Milking frequency modifies DNA methylation at a CSN1S1 gene regulatory region in the bovine mammary gland

M. Nguyen, L. Galio, B. Pétridou, M. Charlier, C. Kress
INRA, UR1196 GPL, F-78352 Jouy-en-Josas, France

A. Gabory, H. Jammes
INRA, UMR1198 BDR, F-78352 Jouy-en-Josas, France

M. Boutinaud, F. Dessauge
INRA, UMR1348 PEGASE, F-35590 Saint-Gilles, France

Eve Devinoy
INRA, UR1196 GPL, France
EpigRAni project funded 2010-2013
Background

- Milk production is not only genetically defined.
- Nutrition, animal health, livestock conditions or even the environment can induce long term effects. Once daily milking (1X) reduces milk production.
- Reductions in milk production during mammary involution\(^1\) or in the case of mastitis\(^2\) have been related to a decrease in CSN1S1 gene expression and associated with an increase in DNA methylation at one of the distal regulatory regions of the gene, located close to two Prolactin Responsive Elements (Stat5RE).

Aims of the project

- What are the consequences of once daily milking in the cow, on CSN1S1 gene expression and on DNA methylation profiles around this gene?
- Which mechanisms can explain the long term effects induced by unilateral once daily milking (1X) versus twice daily milking (2X)?

\(^1\) Singh et al (2010) J. Mammary Gland Neoplasia

Eight Holstein heifers
Well balanced mammary development between left and right half udders

Calving
53 days in milk (2X)

P1, 2X 2X
P2, 1X 2X
P3, 2X 2X

Daily Milk Production/half udder

Biopsies

RNA IHC1 DNA IHC2 MEC IHC3 EM protein

Marion Boutinaud, PEGASE
Once daily milking reduces both milk production and CSN1S1 gene expression

Variations in transcript accumulation (% relative to 2X)

Significant differences:
***, P<0.001
*, P<0.05

Marion Boutinaud, PEGASE
DNA methylation at a distal regulatory region of the CSN1S1 gene

DNA methyltransferases
Methyl-binding proteins
Histone Deacetylases
Transcription repressor

Gene silencing e.g. TSG

-10276 -10216 -10196 -9917

CpG1 CpG2 CpG3

-9917

CpG4

Stat5 Stat5

CSN1S1

DNA Methylation patterns « CpG1-CpG2-CpG3 » for different cows during lactation

Three non-methylated CpG

Three methylated CpG

2x daily milking

1x daily milking

Number of clones for each profile (% , n=96)

***, p<0.0001
1X vs 2X induces a significant increase in DNA methylation at two CpG sites.

**DNA methylation (%)**

![Graph showing DNA methylation levels at CpG sites 1, 2, 3, and 4 for different treatments: MG Lact 2X, MG Lact 1X, MG Pregnancy, and Liver. Significant differences vs MG lactation TDM are indicated with asterisks: *, p<0.05; **, p<0.01; ***, p<0.001. (n=8, Wilcoxon test).**
Conclusions

Unilateral once daily milking induces:

- A significant 37% decrease in milk production. A long term effect with a 7% decrease in milk production when 2X daily milking is resumed
- A significant 50% decrease in CSN1S1 mRNA accumulation in the MG
- A significant 12 to 25% increase in DNA methylation at two CpG sites in a distal region of CSN1S1. A methylation state expected to be maintained until DNA methylation marks are passively erased through cell division.
- No modification in the methylation of a CpG site located within a distal Stat5RE. We are currently studying the affinity of this Stat5RE for Stat5 as compared to its neighbours and whether its methylation state may affect Stat5 binding in vitro (EMSA).
Further questions

- What is the precise role played by this distal region and its epigenetic status as compared to the whole upstream region in CSN1S1 gene expression?

- Epigenetic modifications in this region have been described during involution and related to alteration in Stat5 signaling post weaning. Do we have an alteration of Stat5 signaling during unilateral once daily milking?

- Through modifications in DNA methylation, is the chromatin structure around Stat5RE modified and in turn can it alter chromatin loop formation and the cooperation with other Stat5RE i.e. Stat5 tetramer association?

- How do these epigenetic modifications relate with ncRNA transcription which occurs in both sense and antisense orientation in this 10 kb region?

Minh Nguyen (PhD)
Molecular biology
Animal genetics