Transcriptome Profiling to Predict Piglet Vitality from Umbilical Cord Blood of Purebreds and Crossbreds born in the Same Litter

Comparison of Meishan and Large White Sows

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CONTEXT

- 2/3 of piglet losses in the first 3 days after birth
- Genetic selection against stillbirth efficient but genetic determinism different from that of losses during lactation *Huby et al.. 2003; Roehe et al 2010*

- French modern Large White piglets are bigger but less physiologically mature than in the past *Canario et al.. 2007*
- Biological dysfunction may cause lower vitality and higher mortality in early postnatal period

- Global gene expression profiling of whole blood validated in human identify molecular signatures of development at birth *Cohen et al 2007*

AIM OF THE STUDY

- Can transcriptome profiling predict newborn's survival ability ?
- Identify Molecular predictors of piglet level of development at birth and vitality

GENETIC DESIGN

Mixture of semen from the 2 breeds

First parity sows

LW ♀x (½ LW + ½ MS) ♂

LW x LW MS x LW

MS ♀ x (½ LW + ½ MS) ♂

LW x MS MS x MS

Total production 24 MS + 24 LW sows - 3 mixtures of semen

⇨ Genetic variability maximized

Ho: 50% purebred 50 % crossbred piglets / litter

PHENOTYPIC DATA

BIRTH
Birth weight W0
Body Length BL
BMI and PI

Growth in early lactation W and ADG d1 d3 d7

Mobility
d0 d1 d3 d7

MOBIL
0: no move
1: ½ surface
2: all surface

TRANSCRIPTOMIC DATA

BIRTH

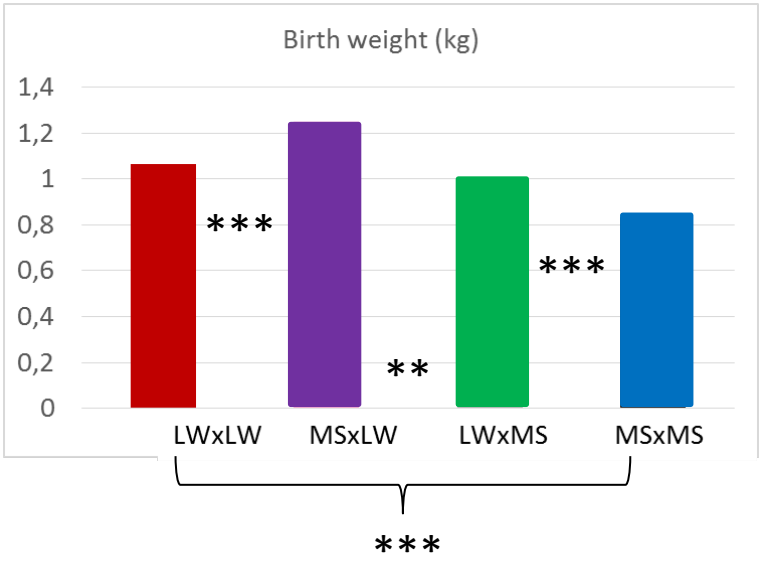
	LWxLW	MSxLW	LWxMS	MSxMS	Total
Female	12	9	11	10	42
Male	9	10	11	9	39
Total	21	19	22	19	81

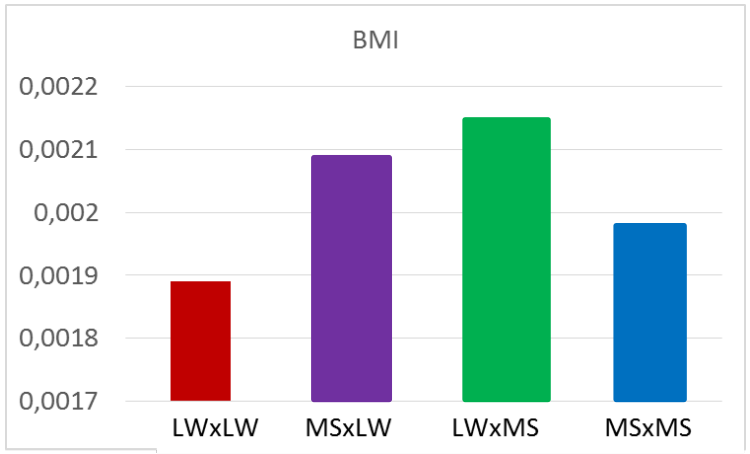
17 sows (3 to 8 piglets per litter) – 3 farrowing batches – 3 mixtures of semen

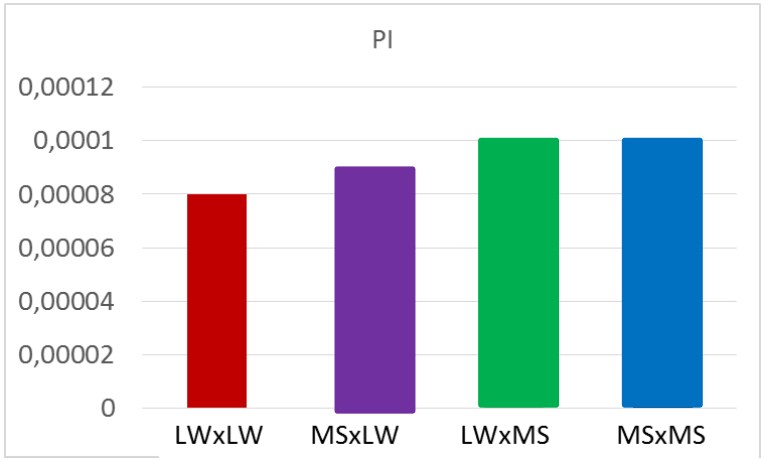
Agilent 60K microarray

COMPARISON OF GENOTYPES WITHIN SOW BREED

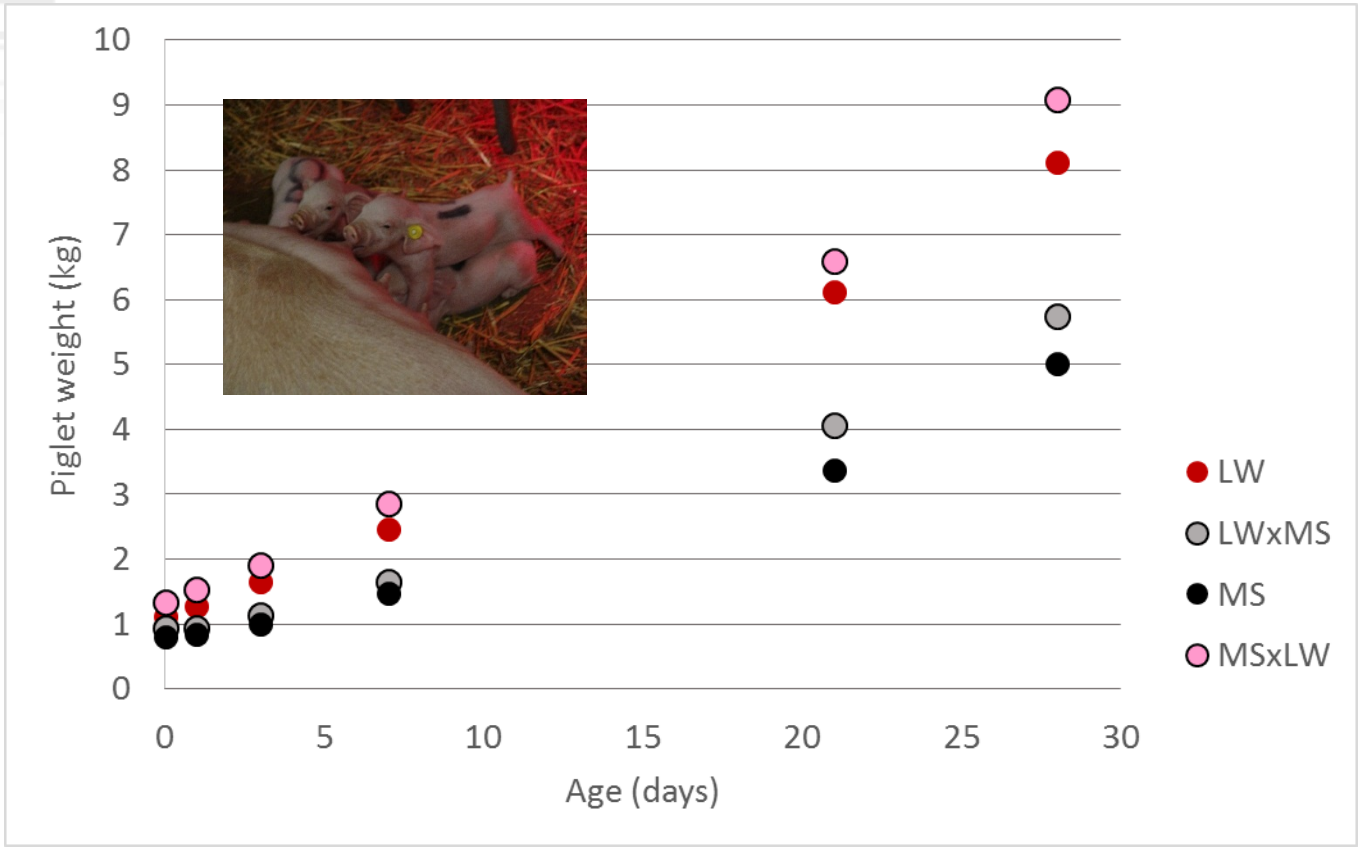
	LWxLW	LWxMS	MSxLW	MSxMS
Mortality 3d	21%	7%	0%	2%





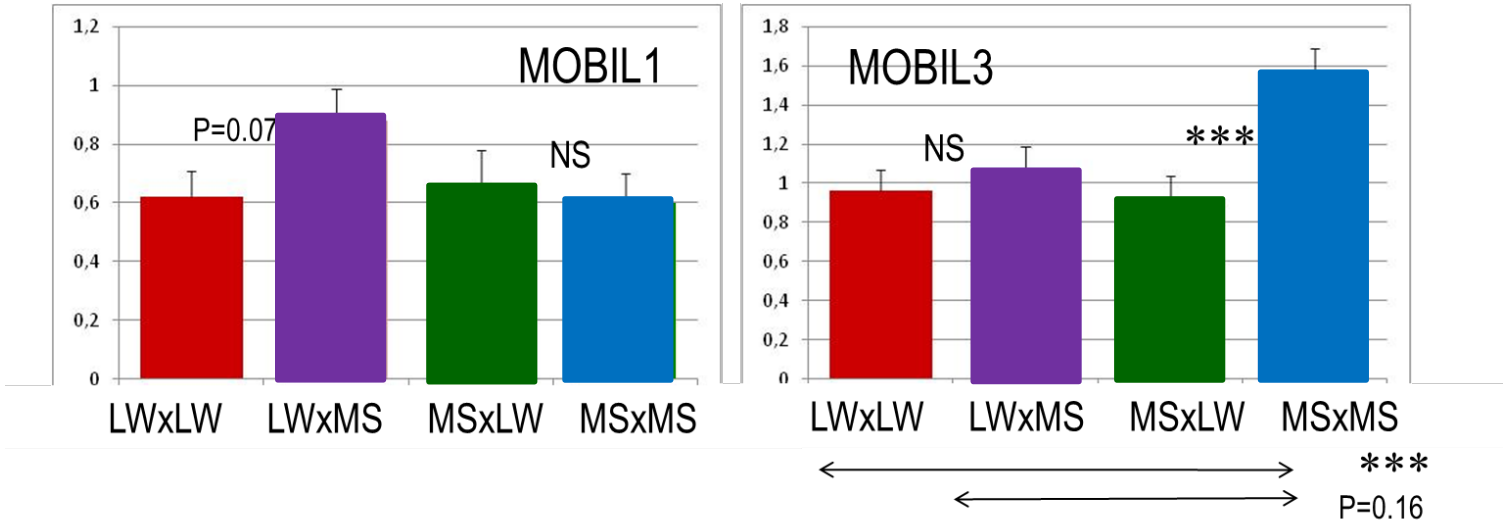


PIGLET EARLY GROWTH



Trait	Sex	LW	MSxLW	LWxMS	MS	RSD
ADG07 (kg/d)	F	0.374	0.516 *	0.300	0.256	0.048
	M	0.440	0.521	0.260	0.282	

PIGLET VITALITY



EAAP 2010 Dauberlieu et al.

DIFFERENTIAL ANALYSES

34.505 spots > differentially expressed probes (DEP)

$y_{ijk} = \mu + sex_i + genotype_j + sex_i{:}genotype_j + batch_i + mixSemen_i + sow_k + \varepsilon_{ijk}$

Benjamini-Hochberg FDR 1 % 2.247 DEP

BIC 39 DEP sex : genotype

\+ 228 DEP sex + genotype

\+ 45 DEP sex

\+ 1962 DEP genotype 86 %

DISCRIMINANT ANALYSES

sPLS-DA : 10 DEP Axis 1 + 5 DEP Axis 2

PHENOTYPIC-TRANSCRIPTOME CO-VARIATION

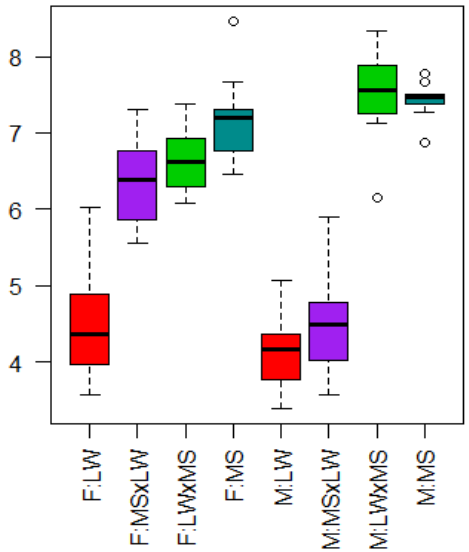
sPLS

GENE ONTOLOGY

GENOTYPE x SEX INTERACTION

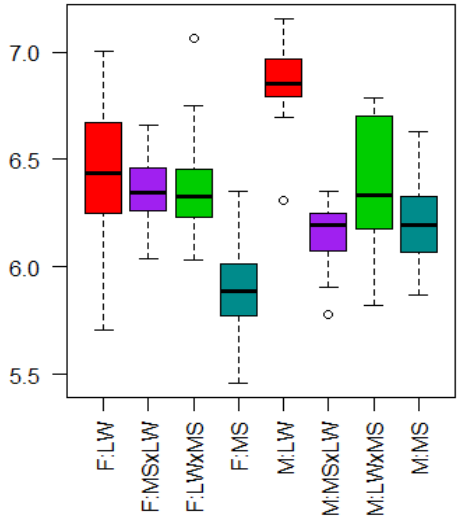
Gene expression

Cox7b SSCX



Mitochondrial respiratory chain

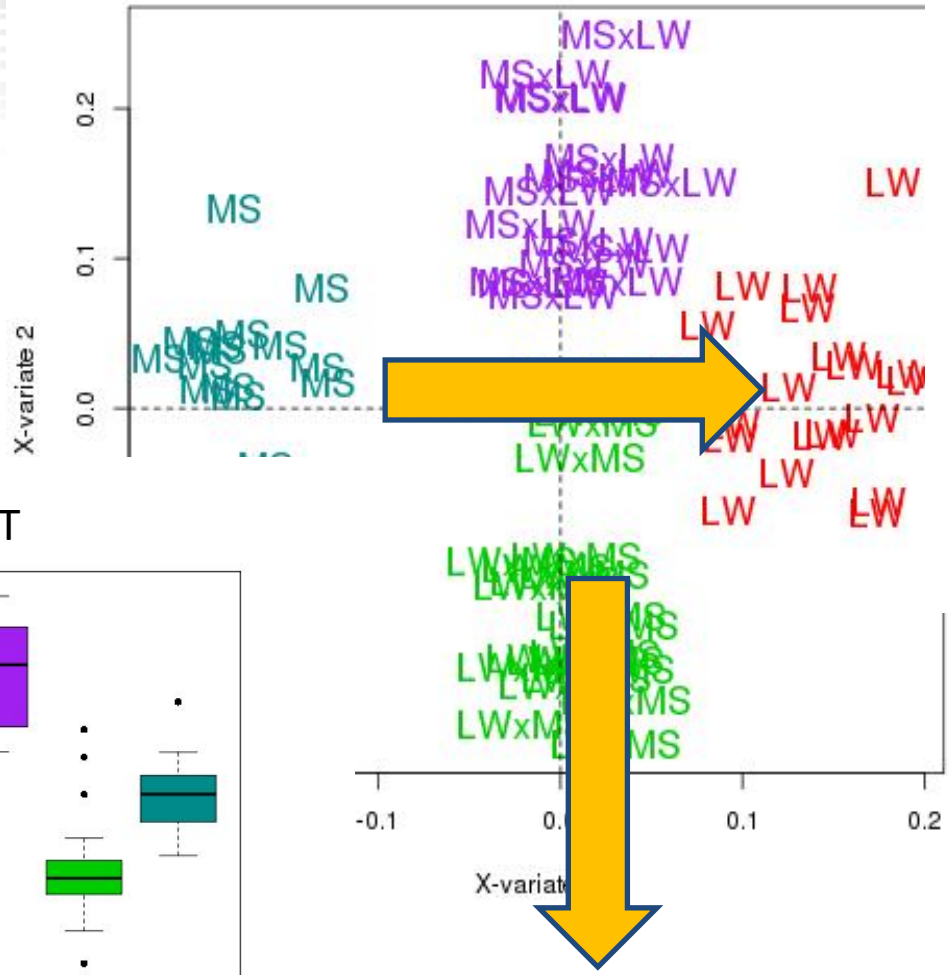
SEPP1 SSC16



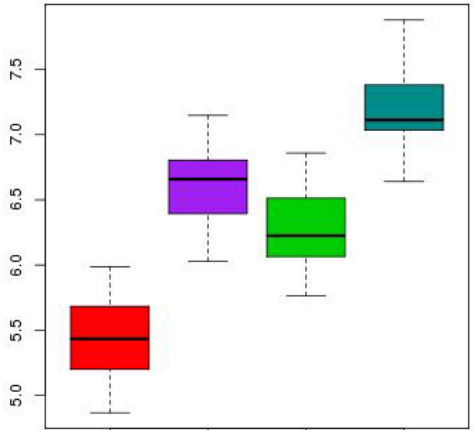
Selenium transport

Is male the weakest sex in LW purebreds ?

GENOTYPE EFFECT



MNF2 MT



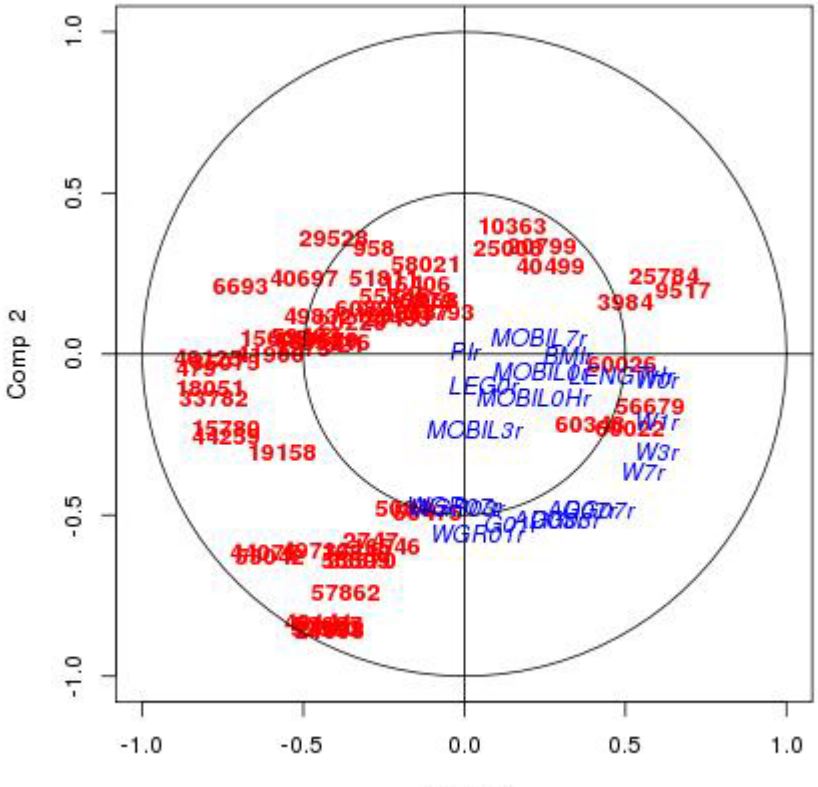
Mitofusin 2, mitochondrial structure

CYTB MT

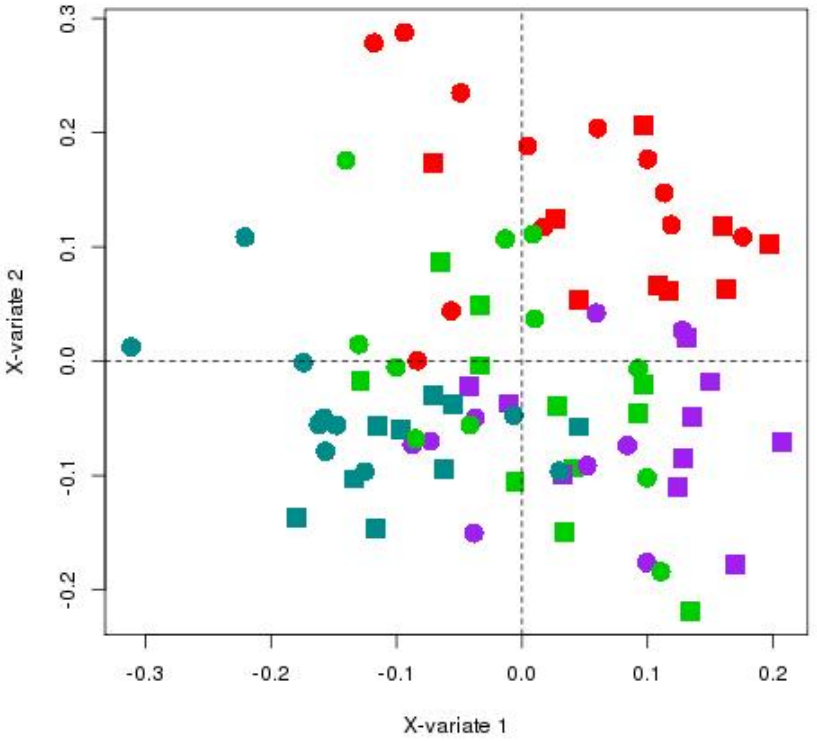
Mitochondrial encoded cytochrome b,
Oxydative phosphorylation

Do the differences refer to vitality or merely differences in body composition?

COVARIATION BETWEEN PHENOTYPE AND GENE EXPRESSION



Clusters of genes that co-vary with growth

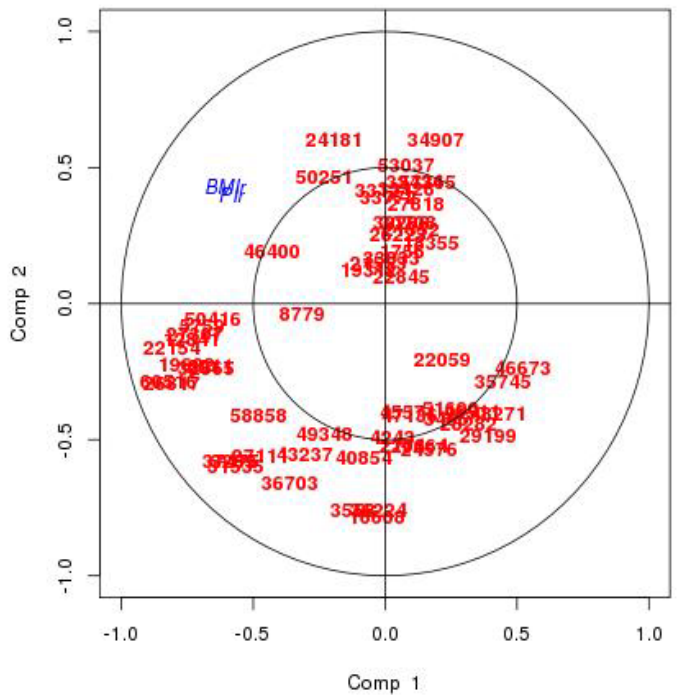


Partitioning according to genotype

LW
MSXLW
LWXMS
MS

Relations with vitality at birth?

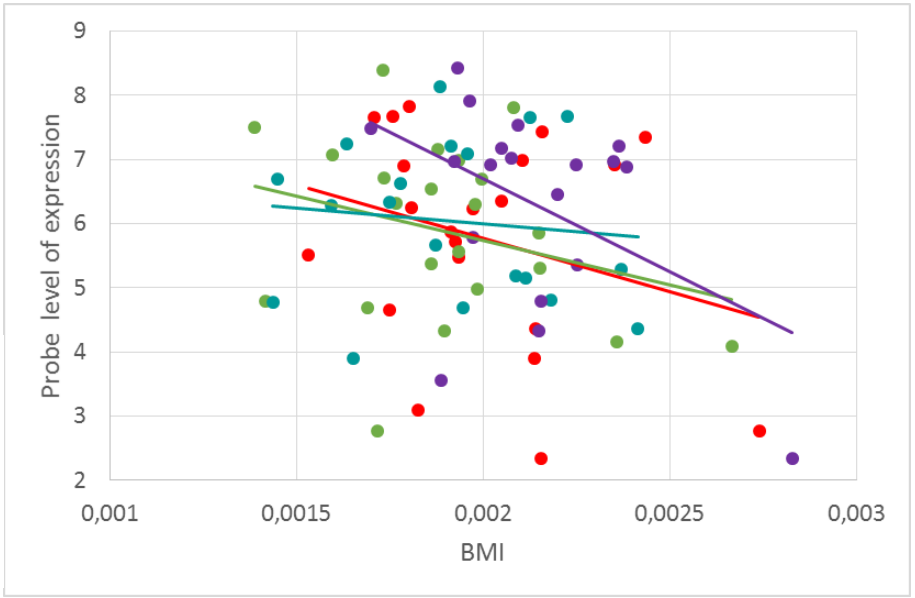
Body mass indexes Probes and Gene expression



6 genes associated with regulation of immune response (HLA-A)

None gene in common with those found for early growth

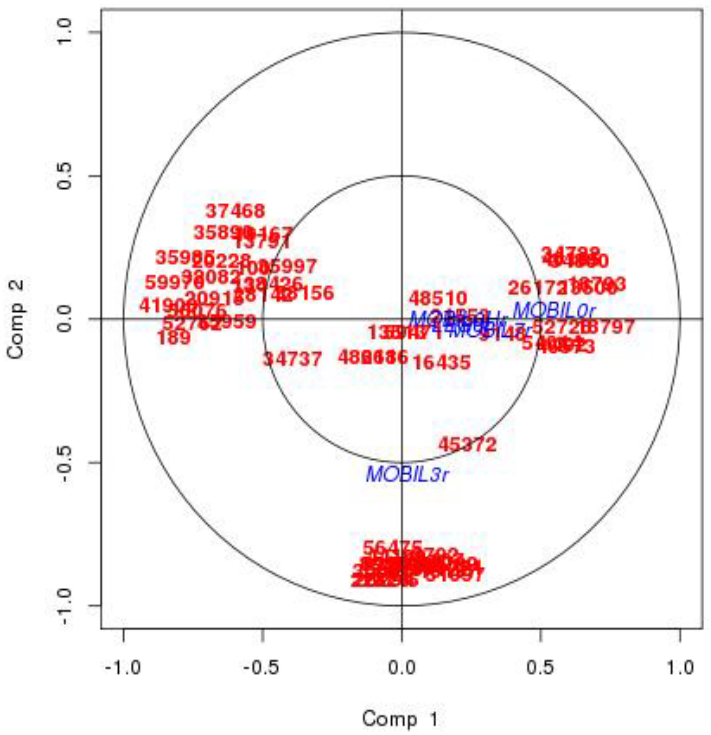
NDRG2 negative regulation of cytokine production

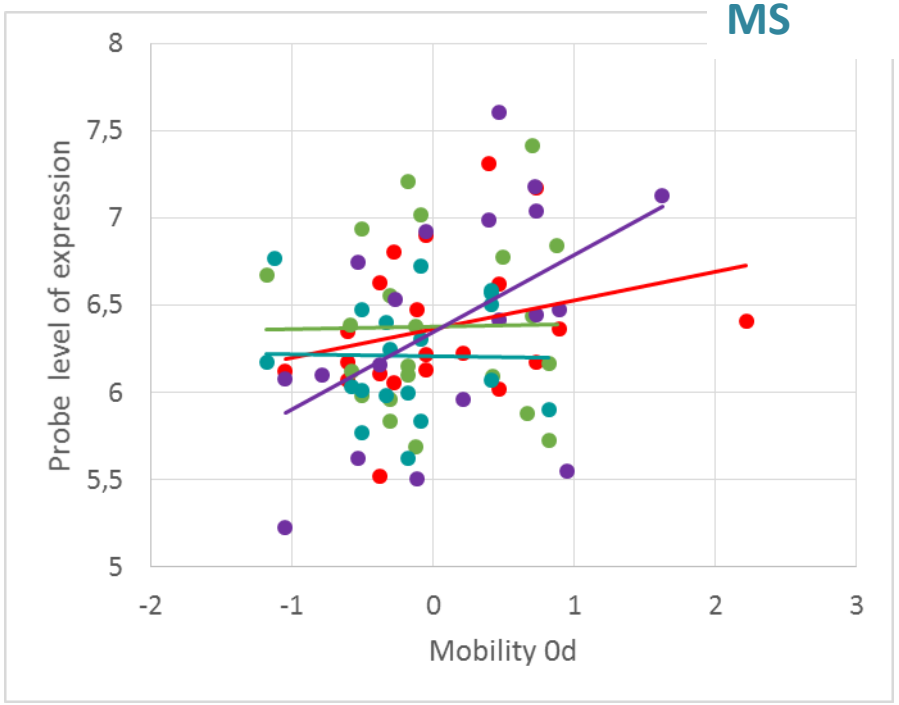


LW
MSXLW
LWXMS
MS

Behavioural traits – Probes and Gene expression

LW
MSXLW
LWXMS
MS





ACTN2 Alpha actinins - diverse group of cytoskeletal proteins

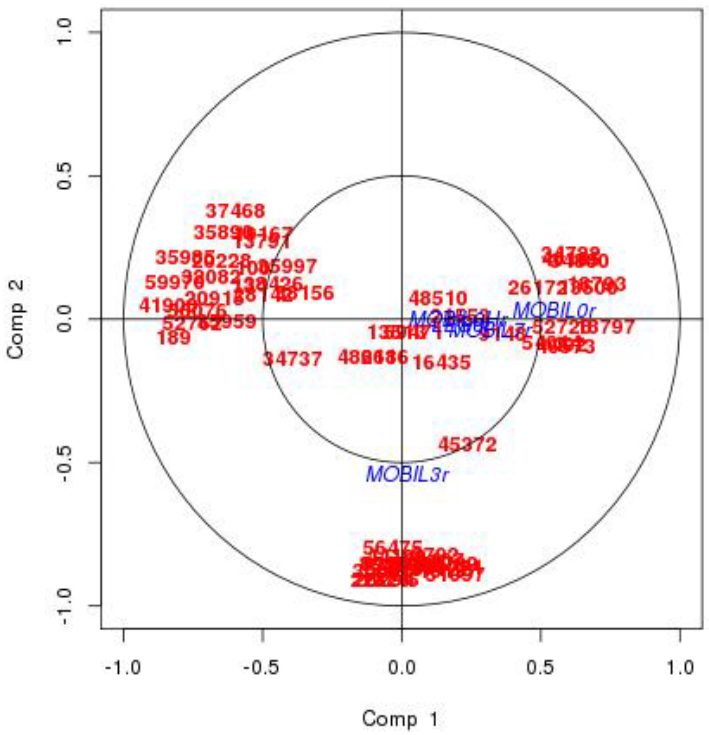
Mobility at birth

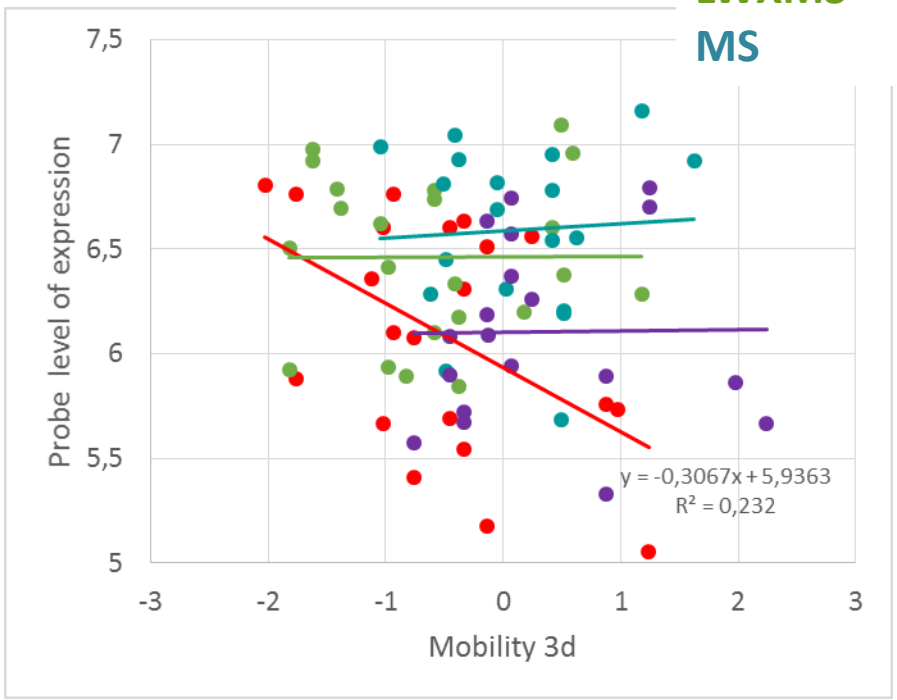
Genes related to

- muscle mass in mice and locomotor activity in rat
- endurance in athletes

Behavioural traits – Probes and Gene expression

LW
MSXLW
LWXMS
MS





Genes related to bone mineral density, bone structure and bone strength in rat

ARL6IP1 ADP-ribosylation factor-like 6 interacting protein 1

⇨ Difficulty / inability to walk

CONCLUSION

From purebreds and crossbreds developped within the same litter

- Different gene expression according to the 4 genotypes
  Maternal and paternal influences on piglet development
- Some relevant DEG according to genotype x sex
- Clusters of genes associated with growth,
  in favour of crossbreds from LW sows
- Promising genes related to immunity for BMI
  and locomotion for early-life behaviour

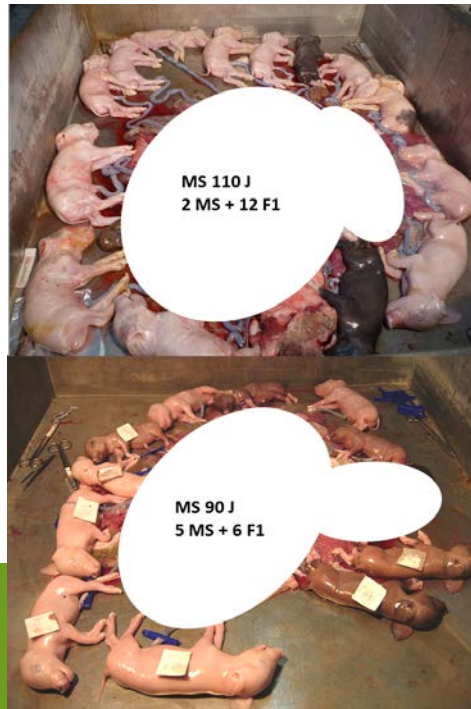
PERSPECTIVES

- Survival at risk mainly in LW PB piglets ; include probes from newborn LW PB dead in early postnatal period

- Further exploration of genes involved in discrimination and relation to identified QTL

- Integrative approach whole blood + specific tissues
- Bridging the gap between piglet and fetus dvp in late gestation

- Validation studies



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Platforms
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Thank you for your attention

INRA
SCIENCE & IMPACT

COMPARISON OF GENOTYPES WITHIN SOW BREED

Piglets alive till d7

Trait	Sex	LW	MSxLW	LWxMS	MS	RSD
W0 (kg)	F	1.036	1.155	1.062	0.853	0.095
	M	1.279	1.322	1.2101	0.913	
LENGTH (cm)	F	23.99 s*	24.13	22.99 *	20.85	0.647
	M	25.58	25.67	23.73 **	21.45	
BMI (%)	F	0.184	0.203	0.207	0.197	0.02
	M	0.193	0.203	0.234	0.205	
W1 (kg)	F	1.102	1.304 **	1.100 *	0.881	0.061
	M	1.364 s****	1.465 s°°	1.122	0.958	
W3 (kg)	F	1.400	1.724 ****	1.299 *	1.060	0.074
	M	1.689 s****	1.902 *	1.327	1.159	
W7 (kg)	F	2.178	2.701 ****	1.972 *	1.604	0.134
	M	2.611 s****	2.928 *	1.992	1.751	

Higher growth in crossbreds, especially the females from LW sows

DATA : 24 sows 282 piglets

Y = genotype * sex + batch + mix + sowID(sow genotype) Lsmeans

COMPARISON OF GENOTYPES WITHIN SOW BREED

Piglets alive till D7

Trait	Sex	LW	MSxLW	LWxMS	MS	RSD
ADG03 (kg/d)	F	0.117	0.193	0.078	0.074	0.031
	M	0.135	0.180	0.035	0.084	
ADG07 (kg/d)	F	0.374	0.516 *	0.300	0.256	0.048
	M	0.440	0.521	0.260	0.282	
WGR03 (%)	F	0.349	0.5334 ****	0.223	0.285	0.045
	M	0.327	0.433 °	0.236	0.296	
WGR07 (%)	F	1.116	1.410 **	0.854	0.947	0.109
	M	1.050	1.212	0.857	0.946	
MOBIL_d3	F	0.98	1.57	1.01	1.44	0.35
	M	1.00	1.57	1.17	1.59	
MOBIL_d7	F	1.60	2.22	1.58	1.62	0.24
	M	1.78	2.11	1.23	1.63	

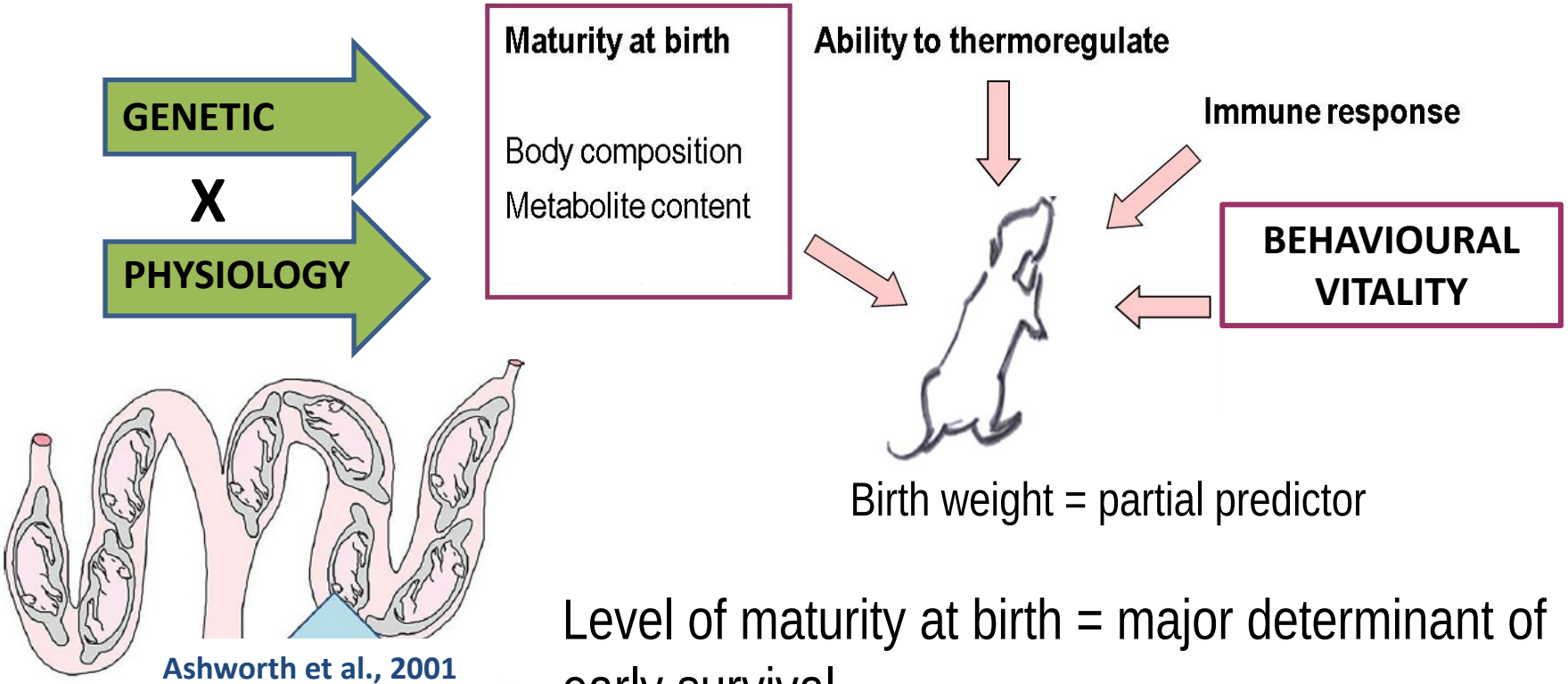
No within-genotype difference according to sex

No difference in behavioural reactivity

SampleName	GeneName	SSC	Description [Source:HGNC Symbol]
42412	ATP4B	11	ATPase, H+/K+ exchanging, beta polypeptide
35894	CHST9	6	carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 9
57627	DEGS1	10	degenerative spermatocyte homolog 1, lipid desaturase (Drosophila)
33624	FAM118A	5	family with sequence similarity 118, member A
19158	GRSF1	8	G-rich RNA sequence binding factor 1
53066	MFN2	*	mitofusin 2
19923	MT-CO2	MT	mitochondrially encoded cytochrome c oxidase II
3187	MT-CYB	MT	mitochondrially encoded cytochrome b
41722	MT-ND2	MT	mitochondrially encoded NADH dehydrogenase 2
13591	PIP4K2A	10	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha
21980	TMPRSS6	5	transmembrane protease, serine 6
60406	MT-ND2	MT	
24149	FAM118A	5	

ROLE OF PIGLET VITALITY ON SURVIVAL-GROWTH

Characteristics of newborn piglet influencing survival and growth



Ashworth et al., 2001

Birth weight = partial predictor

Level of maturity at birth = major determinant of early survival