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EIF2S2 Retroposition into IRF2BP2 Underlies Fleece Variation in Sheep through the Formation of a Long Endogenous Double-Stranded RNA

Julie Demars, Margarita Cano, Laurence Drouilhet, Stéphane Fabre, Bertrand Servin, Philippe Mulsant, Gwenola Tosser-Klopp, Daniel Allain

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EIF2S2 retroposition into IRF2BP2 underlies fleece variation in sheep through the formation of a long endogenous double-stranded RNA

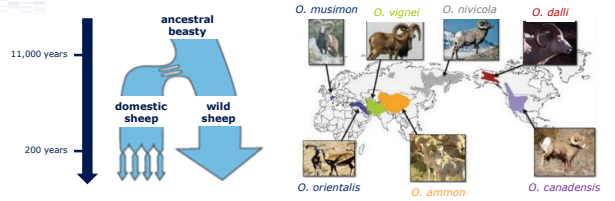


J. Demars, M. Cano, L. Drouilhet, S. Fabre, B. Servin, P. Mulsant, G. Tosser and D. Allain

Abstract W132
PAGXXV - Cattle/Sheep/Goat Workshop
Sunday, January 15, 2017

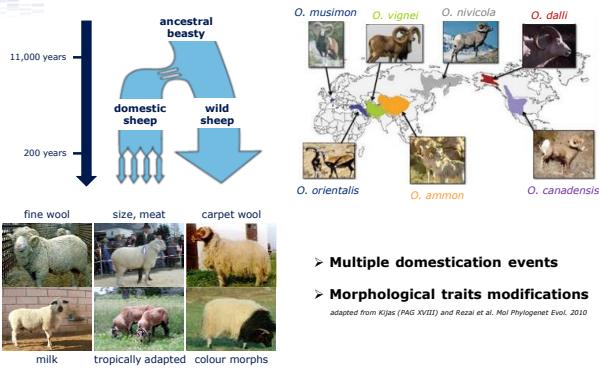


The sheep population history



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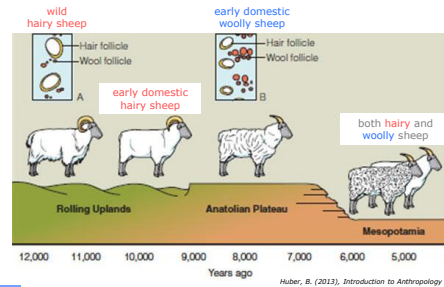
The sheep population history



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Fleece variation under the sheep domestication

- > Variation in composition and structure of the fleece
- > Hairy sheep vs. woolly sheep



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Hairy and woolly sheep in modern breeds

- > Hairy sheep
Sheep Production Handbook : « sheeps whose coats consist of hair, more like that of cattle and goats, rather than wool »

- > Phenotypes (hairy vs. woolly) easily detectable on lambs
 - Hairy long coat \approx 23.1 mm



- Woolly short coat \approx 8.3 mm



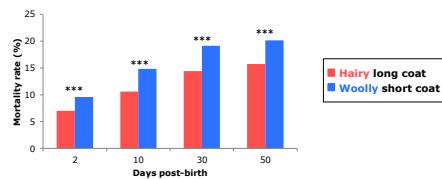
adapted from Allain et al. JAS 2013



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The fleece variation, a major agronomic trait

- > Important survival component
 - Less total mortality rate in long hairy bearing coats lambs



From Allain et al. JAS 2013



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The fleece variation, a major agronomic trait

- **Important survival component**
 - Less total mortality rate in long hairy bearing coats lambs
 - More heat losses in woolly bearing coat lambs
 - Long hairy coat lambs are more resistant to weather changes
- **Heritability of lamb birthcoat type**
 - Binary trait, heritability = 0.85
 - Continuous trait, heritability = 0.56
- **Sheep breeding permanent outdoor**
 - Economical and agronomical trait = Importance to use in selection
 - French Romane breed, high productive potential breed

from Allain et al. JAS 2012

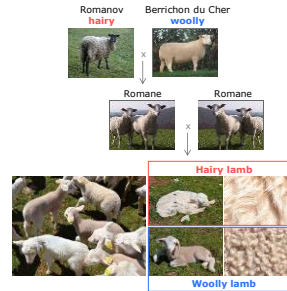


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Description of the study design

A familial strategy

The French Romane breed

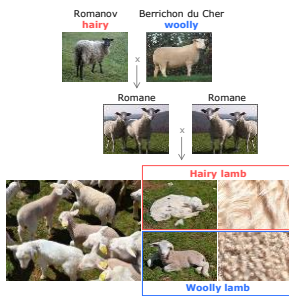


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Description of the study design

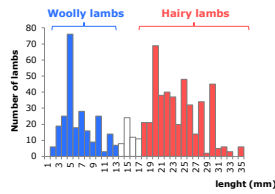
A familial strategy

The French Romane breed



- **Animals**
Half-sib QTL design in Romane
8 sires Romane * dams Romane

- **Phenotype**
Length of lamb's fleece (quantitative)
Type of lamb's fleece (binary)

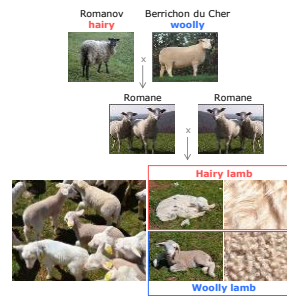


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- **Genotype**
OvineSNP50 Genotyping BeadChip (n = 759)
Ovine Infinium® HD SNP BeadChip (n = 135)

- **Methodology**
Imputation
Genome-Wide Association Analysis (GWAS)
Linkage Analysis

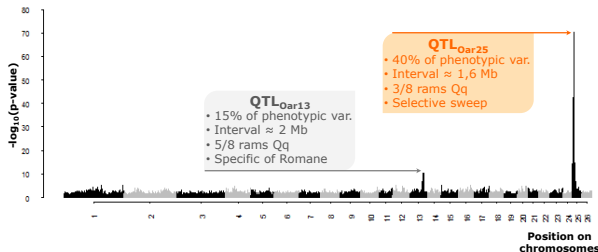


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Two loci underlie fleece variation in Romane

➤ Genetic determinism

- 66% of hairy lambs vs. 33% of woolly lambs
- **Hairy dominant** allele from **Romanov (Q)**
- **Woolly recessive** allele from **Berrichon du Cher (q)**



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Fine-mapping of the QTL_{Oar25}

A familial strategy

The French Romane breed

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- **Methodology**
Identity By State Haplotype Identification

A population strategy

Ancestral species and French breeds

- **Hairy ancestral species**
Ovis orientalis (n = 15)
Ovis musimon (n = 18)
Ovis capadensis (n = 3)
Ovis dalli (n = 2)
- **Hairy domestic breeds**
(6 breeds and 116 individuals)
Causse du Lot, Corse, Limousine, Manech tête rousse, Rava, **Romanov**
- **Woolly modern breeds**
(18 breeds and 402 individuals)
Berrichon du Cher, Blanche du Massif Central, Charmoise, Charollais, Ile-de-France, Lacaune (lait), Lacaune (viande), **Mérino d'Arles**, Mérino de Rambouillet, Mourerous, Ouessant, Préalpes du Sud, Rouge de l'Ouest, Roussin de la Hague, Suffolk, Tarasconnaise, Texel, Vendéen



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Fine-mapping of the QTL_{Oar25}

A familial strategy The French Romane breed

- **Animals**
Half-sib QTL design in Romane
8 sires Romane * dams Romane
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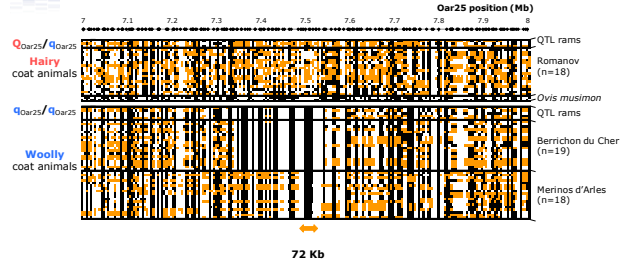
A population strategy Ancestral species and French breeds

- **Animals**
Hairy ancestral species (n = 38)
(*Ovis musimon*...)
Hairy domestic breeds (n = 116)
(Romanov...)
Woolly modern breeds (n = 402)
(Berrichon du Cher, Merino d'Arles...)
- **Phenotype**
None (sheep breed standards)
- **Genotype**
Ovine Infinium® HD SNP BeadChip (n = 554)
- **Methodology**
Haplotypes in the QTL_{Oar25} interval



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The QTL_{Oar25} encompasses the *IRF2BP2* gene



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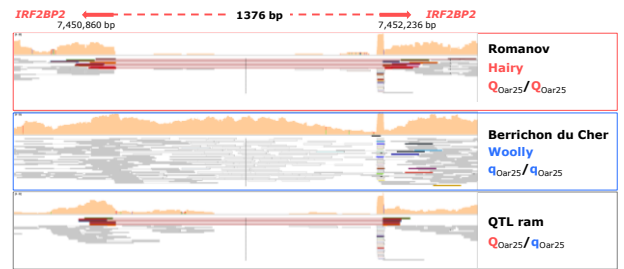
The QTL_{Oar25} encompasses the *IRF2BP2* gene



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The mutation is an insertion within *IRF2BP2*

- **Whole Genome Sequencing**
 - 2 Romanov animals (**hairy** and Q_{Oar25}/Q_{Oar25})
 - 3 Berrichon du Cher animals (**woolly** and q_{Oar25}/q_{Oar25})
 - 2 QTL rams heterozygous (Q_{Oar25}/q_{Oar25})



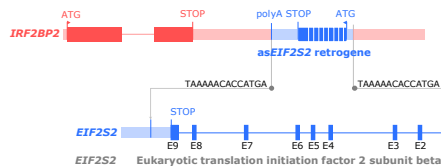
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The insertion is an antisense *EIF2S2* retrogene

• The ancestral hairy *IRF2BP2*^{wt} allele

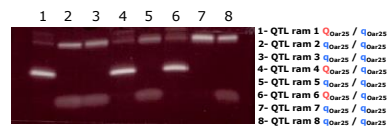


• The modern woolly *IRF2BP2*^{asEIF2S2} allele



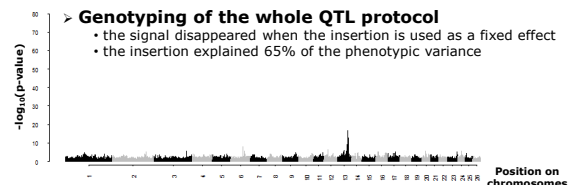
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The insertion segregates in the Romane breed



➢ Genotyping of the whole QTL protocol

- the signal disappeared when the insertion is used as a fixed effect
- the insertion explained 65% of the phenotypic variance



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The insertion discriminates hairy/woolly sheep

Breed	Genotyped animals (n)	Phenotype	Frequency (<i>IRF2BP2^{wt}</i>)	Frequency (<i>IRF2BP2^{asEIF2S2}</i>)
<i>Ovis orientalis</i>	15	Hairy	1.00	
<i>Ovis musimon</i>	18	Hairy	1.00	
<i>Ovis canadensis</i>	3	Hairy	1.00	
<i>Ovis dalli</i>	2	Hairy	1.00	
Causses du Lot	20	Hairy	0.85	0.15
Corse	16	Hairy	1.00	
Limousine	18	Hairy	0.94	0.06
Manech tête rousse	25	Hairy	1.00	
Rava	19	Hairy	0.92	0.08
Romanov	18	Hairy	0.94	0.06
	154		0.96	0.04
Berrichon du Cher	35	Woolly		1.00
Blanche du Massif Central	20	Woolly		1.00
Chamoise	22	Woolly		1.00
Charollais	22	Woolly		1.00
Île-de-France	23	Woolly		1.00
Lacune (lait)	35	Woolly		1.00
Lacune (viande)	34	Woolly		1.00
Mérino d'Aries	18	Woolly		1.00
Mérino de Rambouillet	27	Woolly		1.00
Nourerous	16	Woolly	0.06	0.94
Ouessant	18	Woolly		1.00
Préalpes du Sud	17	Woolly	0.03	0.97
Rouge de l'Ouest	16	Woolly		1.00
Roussin de la Hague	21	Woolly		1.00
Suffolk	18	Woolly		1.00
Tarascennaise	15	Woolly		1.00
Texel	24	Woolly	0.04	0.96
Vendéen	21	Woolly		1.00
	402		0.01	0.99



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The IRF2BP2/asEIF2S2 mRNA is transcribed

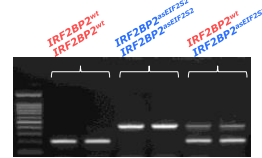
The IRF2BP2 transcript



The chimeric IRF2BP2/asEIF2S2 transcript

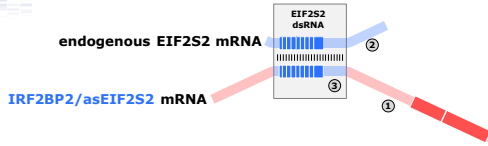


Genotyping of RNA extracted from skin



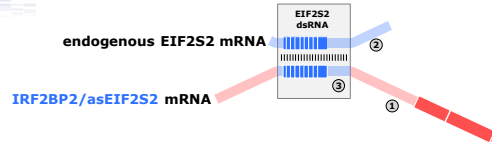
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A double-stranded EIF2S2 RNA is created



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A double-stranded EIF2S2 RNA is created



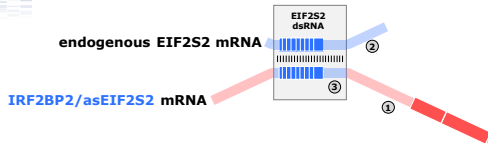
RNAseA protection assay

- ribonuclease that clives only ssRNA (EIF2S2 dsRNA is protected)
- IRF2BP2^{wt}/IRF2BP2^{wt}* versus *IRF2BP2^{asEIF2S2}/IRF2BP2^{asEIF2S2}*



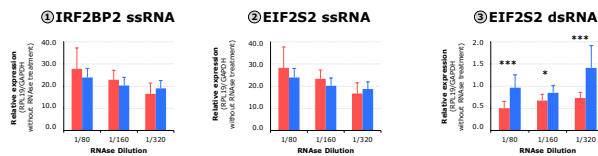
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A double-stranded EIF2S2 RNA is created



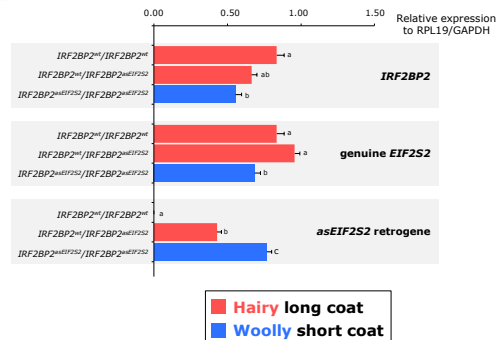
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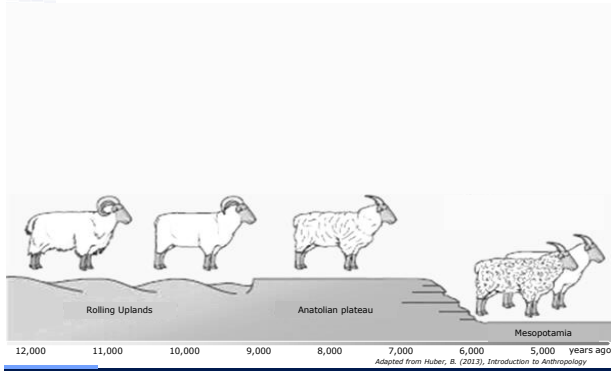
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The dsRNA alters EIF2S2 and IRF2BP2 stability

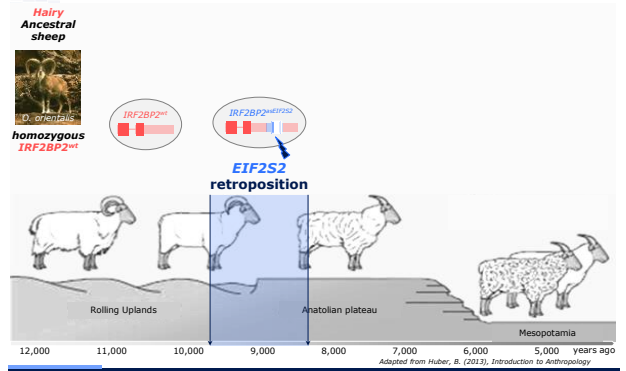


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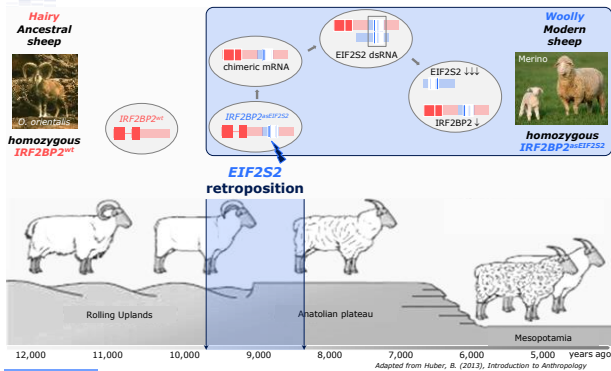
To conclude ...



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> INTA
 M. Cano
 > INRA (GenPhySE lab)
 • GENROC team
 L. Drouilhet, S. Fabre, F. Plisson-Petit, J. Sarry, G. Tosser-Klopp, F. Woloszyn
 • GESPER team
 D. Allain, C. Moreno
 • DYNAGEN team
 B. Servin
 • Experimental farms
 D. Foulquier, F. Carrière, M. Aletru
 • Bioinformatic and Genomic platforms
 P. Bardou, O. Bouchez, H. Berges

A future **permanent position** is still under evaluation
 in the **Animal Genetic Division**
 of the **French National Institute for Agricultural Research**

 How epigenetic mechanisms contribute to
 the determinism of agronomic importance traits ?

- GenPhySE lab, INRA, Toulouse, France
<http://genphyse.toulouse.inra.fr/groups/genepi>
- GenEpi group, Juliette Riquet
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- INRA job opportunities, news at the end of january
<http://jobs.inra.fr/en/Career-opportunities/Researchers/Research-scientists>