Identification of Mutations responsible for Prenatal Mortality in Dairy Cattle

D. Boichard, A. Capitan, A. Djari, S.C. Rodriguez, A.Barbat, A. Baur, C. Grohs, B. Weiss, M. Boussaha, R. Lefebvre, D. Esquerré, C. Klopp, D. Rocha, S. Fritz

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Introduction

- Genomic Selection reference populations are excellent resources for QTL mapping
- Embryonic lethals can be found by looking for deficits in homozygous for some haplotypes
- Demonstration by VanRaden et al, 2011, for 5 regions (HH1, HH2, HH3, BH1, JH1)
- Sequencing technologies make it possible to search for the corresponding causative mutations
- Present study : Analysis of the French Holstein, Montbeliarde et Normande breeds

Material

- 47,878 Holstein, 16,833 Montbeliarde, and 11,466 Normande
- Genotyped with the Illumina Bovine 50k Beadchip
- With sire and maternal grandsire genotyped
- Quality control / Imputation of missing genotypes / phasing done in the process of genomic evaluation

  => Direct use of clean, complete and phased data

Methods

- Analysis of 20-marker haplotypes, on a sliding window over the whole genome
- Haplotypes with frequency > 1%
- Counting the number of homozygous *O(k)* for each haplotype k (within k-carrier sires, to reduce sensitivity to frequency estimation)
- Compare it with its expectation *E(k)* under neutrality, accounting for the pedigree structure

$$E(k) = \sum_{i=1}^{ns} p_{ik} \sum_{j=1}^{nmgs} 0.5\,[q_{jk} + f_k]\, n_{ij}$$

p_{ik} = 0.5 or 1

q_{jk} = 0, 0.5, or 1

f_k = allelic frequency

n_{ij} = # progeny

Detection Results

- 33 regions displaying a lack of or a partial deficit in homozygous ($p<10^{-4}$)
- All of them were breed specific
- A partial deficit could be due to
  - Uncomplete linkage disequilibrium
  - Uncomplete penetrance

Detection Results (a) no homozygous

Breed	Name	BTA	Interval (UMD3.1 Mb)	Expected nb of homoz.	Observed nb of	Haplotype freq. (%)	Chi² test
HOL	BY	21	20.2-			3.6	2.6E-12
HOL	HH1	5	61.4-66			2.6	2.2E-05
HOL	*HH2*	*1*	*93.0-97*			*1.7*	*3.3E-03*
HOL	HH3	8	94.0-96			2.5	4.6E-06
HOL	HH4	1	1.9-3.3	49	0	3.6	2.6E-12
HOL	HT8	7	78.8-80.1	15	0	2.1	1.1E-04
HOL	HT10	11	31.5-33.2	24	1	2.2	2.7E-06
HOL	HT16	26	10.4-12.8	26	2	2.0	2.5E-06
MON	MH1	19	27.6-29.4	131	0	9.0	2.5E-30
MON	MH2	29	27.9-29.1	80	1	7.0	1.0E-18
MON	MH4	4	52.0-53.2	21	1	3.5	1.3E-05
MON	MH10	24	22.9-24.6	26	0	2.5	3.4E-07
NOR	NH1	24	38.1-39.2	12	0	1.8	5.3E-04

BY is already known.
HH1-HH3 were described by VanRaden et al (2011)

Detection Results b) a deficit in homozygous

Breed	Name	BTA	Interval (UMD3.1 Mb)	Expected nb of homoz.	Observed nb of homoz.	Haplotype freq. (%)	Chi² test
HOL	HT5	3	45.8-47.6	68	24	3.9	9.5E-08
HOL	HT6	3	49.4-52.6	91	38	4.6	2.8E-08
HOL	HT7	6	51.6-52.6	202	100	6.9	7.1E-13
HOL	HT9	10	74.8-77	40	13	2.9	2.0E-05
HOL	HT11	12				3.2	3.9E-06
HOL	HT12	15	7			5.5	9.3E-11
HOL	HT13	18	5			3.7	1.8E-06
HOL	HT14	19				2.4	4.2E-07
HOL	HT15	20	5			2.7	1.8E-07
HOL	HT17	26	2			1.8	1.1E-06
MON	MH3	2	3			5.1	1.6E-06
MON	MH5	6	73.3				4E-22
MON	MH6	7	80.1				6E-05
MON	MH7	9	84.6				4E-21
MON	MH8	13	76.4				5E-06
MON	MH9	20	24.2				6E-06
MON	MH11	24	33.4-34.6	159	29	7.2	6.4E-25
NOR	NH2	1	145.7-146.8	49	14	3.8	5.7E-07
NOR	NH3	4	92.3-93.8	41	10	5.9	1.3E-06
NOR	NH4	6	37.7-38.9	38	12	5.2	2.5E-05
NOR	NH5	7	3.6-4.6	58	20	1.9	6.0E-07
NOR	NH6	15	59.8-61.1	45	17	1.9	3.0E-05

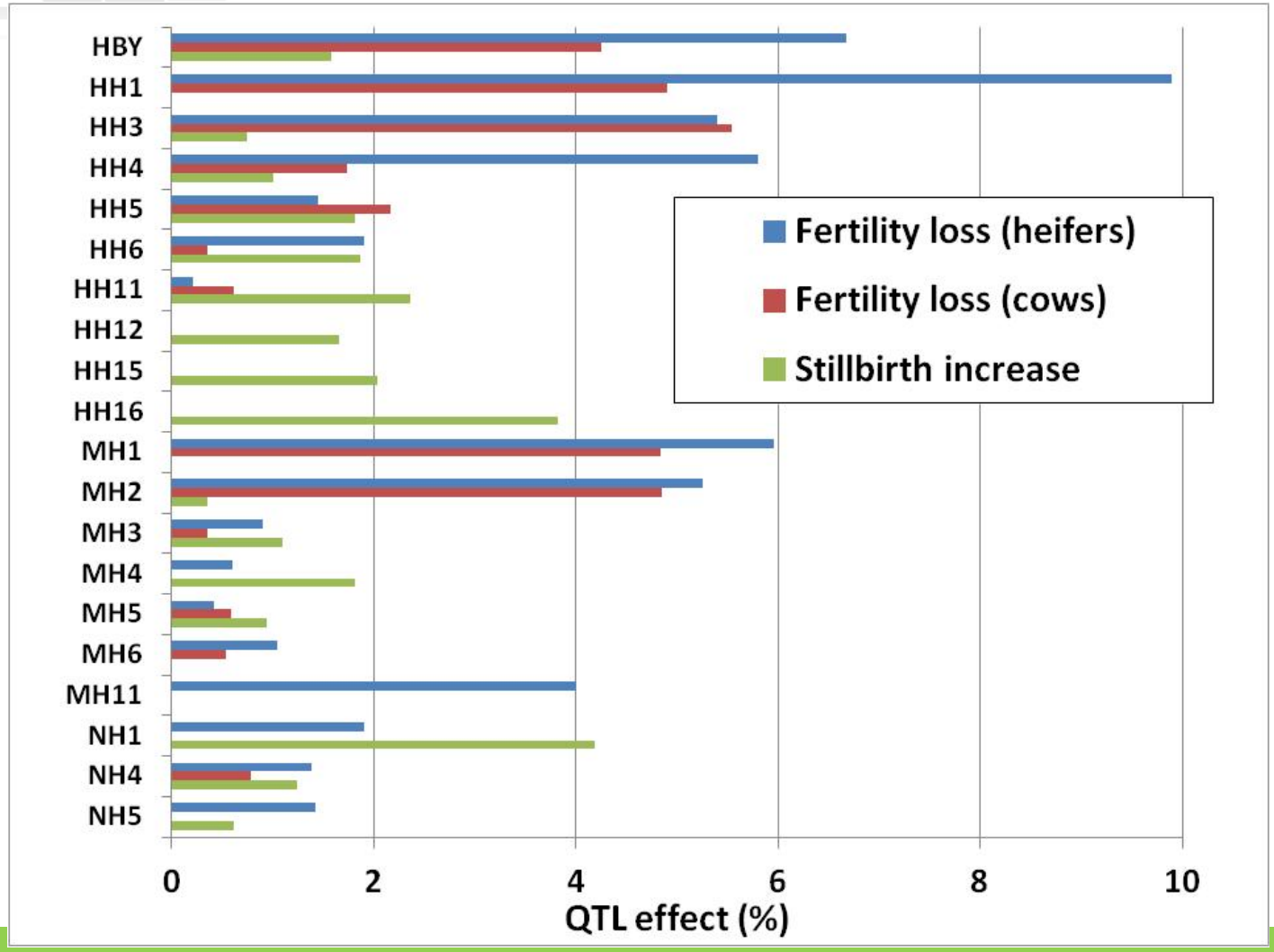
HT5 and HT6 correspond to CVM. Although CVM is lethal, the partial deficit is explained by incomplete linkage disequilibrium

Syndactyly (not lethal, but with a phenotype which explain the elimination from genomic selection

Effects on fertility and stillbirth

- With complete LD and penetrance, a lethal mutation during gestation generates a loss in fertility of ~$\overline{CR}$/8, ie around -5% in cows and -6% in heifers in matings at risk (carrier bull x daughter of carrier sire)
- With complete LD and penetrance, a lethal mutation at birth generates an increase by ~$\overline{LB}$/8, ie around +11-12% in stillbirth rate

Effects on fertility and stillbirth in matings at risk (data from 2000-2010)

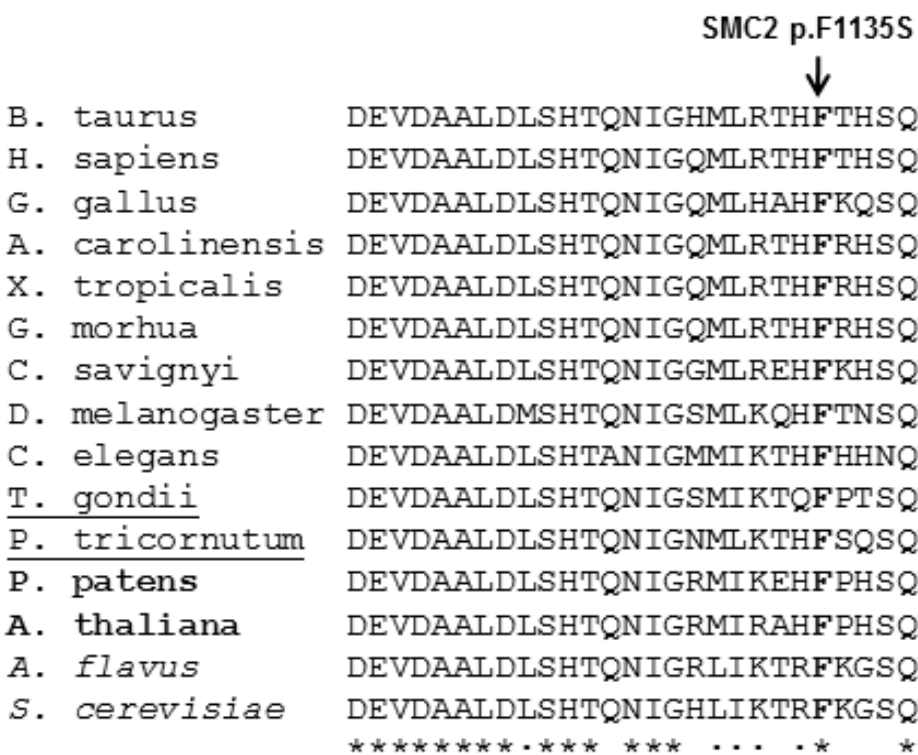


Identification of causative mutations

- Use of 45 bull‘s whole-genome sequences
- Identification of all variants in the intervals +/- 6 Mb
- Filters :
  - Concordant with status
  - No homozygous
  - Allele absent in the other breeds and from the reference sequence
  - In coding sequences
  - Predicted as very deleterious by SIFT and PolyPhen
  - Highly conserved in other species

Identification of causative mutations : HH3

- *nc2)*
- e by a
- n and
- s)



Identification of causative mutations : HH4

- Glycin Amide Ribonucleotide Transformylase gene (GART) on BTA1
- g.1277227A>C
- Substitution of an asparagine by a threonine (p.N290T)
- Asparagine-290 is entirely conserved among eukaryotes
- GART is required for biosynthesis of purines that are key components of molecules as important as DNA, RNA, ATP, etc.

A candidate for MH1

- Sex steroid-binding globulin (SHBG) on BTA19
- g.27956790C>T on BTA19
- Premature stop codon resulting in the loss of 90% of the protein (SHBG p.Q52X)
- SHBG is an androgen transporter that regulates the plasma metabolic clearance rate of steroid hormones

Identification of causative mutations : MH2

- Solute carrier family 37 member 2 protein (SLC37A2) on BTA29, involved in glucose-6-phosphate trafficking
- g.28879810C>T predicted to introduce a stop codon at the very beginning of the protein (SLC37A2 p.R12X)
- Numerous examples of genetic defects caused by deficiency in solute carrier protein have been reported
- Altered trafficking of a particular type of molecule, leading to a lack or an excess of this solute in a given cell compartment
- Lethal KO in mice

Identification of causative mutations : MH5

- NOA1 gene on BTA6
- Encodes for nitric oxide-associated protein 1
- g.74025302del is predicted to cause a frame-shift change of leading to the truncation of half of this protein (p.D363Rfs9X)
- Key role in mitochondrial protein translation
- Mice homozygous for a knock-out allele die during organogenesis and display developmental retardation and abnormal mitochondrial morphology and physiology

Large scale genotyping

- These mutations (among others) were added on the EuroG10K chip
- No homozygous found in 3849 Holstein, 909 Normand and 2931 Montbéliard for HH3, HH4, MH2, NOA1
- One good candidate for MH1 excluded, in SHBG gene

Percentage of heterozygous

QTL	Gene	Polymorphism	HOL	MON	NOR
HH1	APAF1	p.Q579X	**3,5%**	0,0%	0,0%
HH3	SMC2	p.F1135S	**6,7%**	0,0%	0,0%
HH4	GART	p.N290T	**8,0%**	0,0%	0,0%
MH2	SLC37A2	p.R12X	0,0%	**12,1%**	0,0%
MH5	NOA1	p.D363Rfs9X	0.0%	**17.3%**	0.0%

Conclusions

- This study demonstrates the feasibility to detect and identify embryonic lethals
- For more information, Fritz et al, 2013, Plos One, 8, e65550
- Selection against these defects is under way
- Usefulness of large scale genotyping through the LD chip
- Many other regions showing homozygotes deficit, still to characterize

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