Chasing deleterious variants in Holstein dairy cattle

Paolo Ajmone Marsan
Istituto di Zootecnica, Università Cattolica del Sacro Cuore, Piacenza, Italy
paolo.ajmone@unicatt.it

XIX EAAP/Interbull meeting
Tallinn, Estonia - August 28, 2017
Marco Milanesi

Marco Milanesi 1,2,3,4, Stefano Capomaccio 1,4, Yuri T. Utsunomiya 3,5, Lorenzo Bomba 1,6, Licia Colli 1,7, Elisa Eufemi 1,7, Katia Cappelli 4, Ezequiel L. Nicolazzi 8, Stefano Biffani 9,10, Filippo Biscarini 8, Tamiris S. Aguiar 3,5, Raffaele Mazza 10, Jan-Thijs B.C.H.M. van Kaam 11, Raffella Finocchiaro 11, Riccardo Negrini 1,10, José F. Garcia 2,3, Carl J. Rubin 12, Alessandro Nardone 13, Nicolò P.P. Macciotta 14, Alessio Valentini 13, John L. Williams 15

1 Istituto di Zootecnica, Università Cattolica del Sacro Cuore, Piacenza, Italy;
2 Departamento de Apoio, Produção e Saúde Animal, UNESP Araçatuba, São Paulo, Brazil;
3 Collaborating Centre on Animal Genomics and Bioinformatics, IAEA, Araçatuba, São Paulo, Brazil;
4 Dipartimento Medicina Veterinaria, Università degli Studi di Perugia, Perugia, Italy;
5 Departamento de Medicina Veterinária Preventiva e Reprodução Animal, UNESP, Jaboticabal, São Paulo, Brazil;
6 Department of Human Genetics, The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK;
7 BioDNA, Centro di Ricerca sulla Biodiversità e sul DNA Antico, Università Cattolica del Sacro Cuore, Piacenza, Italy;
8 Bioinformatics core facility, PTP Science Park, Lodi, Italy
9 Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche (CNR), Lodi, Italy
10 Associazione Italiana Allevatori, Rome (RM), Italy
11 Anafi - Associazione Nazionale Allevatori Friulana Italiana, Cremona, Italy
12 Science for Life Laboratory Uppsala, Department of Medical Biochemistry and Microbiology, Uppsala University, Husargran, Uppsala, Sweden.
13 Department for innovation in biological, agro-food and forest systems (DIBAF), Universita della Tuscia, Viterbo, Italy
14 Dipartimento di Agraria, Università degli Studi di Sassari, Sassari, Italy
15 School of Animal and Veterinary Sciences, Faculty of Sciences, University of Adelaide, Roseworthy, Australia
16 PRONUTRIGEN, Centro di Ricerca Nutrizionumica e Proteomica, Università Cattolica del Sacro Cuore, Piacenza, Italy;
Fertility index in Italian Holstein

- Negative energy balance
- Increased inbreeding (0.05 per year – Mrode et al., 2005)
- Deleterious recessives
Deleterious variant:

*reduce the reproductive fitness of carriers, and would thus be targeted by purifying natural selection* 

**Unexpected high frequency**

- Demographic events (e.g. bottlenecks)
- Breeding practice
  - In linkage with favourable alleles (hitch-hiking)
  - Spread by important AI sires
- Variants in balancing selection (e.g. beta-globin - malaria)

MacArthur *et al.*, 2014
QTL MAPPING

Fortes et al., 2013

Ajmone Marsan et al.
Harmsful recessive effects on fertility detected by absence of homozygous haplotypes

P. M. VanRaden,*† K. M. Olson,‡ D. J. Null,* and J. L. Hutchison*

*Animal Improvement Programs Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705-2350
†National Association of Animal Breeders, Columbia, MO 66205-1033

• No affected population
• No phenotype is used
Identification of a Nonsense Mutation in CWC15 Associated with Decreased Reproductive Efficiency in Jersey Cattle

Tad S. Sonstegard1, John B. Cole2*, Paul M. VanRaden3, Curtis P. Van Tassell1, Daniel J. Null2, Steven G. Schroeder3, Derek Bickhart1, Matthew C. McClure1

1 Bovine Functional Genomics, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, United States of America, 2 Animal Improvement Programs Laboratories, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, United States of America

Detection of Haplotypes Associated with Prenatal Death in Dairy Cattle and Identification of Deleterious Mutations in GART, SHBG and SLC37A2

Sébastien Fritz1,2, Aurelien Capitan1,2, Anis Djari3, Sabrina C. Rodriguez2,3, Anne Barbaï3, Auréïa Baur1,2, Cécile Grohs3, Bernard Weiss3, Meikki Boussaha3, Diane Esquerre3, Christophe Klopp3, Dominique Rocha3, Didier Boichard4

1 UNCEDA, Genetics Team, Paris, France, 2 INRA, UMR1313 Animal Genetics and Integrative Biology, Jouy-en-Josas, France, 3 INRA, Signae, URBIS Biométrie et Intelligence Artificielle, Castanet-Tolosan, France, 4 INRA, GéT Genomics Facility, UMR444 Laboratoire de Génétique Cellulaire, Castanet-Tolosan, France

Bovine Exome Sequence Analysis and Targeted SNP Genotyping of Recessive Fertility Defects BH1, HH2, and HH3 Reveal a Putative Causative Mutation in SMC2 for HH3

Matthew C. McClure1, Derek Bickhart2, Dan Null3, Paul VanRaden5, Lingyang Xu1, George Wiggans3, George Liu1, Steve Schroeder1, Jarret Glasscock5, Jon Armstrong5, John B. Cole2, Curtis P. Van Tassell1, Tad S. Sonstegard1

1 United States Department of Agriculture, Agriculture Research Service, Bovine Functional Genomics Laboratory, Beltsville, Maryland, United States of America, 2 United States Department of Agriculture, Agricultural Research Service, Animal Improvement Programs Laboratory, Beltsville, Maryland, United States of America, 3 Collegenetics, St. Louis, Missouri, United States of America

Homozygous haplotype deficiency reveals deleterious mutations compromising reproductive and rearing success in cattle

Hubert Pausch1, Hermann Schwarzenbacher2, Johann Burgstaller3, Krzysztof Fisikowski4, Christine Wurmser1, Sandra Jansen1, Simone Jung1, Angelika Schnieke5, Thomas Wittek6 and Ruedi Fries1

1 UNCEDA, Genetics Team, Paris, France, 2 INRA, UMR1313 Animal Genetics and Integrative Biology, Jouy-en-Josas, France, 3 INRA, Signae, URBIS Biométrie et Intelligence Artificielle, Castanet-Tolosan, France, 4 INRA, GéT Genomics Facility, UMR444 Laboratoire de Génétique Cellulaire, Castanet-Tolosan, France, 5 INRA, Genomic and Biotechnological Sciences, UMR1313 Animal Genetics and Integrative Biology, Jouy-en-Josas, France, 6 INRA, Animal Genetics and Integrative Biology, Jouy-en-Josas, France
Use of Sequence

Proceedings, 10th World Congress of Genetics Applied to Livestock Production

NGS-based Reverse Genetic Screen Reveals Loss-of-function Variants Compromising Fertility in Cattle.

C. Charlier¹, W. Li¹, C. Harland¹,², M. Littlejohn², F. Creagh², M. Keehan², T. Druet¹, W. Coppitières¹, R. Spelman² & M. Georges¹

¹Unit of Animal Genomics, GIGA-R & Faculty of Veterinary Medicine, University of Liège, Belgium,
²Livestock Improvement Corporation, New Zealand.

No observed homozygotes

<table>
<thead>
<tr>
<th></th>
<th>Ref/Ref</th>
<th>Alt/Ref</th>
<th>Alt/Alt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
Workflow

Data production

Bulls HD-SNP genotyping

1009 AI Italian Holstein bulls; 800K SNPs

Software used: PLINK 1.9; GHap

62,215 haploblocks; 445,042 haplotypes

HD-SNP phasing and haplotyping

147,209 variants

VEP and SIFT analyses

18 bulls; 54Mbp

Exome sequencing

18 bulls; 54Mbp
Candidate discovery

Homozygous Haplotype Deficiency (HHD) blocks

261 deleterious haplotypes

8,233 deleterious variants

Data production

Bulls HD-SNP genotyping

HD-SNP phasing and haplotyping

Homozygous Haplotype Deficiency (HHD) blocks

Candidate deleterious variants (DelVar)

VEP and SIFT analyses

Exome sequencing

Ajmone Marsan et al. EAAP 2017
Candidate discovery

Data production

- Bulls HD-SNP genotyping
- HD-SNP phasing and haplotyping
- VEP and SIFT analyses
- Exome sequencing

Homozygous Haplotype Deficiency (HHD) blocks

DelVar mapping in HHD blocks (HHDelVar)

Candidate deleterious variants (DelVar)

73 deleterious variants in 56 genes mapping in 51 deleterious haploblocks

Ajmone Marsan et al.

EAAP 2017
In silico assessment

Candidate discovery

Data production

Homozygous Haplotype Deficiency (HHD) blocks
DelVar mapping in HHD blocks (HHDelVar)
Candidate deleterious variants (DelVar)

Proven functional association to fertility/mortality
Conservation in vertebrate evolution
Comapping with QTLs for fertility traits

Bulls HD-SNP genotyping
HD-SNP phasing and haplotyping
VEP and SIFT analyses
Exome sequencing

KRT8; DLAT1
2 good candidates
7 strong candidates
ESPL1; RPTOR; TECRL; SETB2; SPATS2; UBR1; EPB42

Software: PhyloP; FastCons
Database: OMIM; MGI

Workflow

Ajmone Marsan et al.
EAAP 2017
Data production

- Bulls HD-SNP genotyping
- HD-SNP phasing and haplotyping
- VEP and SIFT analyses
- Exome sequencing

Candidate discovery

- Homozygous Haplotype Deficiency (HHD) blocks
- Candidate deleterious variants (DelVar)

DelVar mapping in HHD blocks (HHDelVar)

In silico assessment

- Proven functional association to fertility/mortality
- Conservation in vertebrate evolution
- Comapping with QTLs for fertility traits

Validation

- Good and Strong candidates
- 1000bull Sequence data

Workflow

Ajmone Marsan et al.  EAAP 2017
RUN5 (*Bos taurus* + *B. indicus*):

- 1682 animals
- 70 breed

<table>
<thead>
<tr>
<th>Breed</th>
<th>N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holstein</td>
<td>450</td>
</tr>
<tr>
<td>Fleckvieh</td>
<td>145</td>
</tr>
<tr>
<td>Angus</td>
<td>141</td>
</tr>
<tr>
<td>Brown Swiss</td>
<td>105</td>
</tr>
</tbody>
</table>
## Genotype Frequency in 1000 Bull: 450 Holstein

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Mutation Type</th>
<th>HOM. 1</th>
<th>HET.</th>
<th>HOM. 2</th>
<th>H-W</th>
</tr>
</thead>
<tbody>
<tr>
<td>KR8</td>
<td>5_27217392</td>
<td>T/C Splice variant</td>
<td>10</td>
<td>127</td>
<td>313</td>
<td>N.S.</td>
</tr>
<tr>
<td>BUB1</td>
<td>11_1568943</td>
<td>G/A Splice variant</td>
<td>333</td>
<td>110</td>
<td>7</td>
<td>N.S.</td>
</tr>
<tr>
<td>TECRL</td>
<td>6_81580936</td>
<td>T/C Missense</td>
<td>1</td>
<td>22</td>
<td>427</td>
<td>N.S.</td>
</tr>
<tr>
<td>UBR1</td>
<td>10_38335826</td>
<td>G/A Missense</td>
<td>3</td>
<td>55</td>
<td>392</td>
<td>N.S.</td>
</tr>
<tr>
<td>......</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>......</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8 out of nine candidate mutations not confirmed!
### Genotype Frequency in 1000Bull: Holstein and Entire Dataset

**K→Q** at position 641

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Mutation</th>
<th>Holstein</th>
<th>HOM. 1</th>
<th>HET.</th>
<th>HOM. 2</th>
<th>H-W</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLAT</td>
<td>15_22697929</td>
<td>C/A Missense</td>
<td>Holstein</td>
<td>365</td>
<td>85</td>
<td>0</td>
<td>0.02*</td>
</tr>
<tr>
<td>DLAT</td>
<td>15_22697929</td>
<td>C/A Missense</td>
<td>Entire dataset</td>
<td>1594</td>
<td>88</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Allele frequency of derived allele in Holstein: 9.4%

Derived allele very rare in non Holstein breeds
HAPLOTYPING ALIGNMENT IN HETEROZYGOTES

= reference allele

= alternative allele

--- variants ---

CHR in cis

CHR in trans

chr15_22679557-chr15_22711644 = reference allele

= alternative allele

Missens variant
- *DLAT* (dihydrolipoamide S-acetyltransferase) gene catalyses the conversion of pyruvate to acetyl coenzyme A.
- Up-regulated in the endometrium of pregnant cows
- Expressed in mouse testis and associated with epididymal sperm maturation and with sperm motility
- *DLAT* co-maps with two male fertility QTLs, one associated with semen volume in Holstein and the second with scrotal circumference in Angus.
- The deleterious missense variant changes the 641th amino acid (over 647) in domain associated to energy metabolism.
- The mutation site is highly conserved across vertebrates
CONCLUSIONS
Have we identified a recessive deleterious causal mutation in *DLAT* gene?
May be.........
<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Mutation</th>
<th>Dataset</th>
<th>HOM. 1</th>
<th>HET.</th>
<th>HOM.2</th>
<th>H-W</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBR1</td>
<td>10_38335826</td>
<td>G/A Missense</td>
<td>Holstein</td>
<td>3</td>
<td>55</td>
<td>392</td>
<td>N.S</td>
</tr>
<tr>
<td>UBR1</td>
<td>10_38396251</td>
<td>G/A Intron</td>
<td>Holstein</td>
<td>0</td>
<td>167</td>
<td>283</td>
<td>8,307E-09***</td>
</tr>
<tr>
<td>UBR1</td>
<td>10_38396251</td>
<td>G/A Intron</td>
<td>Entire dataset</td>
<td>0</td>
<td>513</td>
<td>1169</td>
<td></td>
</tr>
<tr>
<td>Intron variant</td>
<td>CHR in trans</td>
<td>CHR in cis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>--------------</td>
<td>------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ajmone Marsan et al.
Institute of Zootechnics

Thank you for your attention!

Ajmone Marsan et al.

EAAP 2017