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Genetic determinism of DNA global methylation rate in sheep

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- DNA or histone biochemical modifications influence adaptation and production traits in plants and animals.

- Whether these epigenetic variations are under a genetic determinism or not remains unknown.

- Chosen epigenetic mark: DNA methylation
- Phenotype: DNA global methylation rate (DGMR) in blood
- Studied species: sheep

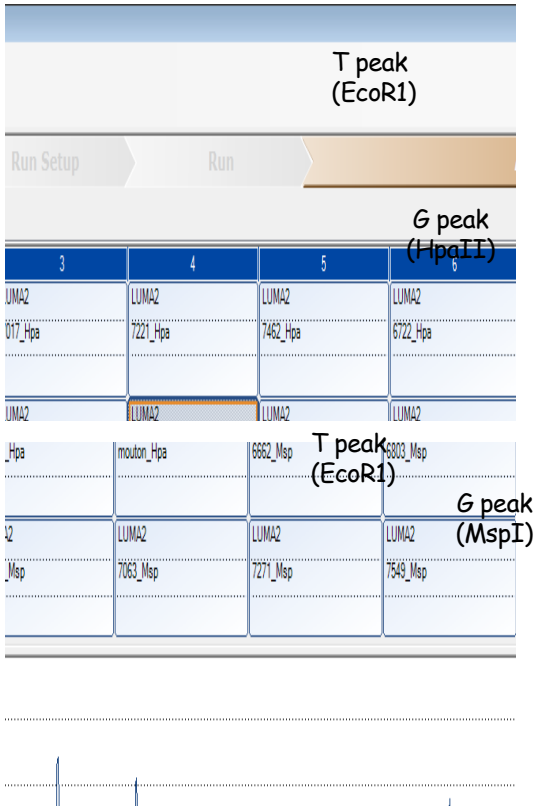
Hypothesis :
DGMR, genetically determined ?

The blood DGMR phenotype

Measured by LUMA (Luminometric Methylation Analysis)

using a pyrosequencing approach

(MspI/HpaII* and EcoRI digestion)



- variable between animals within a breed, between breeds?
- variable with time along animal growth?
- correlated with DGMR from other tissues?

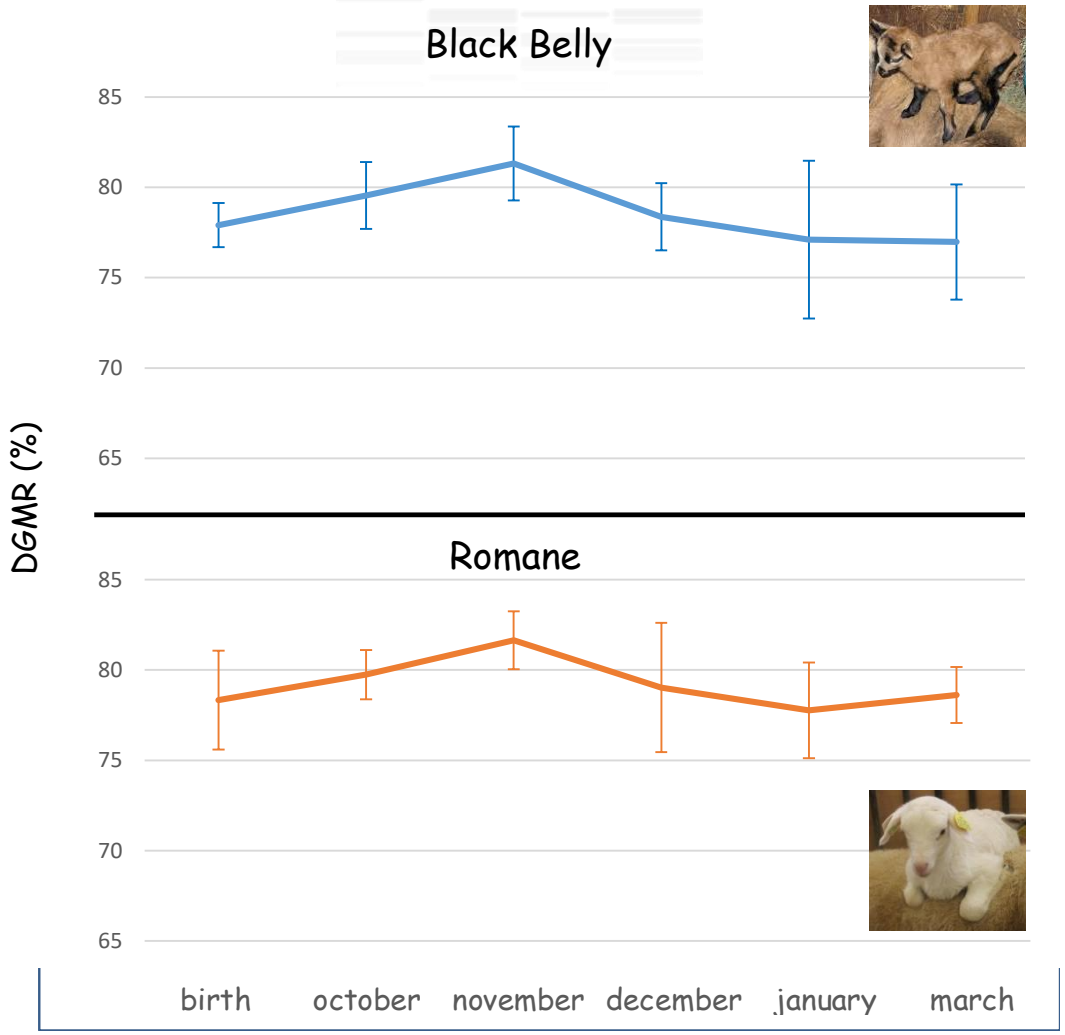
Martinik Black Belly

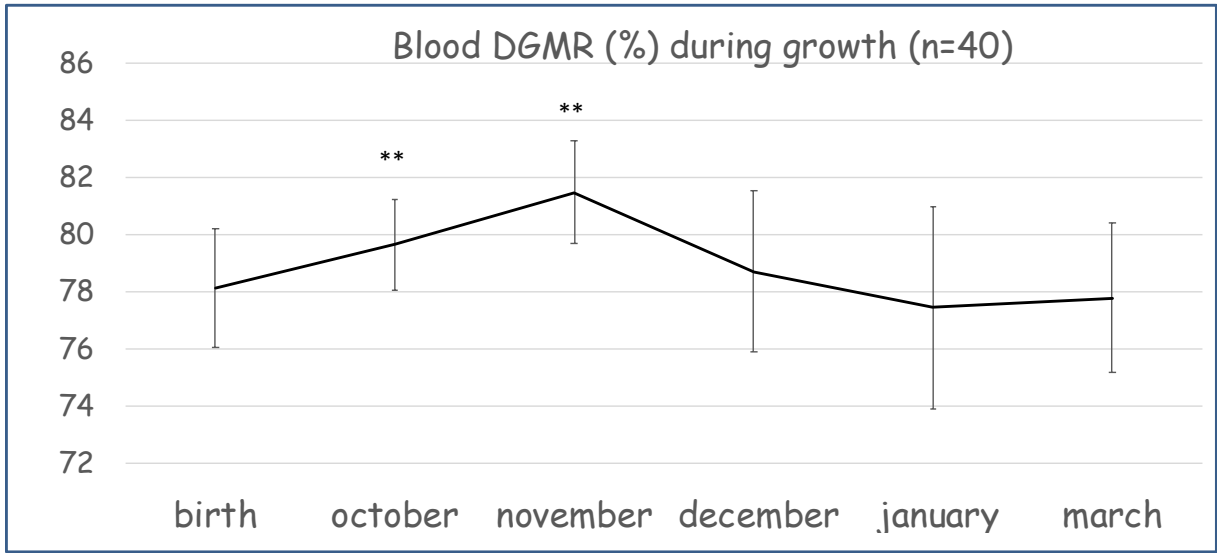
Romane

- Animals
  - 2 breeds
  - 20 lambs (10 females, 10 males) per breed sharing a common environment
  - Monthly blood sampling + complete blood count
  - Slauther at 6 months of age, 17 tissues collected

- Statistical analysis
  - Mixed linear model (repeated measure over time)
    - Blood DGMR = μ + breed + sex + time + animal (random) + ε
  - General linear model
    - Tissue DGMR = μ + breed + sex + sex*tissue + ε

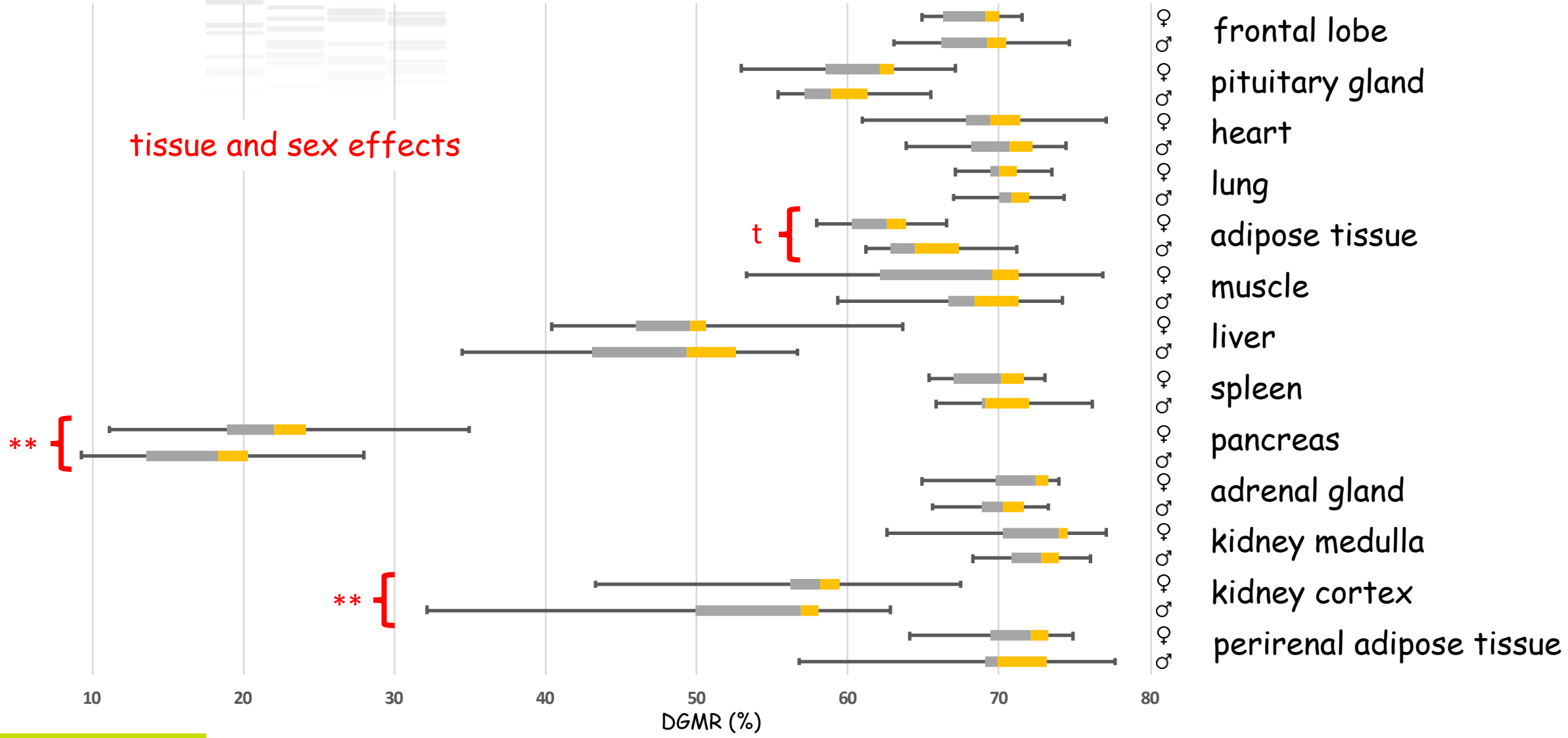
Blood DGMR during lamb growth



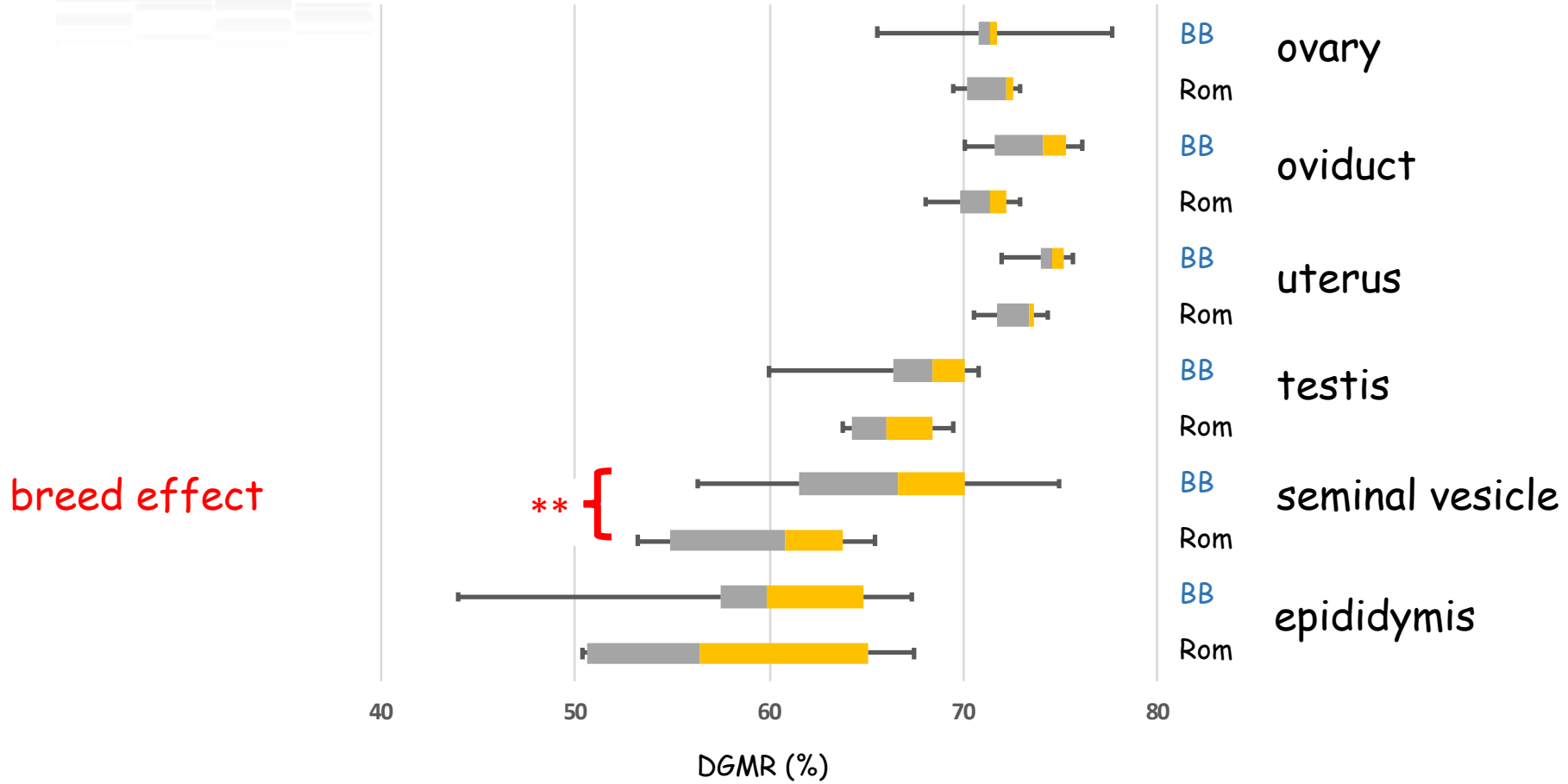


- Only time effect is significant (P<.0001)
- No impact of blood cells count

Non reproductive tissue DGMR at 6 months



Reproductive tissue DGMR at 6 months



DGMR blood-tissues correlation

- Blood and other tissues DGMR are not or poorly correlated

	uterus	frontal lobe	spleen	kidney medulla
Blood at slaughter	0.55	0.31	0.33	0.32
P value	0.042	0.095	0.078	0.081

- Blood DGMR seems not to be a good predictor of other tissue DGMR

Genetic determinism of DGMR in Romane sheep

- Available QTL design (10 families, ~100 individuals/family)
  - ✓ Growth and behavior phenotypes
  - ✓ 50k SNP genotypes
  - ✓ EDTA blood samples from lambs after weaning (~4 months)
    - ➢ DGMR measurement

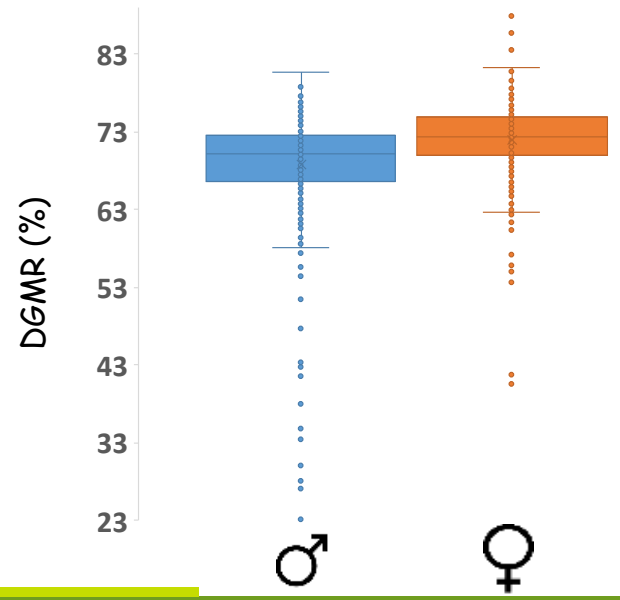
N	mean	SD	min	max
1047	70.71	5.97	23.08	87.94

Variation factors

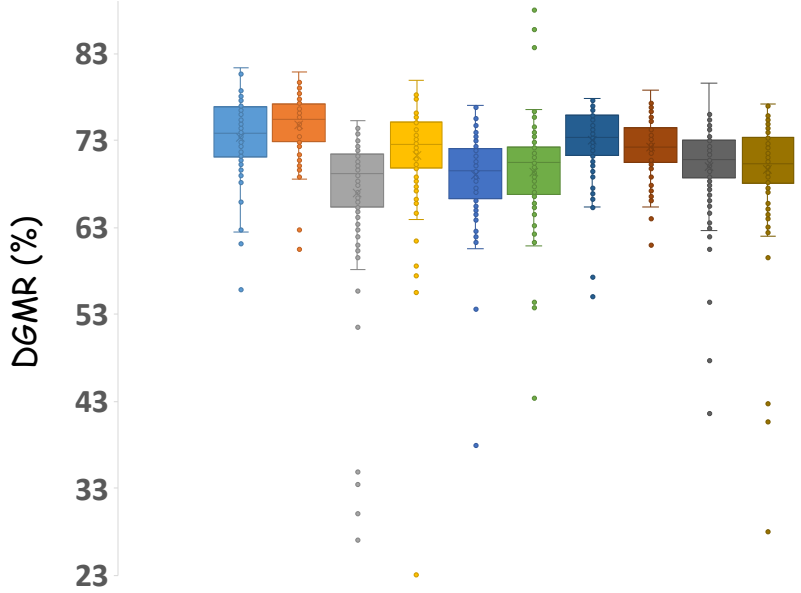
Birth and weaning weight, sex, litter size, suckling type, mother's age, ram (year)

- Significant effect of sex and ram

Sex effect (p<0.0001)



Ram effect (p<0.0001)



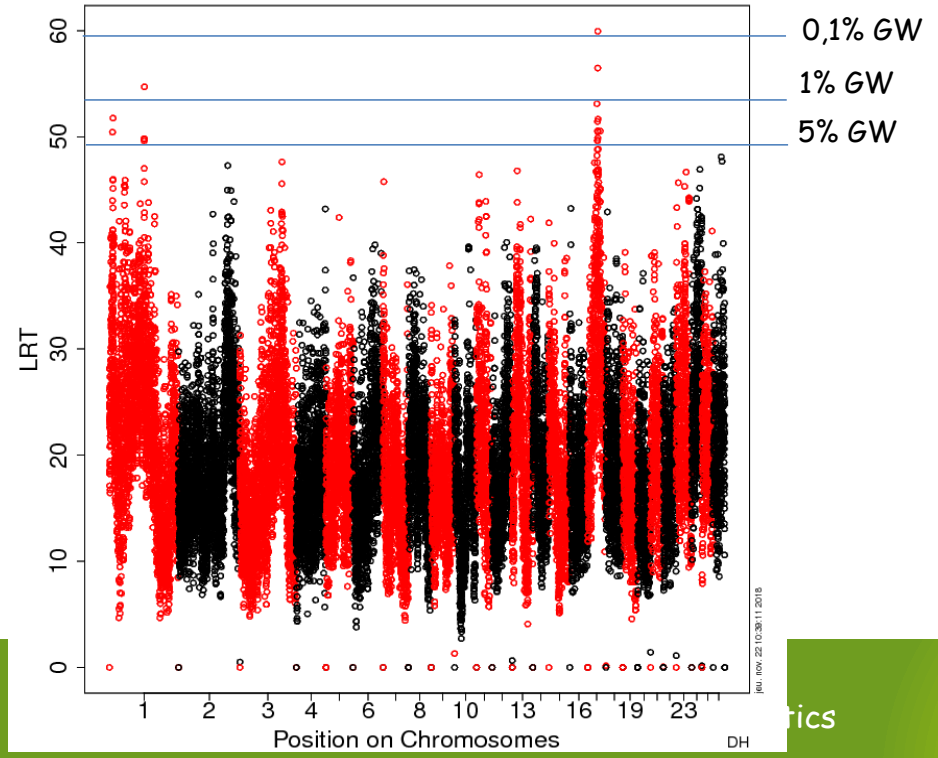
Genetic analyses

Genetic parameters

	Animal (h^2)	Perm	Residual
DGMR	0.20 (±0.05)	0.02 (±0.03)	0.78 (±0.05)

Proportion of total phenotypic variance attributed to the direct additive genetic effect (Animal), the permanent environmental effect of the dam (Perm) and the residual effect (Residual)

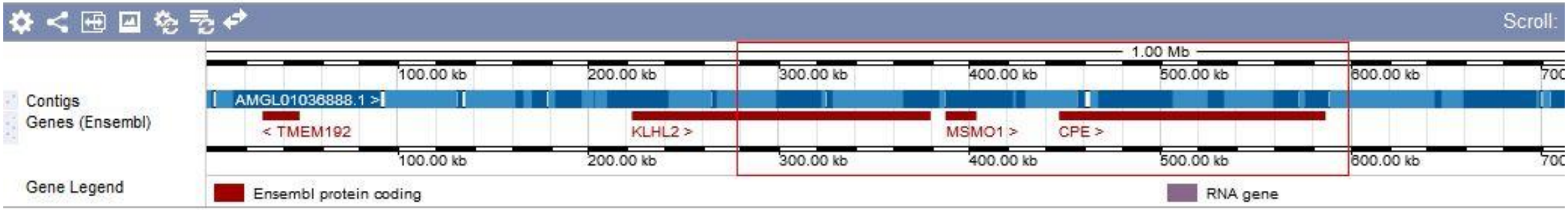
Linkage disequilibrium and association (50k SNP genotypes)



→ significant signals on chr 1 and chr 17

Region in detail

Chromosome 17 (Oar v3.1)



Annotated genes close to the significant genomic region:

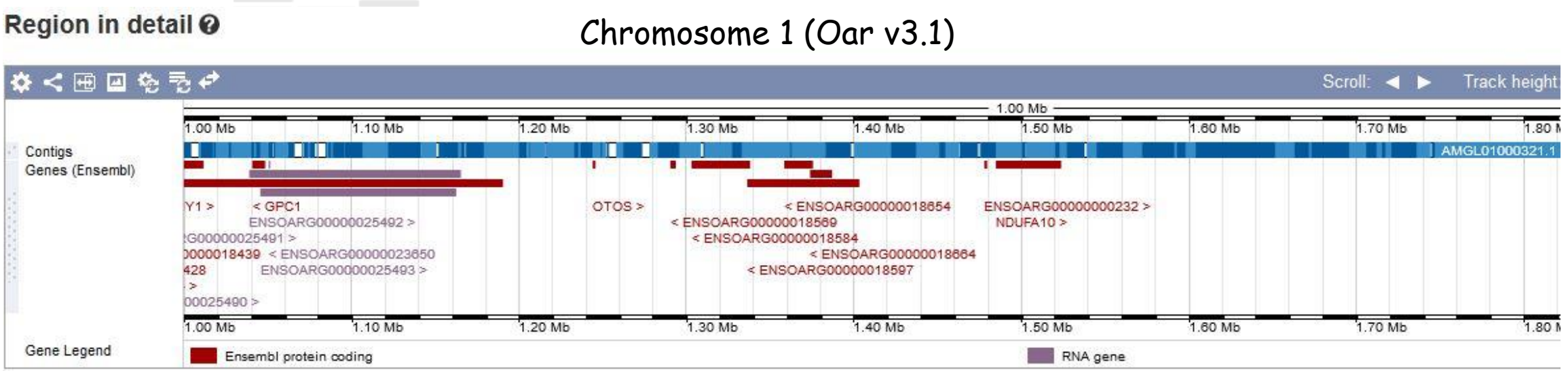
TMEM192: transmembrane protein 192 (lysosomal membrane protein, autophagy)

KLHL2: kelch like family member 2 (reorganization of actin cytoskeleton)

MSMO1: methylsterol monooxygenase 1 (cholesterol biosynthesis)

CPE: carboxypeptidase E (neurotransmitters and hormones biosynthesis, notably Insulin)

Chromosome 1 (Oar v3.1)



Annotated genes close to the significant genomic region:

NDUFA10: NADH:ubiquinone oxidoreductase subunit A10 (electron transport chain of mitochondria) – Hypermethylated in schizophrenia (brain) – A FOXM1-induced differentially methylated genes in cancer.

OTOS: otospiralin (inner ear functioning)

GPC1: glypican 1 (control of cell division and growth regulation, implicated in numerous cancers)

→ no obvious candidate gene(s)

Work in progress…

- DGMR characterization
  - ✓ Bisulfite sequencing (RRBS) on several tissues to confirm the DGMR values and better characterize the difference observed between tissues

- Genetic determinism of DGMR
  - ✓ Genetic correlations between DGMR and other recorded traits (growth, social behavior)
  - ✓ Fine mapping of the QTL signals on Chr17 and Chr1 (whole genome sequencing of chosen animals based on significant haplotypes) → causal mutation(s)
  - ✓ Establishing the link with adaptation traits as functional longevity, environmental change resistance/resilience and trade-off (European SMARTER project).

Thank you for your attention

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