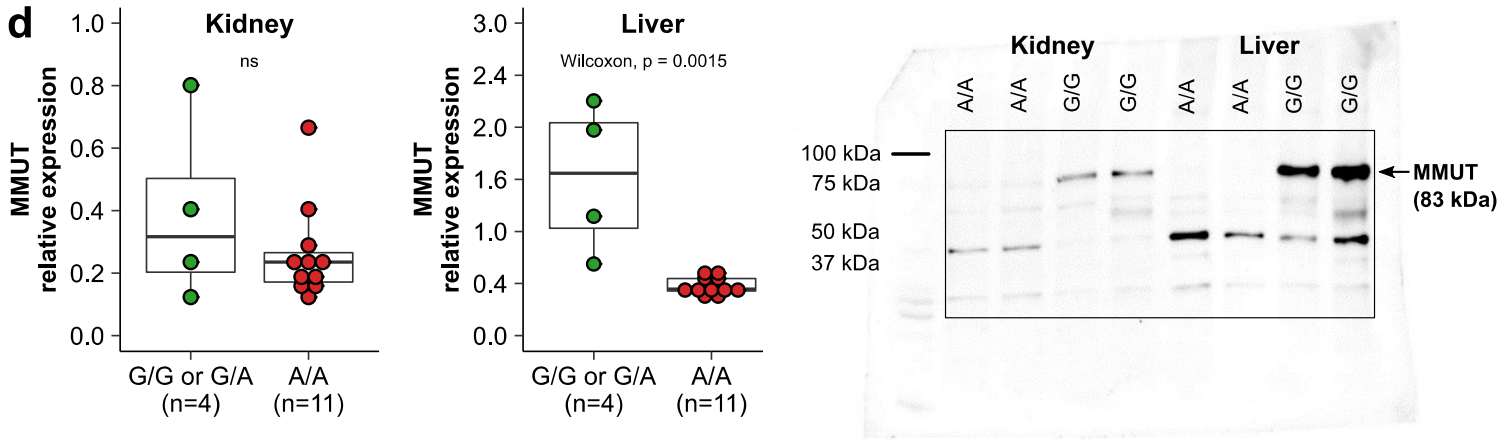
Nb dead lambs (n=25)
Mortality rate ~35%

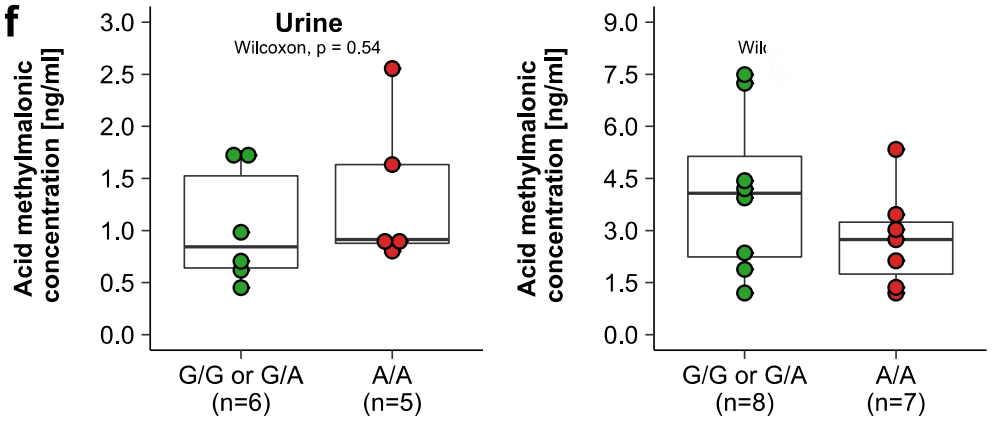




- In utero growth retardation
- Impaired MMUT protein production
- mRNA decay in liver
- No obvious methylmalonic aciduria







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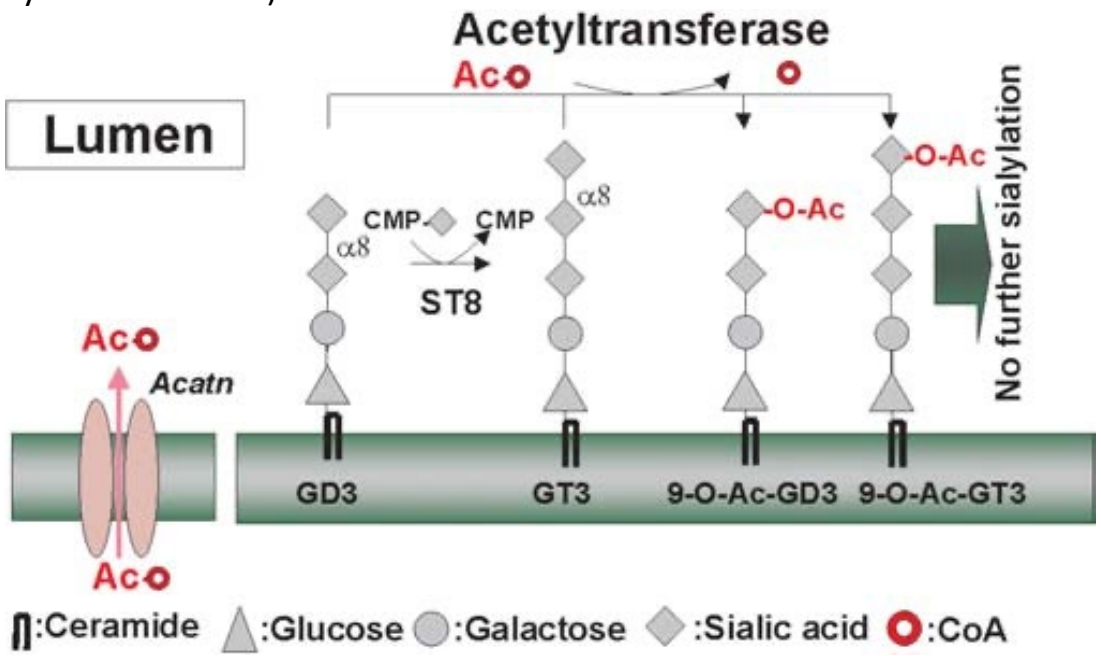
Genetics Selection Evolution 2024

Single base pair duplication in *SLC33A1* gene

(Hirabayashi et al. 2004)



SLC33A1: endoplasmic reticulum transporter required for the formation of O-acetylated (Ac) gangliosides

SCL33A1 mutation in human

- ✓ Huppke-Brendel syndrome (OMIM#614482, congenital cataracts, severe psychomotor retardation, and hearing loss)
- ✓ Spastic paraplegia 42 (OMIM#612539, involuntary muscle stiffness)

SLC33A1 knock-out in mouse (MGI:5661205)

- ✓ Embryo growth arrest/retardation

SLC33A1 knock-down in zebrafish

- ✓ Curved-tail phenotype

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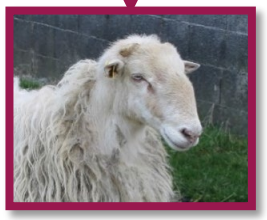
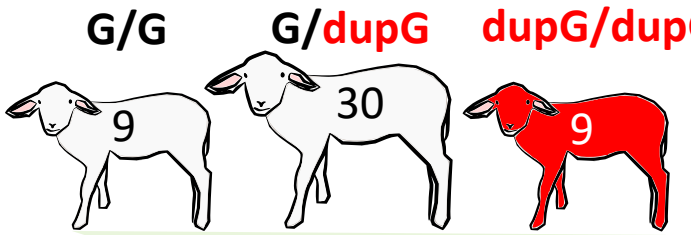
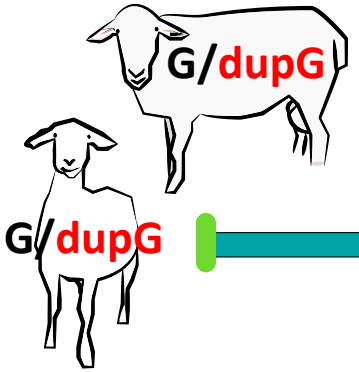


SLC33A1 at-risk mating



48 lambs



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Daughters of
MTRDHH2 carrier rams

AIS: 48%

Monitoring of 31 gestations

born
d0

Weaning
d30

≠ pop mean: 60%

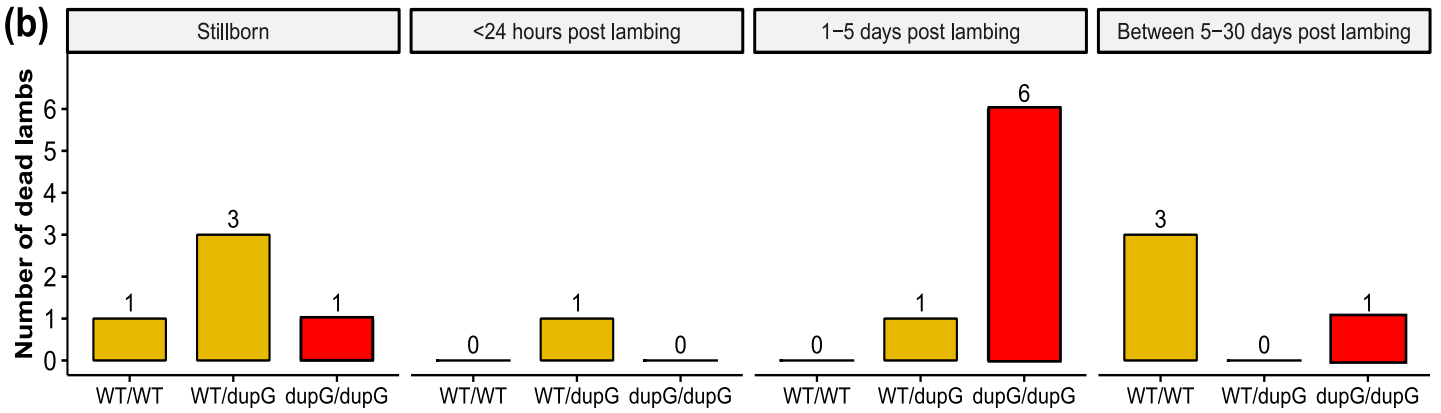
Viability <30 days

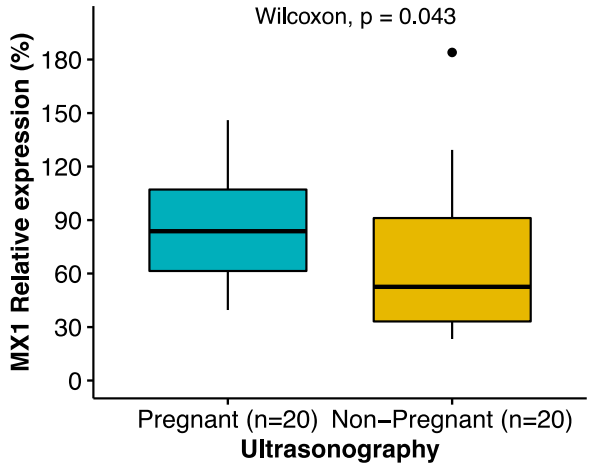
Nb dead lambs (n=17)
Mortality rate ~35%

47%← dupG/dupG

G/dupG

G/G





- Pregnancy monitoring

- D15 post-AI : molecular test based on circulating expression of Interferon Tau-stimulated genes (MX1 and STAT1)

- D45-60 post-AI : ultrasonography diagnostic

→ 4 heterozygous (G/dupG) ewes experienced early embryonic losses.

- Homozygous(dupG/dupG) phenotyping at lambing

Mummified fetus

Stillbirth- no clinical defect

Alive – morphological defect

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Conclusion et perspectives

- Reverse genetic screen: "easy way" to find relevant genomic regions hosting highly deleterious variants (thanks to ↗ GS genotyping data), but associated phenotype more complex to obtain (metabolic defects).
  - Identification of 3 recessive lethal variants in *CCDC65*, *MMUT* and *SCLC33A1* with a huge impact on lamb viability, health and welfare before weaning age.
  - Supposed recessive variants in candidate genes to be confirmed as deleterious/lethal (*IDI1*, *ORC5*, *PREB*, *GPN1*, *EDC3*), deleterious/health disorder (*FCGR1A*) or affecting breed standard/morphology (*ASIP*, *RXFP2*).

- Genetic markers to manage the identified defects in French dairy sheep (available genetic diagnosis, implementation on the Sheep Genome Consortium SNP chip)
  - Improving breeding scheme: limiting lamb mortality and welfare issues
  - Allele sharing with other national or international sheep breeds

- Potential animal models for human diseases (Ciliary dyskinesia, Methylmalonic aciduria, Spastic paraplegia, Huppke-Brendel syndrome, …)

Thank you for your attention !

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Contact:
stephane.fabre@inrae.fr
maxime.ben-braiek@inrae.fr